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RESEARCH ARTICLE
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Protein Concentrate from White Lupine of Dega Variety

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ABSTRACT

The global annual deficit of feed protein exceeds 30 million tons, with Russia accounting for approximately 2–2.5 million tons of this shortfall. A notable trend in contemporary animal husbandry is the increasing substitution of conventional animal-derived protein sources with plant-based and single-cell microbial proteins, aiming to enhance sustainability and reduce reliance on traditional feed components. The main sources of plant protein are legumes (soybeans, peas, fodder beans, and lupine, etc.). Lupine - a legume with a high content of protein and fiber is of great interest. This article presents the results of obtaining a plant protein concentrate by enzymatic treatment of lupine and assessing its nutritional value for use in animal husbandry. White lupine of Dega variety (Republic of Tatarstan) was used in the work. Lupine seeds were pre-treated in an EM-150 feed extruder (Russia) at the production site of "GreenTex Scientific and Technical center" LLC (Kazan). The concentration of total nitrogen, true protein, fiber, fat, ash, pectin, and starch was determined in the seeds before and after extrusion. Enzymatic hydrolysis of lupine was carried out with enzyme preparations from Novozymes (Denmark) and Production Association "Sibbiopharm" LLC (Russia) in a laboratory shaker-incubator Kuhner ISF1-X (Switzerland) with subsequent separation of the solid and liquid fractions by centrifugation. The solid sediment (protein concentrate) was dried, and both total and true nitrogen contents were quantified. During enzymatic hydrolysis, the majority of lipids present in the extruded lupine were released into the hydrolysate. When using Novozymes enzyme preparations, the maximum yield of reducing substances (RS) reached 1.66% after 5–6 hours of hydrolysis, compared to 1.55% after 10–12 hours in the absence of extrusion pretreatment. In contrast, enzymatic treatment with Sibbiopharm preparations resulted in a higher RS yield of 2.2%, achieved within just 1.5 hours. Fourier-transform infrared (FTIR) spectroscopy (Shimadzu, Japan) revealed structural modifications in the carbohydrate and protein components, indicating significant biochemical transformations during hydrolysis. The protein concentration in the final product increased from 39.8% to 47.58%. Evaluation of the amino acid composition on the NIRS™DS 2500 analyzer showed that enzymatic treatment of lupine seeds allowed increasing the amount of amino acids from 25.95 to 42.08%, including replaceable amino acids increase from 9.97 to 15.21% and essential amino acids increase from 15.98 to 26.87%. The resulting protein concentrate from lupine can be used for feeding farm animals and poultry in order to balance diets in terms of protein and amino acids content.

Keywords: Lupine, Protein concentrate, Extrusion, Enzymatic hydrolysis, Amino acids, Protein, Feed protein.

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INTRODUCTION

The development of animal husbandry as one of the priority sectors of the agro-industrial complex in Russia, taking into account the implementation of import substitution programs and the constant growth in prices for protein components of feed, will require a significant increase in feed production, an increase in feed quality and improvement of production technologies using integrated approaches to the processing of alternative plant raw materials in the near future. In addition to the general feed deficit, it is necessary to eliminate the chronic deficit in such substances as protein and amino acids which are essential for the farm animal's nutrition. The annual global deficit of feed protein exceeds 30 million tons, and in Russia it is about 2-2.5 million tons (Цыгуткин, 2023).

In modern animal husbandry there is a tendency to increase the consumption of plant and single-cell microbial protein. At the same time, the main source of feed protein is plant protein obtained from legumes. Soybeans are of undoubted practical interest for companies engaged in the deep processing of plant agricultural raw materials. As a result of the limited diversification of protein feed products, livestock specialists compensate for the deficit of feed protein by using soybeans, which are genetically modified in most cases. At the same time, the price of such products (soybean meal, full-fat extruded soybeans) is extremely high and makes up a large share of the compound feed cost (Серегина et al., 2021).

