

## Some functional properties of lupin proteins modified by lactic fermentation and extrusion

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### Abstract

The aim of this work was to determine modifying effects of lactic fermentation and extrusion processes on functionality of lupin proteins. Protein content, surface hydrophobicity, water absorption capacity (WAC), water solubility index (WSI) and emulsifying properties (EAI, ESI) of protein preparations obtained from lupin seeds (*Lupinus luteus*, *Lupinus albus*, *Lupinus angustifolius*), with various contents of hull, were analyzed. Changes of protein properties were affected by lupin cultivar, hull content and applied processing method. An increase of soluble protein content after controlled lactic fermentation of lupin seeds, and changes of surface protein hydrophobicity, WAC and WSI values after each treatment and significant worsening of protein emulsifying properties were observed. Correlations were found between parameters examined in this study.

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**Keywords:** Lupin seeds; Proteins; Functional properties; Surface hydrophobicity; Lactic acid fermentation; Extrusion

### 1. Introduction

Quality and technological usability of food proteins are determined by their nutritional values and functional properties. When the protein is considered, not as a main food component, but as one of many constituents, its functionality can be an even more important evaluation criterion than the nutritional value.

Some changes in the methodology of evaluation of protein functionality introduced by Kinsella (1976) have been suggested by Schwenke (2001). In his opinion, the term “functional potential” better reflects the relation-

ship between structure and functional properties of proteins. Functional properties of proteins in food products result from interactions between a protein and other food components, such as other proteins, polysaccharides, lipids, phenols, and phytic acid.

Solubility, water, lipids or aroma-binding ability, as well as some surface-active and interfacial protein properties, such as emulsifying and foaming, also result from similar interactions. Viscosity and gelling reflect hydrodynamic properties of protein macromolecules and, in particular, their true shape and size. Therefore, it is generally accepted that the so-called “functional potential” of a protein means its ability to create, during processing, all physicochemical properties which characterize the protein structure, including ability to change conformation. The term “functional properties” should rather be replaced by “techno-functional properties” since it describes the behaviour of food component blends (food

*Abbreviations:* EAI, emulsifying activity index; ESI, emulsion stability index; WAC, water absorption capacity; WSI, water solubility index.

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system) under optimal conditions of a technological process (Schwenke, 2001).

Numerous factors should be taken into consideration to explain behaviour of proteins in food systems (Sikorski, 2001). Among primary factors, there are amino acid composition, their sequence and molecular weight. These factors affect secondary structure of protein, its hydrophobicity, the net charge and charge distribution, flexibility of the molecule and isoelectric point. Surface hydrophobicity is a unique property of proteins, correlating with their functional properties, such as solubility, water absorption, gelation, emulsifying and foaming properties (Kato & Nakai, 1980; Kohnhorst & Mangino, 1985; Nakai, Ho, Tung, & Quinn, 1980; Nakai, Li-Chan, & Hayakawa, 1986; Townsend & Nakai, 1983; Tsutsui, Li-Chan, & Nakai, 1986; Voutsinas & Nakai, 1983).

Protein functionality also depends on the so-called extrinsic factors, such as character of the solvent, temperature, pH value, ionic strength, divalent cations, denaturants, other macromolecules, lipids and activities of enzymes (Zayas, 1996).

Processing of food leads to a variety of desirable and undesirable changes resulting from chemical, enzymatic and physical, both intramolecular and intermolecular, interactions. Our successful approach to improve nutritional value and organoleptic properties of lupin protein preparations is only one example among numerous studies done in this area. Lactic acid fermentation and extrusion, widely used in the food industry, resulted the removal of the undesirable “beany” flavour well recognized in legumes. However, not all changes caused by fermentative and hydrothermal processes of lupin seeds were desirable. For instance, significant qualitative and quantitative changes of the oligosaccharide profile were observed (Lampart-Szczapa et al., 2003). Similar changes were noticed with the tocopherol content (Nogala-Kańska, Lampart-Szczapa, Janczak, Malinowska, & Kossowska, 2003).

