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Studying the Features of the Protein Extraction from Oat Grains

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Abstract

Oat is one of the most common cereals and belongs to one of the most important fodder grains, since its acreage is only slightly inferior in size to the plantings of wheat, rice, corn and barley. The high biological value of this plant raw material, due to the high content of proteins, fats, vitamins, minerals, explains the interest of scientists in finding new solutions for the isolation of such substances and their further use in the agricultural and food industries, for example, to create enriched foods. The features of the process of extraction of protein from oat grains are studied in this article. Optimal parameters of acid and alkaline hydrolysis were selected in the course of the work. It was found that with the same technological parameters of acid and alkaline extraction, the greatest yield of protein was observed during the use of the 1 M sodium hydroxide aqueous solution. It has been proved that the use of alkaline hydrolysis allows achieving maximum protein yields from plant raw material with minimal losses (protein yield was 93.65 ± 5.62%).

Keywords: Oat, biological value, protein, alkaline hydrolysis, acid hydrolysis, protein yield.

INTRODUCTION

Russia is rightfully considered as one of the largest producers and consumers of oats. In the Siberian Federal District, oat is one of the main fodder grains, here its planting area reaches 1337.1 thousand hectares. To date, oats has played a major role in the agricultural and food industry of the country [1].

The widespread use of oat in the industry is explained by its high nutritional value due to the content of such components as proteins, carbohydrates, fats, vitamins and minerals in it. Oats are rich in complex carbohydrates, high-quality proteins and dietary fibers [2, 3, 4].

From all cereal crops the oat protein has the highest biological value, followed by rye, corn and wheat [5, 6]. The high nutritional value of oat proteins is determined by the presence of essential amino acids (methionine, lysine, valine, tryptophan, threonine, leucine, isoleucine, phenylalanine), which are not synthesized in the human body and animals from other amino acids, and should be administered together with food. Oat protein is considered to be balanced by amino acid composition, and the proportion of assimilable proteins in the oat grain reaches 90-95% of the total protein [7, 8]. The fractional composition of oat grains is represented by albumins, prolamines, glutelins and globulins [9].

Oat proteins or protein substances are multifunctional in terms of chemical composition and physicochemical properties. They can be used, absolutely, like all vegetable proteins, as moisture-, fat-binding agents, emulsifying agents in the food industry when designing and producing innovative functional foods, or specialized products [10].

It is worth noting that the total production of vegetable protein worldwide is less than needed about 1.5 times, and animal protein - almost 3 times. According to the International Food Commission at the UN, protein deficiency affects about the half of the world's population [11].

Vegetable raw materials contain proteins in the form of complex aggregates, and it is necessary to choose the correct method of isolation in order to isolate them in a biologically active state. There are a variety of methods for the isolation of protein substances in fundamental and practical science, but at present the most widely used ones are the methods of extracting useful components from plant raw materials [12]. The extraction is a complex process of extracting organic substances from animal, vegetable and mineral material in the presence of organic solvents in extractors. Currently, extraction is one of the main methods of extracting useful substances for the food, agricultural and pharmaceutical industries [13, 14].

Extraction of plant protein from the raw material is a laborious process and is complicated by the presence of water-soluble complex components, such as carbohydrates, lipids, pigments, inorganic ions, and other non-protein substances [15].

Aqueous solutions of polar and incomplete organic solvents, distilled water, aqueous solutions of neutral salts and buffer solutions, as well as solutions of acids and alkalis and enzyme preparations are used as extractants of protein substances. The above extraction methods are widely used for the production of protein substances in the food, feed and pharmaceutical industries. Each method has a number of advantages and disadvantages. Extraction using enzyme preparations is effective, but has a high net cost [16, 17]. Extraction with the use of aqueous solutions and distilled water allows for the fullest extraction of enzymes from plant raw material. Alkaline and acid extractions are the most preferred in the industry for the production of proteins and protein isolates [6]. Two extraction methods had been investigated and the most optimal one for the protein isolaton from oat grain was selected.

MATERIALS AND METHODS

The following were used as research objects:

- huskless oat grains of the "Sibirskiy Golozerniy" variety;
- glumiferous oat grains of the "Skakun" variety;

In order to isolate protein from oat grains, the following methods were used:

The mass fraction of protein in the oat grains was determined in accordance with GOST 10846-91 "Grain and its derivatives. Protein Determination Method".

