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Investigation of the chemical composition and physicochemical properties of *Chlorella vulgaris* biomass treated with pulsed discharges technology for potential use in the food industry

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ABSTRACT

The use of chlorella as a dietary supplement has great prospects. Nevertheless, the processing of chlorella is associated with certain difficulties that limit its use on an industrial scale. Problems with the processing are primarily related to the thick and strong cell wall of chlorella (50-100 nm), which is poorly digested by most vertebrate species due to its complex multilayer structure. Our experiments have shown that discharge pulse treatment contributes to the destruction of the strong cell wall of chlorella. The results of atomic force microscopy and the determination of the antioxidant activity of the suspension confirm this. A study of the chemical composition of dried chlorella biomass showed a content of 56.8% protein and 12.6% fat, which causes a high nutritional value of chlorella. The study of the physicochemical properties of the prepared chlorella preparation showed pronounced hydrophilicity of proteins. Observation of gels with different contents of chlorella preparation, formed during heating and subsequent cooling and stored for seven days at +8 °C, showed that the gels do not emit a synergistic liquid. Total gels based on the chlorella preparation are characterized by high stability. Based on the results obtained, we concluded that the preparation based on disintegrated chlorella has a high potential for functional and technological application in food technologies.

Keywords: *Chlorella vulgaris*, protein, gel, food products, pulsed discharges technology

INTRODUCTION

Currently, to ensure food security, the vector of development of the food industry is aimed at finding an alternative raw material base that will provide the population with essential nutrients and biologically active substances **[1]**, **[2]**. Many works consider non-traditional plant raw materials, insects, and seaweed as the most promising raw materials for processing and use in food products **[3-7]**.

In our opinion, microalgae represent a high raw material potential for the food industry. The basis providing a multifunctional complex of mechanisms of adaptation of these plant species to sudden changes in environmental conditions is a highly complex metabolism of unicellular phototrophs. This contributes to the widespread settlement of microalgae in the biosphere and provides simple cultivation of microalgae biomass **[8]**, **[9]**. The use of microalgae biomass in the food industry is also interesting from the point of view of high protein content and biologically active substances **[10]**, **[11]**. It is by the content of protein and biologically active substances that *Chlorella vulgaris* stands out among many microalgae. The planktonic strain of *Chlorella vulgaris* has been studied for several decades. Scientific databases have many data on its chemical composition and physicochemical properties **[12-16]**. However, most of the work was carried out with the native form of chlorella, although the cell wall is a strict limiting factor that reduces the degree of chlorella digestion and, consequently, the digestibility of *Chlorella vulgaris* biomass **[17]**, **[18]**. Therefore, chlorella with a destroyed cell wall is of great practical value.

Technologies based on biological, mechanical, chemical and electrophysical exposure methods can destroy the chlorella cell wall **[19]**. Electrophysical methods are the most promising for processing chlorella biomass into food products since they are inert and do not have additional effects on biological objects. Among the

electrophysical methods, the discharge-pulse technology proposed by Oboturova et al. (2015) is interesting **[20]**. The authors studied the effect of discharge-pulse treatment on the structure of muscle fibers of animal products and the hydration of biopolymers. The work results showed that during discharge-pulse processing in the established modes, the destruction of myofibrils of meat raw materials with a hardness of 10 to 150 kPa occurs, as well as the rupture of the cell walls of microorganisms with a tensile strength of 60-120 kPa. Consequently, the discharge-pulse technology can also be used for the disintegration of the cell wall of *Chlorella vulgaris*.

This work aimed to study the chemical composition and physicochemical properties of *Chlorella vulgaris* biomass treated with discharge-pulse technology for potential use in the food industry.

Scientific Hypothesis

A protein preparation based on chlorella treated with discharge-pulse technology has high biological activity and can be used in food products.

MATERIAL AND METHODOLOGY

Samples

Suspension of *Chlorella vulgaris* strain CALU 157 (3 g/l) obtained from Bionics Inc. (Stavropol, Russia).