This research was done to characterize selected functional properties of protein preparations obtained by the use of lactic acid fermentation and extrusion of lupin seeds.

## 2. Materials and methods

### 2.1. Samples and chemicals

Lupin seeds were collected in the same year, 2001, and purchased from the Plant Breeding and Acclimatization Station at Przebedowo near Poznan (Poland). Protein preparations were prepared from seeds of the following lupin cultivars: *Lupinus luteus* Juno and Parys, *Lupinus albus* Boros, Butan and *Lupinus angustifolius* Baron, Cesar. Folin-Ciocalteu reagent was from Merck (Darmstadt, Germany), while ANS (1-anilino-8-naph-

thalenesulfonate magnesium salt) was from Sigma Chemical Co, St. Louis, MO, USA. Commercially produced soybean oil, purchased in Poland, was used for determination of emulsifying properties. Bovine albumin fraction V from SERVA Co, Germany was used as a protein standard. All reagents were of analytical grade.

### 2.2. Treatment

#### 2.2.1. Preparation

Lupin seeds were ground under lab conditions with a Record impact mill and divided with a pneumatic separator and proper sieves into fractions with particles having maximal diameters below 2 mm and various hull contents. Fermented, extruded or fermented and then extruded samples were examined in this study.

#### 2.2.2. Lactic acid fermentation

Blend of the strains, *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Lactobacillus brevis* were used for fermentation of lupin seeds under conditions recommended as optimal for their development. Fermentation was carried out with inoculum in an amount equal to 10% of sample weight and raw material humidity of 60%, in hermetic flasks, incubated at 30 °C, until pH reached values in range of 4.0–4.2, for approx. 20–22 h.

#### 2.2.3. Extrusion

Extrusion was accomplished using laboratory double-screw extruder, ZSK 25 P8.2 E (KruppWerner & Pfeiderer GmbH). Humidity of raw material was kept at 35% by temperatures 95/120/140/130 °C at individual sections of the extruder.

### 2.3. Analysis

#### 2.3.1. Protein content

The soluble protein content was estimated colorimetrically with the Folin-Ciocalteu reagent according to the adopted procedure of Lowry, as described by Ładoński and Gospodarek (1986). 0.01 M phosphate buffer, pH 7.0, was the solvent under following conditions: sample to buffer ratio, 1:100, extraction time, 30 min, temperature, 20 °C. Absorption of protein solutions was measured at 750 nm.

#### 2.3.2. Surface hydrophobicity

Surface hydrophobicity of examined protein preparations was determined on soluble protein using a fluorescence probe ANS (1-anilino-8-naphthalenesulfonate magnesium salt), according to the modified procedure described by Kato and Nakai (1980).

Protein solutions were diluted to concentrations between 0.001% and 0.020% protein using 0.01 M phosphate buffer, pH 7.0, 15 µl of ANS methanol

solution was added to 3 ml of diluted protein. Fluorescence intensity was measured with a spectrofluorophotometer, SHIMADZU RF-5001 PC, at excitation wavelength  $\lambda_{\text{ex}} = 390$  nm and emission wavelength  $\lambda_{\text{em}} = 480$  nm. Pure methanol and diluted ANS solution were used in the calibration procedure. The initial slope of the fluorescence intensity versus protein concentration (%) plot was calculated by linear regression analysis and used as an index of the protein hydrophobicity.

### 2.3.3. Emulsifying properties

The emulsifying activity index (EAI) and emulsion stability index (ESI) of protein solutions (0.1% in 0.01 M phosphate buffer, pH 7.0) were determined by the method of Pearce and Kinsella (1978) which measures the turbidity of an oil/water emulsion diluted with 0.1% SDS; 4 ml of protein solution and 4 ml of soybean oil were homogenized for 2 min with an Ultraturrax macerator at maximum speed. The emulsion thus prepared was diluted with 0.1% SDS and turbidity was measured at 500 nm. EAI was calculated and expressed in  $\text{m}^2/\text{g}$ . Emulsion stability index (ESI, min) of the emulsions prepared for EAI determination was defined as the time in minutes for  $A_{500}$  to decrease to one-half the value at zero-time.