White lupine (*Lupinus albus*) is successfully considered an alternative to soybeans (Gaponov and Yagovenko, 2021). Of all the legumes, lupine has a higher protein content (Chukwuejim et al., 2024). Composition rich for amino acid allows this crop to be considered as an alternative to soybeans (Abilov, 2021). At the same time, the crop exhibits low cultivation requirements and adapts well to the agroclimatic conditions of the country's mid-latitude regions. The fat content of lupine varies from 5 to 20%, including triglycerides with a high proportion of unsaturated fatty acids (83-90%), including 30-39% of linoleic acid, which is essential for poultry (Ferchichi et al., 2021). In terms of the amount of tocopherols and phospholipids in oil, lupine is also not inferior to soybeans. An increased content of vitamins B, C, carotenoids, and minerals has been noted in lupine (Sadak, 2023). This unique composition determines the high nutritional value of this crop and its role in reducing the deficiency of plant protein (Huamaní-Perales, 2024). White lupine responds well to the use of selenium, serves as a concentrate for manganese and cobalt (Akonaç and Canal, 2023), which makes it attractive for normalizing mineral metabolism in highly productive poultry crosses.

Unlike soybeans, lupine seeds contain virtually no trypsin inhibitors and can be used in feed without heat treatment (Pereira et al., 2022, Ohanenye et al., 2022). However, lupine contains quinolizidine alkaloids, which can be accumulated to toxic levels depending on the species (Tsygutkin, 2023). Therefore, lupine is currently little used in animal feed (Gresta et al., 2023). According to the literature, the removal of antinutrients is achieved by water treatment (Chamone, 2023) or extrusion (Manzocchi, 2023). In addition, extrusion improves protein digestibility and protects protein from degradation in the rumen (Mendowski, 2019). Today, methods of pre-heat treatment for lupine can be used not only for the purpose of removing anti-nutritional substances, since modern breeding programs have already ensured the selection of sweet lupine varieties with reduced alkaloid content (≤ 0.2g/kg of dry matter) (Boukid and Pasqualone, 2022). Such treatments are necessary for biotechnological processes, as they allow the specific surface area of seeds to be revealed, which greatly accelerates hydrolytic reactions and enzyme treatment.

Despite the prospects for using lupine as a source of feed protein, there are still no comprehensive solutions for deep processing of this crop to increase its nutritional value in feed (Tarek, 2024). Numerous methods have been proposed for the production of lupine protein isolates, concentrates and hydrolysates (Shrestha et al., 2021). In almost all cases, the most effective methods are those that involve some kind of pre-treatment. As a rule, this is thermal extrusion and water cooking. Enzymatic hydrolysis is no exception. Here, extrusion helps to increase the soluble carbohydrates in the lupine seed coat (Zhong, 2021), giving enzymes more complete access to difficult-to-hydrolyze carbohydrate fractions (Pasarin, 2023).

The selection of enzyme compositions (Samel and Wojciechowski, 2020), taking into account the carbohydrate content of lupine, in order to obtain protein concentrates by selective enzymatic hydrolysis, is not well studied. In the literature, most of the works are devoted to the hydrolysis of the protein fraction of lupine seeds (Opazo-Navarrete et al., 2022), in particular with proteolytic enzymes (Garmidolova et al., 2022). The purpose of such treatments is to obtain protein hydrolysates for the food industry (Großmann, 2019). Protein availability in the food industry is directly related to the global deficit of feed protein, which is a pressing problem in animal husbandry. Therefore, it is of great interest to obtain cheaper protein concentrates from lupine specifically for feed production.

Currently, some practical experience has been accumulated in the sphere of the use of lupine seeds as a feed additive. There are reports in foreign and domestic literature on the positive effect of supplementation with lupine on the productivity of poultry. For example (Spina, 2022), 5-15% of the seeds of white lupine of Dega variety can be used to replace soy products in the diets of laying hens, which does not reduce the zootechnical and incubation indicators. According to David et al., (2024), instead of animal feed and soybean meal in complete compound feeds, white lupine seeds can be used for broilers in an amount of up to 20%, and for laying hens in an amount of up to 15%. Experiments on the use of white lupine in feeding quails also showed the effectiveness of including it in the diet in an amount of 10% during growing and fattening poultry for meat. Live weight and average daily gain increased by 10-11% compared to the control, and feed costs decreased by 5.8-7.1%. This is probably due to a slightly higher level of crude protein (by