Chemicals

We used reagents of recognized analytical purity and distilled water. The following chemicals were used in the work: Acetone, Glycerin, Ethanol, Sodium carbonate, Sodium sulphate, Magnesium acetate, Nitric acid, Sulfuric acid, Hydrochloric acid, Gallic acid, Oxalic acid, Sodium hydroxide, Potassium permanganate, Phenolphthalein. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality).

Animals and Biological Material

We have used *Chlorella vulgaris* strain CALU 157 (3 g/l) in this research.

Instruments

Geppler consistometer (Fungilab, Madrid, Spain), Laboratory water bath UT-4302E (ULAB, Saint Petersburg, Russia), universal testing machine Instron 3342 (Instron Engineering Corporation, Cambridge, USA), atomic force microscope Ntegra Spectra (NT-MDT, Zelenograd, Russia), Soxhlet extraction apparatus (IsoLab, Moscow, Russia), Kjeldahl apparatus (Gerhardt GmbH & Co. KG, Königswinter, Germany), homogeniser (Witeg Labortechnik GmbH, Wertheim, Germany), resistivity meter Fann 88C (Fann Instrument Company, Aberdeen, UK), centrifuge CNL-8 (AnalytPromPribor, Moscow, Russia), pH-meter F20-Standard (Mettler Toledo, Moscow, Russia), UV-1800 spectrophotometer (Shimazy Inc., Tokyo, Japan), Climate camera BINDER KBW 240 (BINDER GmbH, Tuttlingen, Germany), pulsed discharges generator PDG-6 (Unique Pulse, Stavropol, Russia).

Laboratory Methods

Antioxidant activity: by the spectrophotometric method according to GOST R 53160-2008 **[21]**.

The mass fraction of protein is determined by the Kjeldahl method according to GOST 26889-86 **[22]**.

The mass fraction of moisture: drying the suspension to a constant mass in the drying cabinet at a temperature of 103 ±2 °C.

The mass fraction of fat is determined by determining fat using the Soxhlet extraction apparatus according to GOST 13496.15-97 **[23]**.

The mass fraction of ash is determined by the accelerated mineralization method in the presence of a solution of magnesium acetate.

Microstructural studies were conducted on the atomic force microscope Ntegra Spectra (NT–MDT, Zelenograd, Russia) **[24]**.

Preparation of protein preparation gels: 20 ml of the protein preparation solution with the studied concentration was dispersed in water in 25 ml beakers. The dispersions were thoroughly mixed with a glass rod and kept for 30 minutes. The beakers were covered with aluminium foil to prevent moisture loss and heated in a water bath to plus 85 °C for 30 minutes. The hot samples were immediately placed in the refrigerator and kept at 4 °C for 24 hours.

Gel-breaking stress limit: measurements were carried out on a universal testing machine, Instron 3342 (Instron Engineering Corporation, Cambridge, USA), using a set of cylindrical indentors. The force expended by the indenter on uniaxial compression of the gel sample before its destruction (P_p), related to the sample area (Fo), was taken as the value of the ultimate fracture stress (σ):

$$\sigma = P_p / Fo, N/m^2 (Pa)$$

Solubility of dry protein preparations: sample preparation - according to the method used by S. Tian **[25]**: 20 ml of 0.4% aqueous solution of the protein product under study was suspended in a 25 ml test tube for 30 minutes.

The temperature of the solutions depends on the objectives of the experiment. The suspension was then centrifuged at 4000 rpm for 20 minutes.

The water absorption capacity was determined by the water saturation method of samples of dry protein preparations. The method is based on determining the absorption capacity of the drug by introducing a sufficient amount of moisture that can retain the material due to the capillary structure. An equal amount of the drug was placed in centrifuge tubes, and water was added, gradually increasing its amount for each subsequent tube. After mixing, the suspensions were kept for 30 minutes and centrifuged at 4000 rpm. The maximum amount of added water that did not separate after centrifugation was taken as the value of the water absorption capacity. The indicator was expressed as the ratio of absorbed moisture to the weight of the dry sample, expressed in %.