### 2.3.4. Water absorption capacity (WAC) and water solubility index (WSI)

Water absorption capacity (WAC) and water solubility index (WSI) were measured according to a modified method of Smith, Juhn, Carpenter, Mattil, and Cater (1973). 1.5 g of sample were mixed with 15 ml distilled water, using a Vortex type mixer, for 2 min. The sample was then allowed to stand at ambient temperature for 30 min before centrifuging at ca 12,500g. Water absorption capacity (WAC) was expressed as the volume (ml) of supernatant noted in a graduated cylinder. Then, the supernatants were transferred to tubes of known weight and dried to achieve constant weight in a blast drier at 105 °C. WSI (%) value was calculated as (weight of dissolved solids in supernatant/weight of dry sample solids in the original sample)  $\times$  100.

## 2.4. Statistical analysis

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean  $\pm$  SD.

## 3. Results and discussion

### 3.1. General

Analyzed lupin types represent three cultivars having significant differences in their chemical compositions

(Jasińska & Kotecki, 1993). Yellow lupin seeds (*Lupinus luteus*) are characterized by the highest total protein content ( $\sim$ 46%); however, white lupin (*Lupinus albus*) contains the highest amounts of fat ( $\sim$ 9%). In seeds of narrow-leaved lupin (*Lupinus angustifolius*), the protein content ( $\sim$ 32%) corresponds well to white type ( $\sim$ 6%) and fat content is similar to that in yellow lupin ( $\sim$ 5%).

Lupin underwent technological processes, involving fermentation with a lactic bacterium, as well as extrusion; however, preparations fermented earlier were also extruded. Obtained results were statistically analysed and are presented in the tables.

### 3.2. Protein content

Solubility is a very important protein feature, suggested as an indicator of protein status, often deciding technological usefulness (Schein, 1990). It can be correlated with other functional features, as for example surface hydrophobicity and emulsifying properties (Konieczny, 2001; Nakai et al., 1986). Lupin protein solubility was estimated, based on its extract ability under analytical conditions as defined for the Lowry method, and these results are presented in Table 1. In accordance with cultivar features of lupin seeds, among unmodified samples, *Lupinus luteus*-type samples were characterized with the highest protein content, while the lowest amounts of protein were extracted from *Lupinus angustifolius* samples. Independently of lupin type and modification method, higher amounts of protein were always extracted from hull-free samples. Change tendency of protein content in samples was characteristic of the applied technology, which proves that each of the adopted processes modified protein extraction ability in a different way. Significant increase of lupin protein extraction was an effect of fermentation with participation of the lactic acid bacterium. In all samples after fermentation, there were higher amounts than before. This was predictable and it is mostly connected with the hydrolytic activity of proteolytic enzymes produced by the lactic bacterium (Chmiel, 1994).

Extrusion conversely to fermentation, caused decrease of the protein content extracted from lupin samples. The lowest protein content was observed in samples that underwent the extrusion process only; however, the protein extraction decrement was greater when fermented samples were extruded (with higher, than unmodified sample, soluble protein contents). Evidently fermentation weakens lupin protein resistance to denaturing factors. The observed decrease of lupin protein extraction ability was caused by factors occurring during the extrusion. During this hydrothermal process, changes caused by high temperature and pressure occur in protein particles. Presented results show, that under these conditions, partly modified protein from fermented samples was more labile than native

Table 1  
Soluble protein content in samples of different lupin varieties (Lowry method, g/100 g d.m.)