0.57-1.22%) in the experimental group (Fedorova et al., 2023). The experiments (Struți, 2023) on the use of white lupine in growing and fattening quails for meat confirmed the effectiveness of using 10% whole and 7% hulled lupine grain. The average live weight of the bird was 7.2-7.5% higher than in the control, and feed conversion was 5.5% better with the highest meat productivity index. Similar results were obtained by (Pietras et al., 2021), who assessed the addition of lupine seeds (*Lupinus luteus L.*) to the diet of broiler chickens on growth performance, carcass and meat quality. Some researchers suggest adding enzymes to the diet along with lupine. For instance, Fedorova et al. (2023) reported that replacing soybean with low-alkaloid lupine, either alone or in combination with the enzyme preparation Protosubtilin A-250 G3X, in quail diets did not negatively impact growth performance compared to the control group. Notably, the group receiving the enzymatically treated lupine achieved the highest total weight gain of 271g. Furthermore, the inclusion of 29% lupine in compound feed for quail significantly reduced overall feed costs. Based on these findings, the authors concluded that low-alkaloid lupine represents a promising alternative protein source to soybean in poultry nutrition.

Taking in consideration the above, and the fact that almost all livestock farming has switched to diets with plant and microbial protein, complex biotechnological processing of legumes, in particular lupine, is a relevant and primary practical task for the production of feed protein. Most studies are devoted to the use of lupine for food purposes (protein isolates and concentrates), although there are isolated reports on the use of this crop in animal husbandry. Given the presence of anti-nutritional substances in lupine seeds (mainly fiber and fat) with a high protein content, the use of this crop is not always successful in feeding farm animals and poultry, and requires preliminary seed treatment. Attempts to reduce the anti-nutritional properties of lupine using enzyme preparations are very rare in the literature, although biotechnological processing is a fairly promising and cheap method of influencing high-protein raw materials. In this regard, the purpose of this work was to obtain a plant protein concentrate by enzymatic processing of lupine and assess its nutritional value for use in livestock farming.

MATERIALS & METHODS

The work used white lupine of Dega variety, bred by the Russian State Agrarian University - Moscow Agricultural Academy named after K. A. Timiryazev and provided by the Tatar Research Institute of Agriculture, Federal Research Center "Kazan Scientific Center of the Russian Academy of Sciences". The presented sample contains 8-10% of fat, 37-38% of protein, 0.05% of alkaloids. Lupine seeds were crushed in "V'YUGA 3MT" laboratory mill (Russia) to a small (0.5-1mm) fraction. Additional processing was also carried out: lupine seeds were extruded in uncrushed form in a single-screw extruder for feed EM-150 (Russia) at the production site of the "GreenTex Scientific and Technical center" LLC (Kazan). The extrusion temperature was 150°C, the processing time was 8-10s (Prosvirnikov, 2024). The extruded lupine was also crushed in "VYUGA 3MT" mill (Russia) into 3 fractions: small (0.5-1mm), medium (1-2mm) and large (2-5mm). The moisture content of the samples was measured using an AND MX-50 automatic moisture analyzer (Japan). The protein concentration (based on total nitrogen) in lupine samples was determined using the Kjeldahl method, employing a SELECTA (Spain) wet digestion system with a remote temperature-controlled block operated under a fume hood, followed by ammonia distillation. True protein content was assessed using the Barnstein method. Fiber content was measured according to the Henneberg and Stohmann method, while fat was quantified using the Soxhlet extraction method in accordance with GOST 13496.15-2016. Ash content was determined following GOST 26226-95, pectin content according to GOST 54066-2010, and starch content using GOST ISO 6493-2015. Foreign enzyme preparations Novozymes (Denmark) for hydrolysis of polysaccharides were used to perform enzymatic hydrolysis: cellulase complex NS22074 (activity 1,000EGU/g), β-glucosidase NS50010 (activity 250CBU/g), β-glucanase and xylanase NS22002 (activity 45FBG/g (~470FXU/g)), glucoamylase NS22035 (activity 750AGU/g). Based on the data on the quantitative content of starch, fiber and free sugars in the raw materials, the dosages of enzyme preparations were determined and the enzyme composition was made. Enzymatic hydrolysis with Novozymes preparations was carried out as follows. The prepared raw materials (initial lupine of small fraction, extruded lupine of small fraction, extruded lupine of medium fraction, extruded lupine of large fraction), the enzyme composition and distilled water were placed in sterilized 1000ml flasks in the calculated amount, the flasks were covered with a cotton-gauze swab. Enzymatic hydrolysis was carried out in a laboratory shaker-incubator Kuhner ISF1-X (Switzerland) at t=50-60°C, 100min^{-1}, pH=5.5, for 24 hours (Fig. 2).