The mass fraction of carbohydrates in oat grains was determined in accordance with GOST 31683-2012.

The mass fraction of fiber and fat in oat grains was determined in accordance with GOST 32749-2014 "Oilseeds, oil cakes and meals. Determination of moisture, fat, protein and fiber by near-infrared spectroscopy."

The mass fraction of ash was determined in accordance with GOST 10847-74 "Grain. Methods for determination of ash content".

The mass fraction of water was determined in accordance with GOST 13586.5-93 "Grain. Humidity Determination Method".

The optical density of the solutions (extinction) was determined using the UV 1800 spectrophotometer (Shimadzu, Japan).

The total protein yield was determined by the Dumas method using the RAPID N Cube protein nitrogen analyzer.

RESULTS

The Sibirskiy Golozerniy huskless oat and the Skakun glumiferous oat are the most widespread types of oats in the Siberian Federal District. To confirm the high protein content in the oats, studies were conducted to determine the chemical composition of the aforementioned varieties of oat grains (Table 1).

Table 1 - Chemical composition of the Sibirskiy Golozerniy oat grains

Indicator Name	Indicator Value	
	glumiferous oat	huskless oat
Mass fraction of protein,%	10.6±0.6	17.3±0.9
Mass fraction of carbohydrates,%	59.8±3.6	62.7±3.8
Mass fraction of cellulose,%	10.7±0.6	1.6±0.1
Mass fraction of fat,%	4.8±0.3	5.8±0.5
Mass fraction of ash,%	3.1±0.2	2.2±0.1
Mass fraction of water,%	11.0±0.7	10.9±0.7

Since the grains of the Sibirskiy Golozerniy huskless oat exceed the glumiferous oat by mass fraction of protein, and also are the leader in the content of replaceble and irreplaceable amino acids, it was selected for further research on the selection of the optimal method of protein extraction. A special feature of the variety is the absence of a film near the grain, which determines its high quality characteristics.

Alkaline extraction makes it possible to obtain a protein extract without mineral impurities. This method is disadvantaged by the formation of D-amino acids in toxic forms, as well as an increased color value of the extract (Maillard reaction). However, it is necessary to take into account the quality of the resulting protein concentrate or isolate, since the quality parameters of the protein concentrate are the main criteria for selecting the technological regimes for obtaining the finished product. Thus, the quantitative regularities of the extraction process - the maximum protein yield were studied.

The protein was determined by the biuret reaction. This reaction forms complexes of peptide bonds with copper (II) ions colored in violet in an alkaline medium.

The study started with the construction of a calibration graph. For this purpose a standard protein solution (albumin) containing 10 mg protein in 1 ml was prepared, a number of solutions of known concentration were prepared from a standard protein solution, according to Table 2.

Table 2. Dilution of a standard protein solution

Sample No.	Volume of standard protein solution, ml	Water volume, ml	Protein weight in sample, mg	Optical density of the solution
1	–	1.00±0.06	Control	–
2	0.20±0.01	0.80±0.04	2.0±0.1	0.265±0.010
3	0.40±0.02	0.60±0.03	4.0±0.2	0.328±0.020
4	0.60±0.03	0.40±0.02	6.0±0.3	0.385±0.020
5	0.80±0.04	0.20±0.01	8.0±0.4	0.471±0.030
6	1.00±0.06	–	10.0±0.6	0.585±0.030

4 ml of biuret reagent were added to the control tube and to samples with the known protein concentration, and left at room temperature for 30 minutes to develop color.

The measurements were carried out on a UV 1800 spectrophotometer (Shimadzu, Japan) at a wavelength of 550 nm against a blank sample. The results obtained for protein solutions of the known concentration are shown in Figure 1.

To select the parameters of alkaline extraction of protein from oat grains, the pH values of the medium, the hydromodule, the temperature and the duration of the extraction process varied.

At the first stage, the yield of protein from oat grains of "Siberian holographic" variety was studied depending on various pH values for alkaline extraction. The value of active acidity affects the extraction of solely organic compounds that are capable of ionization. In this case, charged organic compounds are hydrated much better than neutral molecules, and the latter, in turn, are better solvated by nonpolar organic solvents.