The degree of swelling of dry protein preparations was determined by the method of excess water **[26]**, according to which a sample weighing 0.25 g was placed in a 10 ml centrifuge tube, 5 ml of water was added, stirred for 30 minutes, weighed and centrifuged at 4000 rpm for 20 minutes. The infusion liquid is drained, and the test tube is weighed. The weight of the test tube is subtracted from the weighing results. The ability to swell is calculated as the ratio of the absorbed substance to the mass of the sample and expressed as a percentage:

$$\text{Degree of swelling} = ((M_1 - M_2)/M_2) \times 100\ \%$$

Where:
M_1 is the mass of the sample after absorption of water/oil (sediment), g; M_2 is the mass of the dry sample, g.

Description of the Experiment

Sample preparation: Suspension of *Chlorella vulgaris* strain CALU 157 (3 g/l). The population density is 50-60 million cells in 1 ml (Figure 1).

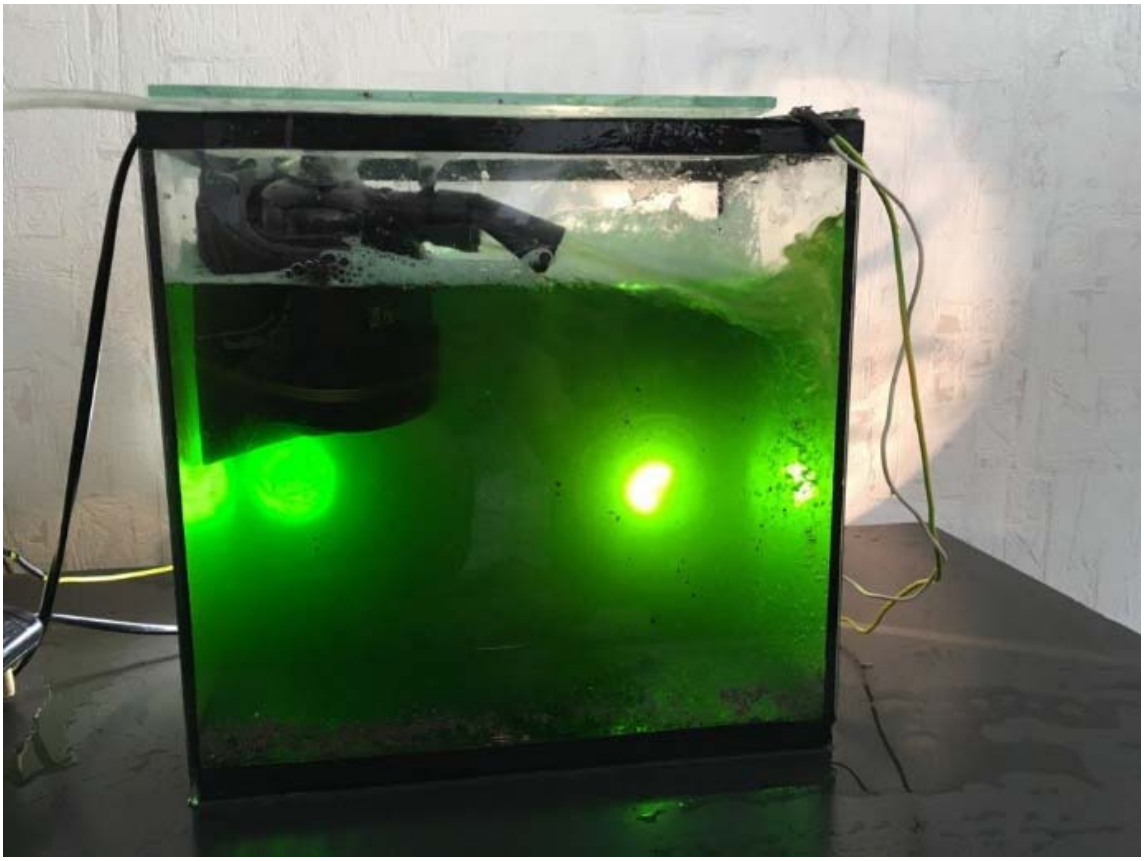

Figure 1 Preparation of *Chlorella vulgaris* strain CALU 157 (3 g/l) suspension.