Every 2 hours, hydrolysate samples were collected from the flasks and centrifuged using a Biobase centrifuge (China) at 10,000rpm for 5 minutes. The pH of the supernatant was measured using a Multitest IPL-311 analyzer (Russia). The concentration of reducing substances (RS), expressed as glucose equivalents, was determined by the Benedict–Bertrand method. Additionally, the dry weight of reducing substances was assessed by drying the samples in an ESCO Isotherm laboratory drying cabinet (China), followed by gravimetric analysis. All measurements were performed in duplicate. After the enzymatic hydrolysis was completed, the content of the flasks was centrifuged, the supernatant (4 samples) was separated from the solid residue, sterilized by boiling and prepared for further cultivation. The solid sediment (four samples) was dried in an oven at 103-105°C for 12 hours, after which it was crushed. The quantitative content of total and true nitrogen was determined in the obtained solid samples (Fig. 3). Based on the results of enzymatic hydrolysis, the optimal fraction of the crushed raw material was determined. Also, powder samples were analyzed on an IR analyzer at

the Department of Synthetic Rubber Technology of the Kazan National Research Technological University.

Analytical studies of the nutritional value and amino acid composition of protein raw materials were carried out in laboratory conditions at the Tatar Research Institute of Agriculture of the Federal Research Center of the Kazan Scientific Center of the Russian Academy of Sciences. The samples were analyzed using a NIRS™DS 2500 analyzer calibrated using global data. ISIscan Nova software was used for control. The objects of the study were: 1) extruded soybean seeds of Milyausha variety (sample 1 - control); 2) extruded lupine seeds of Dega variety (sample 2); 3) lupine protein concentrate (sample 3). Soybean of Milyausha variety was bred jointly by the Siberian and Tatar Research Institute of Agriculture, and white lupine of Dega variety was created at the Russian State Agrarian University - Moscow Agricultural Academy named after K. A. Timiryazev. Statistical processing of the experimental data was performed in MS Excel, statistical significance of the results was calculated using Student's t-test, the difference was considered significant at $P < 0.05$.

RESULTS

Table 1 presents the component composition of lupine before and after processing, expressed as a percentage of absolutely dry matter (a.d.m.).

As can be seen from Table 1, extrusion helps to reduce the amount of pectin, starch and slightly fiber. After enzymatic hydrolysis, extruded lupine contained a reduced amount of polysaccharides and fat. Most of the fat was released into hydrolysate during enzymatic hydrolysis. The data of component analysis showed changes in the composition of lupine after heat treatment, and these changes (in particular, a decrease in the proportion of starch, pectin) will further contribute to a reduction in the duration and an increase in the efficiency of the enzymatic hydrolysis process.

Fig. 1 shows the results of changes in the concentration of reducing substances C_{RS} (in terms of glucose) during enzymatic hydrolysis (τ) with Novozymes preparations.

The obtained IR spectra of lupine before and after treatment confirmed chemical changes in the structure of proteins, fats and carbohydrates that occur during heat treatment of lupine seeds (Fig. 2).

The contents of crude protein (calculated from total nitrogen) and true protein nitrogen (determined via the Barnstein method) were quantified as mass percentages of absolutely dry matter (a.d.m.) using the classical Kjeldahl method, which is considered the most accurate for nitrogen determination. The corresponding results are illustrated in Fig. 3.

A comparative assessment of the amino acid composition of extruded soybean and lupine seeds, as well as lupine protein concentrate obtained by fermentation of lupine seeds, was carried out for further use in feed additives and compound feeds for farm animals, poultry and aquaculture. The amino acid composition of the studied samples is presented in Fig. 4.