Sample	<i>Lupinus luteus</i>		<i>Lupinus albus</i>		<i>Lupinus angustifolius</i>	
	Juno	Parys	Boros	Butan	Baron	Cesar
<i>With hull</i>						
Non modified	33.1 ± 0.07 <sup>a</sup>	40.5 ± 0.07	29.0 ± 0.69	28.1 ± 1.27	24.8 ± 0.36	26.0 ± 0.08
Fermented	36.7 ± 0.73	41.3 ± 0.03	33.5 ± 0.07	33.6 ± 0.10	29.2 ± 0.39	29.0 ± 0.03
Fermented and extruded	28.4 ± 0.35	26.2 ± 0.02	25.2 ± 0.09	25.0 ± 0.02	20.90 ± 0.17	24.53 ± 0.12
Extruded	21.8 ± 0.44	24.5 ± 0.04	23.3 ± 0.05	22.0 ± 0.08	20.9 ± 0.17	24.5 ± 0.12
<i>Without hull</i>						
Non modified	38.4 ± 0.94	42.7 ± 0.04	34.3 ± 0.10	34.1 ± 0.04	31.4 ± 0.50	32.2 ± 0.05
Fermented	45.6 ± 1.10	47.5 ± 0.11	43.6 ± 0.07	37.7 ± 0.07	36.4 ± 0.58	41.1 ± 0.05
Fermented and extruded	35.1 ± 0.19	29.5 ± 0.05	32.2 ± 0.08	32.3 ± 0.11	24.1 ± 0.07	31.2 ± 0.13
Extruded	33.1 ± 1.07	29.1 ± 0.09	27.6 ± 0.03	26.3 ± 0.07	23.6 ± 0.58	26.5 ± 0.12

<sup>a</sup> Mean ± SD.

protein from unmodified samples. The most sensitive, under conditions occurring during extrusion, seem to be proteins from yellow varieties of lupin, which proves that lupin protein modification character depends on cultivar features. Extrusion causes protein conformation modification; numerous noncovalent and covalent bonds stabilizing secondary structure are destroyed, and new intermolecular bonds can occur between forming subunits. After extrusion, proteins have a more fibrous structure and that is why it is harder to extract them and estimate their contents (Obuchowski, 1991). Protein contents in our samples are related to protein conformations before and after technological modifications. Protein secondary structure of fermented and extruded preparations is potentially exposed to maximal changes, which is well illustrated by protein contents estimated in selected samples.

### 3.3. Surface hydrophobicity

Lupin proteins were also studied by surface hydrophobicity estimation, because it is also a determinant of protein techno-functional properties. Hydrophobic reactions are very important in stabilizing intramolecular protein structure. Protein functionality is mostly connected with hydrophobicity, resulting from distribution, on its surface, of nonpolar aliphatic and aromatic residues of aminoacids. Lupin protein surface hydrophobicity was estimated in this study using a fluorometric method with ANS, and obtained results are presented in Table 2. As is evident, this property was different for protein of each analyzed lupin preparation. Samples without hull had higher surface hydrophobicity than those with hull, and each of the adopted technologies caused at least a double decrement of this lupin protein functional property.

Different factors related to protein structure, environmental conditions (temperature, pH, ion strength), as well as reactions with other components (different proteins, saccharides, lipids), influence protein hydro-

Table 2  
Surface hydrophobicity of lupin protein preparations determined by ANS on soluble protein (0.01 M phosphate buffer pH 7.0)

Sample	JUNO <i>Lupinus luteus</i>	BOROS <i>Lupinus albus</i>	BARON <i>Lupinus angustifolius</i>
<i>With hull</i>			
Non modified	715 ± 13.31 <sup>a</sup>	758 ± 3.79	733 ± 1.96
Fermented	347 ± 0.86	207 ± 3.98	365 ± 2.67
Fermented and extruded	370 ± 0.92	242 ± 1.26	464 ± 3.33
Extruded	321 ± 2.50	227 ± 2.20	511 ± 0.12
<i>Without hull</i>			
Non modified	822 ± 2.09	959 ± 6.66	813 ± 1.46
Fermented	452 ± 0.55	264 ± 2.58	541 ± 3.73
Fermented and extruded	406 ± 2.23	243 ± 2.12	513 ± 1.69
Extruded	358 ± 0.46	210 ± 1.64	671 ± 5.93

<sup>a</sup> Mean ± SD.

phobicity (Konieczny & Uchman, 2002). This observation was also confirmed by results that we obtained.