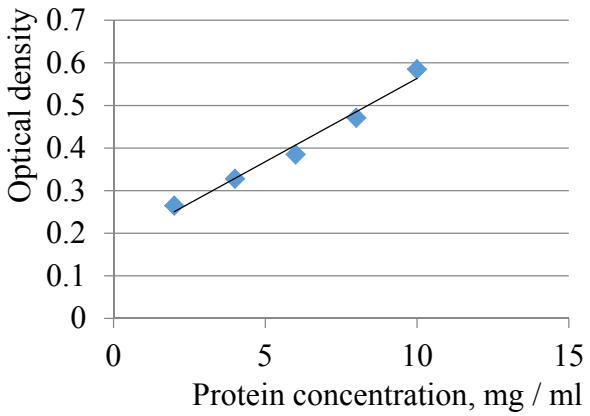

Figure 1 - Calibration chart for protein determination by biuret method

According to the theoretical aspects of extraction with the addition of acids to organic substances, the latter form salts of organic compounds. This reduces the number of undissociated molecules and increases the number of ions. This leads to a decrease in the degree of extraction of organic substances by the solvent. When adding the alkalis to organic compounds, the growth of undissociated molecules of these compounds is observed. As a result, the degree of extraction of organic compounds increases in alkaline medium.

The active acidity of the reaction medium varied between 9.0 and 13.0. At the same time, the 1 M sodium hydroxide aqueous solutions were used in accordance with GOST 4328-77, and 1 M potassium hydroxide - in accordance with GOST 24363-80. Grains of Sibirskiy Golozerniy oat were preliminarily chopped. Extraction was carried out with the following technological parameters: the process hydromodule was 1:10, the operating temperature of the process was 30 ± 2 °C, and the duration was 60.0 ± 0.3 min. During the experiment the test samples were constantly mixed.

The samples were then centrifuged at 7,000 ± 200 rpm for 15.0 ± 0.3 min for sediment detachment, 1 ml was withdrawn, and 4 ml of the biuret reagent were added, incubated for 30 minutes to color formation, and measured on a spectrophotometer at a wavelength of 550 nm. The protein concentration was determined using the calibration curve. All measurements were carried out in duplicate. The results of the study are shown in Table 3.

Table 3 - Effect of pH of the medium on the protein yield

Active acidity value	**1 M potassium hydroxide water solution**	**1 M sodium hydroxide water solution**
	protein yield, %	
9.0	8.94±0.53	12.75±0.76
9.5	12.81±0.76	14.56±0.87
10.0	14.70±0.88	18.78±1.12
10.5	19.83±1.18	23.22±1.39
11.0	23.28±1.39	34.77±2.08
11.5	72.99±4.38	81.18±4.87
12.0	67.06±4.02	77.71±4.66
12.5	68.09±4.08	74.61±4.47
13.0	89.34±5.36	-

Analysis of the results given in Table 3 shows that it is advisable to use aqueous alkaline solutions for the extraction of protein substances from plant matter. With an increase in the active acidity of the reaction medium (addition of solutions of potassium hydroxide and sodium hydroxide), the yield of protein from oat grains, as well as the mass fraction of protein in the extract increase. The use of the sodium hydroxide aqueous solution as an extractant results in a higher yield of protein from oat grains compared to the potassium hydroxide aqueous solution. Thus, for example, with an active acidity of 9.5, when extracting with KOH solution, the mass fraction of protein was 1.300 ± 0.07%, which was 13.7% lower than the yield of protein in case of sodium hydroxide aqueous solution. The highest yield of protein (in case of the sodium hydroxide aqueous solution) was observed with an active acidity of 11.5. In case of using an aqueous solution of potassium hydroxide, two maxima of the protein yield and two maxima of the protein mass fraction were observed. The first peak was observed with an active acidity of 11.5. At a given pH, the yield of the protein was 72.99 ± 4.38%, and the mass fraction of the protein was 7.409 ± 0.440%. The maximum yield of protein was observed at a pH of 13.0. At a given active acidity value, the protein yield was 89.34 ± 5.36%, and the weight fraction of protein in the resulting alkaline extract was 9.068 ± 0.540%.