Table 1: Component composition of lupine before and after treatments, % a.d.m. [1]

Indicator	Lupine initial	Lupine extruded	Extruded lupine after enzymatic hydrolysis (Novozymes)	Extruded lupine after enzymatic hydrolysis (Sibbiopharm)
Moisture	8.8±0.2	4.4±0.11**	3.6±0.14**	8.73±0.2
Total -nitrogen	39.8±1.1	40.2±0.72	47.6±0.66**	45.4±0.92*
Protein according to Barnstein method	18.9±0.9	19.0±0.91	19.5±0.82	19.8±0.93
Fiber	10.0±0.62	9.3±0.4	4.2±0.31*	6.2±0.38*
Fat	12.0±0.22	11.5±0.13	3.5±0.11***	2.4±0.08***
Ash	3.2±0.1	3.2±0.12	3.1±0.1	3.0±0.13
Pectin	10.1±0.1	6.6±0.1*	0.2±0.05***	1.72±0.06***
Starch	8.2±0.6	4.11±0.45*	0.15±0.03**	0.05±0.06**

Note: Data are presented as mean±SD. Statistical significance is relative to the previous treatment stage. *- P<0.05; **- P<0.01; ***- P<0.001.

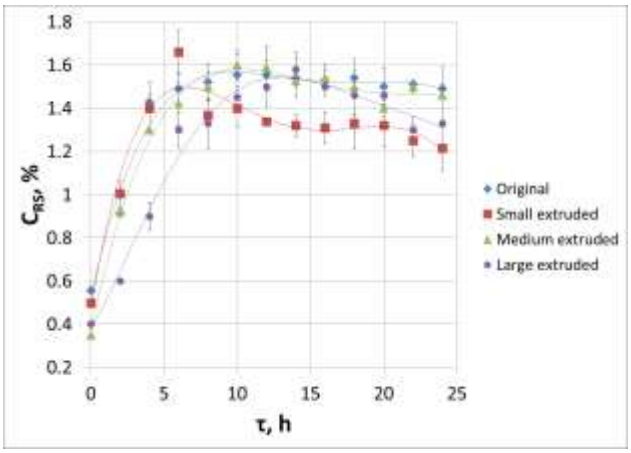

Fig. 1: Concentration of reducing substances C_{RS} (in terms of glucose) (enzymatic hydrolysis with Novozymes preparations).

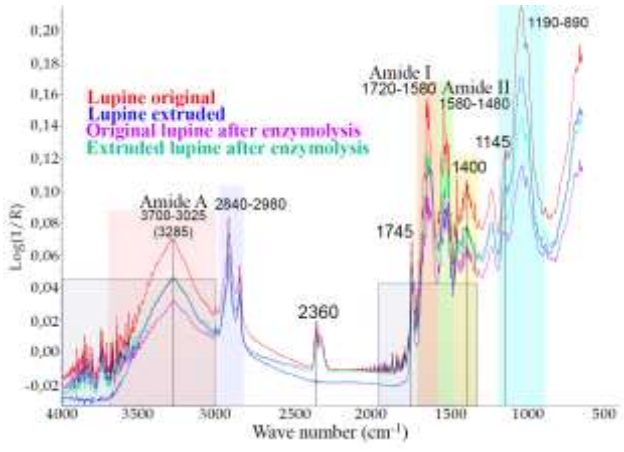

Fig. 2: Comparative IR spectra of lupine before and after treatments.

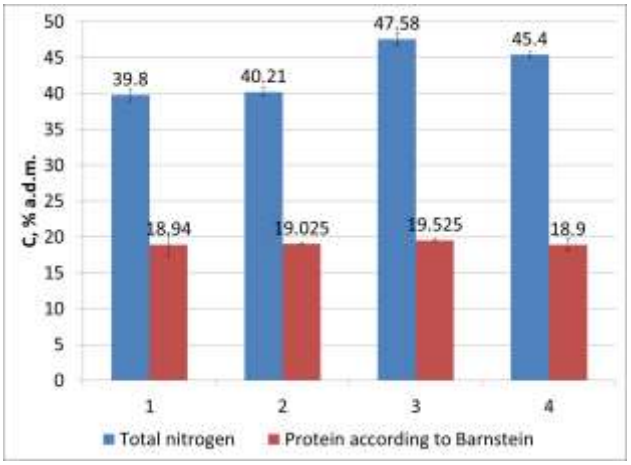

Fig. 3: Concentration of crude protein and true protein (determined by the Barnstein method) in solid lupine products, expressed as mass percentage of absolutely dry matter (a.d.m.). 1) Initial lupine; 2) Extruded lupine; 3) Lupine after enzymatic hydrolysis with Novozymes preparations; 4) Lupine after enzymatic hydrolysis with Sibbiopharm preparations (Russia).