Number of samples analyzed: 5.
Number of repeated analyses: 3.
Number of experiment replication: 2.

Design of the experiment

In the first stage of the experiment, we studied *Chlorella vulgaris* in the native state in the form of a suspension and a dried mass obtained by holding the homogenate and centrifugate of the suspension in a heat-drying cabinet for 24 hours at a temperature of 105 °C. A technology of chlorella disintegration has been developed to improve its digestion utilizing discharge-pulse processing. The antioxidant activity of chlorella suspension and the microstructure of chlorella cells after discharge-pulse processing were studied to obtain information on the degree of disintegration of chlorella cells. Next, we studied the chemical composition of the prepared protein preparations. We also conducted a study of the physicochemical properties of the obtained protein preparations, including determining: the effect of temperature on the solubility of chlorella proteins, the effect of NaCl concentration on the solubility of chlorella proteins, the effect of the concentration of chlorella preparation on the strength of gels, as well as the water-absorbing ability and swelling of protein preparations based on chlorella biomass.

Statistical Analysis

Statistical processing of experimental data was carried out using the NSR program.BASE Excel and analysis of variance using Student's criterion with confidence probability ($\alpha = 0.95$).

RESULTS AND DISCUSSION

The Suspension of *Chlorella vulgaris* strain CALU 157 samples were sampled when a concentration of 3 g/l was reached, corresponding to a population density of 50-60 million cells in 1 ml (Figure 2).