The results show that, with an active acidity of 11.5, the mass fraction of protein in the alkaline extract obtained by the sodium hydroxide aqueous solution is 10% higher than that in the extract obtained by the potassium hydroxide aqueous solution. A further increase in the pH value leads to a more intense coloring of the solutions in yellow and contributes to an increase in viscosity. When extracting the protein with the sodium hydroxide aqueous solution at pH 13.0, the alkaline extract acquired a gel-like state, which resulted in the subsequent failure of the experiment, and a similar process was observed in samples with a pH of 12.5.

Thus, 11.5 was chosen as the optimum value of active acidity for the alkaline extraction process.

The change in temperature affects the distribution constant of the extracted substance. This is because when the temperature changes, the solubility of the extracted substances in each phase changes, and the mutual solubility of the organic and aqueous phases changes. Moreover, as the temperature varies, the solubility of the substance in each phase varies unequally.

When the temperature changes, the dissociation and association of the substance in the relevant phase also change. Therefore, when the temperature varies, hydration (solvation) and extractability of chemical compounds change.

In this regard, further research is aimed at studying the optimum temperature of alkaline extraction of protein from oat grains. To do this, the temperature varied from 35 to 65 °C during the experiment. A higher temperature leads to a decrease in the protein yield in the alkaline extract, due to hydrolyzing it to monomers. The influence of the temperature regime on the extraction process was studied at an active acidity of 11.5, and a hydromodule of 1:10. The

extraction time was 60.0 ± 0.3 min. The results are shown in Table 4.

Table 4 - Effect of extraction temperature on the protein yield

Extraction temperature, °C	Extractant solution 1 M KOH	Extractant solution 1 M NaOH
	protein yield, %	
35	71.17±3.56	83.69±4.18
40	72.11±3.61	94.04±4.70
45	61.77±3.09	79.80±4.00
50	63.74±3.19	67.37±3.37
55	68.31±3.42	81.01±4.05
60	69.84±3.49	79.18±3.96
65	71.33±3.57	70.68±3.53
70	68.22±3.41	69.87±3.49

The results of the empirical data presented in Table 4 show that the temperature of the alkaline extraction process affects the protein yield. The highest protein yield was observed at a temperature of 40±2 °C. Thus, when extracted with the potassium hydroxide aqueous solution, the protein yield at a temperature of 40 ± 2 °C was 72.11±4.33%, and in case of the sodium hydroxide aqueous solution, the protein yield was 94.04 ± 5.64%, which was 1.3 times higher than when extracted with the potassium hydroxide aqueous solution. A sharp decrease in the yield of the extracted substance at temperatures above 40 ± 2 °C is most likely due to the thermal hydrolysis of natural polymers to peptides of low molecular weight. In addition, as the temperature of the process increases, the rate of molecular diffusion increases due to an increase in the kinetic energy of the molecules and a decrease in the extractant viscosity. Thus, the optimal temperature for alkaline extraction of proteins from oat grains of the Sibirskiy Golozerniy variety is 40 ± 2 °C.

Extraction refers to diffusion processes and obeys the laws of mass transfer. If to represent the extraction in general form, it can be divided into four interrelated stages. At the first stage of mass transfer, an organic solvent penetrates into the pores of the plant material. At the second stage, the extractable substance is dissolved in the organic extractant. The third extraction stage consists in transferring the extracted organic matter from within the plant material to the phase interface.

The last extraction stage consists in convective diffusion (transfer of the extracted substance from the phases interface to the extractant). Immediately after the interaction of the organic solvent and the biologically active substance extracted, the molecules of the extracted substance pass into the extractant and, conversely, the solvent penetrates into the particles of the plant material. In this mass transfer process, the difference in the concentrations of the extracted substance and the organic extractant plays a leading role. Therefore, an important indicator of the extraction of biologically active substances into a chemical solvent is the hydromodule, due to the fact that the difference in the concentrations of chemical reaction agents is the driving force of the diffusion process. The greater the difference in the concentrations of the extractable substance in the cell and the solvent is, the more intensive the diffusion is. In addition, the larger the contact surface of the extractable substance with the solvent and the duration of contact is, the greater the amount of the substance will diffuse per unit of time in this system.