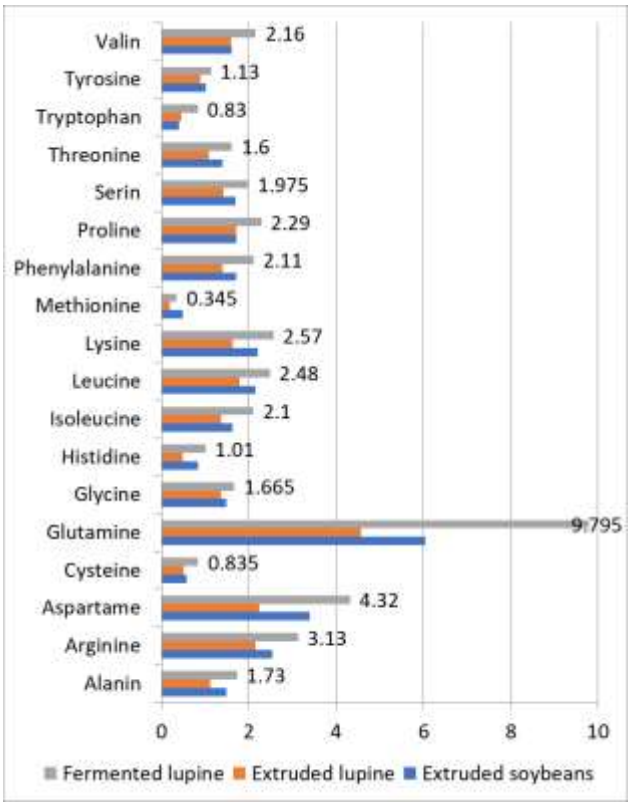

Fig. 4: Comparative amino acid composition of samples (% of total protein).

Amino acid analysis of the samples showed that the content of replaceable and essential amino acids in sample 1 was at the level of 32.33%, in sample 2 – 25.95%, in sample 3 it was higher compared to other samples and amounted to 42.0% (of the total amount of protein). In extruded soybean seeds (sample 1), the fraction of replaceable amino acids contained the highest level of glutamine – 6.04%; in the fraction of essential amino acids, the highest content of arginine – 2.54% and lysine – 2.21% was observed. The amount of sulfur-containing amino acids was: methionine – 0.49% and cysteine – 0.57%. In extruded seeds of white lupine (sample 2), the fraction of replaceable amino acids contained the highest level of glutamine – 4.57% and aspartame – 2.24%, in the fraction of essential amino acids: arginine – 2.16%, leucine – 1.78%, valine – 1.60%. The amount of sulfur-containing amino acids was: methionine – 0.2% and cysteine – 0.51%. In the lupine protein concentrate (sample 3) the fraction of replaceable amino acids contained the highest level of glutamine – 9.80% and aspartame – 4.32%, the fraction of essential amino acids: arginine – 3.13%, lysine – 2.57%, leucine – 2.48%, valine – 2.16%. The amount of sulfur-containing amino acids was: methionine – 0.35% and cysteine – 0.84%.

DISCUSSION

The main advantage of extrusion is the disclosure of the specific surface area of lupine seeds, especially their shell, consisting mainly of cellulose and hemicelluloses. Another important factor is the release of fat, which content in the seeds reaches up to 12%. These physicochemical transformations, in our opinion, contribute to the intensification of enzymatic treatment due to the availability of the substrate for enzyme penetration. The reactions of hydrolytic destruction of carbohydrates, especially easily hydrolyzed ones, can be accelerated by increased processing temperature during extrusion and the moisture contained in lupine seeds. This is also confirmed, for example, in the work of (Chamone, 2023).

Our study showed that extrusion (especially when using a small fraction) allows achieving the maximum yield of RS (1.66%) after 5-6 hours, while raw materials not processed by extrusion get the maximum amount of RS after 10-12 hours (1.55%). Obviously, the open surface of lupine seeds and preliminary chemical transformations of polysaccharide components during heat treatment play an important role here. The maximum yields of RS for larger fractions of extruded lupine are close to the original raw material. However, the rates of RS yield here are explained differently. In the original lupine of small fraction, the lower rate is caused by the presence of a larger amount of carbohydrates subjected to enzymatic hydrolysis and an unopened specific surface. In larger fractions of extruded lupine, the rate slows down due to the particle size, but the yields of RS are comparable with the yield from untreated lupine. Since the maximums of RS are observed on average after 4-6 hours, there is reason to believe that the main share of sugars is formed by the products of cellulose hydrolysis (lupine seed coat). Therefore, in this Novozymes composition, the limiting enzymes are NS22074 and NS50010.