Presence of hull in samples was a reason for considerable differences in surface hydrophobicity of lupin protein. After fermentation in the case of the narrow-leaved cultivar, this difference grew from 10% to 33% and, in the case of yellow one, from 14% to 25%. The influence of hull presence on protein hydrophobicity of a white type was not typical of the remaining two cultivars. The difference (which for unmodified samples was the highest and equal 21%, after fermentation) decreased to 12% and, after extrusion, to zero. The main component of protein hull is fibre, built from compounds with polysaccharide character (cellulose, hemicellulose, pectins), as well as gums. Enzymatic as well as thermal processes can influence content and composition of fibre (Oh & Grundleger, 1990; Orue, Burton, Alonso, Ballaz, & Marzo, 1998; Rzędzicki & Mościcki, 2000; Vidal-Valverde & Frias, 1991). The estimation of fermentation and extrusion influence on modification of lupin hull components will be the object of separate investigations.

According to Nakai, Li-Chan, and Arteaga (1996), numerous hydrophobic groups take part in protein-

protein and protein–lipids interaction on the protein surface. In our investigations, the modifying influence of the adopted technological processes was clearest in samples of white lupin - Boros, whose seeds present the highest lipid contents among lupins. Before modification, hydrophobicity of Boros samples was higher than that of others (about 15% higher); however, after each technological process, it was lower (about 30% lower). Hydrophobicities of unmodified samples of yellow and narrow-leaved lupin were similar; however, between those two species, protein of yellow lupin seemed to be the more labile. The smallest changes in protein composition represented by surface hydrophobicity were observed in narrow-leaved lupin samples. In comparison with results for white lupin, this feature was, on average, twice higher after each technological process. From comparison of properties of lupin protein composition based on its hydrophobicity, it seems, that the most susceptible to activity of modifying factors are *Lupinus angustifolius* proteins, while *Lupinus angustifolius* proteins are the most stable. The results mentioned above allow us to conclude that under both fermentation and hydrothermal conditions, surface hydrophobicity of lupin protein was mainly formed with participation of lipids.

Mechanisms of physical, chemical and enzymatic reactions causing change of protein hydrophobicity during food processing are complicated, varied and often equivocal. In the case of the studied lupins, both hydrolysis, with participation of proteolytic enzymes produced by the lactic bacterium and protein denaturation caused by hydrothermal processes during extrusion, occurred. As we have affirmed, each of these processes decreased lupin protein hydrophobicity. Differences resulting from conditions of a given method of modification were significantly connected with lupin species; however, change tendencies cannot be unequivocally estimated. Results of our hydrophobicity estimation of fermented samples confirm other investigations concerning modification of protein properties caused by fragmentation of protein particles and destruction of primary structure as an effect proteolytic enzyme activity (Koniczny, 2001; Mahmoud, 1994). As is frequently observed, thermal denaturation causes increase of both vegetable and animal protein surface hydrophobicity (Ju, Hettiarachchy, & Rath, 2001; Marin, Casas, & Cambero, 1991; Nakai & Li-Chan, 1987). However, each of the studied lupin preparations presented lower hydrophobicity after extrusion than before.

### 3.4. Emulsifying properties

Emulsifying properties of our preparations were also studied technological modifications (Table 3). As in the case of the protein content and its surface hydrophobicity, results obtained for samples without hull showed higher values. Among unmodified samples, protein

emulsifying activity of the white variety was the worst. Generally speaking, adopted technological processes worsened emulsifying properties of lupin protein. Extrusion caused the largest changes, and fermentation the smallest. These results also prove interreaction between chemical composition of lupin samples and their properties. Emulsifying properties of protein of the narrow-leaved variety worsen the most; however, the character of this change (as in the case of hydrophobicity) was different than from that of the protein of white and yellow lupin. As a result of extrusion of fermented samples, preparations with better emulsifying activity and emulsion stability than those obtained by the separate usage of these processes were obtained. Most often, protein preparations with higher hydrophobicities had better emulsifying properties (Sikorski, 2001). In our research, conducted with lupin, we observed the same relationship. Results of statistical analysis show relatively high correlation between these functional properties of lupin protein preparations (Table 4). Both, correlation and determination coefficients, determining emulsifying activity of examined preparations, are highest for narrow-leaved lupin and lowest – for the white variety.