Further research is aimed at determining the optimum ratio of plant raw materials and chemical extractant. The results of the study are shown in Table 5. Extraction was carried out at an active acidity of 11.5, a temperature of 40 ± 2 °C, and duration of 60 ± 3 min.

Table 5 - Effect of hydromodule on the protein yield

Hydromodule	Extractant solution 1 M KOH	Extractant solution 1 M NaOH
	protein yield, %	
1:5	41.31±2.07	48.28±2.41
1:10	75.25±3.76	94.98±4.75
1:15	86.86±4.34	84.93±4.25
1:20	91.95±4.60	83.54±4.18

According to the results of Table 5, it can be established that the greater the difference in concentration of chemical reaction agents is, the higher the yield of protein from plant material is. The greatest yield of protein was observed with the hydromodule 1:10. Thus, for a given difference in the concentration of reagents, in case of the potassium hydroxide aqueous solution, the protein yield was 75.25 ± 3.76%, and in case of sodium hydroxide, the protein yield from the plant raw material was 94.98 ± 4.60%, which was 1.26 times more than the extraction of protein in the presence of the potassium hydroxide solution. The use of a higher hydromodule (more aqueous solution of alkali) led to a decrease in the protein isolate yield, denaturation of protein substances, and the change in the consumer indices of protein isolates, due to the formation of dark-colored compounds.

When determining the optimal regimes of protein extraction from oat grains of the Sibirskiy Golozerniy variety, besides temperature, it is necessary to take into account the active acidity and the ratio of the concentrations of the extracted substance and the solvent, and also the process duration. During the extraction, it is necessary to strive for the maximum completeness of extracting the extracted substances in a short period of time. A long extraction process contributes to the contamination of extracts and isolates by concomitant high-molecular compounds, the diffusion rate of which is much lower than that of the biologically active substances extracted.

For this purpose, the dependence of the yield of extractive substances on the duration of the process was determined. The process of protein extraction from oat grains was carried out for 30, 60, 90, 120 and 150 minutes. Extraction was carried out with the sodium hydroxide aqueous solution at an active acidity of 11.5, a temperature of 40 ± 2 °C, and a 1:10 hydromodule. The results of the study are shown in Table 6.

Table 6 - Effect of the duration of alkaline extraction on protein yield

Process duration, min	Protein yield, %
30	80.99±4.85
60	91.78±5.50
90	93.65±5.62
120	93.95±5.64
150	94.25±5.65

The results of determining the optimal duration of protein extraction from oat grains of the Sibirskiy Golozerniy variety, presented in Table 6, show that the amount of the extracted substance diffused through the layer of the organic membrane of the plant material is directly proportional to the extraction time. The highest protein yield (94.25 ± 5.65%) was noted with the duration of alkaline extraction of 150 min. In the extraction process, the first 90 minutes saw a sharp increase in protein yield (this value was 93.65 ± 5.62%). The subsequent increase in the duration of protein extraction with alkali aqueous solution up to 150 min allowed increasing the protein yield by only 0.64%. Given the fact that the duration of any technological process should be determined by economic considerations, it is advisable to select the optimal time period. It was found that the optimal extraction time with the alkali aqueous solution of alkali at a temperature of 40 ± 2 °C, a 1:10 hydromodule and an active acidity of 11.5 is 90 ± 2 min.

Acid extraction makes it possible to obtain an extract with the lowest degree of amino acids destruction, as well as a low content of toxic substances, such as enantiomers.

In order to select the optimal technological parameters for acid extraction of protein from oat grains of the Sibirskiy Golozerniy variety, the same parameters as for the alkaline extraction (active acidity, concentration difference, temperature and duration of the process) varied.

In order to vary the pH of the reaction medium, aqueous solutions of inorganic acids such as hydrochloric acid and sulfuric acid were used. The active acidity of the reaction medium varied between 1.0 and 3.0. Extraction was carried out with the following technological parameters: the process hydromodule was 1:10, the operating temperature of the process was 30 ± 2 °C, and the duration was 60.0 ± 0.3 min. During the experiment the test samples were constantly mixed. The results of the study are shown in Table 7.