Based on the results of the RS yield assessment, the optimal fraction for enzymatic hydrolysis by Novozymes enzymes – a small one with a particle size of 0.5-1mm – was determined, which showed the maximum RS yield in a shorter hydrolysis period while maintaining the pH in the medium at a level sufficient for the effective operation of enzymes. Particle size during enzymatic hydrolysis does significantly affect the rate of carbohydrate degradation. For example, in the work (Luis and Einfalt, 2021) it was shown that small particle size also has a positive effect on

the viscosity of the suspension and, consequently, on its miscibility. The key conclusion of this study was that enzymatic hydrolysis at high gravity with a solids content of 30-35% dry matter was indeed successfully applied when using wheat straw in the form of small particles. These data are quite consistent with our data, which confirm an increase in the enzymatic hydrolysis rate by almost two times.

As evident from the infrared (IR) spectra of untreated lupine and lupine subjected to enzymatic hydrolysis, there is a marked reduction in the intensity of absorption bands associated with polysaccharide bond vibrations, particularly within the regions of 1190–890cm^{-1}, 1145cm^{-1}, and 4000–3000cm^{-1}. However, precise identification of the specific polysaccharide groups (e.g., hexosans, pentosans) undergoing hydrolytic degradation remains challenging due to the overlapping of characteristic absorption bands within these spectral ranges. The band 1190-890cm^{-1}, including vibration of glycosidic bonds (1175-1140cm^{-1}) characterizes the destruction of cellulose, which is compared with a decrease in the intensity of vibrations of H-bonds of cellulose in the range of 3700-3000cm^{-1}. In the whole, a decrease in the intensity of this band is mainly associated with the hydrolysis of polysaccharides. In general similar changes were observed during cellulose degradation, for example, in the work (Hong, 2021) similar changes were noted in the same absorption ranges. In fermented samples, a decrease in intensity in the Amide 1 and Amide 2 bands was also observed. This may be due to the transition of some soluble fractions of proteins and non-protein nitrogenous compounds to the hydrolysate during long-term enzymatic hydrolysis. In the work (Malinowska-Pańczyk, 2023) similar results were observed, however, they were obtained under different conditions, but the mechanism is obviously similar. The shape and structure of proteins changing during treatments was discussed above. The concentration of fats also tended to decrease in samples after enzymolysis (band 2980-2840cm^{-1} and carbonyl band at the peak of 1745cm-^{1}). The peak at 2360cm^{-1} was characterized by the C=O bond of carbon dioxide in many sources (Maciejewska et al., 2024). Its absence in the spectrum of extruded lupine may be due to heat treatment and desorption during pressure release, although this has not yet been confirmed by our study. High-frequency vibrations appearing in the regions of 4000-3500cm^{-1} and 1900-1350cm^{-1} corresponded to water, which always accompanies products in bound or free form after enzymatic hydrolysis and significantly affects the shape of the spectra, which causes difficulties in interpreting the ranges characterizing the amide bonds of the main chain of proteins. Nevertheless, the preservation of symmetry in the spectra in the regions responsible for the bonds in amides indicates the preservation of the general structure of plant proteins in lupine. Indeed, the symmetrical arrangement of band intensities was often interpreted in studies as an unchanged protein structure, for example, in (Lopes, 2021). Comparing the spectra of extruded lupine before and after enzymolysis, it is clear that the protein concentration increases (characteristic ranges of Amide 1 and Amide 2), indicating the possibility of obtaining protein concentrates. Obviously, the final protein concentration in the product will depend on the quality of the selective hydrolysis of carbohydrates, so IR spectrometry of the samples is an important tool in this study.

