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EVALUATION OF THE PROSPECTIVE OF PRODUCTION AND USING OF PROTEIN FROM WHITE NUTRITION LUPINE

Nowadays in the whole world the mankind lacks protein foods. The average protein deficiency in the world is 56.1 million tons or 25%, incl. for Ukraine – about 255 thousand tons [3]. There are many reasons for that, but the main ones are ecological, social, economic, etc. The annual increase in the number of the world's population by 75 million people who need food products also influences the issue greatly [1]. Climate change and ecological problems lead to the disappearance of traditional and the emergence of new types of crops that are able to adapt to new weather conditions. The decrease in the purchasing power of the domestic consumer reduces the consumption of animal protein in meat, dairy and fish products in the daily rations. With a balanced diet, a person should consume animal and vegetable proteins in a ratio of 55% and 45% respectively, which is not consistent with reality [4]. That is why, in our opinion, it is expedient to search for alternative sources of protein among the crop production.

Today, the basic raw material for the production of protein concentrates and isolates from plant raw materials is soybean, the production of which has increased 9 times over the past 50 years, its average selling price being 343.22 UAH/per centner [5]. However, the populations become concerned with the widespread use of this crop in food technology, due to the large amount of genetically modified raw material and its high cost [6]. In addition, the plant itself is very demanding to the acidity of soils, nitrogen and phosphorus fertilizers [7].

Therefore, scientists are looking for alternative sources of complete protein among other types of crops, first and foremost among grain and legumes. Particular attention is paid to dietary lupine, which, unlike soy, is much cheaper and characterized by high yielding capacity [4, 11].

The object of the study was Ukrainian varieties of white lupine –

"Chabanskyi", "Makarivskyi", "Veresnevyi", "Serpnevyi", "Rhapsody", "Lybid" and "Shchedryi". These varieties are selected at the National Scientific Centre “Institute of Agriculture of NAAS” (Chabany village, Kyiv region) and the Institute of Agricultural Microbiology and Agro-Industrial Manufacture of NAAS (Progress village, Kozeletskyi district, Chernihiv region). For all sorts, the round shape and cream and white colour of the seeds are characteristic, they are listed in the "Register of Plant and Hybrids Varieties of NSC ‘Institute of Agriculture of NAAS’" and recommended for cultivation in the forest-steppe and Polissia zone of Ukraine [9].

To compare the chemical composition of lupine seeds with other leguminous plants, the "Success – 2" variety of soybeans was chosen [16].

Protein concentrates containing more than 50% of protein are one of the most commonly produced proteiniferous products, which are a powdered product from which sugars, oligosaccharides and other soluble fractions are removed [4]. Protein concentrates are obtained by acid hydrolysis, heat treatment and enzymatic hydrolysis [10].

Thus, a Russian scholar from St. Petersburg State University of Trade and Economics I.A. Pankina has developed a method for obtaining protein concentrate from lupine seeds, which provides for preliminary soaking of raw materials for 3.5 hours at a temperature of 90°C in 1% of sodium bicarbonate solution, washing, cooking grain for 2 hours at a hydromodule 3, drainage of the liquid, rough grinding with the addition of water , homogenization, pasteurization at a temperature of 85 ± 5°C for 5 minutes and cooling the finished paste. The result is a protein product with a protein content of 43.8% RS [11].

It is possible to improve the nutritional value and reduce the detoxification properties of protein paste by adding up to 10% of oil, water and chitosan, followed by homogenization for 2-5 minutes [12].

Russian scientists have also developed a method for obtaining a protein product by a physical method, namely the treatment of seeds with a superatmospheric pressure. Researchers argue that with periodic changes in pressure and seed treatment with water saturated with carbon dioxide, the length of the process is significantly reduced. Further, the protein extraction was carried out in an aqueous alkaline solution at 9pH and a temperature of 45°C. The obtained protein was isolated from an alkaline solution by isoelectric precipitation at 4.45 ± 0.05 pH [13]. The content of this nutrient in the finished product adds up to 42% of SR.

Since the methods of hydrothermal treatment, the physical method, alkaline and acid hydrolysis do not allow obtaining a protein product with a protein content of more than 44%, it is rational to use the method of enzymatic hydrolysis. The effect of amylolytic, proteolytic, cellulolytic and hemicellulotitic enzymes on the quality and nutritional value of protein lupine concentrates was studied by scientists [14,15].