Table 7 - Effect of pH of the medium on the protein yield

Active acidity value	Hydrochloric acid aqueous solution	Sulfuric acid aqueous solution
	protein yield, %	
1.0	35.98±2.16	33.12±1.98
1.5	43.56±2.61	39.44±2.37
2.0	46.77±2.81	44.23±2.65
2.5	44.12±2.65	41.86±2.51
3.0	42.33±2.54	30.88±1.85

It can be concluded that in case of using acid extraction of protein from oat seeds, the optimal value of active acidity is 2.0 units. The greatest protein yield from the prototype is achieved when using the hydrochloric acid aqueous solution.

Further research is aimed at studying the optimum temperature of acid extraction of protein from oat grains.

The influence of the temperature regime on the extraction process was studied at an active acidity of 2.0 units, and a hydromodule of 1:10. The extraction time was 60.0 ± 0.3 min. The results are shown in Table 8.

Table 8 - Effect of extraction temperature on the protein yield

Extraction temperature, °C	Hydrochloric acid aqueous solution	Sulfuric acid aqueous solution
	protein yield, %	
35	34.15±1.71	32.27±1.61
40	46.98±2.35	44.65±2.23
45	43.26±2.16	41.88±2.09
50	42.11±2.10	38.97±1.95
55	41.58±2.08	36.40±1.82
60	39.17±1.96	35.42±1.77
65	35.19±1.76	32.63±1.63
70	33.44±1.67	31.19±1.56

It can be concluded that in case of using acid extraction of protein from oat seeds, the optimal temperature is 40±2 °C. The greatest protein yield from the prototype is achieved when using the hydrochloric acid aqueous solution.

Then, the optimum ratio of plant raw material and chemical extractant was determined. The results of the study are shown in Table 9. Extraction was carried out at an active acidity of 2.0 units, a temperature of 40 ± 2 °C, and a duration of 60 ± 3 min.

Table 9 - Effect of hydromodule on the protein yield

Hydromodule	Hydrochloric acid aqueous solution	Sulfuric acid aqueous solution
	protein yield, %	
1:5	41.31±2.06	43.26±2.16
1:10 AM	46.96±2.35	45.01±2.25
1:15 AM	42.77±2.14	39.33±1.97
1:20	35.19±1.76	32.87±1.64

In case of acid extraction of protein from oat seeds, the optimum difference in reagent concentrations is 1:10. The greatest protein yield from the prototype is achieved when using the hydrochloric acid aqueous solution.

To determine the duration of the acid extraction, the extraction was carried out with the hydrochloric acid aqueous solution with an active acidity of 2.0, a temperature of 40 ± 2 ° C and a 1:10 hydromodule. The results of the study are shown in Table 10.

Table 10 - Effect of the duration of acid extraction on protein yield

Process duration, min	Protein yield, %
30	41.19±2.06
60	44.32±2.22
90	46.96±2.35
120	47.74±2.39
150	48.43±2.42

It has been found that the optimal extraction time with the acid aqueous solution of alkali at a temperature of 40 ± 2 °C, a 1:10 hydromodule and an active acidity of 2.0 is 90 ± 2 min.

DISCUSSION

By comparing the results of studies on the qualitative and quantitative indicators of the protein extraction process from oat grains by acid and alkaline methods, it can be concluded that for two types of extraction the optimum technological parameters are the temperature of 40 ± 2 ^{o}C, the 1:10 hydromodule, the 90 ± 2 Min duration, the active acidity of acid extraction 2.0 and active acidity of alkaline extraction 9.0. It had been found that at these extraction parameters, the greatest protein yield was observed with the 1 M sodium hydroxide aqueous solution (protein yield was 93.65 ± 5.62%) and the 1 M hydrochloric acid aqueous solution (protein yield was 46.96 ± 2.80%).

CONCLUSION

The results obtained indicate that in order to obtain a protein isolate from the Sibirskiy Golozerniy oat grains and subsequent production of enriched food products based on oat grain proteins, the use of alkaline extraction of biological substances from plant material will allow for the maximum protein yield from plant material with minimal losses (the protein yield is 93.65 ± 5.62%).

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