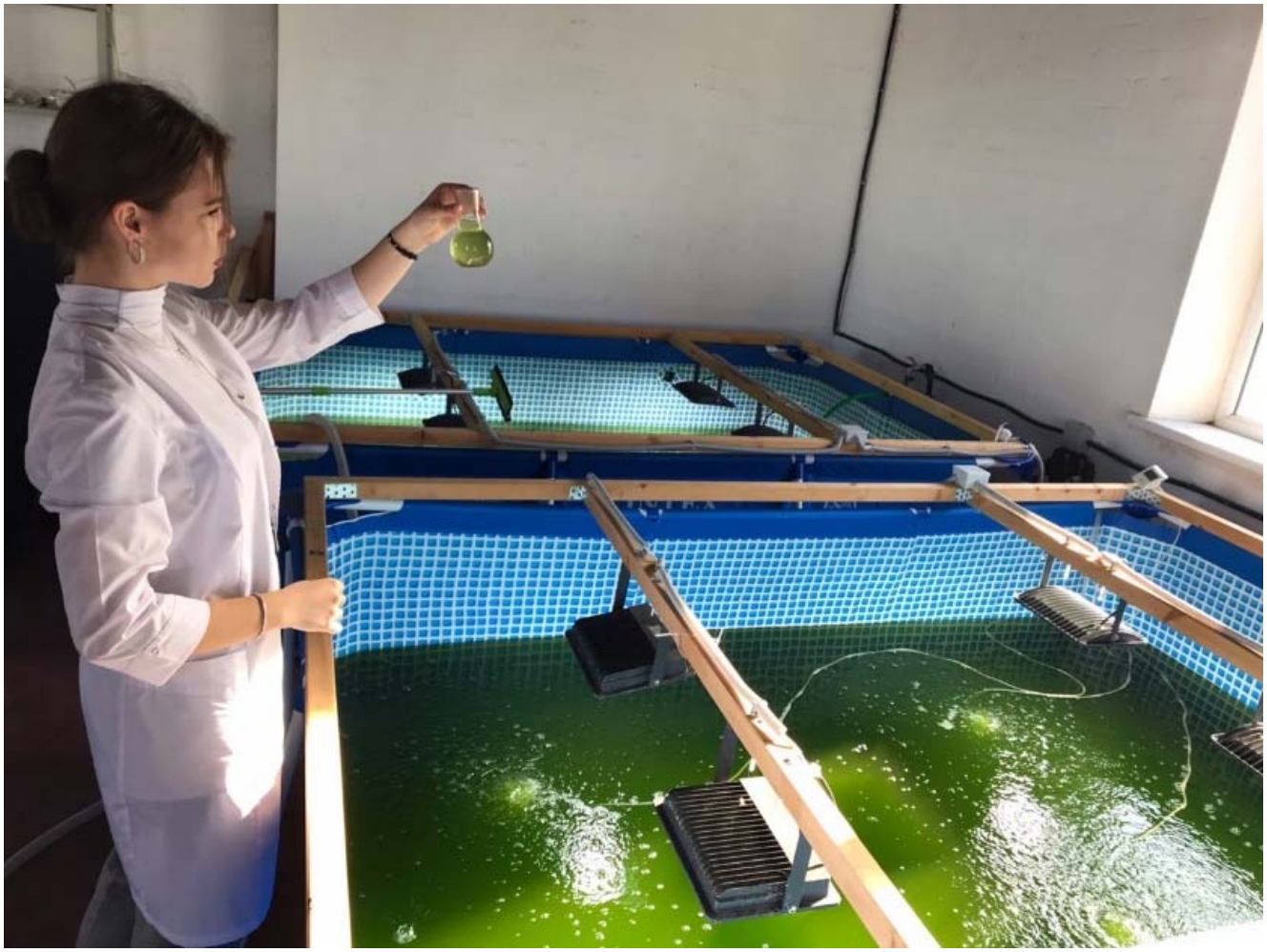

Figure 2 A sampling of samples Suspension of *Chlorella vulgaris* strain CALU 157.

Discharge-pulse treatment of chlorella was carried out using pulse discharge generator PDG6 in the energy mode of 5 kJ in 1-100 pulses.

The disintegration results were determined by microstructural analysis and investigation of changes in the antioxidant activity of the suspension, which indirectly shows the presence of free antioxidants in the system – chlorophyll and chlorellin **[27-29]**.

The results of atomic force microscopy are shown in Figure 3.

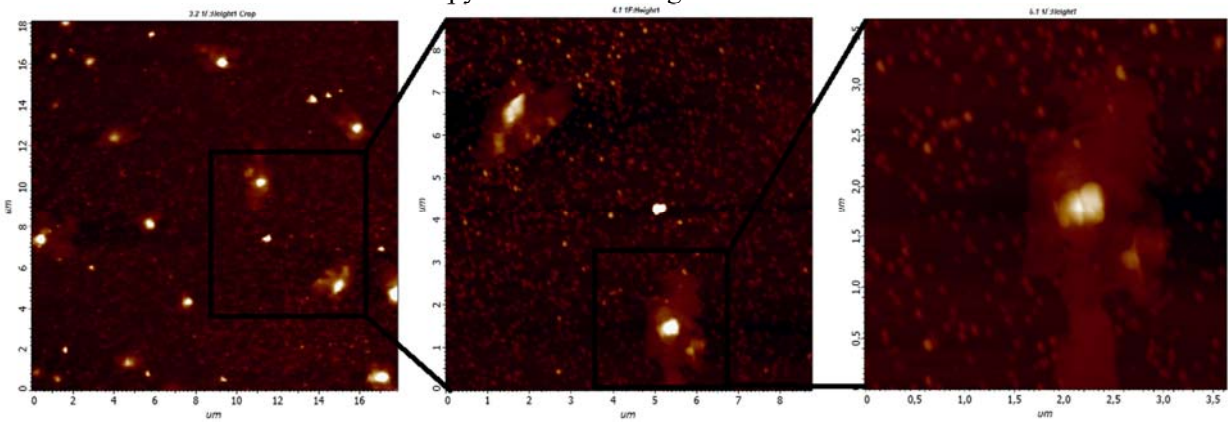

Figure 3 Atomic force microscopy of the treated chlorella suspension.