Russian scientists have also developed a method for obtaining a protein concentrate using amylolytic enzymes, namely starch hydrolysis with amilosubtilin and glucoadamorine. Enzymes were added in the amount of 2.4 units AC/g of flour starch and 2.8 units GlA/g of flour starch, respectively, at 37°C. Hydrolysis was carried out for 3 hours, after which the mixture was centrifuged, the centrifuge was decanted, and the protein was precipitated in it at an isoelectric point (3.0-3.2 pH). The chemical composition of the product obtained is as follows: protein – 64.2%, fat – 7.9%, carbohydrates – 17.1%, humidity 8.3%, ash – 2.5% [14].

In Plekhanov Russian University of Economics [15] the induced autolysis to produce protein concentrate from the seeds of lupine was proposed. At the first stage of obtaining the protein product, organic proteolytic was carried out, where acidic protease was used as exoenzymes. The process was carried out at 4.0 pH for three hours with the hydromodule 5. The second stage, autolysis, was carried out using endoenzymes at a temperature of 22°C, 7.0 pH, for 72 hours. As a result, a protein product with a protein content of 43.0% SR was obtained.

Scientists have also created many multi-enzymatic compositions that would possibly increase the protein content of the finished product. The researchers selected the following enzyme preparations: Celluclast BG, Celoviridine G20x, Cellulase-100 (cellulolytic activity), Pentopan Mono (hemicellulolytic activity), α-amylase, Distitsim BA-T Special (amylolytic activity). Thus, when using enzymatic hydrolysis with the indicated enzymes, the amount of protein in the finished product was 55% [4].

Therefore, for the production of protein concentrates with the enzymatic hydrolysis method, selection of enzymes that have a complex effect on the macronutrients raw materials is a priority. For this purpose it is necessary to thoroughly investigate the contents of the main components of the studied varieties of lupine seeds.

The main indicator of the prospect of using lupine for the production of protein products is the protein content. Accordingly, it is appropriate to study the amount of this nutrient in domestic lupine varieties and compare it with the domestic soybean variety [16] (fig. 4.1).

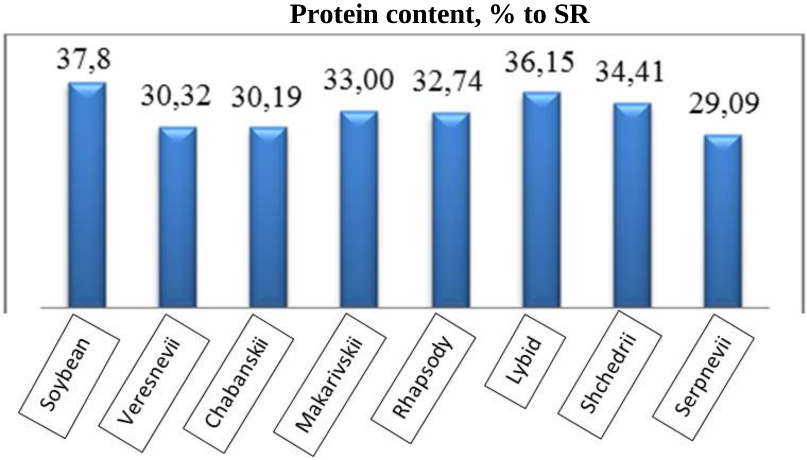


Figure 4.1. Protein content in lupine and soy

From fig. 4.1 it can be concluded that the amount of protein in soybeans is 37.80% to RS. In domestic varieties of lupine, this indicator is volatile between 29.09 and 36.15% to RS. According to the data received, the protein content of the lupine seed of the "Lybid" variety is less than in soy by 1.65%. Other varieties contain a significantly lower amount of this nutrient. Thus, the “Serpnevyi” variety is inferior to soybean by 8.71% of protein. These results indicate that the protein content of the lupine seed in not the same different varieties and is 32.62% on average. The indicators identified make it possible to confirm that lupine is a high-protein crop.

An analysis of all varieties of lupine for the alkaloid content has also been carried out. These compounds are natural organic substances of plant origin, which have alkaline properties and when interacting with acids form salts [17]. According to the research results, the content of alkaloids in domestic varieties of lupine is 0.001% ("Veresnevyi", "Lybid", Shchedryi), 0.002% ("Serpnevyi", "Rhapsody") and 0.003% ("Chabanskyi", "Makarivskyi"). The resulting values make it possible to confirm that the domestic lupine varieties are low alkaloid, since the content of this anti-nutrient compound does not exceed the regulatory value of 0.025%. This indicator shows the possibility of using lupine for food production.