### 3.5. Water absorption capacity (WAC)

Samples without hull showed the highest water absorption ability among unmodified samples; however, the highest values were shown by the yellow species and the lowest by the white (Table 5). As was expected, lupin modification also affected water absorption ability. This property was not only correlated with changes caused by adopted technological process, but also with hull content and sample chemical composition, resulting from specimen features. Fermented samples showed higher water absorption abilities than non-modified samples. However, extrusion by itself, especially in the case of samples without hull, caused worsening of water absorption ability. These results also reflect changes of protein composition under the influence of enzymes produced during fermentation and high temperature as well as pressure occurring during extrusion. Higher water absorption ability should be connected with more protein released from protein complexes during fermentation. However, worsening of this property, in the case of extruded preparations, is a consequence of forming new, intermolecular bonds, structures and insoluble protein complexes. Avin, Kim, and Maga (1992) observed that the WAC value depends on extrusion temperature and may increase with temperature rise.

### 3.6. Water solubility index (WSI)

In comparison with non-modified samples water-soluble substance content, determined after fermentation, was lower, while, after extrusion, it was higher (Table 6).

Table 3  
Emulsifying properties of examined lupin samples

Sample	Emulsifying properties	Non modified	Fermented	Fermented and extruded	Extruded
<b>With hull</b>					
Juno <i>Lupinus luteus</i>	EAI (m <sup>2</sup> /g)	11.32 ± 0.05 <sup>a</sup>	10.56 ± 0.04	8.71 ± 0.03	8.53 ± 0.03
	ESI (min)	8.34 ± 0.03	7.06 ± 0.02	6.50 ± 0.04	5.16 ± 0.02
Boros <i>Lupinus albus</i>	EAI (m <sup>2</sup> /g)	10.73 ± 0.08	9.23 ± 0.26	6.84 ± 0.07	5.64 ± 0.12
	ESI (min)	7.44 ± 0.37	5.90 ± 0.39	5.62 ± 0.10	3.91 ± 0.09
Baron <i>Lupinus angustifolius</i>	EAI (m <sup>2</sup> /g)	11.46 ± 0.03	4.65 ± 0.04	6.20 ± 0.02	5.12 ± 0.03
	ESI (min)	7.37 ± 0.03	3.68 ± 0.04	5.74 ± 0.08	2.87 ± 0.02
<b>Without hull</b>					
Juno <i>Lupinus luteus</i>	EAI (m <sup>2</sup> /g)	12.21 ± 0.01	11.63 ± 0.07	9.77 ± 0.04	9.05 ± 0.04
	ESI (min)	8.41 ± 0.06	7.16 ± 0.01	5.88 ± 0.06	4.95 ± 0.04
Boros <i>Lupinus albus</i>	EAI (m <sup>2</sup> /g)	11.48 ± 0.27	11.05 ± 0.18	9.80 ± 0.13	8.32 ± 0.06
	ESI (min)	7.51 ± 0.07	7.18 ± 0.08	7.06 ± 0.11	5.35 ± 0.13
Baron <i>Lupinus angustifolius</i>	EAI (m <sup>2</sup> /g)	12.16 ± 0.04	4.84 ± 0.03	6.59 ± 0.04	5.33 ± 0.04
	ESI (min)	7.44 ± 0.01	2.46 ± 0.04	5.61 ± 0.07	4.62 ± 0.04

EAI, emulsifying activity index; ESI, emulsifying stability index.

<sup>a</sup> Mean ± SD.