According to the scans of the chlorella suspension, the shape of the studied microflora changed discharge-pulse treatment. Some objects have passed into the coliform (elongated) from the coccoid form. At the same time, an area with culture fluid is visible around the chlorella units, apparently, at high hydraulic pressures, a rupture of the strong cell wall occurred, and the culture fluid passed into suspension. This assumption is confirmed by the results of antioxidant activity studies (Figure 4).

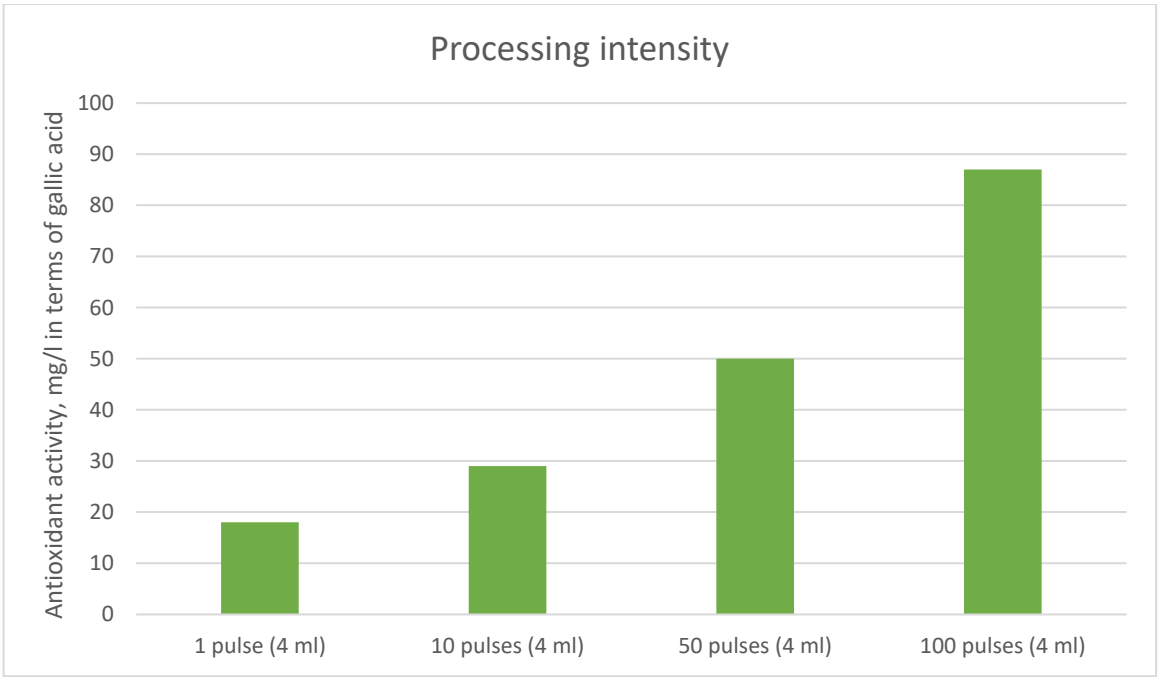

Figure 4 Antioxidant activity of the treated suspension.

When processing the chlorella suspension, the optical density varied from 0.109 (1 pulse discharge) to 0.205 (100 pulse discharges). Antioxidant activity increased along a steeper trend line from 17.2 mg/l (1 pulse discharge) to 87.3 mg/l (100 pulse discharges).

Thus, during discharge-pulse processing, the main goal associated with the disintegration of the cell wall of chlorella was achieved. This is also justified by the study of antioxidant activity and atomic force microscopy.

After the discharge-pulse treatment, the chlorella suspension was filtered through a filter membrane with a pore size of 2 microns. The filtrate was dried in the Climatronic climate chamber at 105 °C for 24 hours.

We examined the resulting chlorella biomass preparation for the content of protein, moisture, fat, and ash (Table 1).