An important indicator of the protein quality is its biological value. This indicator reflects the degree of compliance of its amino acid composition with the body amino acids needs. Therefore, it is advisable to study the amino acid composition of lupine proteins and compare it with soy (table 4.1).

Table 4.1

Amino acid composition of the studied varieties

	Soy	Makarivskyi	Serpnevyi	Veresnevyi	Chabanskyi	Lybid	Shchedryi
Amino acid composition of protein, mg/g							
Key:							
Valine	48.3	31.06	27.39	35.47	30.02	34.50	35.35
Isoleucine	32.7	33.44	25.58	32.05	28.58	32.96	32.72
Leucine	65.0	111.80	91.80	109.94	98.52	112.77	112.14
Lysine	63.8	65.16	55.61	64.68	59.68	65.67	66.33
Methionine	11.6	9.55	8.69	10.63	9.53	9.12	9.23
Threonine	50.4	44.48	38.53	43.88	41.15	46.08	45.00
Phenylalanine	45.1	48.68	39.46	46.90	42.28	45.57	46.14
Dispensable:							
Alanine	53.9	49.39	42.21	49.97	46.27	37.28	33.31
Aspartic acid	96.6	104.50	91.57	98.01	96.20	91.65	89.97
Arginine	98.8	122.30	86.67	106.68	97.34	100.99	107.71
Histidine	31.7	28.75	24.14	28.41	26.47	19.61	20.44
Glycine	52.9	59.47	51.50	58.00	55.08	41.52	43.81
Glutamic acid	197.2	382.40	335.02	365.02	332.84	208.41	210.00
Proline	50.9	76.15	76.01	74.09	72.91	39.73	25.36
Serine	56.8	73.34	62.84	71.43	66.45	53.75	48.76
Tyrosine	34.4	43.76	31.22	41.46	32.88	37.79	36.03
Cystine	9.8	25.15	18.85	21.32	18.68	24.78	25.69
Key amino acids scores, %							
Lysine	116.0	118.5	118.2	128.0	118.6	119.4	120.6
Threonine	126.0	111.2	112.5	119.4	112.4	115.2	112.5
Methionine+ cystine	61.1	99.1	93.6	99.3	88.2	96.85	99.7
Valine	96.6	62.1	64.0	77.2	65.6	68.3	70.7
Isoleucine	81.8	83.6	74.7	87.2	78.1	82.4	81.8
Leucine	92.9	159.7	153.3	170.9	153.8	161.1	160.2
Phenylalanine + Tyrosine	132.5	154.0	137.6	160.0	136.9	138.9	136.9

According to the table 4.1, the limiting amino acids in soy are sulphur-containing methionine and cystine, which scores are respectively 61.1%. For all lupine varieties, the first limiting acid is valine (62.1-77.2%). According to the content of leucine, virtually all lupine varieties exceed soy significantly, namely, the variety "Makarivskyi" to 71%, "Serpnevyi" and "Chabanskyi" – to 65%, "Veresnevyi" – to 83%, "Lybid" – to 73%, and "Shchedryi" – to 72%. The content of isoleucine, threonine and lysine in all lupine varieties is identical. Amino acid scores for phenylalanine + tyrosine in all lupine varieties exceed soy, but the largest amount of amino acids is contained in "Veresnevyi" variety.

After studying the content of alkaloids and protein in domestic lupine varieties, and its amino acid composition for further research, we have selected 3 varieties: "Makarivskyi", "Lybid" and "Shchedryi".

The next step was to study the fat content (fig. 4.2).

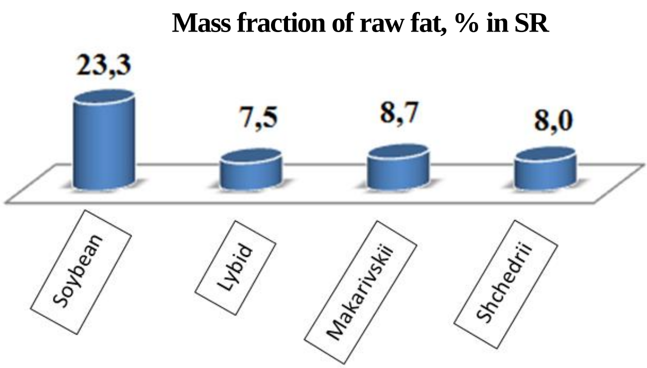


Figure 4.2. Fat content in lupine and soy

According to the research results, the fat content of the studied lupine seeds varieties is volatile between 7.5 and 8.7% to RS, which is significantly lower than in soybean seeds (23.3% to RS) [16]. That is why this nutrient is removed in soy protein concentrates production. As for lupine, the removal of lipids is not entirely appropriate. This is due to the fact that their amount is almost three times lower than in soy and the cost of the final product is significantly increased.