As for the quantitative yield of proteins, their determination by IR spectra is possible, however, given the overlap of bands from polysaccharides, water and triglycerides, such a task is difficult at this stage, although it is possible when obtaining purer concentrates free of carbohydrates. In the sample of extruded lupine treated with Novozymes preparations, the yield of crude protein after enzymatic hydrolysis in the solid residue reached 47.58% of a.d.m. It was possible to increase the protein concentration in the final product from 39.8%, that is, by 7.8 absolute percent or by 19.54 relative percent. This indicates partial destruction of the carbohydrate fraction of lupine seeds, which confirms our hypothesis about obtaining protein concentrates from lupine. The mechanism of the effect of fats on enzymatic hydrolysis has not been fully studied, although according to some data (Baena, 2022), fats do not have a significant effect on the functionality of enzymes. In general, the product after removal of fat and carbohydrate fraction is close in composition to soybean meal, and may possibly be called "bio meal", since its processing, unlike the technology of classical meals, does not involve extraction stages with organic solvents, but is obtained using biotechnology.

Studies have shown that enzymatic treatment of lupine seeds allows increasing the amount of amino acids from 25.95 to 42.08%, including replaceable amino acids from 9.97 to 15.21%, essential amino acids from 15.98 to 26.87%. At the same time, the content of glutamine increases from 4.57 to 9.80%, aspartame from 2.24 to 4.32%, arginine from 2.16 to 3.13%, lysine from 1.62 to 2.57%, leucine from 1.78 to 2.48%, cysteine from 0.51 to 0.84%, methionine from 0.2 to 0.35%. Enzymatic hydrolysis of the carbohydrate fraction allows obtaining lupine protein concentrate, which, compared to extruded soybean seeds, contains 2.8% more essential amino acids and 6.95% more replaceable amino acids. The lupine protein concentrate demonstrated higher levels of several key amino acids compared to extruded soybean protein, with increases of 3.76% in glutamine, 0.92% in aspartic acid, 0.59% in arginine, 0.36% in lysine, and 0.27% in cysteine.

Conclusion

The results of the study of obtaining protein products from white lupine of Dega variety using enzyme complexes showed the effectiveness of preliminary seed extrusion, which allowed to reduce the duration of enzymatic hydrolysis of the carbohydrate fraction of lupine by almost two times. The particle size of extruded lupine of 0.5-1mm was the optimal fraction for hydrolysis. The most effective hydrolysis was achieved using enzyme compositions consisting of Novozymes preparations, especially in terms of cellulose polysaccharides, but domestic preparations have shown high efficiency in the hydrolysis of starchy polysaccharides. Taking into account these data, it makes sense to create a complex enzyme composition that will

allow performing a more complete hydrolysis of the carbohydrate fraction of lupine and obtaining products with higher protein content. The residual fat content in the protein product should also be reduced by preliminary extraction. In the extruded lupine sample (treated with Novozymes preparations), the yield of crude protein after enzymatic hydrolysis in the solid residue reached 47.58% a.d.m. It was possible to increase the protein concentration in the product from 39.8%, i.e. by 7.8 absolute percent or by 19.54 relative percent. The conducted studies on the enzymatic hydrolysis of extruded lupine and the consequent results confirm the possibility of obtaining protein concentrates by selective enzymatic hydrolysis of carbohydrate components included in lupine. The comparative amino acid composition showed the possibility of using the obtained protein concentrate in feeding farm animals, poultry and aquaculture in order to balance rations for amino acids.

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Data Availability: All the data is available in the article.

Ethics Statement: This study did not involve humans or animals, and therefore it did not require official ethical approval. All experimental procedures related to crop cultivation and sampling were conducted in accordance with relevant institutional, national and international guidelines and legislation.

Author's Contribution: Denis Tuntsev prepared the raw materials and developed the methodological part of the study. Liliya Ismagilova, Anna Brodneva conducted experiments on the analysis of the original raw materials. Rauza Valeeva conducted experiments on enzymatic hydrolysis. Elina Vasileva conducted studies on IR spectroscopy. Oleg Yakimov, Almaz Salyakhov conducted studies on the analysis of protein fractions. Munira Gainullina, Evgeny Krupin conducted studies on the amino acid composition of concentrates. Dmitry Prosvirnikov, Munira Gainullina, Alina Battalova Sergey Smolentsev analyzed the obtained data and made conclusions, and wrote the article based on the obtained data. The final version of the manuscript was revised by all authors. All authors edited, read and approved the final version of the manuscript.

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