Table 4  
Linear correlation coefficients between surface protein hydrophobicity and emulsifying properties of lupin protein preparations

Sample	Surface hydrophobicity			
	EAI (m <sup>2</sup> /g)		ESI (min)	
	R	R <sup>2</sup>	R	R <sup>2</sup>
<b>Juno <i>Lupinus luteus</i></b>				
With hull	0.74	0.65	0.83	0.80
Without hull	0.80	0.56	0.89	0.70
<b>Boros <i>Lupinus albus</i></b>				
With hull	0.73	0.54	0.78	0.61
Without hull	0.66	0.44	0.55	0.30
<b>Baron <i>Lupinus angustifolius</i></b>				
With hull	0.93	0.88	0.72	0.52
Without hull	0.80	0.64	0.68	0.47

EAI, emulsifying activity index; ESI, emulsifying stability index; R, correlation coefficient; R<sup>2</sup>, determination coefficient.

Table 5  
Water absorption capacity (WAC) (ml) of lupin protein preparations

Sample	Juno <i>Lupinus luteus</i>	Boros <i>Lupinus albus</i>	Baron <i>Lupinus angustifolius</i>
<b>With hull</b>			
Non modified	9 ± 0.29 <sup>a</sup>	7 ± 0.35	8 ± 0.00
Fermented	13 ± 0.71	11 ± 0.58	9 ± 0.58
Fermented and extruded	10 ± 0.26	11 ± 0.32	11 ± 0.00
Extruded	12 ± 0.35	14 ± 0.41	13 ± 0.00
<b>Without hull</b>			
Non modified	12 ± 0.15	8 ± 0.35	10 ± 0.15
Fermented	14 ± 0.58	11 ± 0.58	10 ± 0.50
Fermented and extruded	13 ± 0.29	13 ± 0.00	11 ± 0.50
Extruded	11 ± 0.29	8 ± 0.58	8 ± 0.58

<sup>a</sup> Mean ± SD.

Similar results was observed (Czarnecka, Czarnecki, Nowak, & Roszyk, 1998) during investigations of pea and bean. These results show a dependence between

Table 6  
Water solubility index (WSI) (%) of lupin protein preparations

Sample	Juno <i>Lupinus luteus</i>	Boros <i>Lupinus albus</i>	Baron <i>Lupinus angustifolius</i>
<b>With hull</b>			
Non modified	15.2 ± 0.25 <sup>a</sup>	12.0 ± 0.59	10.9 ± 0.78
Fermented	10.4 ± 0.62	11.0 ± 0.25	9.7 ± 0.41
Fermented and extruded	9.2 ± 0.44	10.0 ± 0.46	9.8 ± 0.04
Extruded	16.5 ± 0.14	15.9 ± 0.30	9.1 ± 0.34
<b>Without hull</b>			
Non modified	18.1 ± 0.34	12.1 ± 0.17	13.6 ± 0.78
Fermented	11.6 ± 0.30	12.2 ± 0.21	11.3 ± 0.41
Fermented and extruded	11.4 ± 0.30	10.2 ± 0.05	11.9 ± 0.04
Extruded	18.9 ± 0.21	15.5 ± 0.26	14.4 ± 0.34

<sup>a</sup> Mean ± SD.

WSI and (determined in earlier investigations) oligosaccharides. As was observed, in samples after fermentation, percentage participation of oligosaccharides was lower than that before; however, in extruded samples, it occurs in higher amounts. Decrease of saccharide content during fermentation may be explained by their metabolism by lactic bacteria. Higher saccharide contents determined in extruded preparations, are probably connected with their release from cellular structures under the critical conditions of this process.

#### 4. Conclusions

Lactic acid fermentation and extrusion processes, which improve sensorial properties of lupin protein preparations are not desirable for their selected techno-functional properties. Examined protein is characterized by a lower hydrophobicity and, in consequence, by worse emulsifying parameters expressed as EAI and ESI values. Extrusion worsens the water absorption index of

lupin samples, while the lactic acid fermentation lowers the content of water-soluble substances.

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