Since lipids are one of the main components of lupine seed, it is necessary to study its fatty acid content (table 4.2).

According to table 4.2, more than half of the fatty acids in all lupine varieties are monounsaturated fatty acids, namely oleinic acid (52.42-52.62%), the use of which is only 46% of the daily ration.

Table 4.2

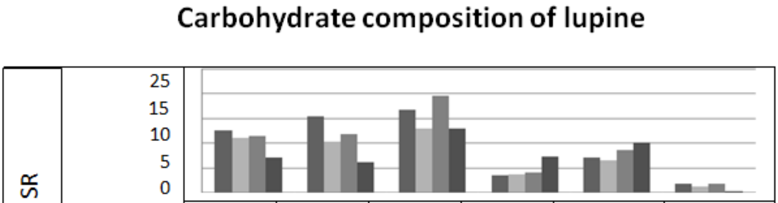
Fatty acid content of lupine and soy seeds

	Soy “Success”	Variety “Shchedryi”	Variety “Lybid”	Variety “Makarivskyi”
Mass fraction of fatty acid, % to the amount of fatty acids:				
Polyunsaturated fatty acids **(PSFA)**				
Linoleic acid (C18:2)	51.2	14.95	14.12	16.25
Linolenoic acid (C18:3)	7.3	9.09	8.41	8.00
Docosadienoic acid (C 22:2)	-	0.07	0.15	0.11
∑	58.5	24.11	22.68	24.36
Monounsaturated fatty acids **(MSFA)**				
Oleinic acid (C18:1)	22.4	52.54	52.62	52.42
Palmitoleic acid (C16:1)	7.9	0.30	0.26	0.31
Erucic acid (C22:1)	-	0.78	1.14	1.70
Cis-11-eicosenoic acid (C20:1)	-	3.36	3.36	3.56
∑	30.3	56.98	57.38	57.99
Saturated fatty acids **(SFA)**				
Palmitic acid (C16:0)	9.0	9.54	9.79	9.07
Myristinic acid (C14:0)	0.1	0.11	0.11	0.15
Stearic acid (C18:0)	1.8	2.57	2.86	2.22
Arachic acid (C20:0)	0.3	1.28	1.32	1.04
Behenic acid (C22:0)	-	4.03	4.41	4.20
Lignoceric acid (C 24:0)	-	1.29	1.34	0.89
Heptadecanoic acid (C 17:0)	-	0.02	0.03	0.02
Pentadecanoic acid (C15:0)	-	0.07	0.08	0.06
∑	11.2	18.91	19.94	17.65

The content of polyunsaturated fatty acids is 22.68-24.36%, more than half of which is linoleic acid (14.12-16.25%). The amount of saturated fatty acids is 17.65-19.94%, among which palmitic acid predominates (9.07-9.79%).

According to the literature [16] the soybean variety "Success-2" contains 58.5% of PSFA, 30.3% of MSFA and 11.2% of SFA. When comparing the fatty acid composition of soybean and lupine, it was found that the content of polyunsaturated fatty acids in soy is 2.4 times greater than in lupine. However, the amount of monounsaturated fatty acids is 1.9 times less than that of lupine. These results allow us to confirm that mono- and polyunsaturated fatty acids predominate in lupine seeds (74.54-77.96%). That is why lupine seeds can be considered a promising source of biologically active lipids.

An important place in lupine chemical composition is occupied by carbohydrates (fig. 4.3). The main representatives of this class of organic compounds in legumes are starch, sugars, fiber, pentosans, pectin substances, etc. Therefore, it is advisable to analyze the content of the above mentioned compounds in the studied lupine varieties (fig. 4.3).