According to the data obtained, the dried biomass of chlorella contains about 56.8% protein with 12.6% fat. The high ash content is due to the high content of carbohydrates included in the structure of the cell wall of chlorella in the form of composite glycoproteins **[30]**, **[31]**.

Table 1 Results of the study of the chemical composition of dried chlorella biomass (p <0.05).

Indicator	Meaning
Mass fraction of crude protein, %	56.8 ±1.1
Mass fraction of moisture, %	6.2 ±0.05
Mass fraction of crude ash, %	24.4 ±0.1
Mass fraction of crude fat, %	12.6 ±1.3

The most significant property of proteins is their ability to interact with water **[32]**, **[33]**. Solubility is one of the main indicators characterizing such an interaction **[34]**. It is known that solubility is determined by the specific properties of proteins – the size and shape of molecules, molecular weight, and the value of the isoelectric point **[35]**, **[36]**. This indicator is significantly influenced by external factors – pH, temperature, ionic strength, amount of solvent, presence of salts, and other environmental conditions **[37-39]**. Therefore, the same protein product under different hydration conditions may show a different dissolved ability. In this regard, we determined the effect on the solubility of chlorella protein of the most important technological factors: temperature (Figure 5) and sodium chloride (Figure 6).

Solubility is the ratio of the protein content transferred to the solution during extraction to the total protein content (2.592 mg/ml), expressed as a percentage.

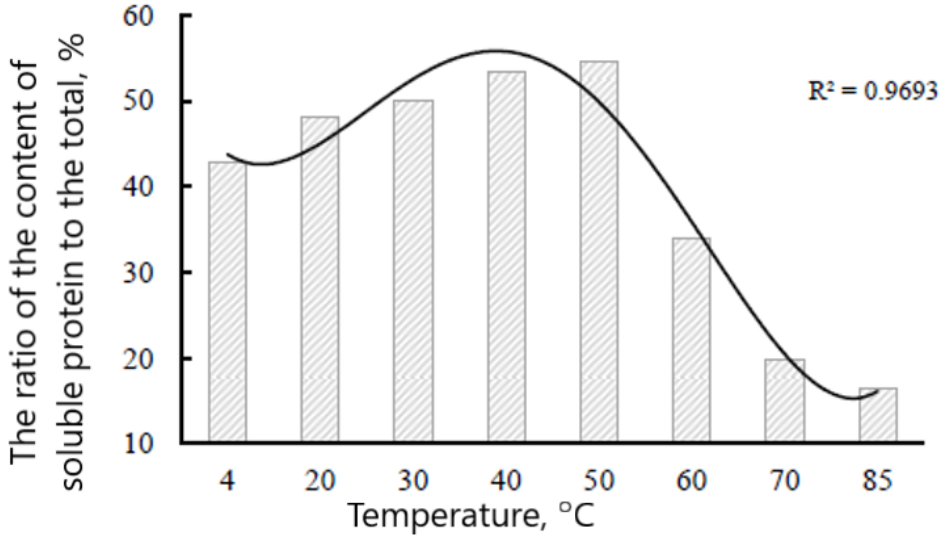

Figure 5 The effect of temperature on the solubility of chlorella proteins.

It is known that in the temperature range at which the main technological processes of meat processing take place – from +4 °C to +20 °C, from 1.11 to 1.24 mg/ml of protein passes into the solution, which is 42.9 and 48%, respectively, to their total content determined at +20 °C.

As the temperature increases, the protein solubility of the protein mixture increases and reaches maximum values at a temperature of about +50 °C and is 127% of the initial level at +4 °C. The subsequent increase in temperature leads to a decrease in the indicator, and at +8 °C, its value is 38.5% of the initial level. When the solvent temperature rises to +50 °C, the indicator's values increase is most likely due to an improvement in the solubility of the collagen ingredient, which is consistent with the data of Post et al. (2011) **[40]**. The observed decrease in the solubility of the mixture in the temperature range from +50 to +85 °C is probably due to the development of denaturation and aggregation processes in non-collagen proteins, which was also declared in the works of other researchers **[41-44]**.