	Sugar, %	Cellu-lose, %	Starch, %	Pectin, %	Hemicel-lulose, %	Lingine, %
Shchedrii	12.5	15.5	16.8	3.5	7.2	1.9
Lybid	11	10.3	13	3.7	6.5	1.2
Makarivskii	11.15	11.8	19.5	4.1	8.7	1.8
Soy	7.1	6.1	12.9	7.3	10.2	0.4

Figure 4.3. Carbohydrate composition of the studied lupine varieties

According to fig. 4.3 the basic components of the carbohydrate fraction are cellulose, starch and sugars. "Shchedryi" variety contains the largest share of sugar – 12.5%, and "Lybid" has the smallest share – 11.0%. Soy contains 7.1% of sugar, which is significantly lower than in any of lupine varieties. Almost half of the total amount of carbohydrates in lupine and soya seeds is food fibers such as cellulose, hemicellulose, lignin and pectin. In “Shchedryi” variety, the polysaccharides share is 44.9% of the total content of carbohydrates, in “Lybid” it is 34.7%, in "Makarivskyi" variety the share is 45.9% and in the soybean "Success-2"variety – 36. 9%.

Having examined and analysed the chemical composition of white lupine seeds, we found out that the share of protein content is 29.09 - 36.15% to RS, fat is 7.5-8.7% to RS, and carbohydrates – 45.7-57.4% to RS.

Having carried out a thorough study of the chemical composition of lupine, it can be stated that it is a promising raw material for the production of protein products. In order to obtain protein concentrates with maximum protein content, we have chosen enzymatic hydrolysis, where the selection of enzymes is a necessary component. It was precisely for this purpose that a thorough analysis of the carbohydrate component was carried out, which made it possible to confirm the necessity of using enzymes with amylolytic, cellulolytic and hemicellulolytic activity. Having examined the market for enzyme preparations, the following enzymes deserve particular attention: Cellulase, Pentopan 500 BG, α-amylase, Glucoamylase, Viscoflow MG, Ultraflo XL. Their characteristics are presented in table 4.3.

Table 4.3

Selected enzyme preparations characteristics

No.	Name of the enzyme preparation	Basic activity	Brand name, country of manufacture	Optimal temperature
1	Cellulase	Cellulolytic activity	S Bio, Ukraine	50-70°C
2	Pentopan 500 BG	The preparation has xylanase and hemicellulase activity.	Novozymes, Denmark	40°C.
3	α-amylase	Amylolytic activity	S Bio, Ukraine	90-95°C
4	Glucoamylase	Amylolytic activity	S Bio, Ukraine	55-62°C
5	Viscoflow MG	A mixture of beta-glucanase, xylanase, arabinoxylanase, pentosanase, cellulase, hemicellulase and bacterial α-amylase	Novozymes, Denmark	55 - 70°C
6	Ultraflow XL	Beta-glucanase preparation including the related activities - xylanase, pentosanase, cellulase and α-amylase.	Novozymes, Denmark	65-70 °C

These preparations have the necessary enzymes for the hydrolysis of both starch and non- starch polysaccharides. Thus, α-amylase and Glucoamylase break up starch to form dextrin and maltose. With simultaneous action of α-amylase and Glucoamylase starch is hydrolyzed by 95% [18]. Enzyme preparation Cellulase breaks down the internal bonds and the crystalline structure of cellulose to form the separate chains of disaccharides. Pentopan 500 BG has a xylanase activity that modifies pentosans [19]. Enzyme preparations Viscoflow MG and Ultraflo XL have all the necessary activities for the complex action on a substrate in their composition.

In the production of protein concentrates, important components of the process are also the duration of hydrolysis, temperature, pH of the medium, the hydromodule, application amount of enzyme preparation, whether it was mixed or not, etc. Due to combining a number of enzymatic hydrolysis aspects, there is a probability of obtaining a protein product with more than 45% of protein content.

Conclusions

Having examined the Ukrainian lupine varieties we found out that basically it is not inferior to soy as far as protein content and amino acid composition are concerned.

The amount of lipids in lupine is almost three times less than in soy. The fatty acid composition of lupine seeds is dominated by polyunsaturated and monounsaturated fatty acids, such as oleic and linoleic.

The carbohydrate composition study makes it possible to confirm that almost half of it is non-starch polysaccharides such as cellulose, hemicellulose, lignin and pectin substances.

Taking into consideration all the above-mentioned methods of obtaining protein concentrates, in our opinion, the most appropriate method is enzymatic hydrolysis. Our choice is due to the production of concentrates with the highest content of protein.

For enzymatic hydrolysis, we have chosen a number of enzyme preparations that, due to their enzymes, can have a complex effect on the carbohydrate component, releasing the protein bound to carbohydrates.

Thanks to the new studies, in the long run lupine products can be used in the food production, including in dining outlets technology.

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