Studies of sodium chloride's effect on the protein mixture's solubility were carried out at +4 °C. Also, according to the data obtained, the differences in the solubility of the protein mixture at +4 and +20 °C do not exceed 5% (Figure 6).

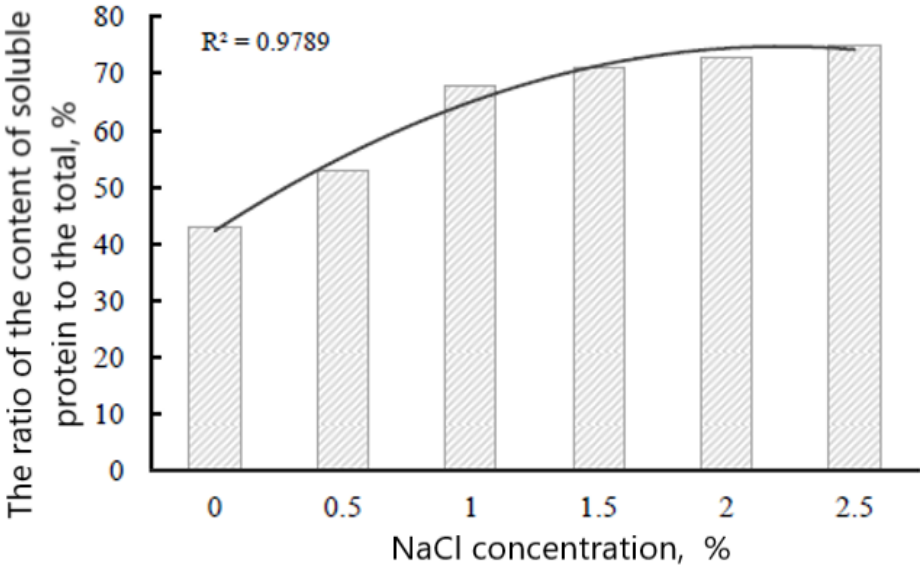

Figure 6 Effect of NaCl concentration on the solubility of chlorella proteins.

We found that in the presence of sodium chloride, the solubility of the proteins of the studied mixture increases significantly. The most noticeable changes were noted when the NaCl content was up to 1% by weight of the solution. With an increase in the NaCl content in the system, the solubility of proteins also increases, but less intensively. Comparison of data on the solubility of chlorella proteins at +4 °C without the addition of NaCl (Figure 5) and in the presence of 0.5; 1.0; 1.5; 2.0, and 2.5% sodium chloride (Figure 6) showed that the values of the indicator increased by 1.23; 1.59; 1.66; 1.70 and 1.75 times, respectively.

Along with solubility, protein ingredients' most important functional and technological properties include moisture absorption capacity and degree of swelling **[45]**, **[46]**.

The scheme of water binding by dry protein includes the concept of water retained by the texture of the drug due to capillarity and the idea of swelling, which is expressed by an increase in the volume of water adsorption in this type of product **[47]**, **[48]**. The study of the ability of dry protein preparations to retain or adsorb moisture is critical since it is this moisture that gives juiciness to products **[49]**.

From a practical point of view, the rapid and effective swelling of ingredients contributes to the immobilization of moisture, which is extremely important at those stages when the process of absorption and binding of moisture by muscle proteins takes time **[20]**, **[50]**.

The moisture absorption capacity was determined at a temperature of +4 °C. It was found that the moisture-absorbing capacity of chlorella proteins was 642%, which means that 1 part of the dry preparation, with the application of some physical activity, can retain up to 6.4 parts of water in its structure.

The determination of the degree of swelling showed that under conditions of excess water, moisture retention by the structure of the mixture loses its significance; part of the proteins dissolves, and the remaining part swells. After 30 minutes of exposure to protein dispersion at +4 °C, the amount of moisture that did not separate after centrifugation was 880.4% by weight of the dry sample.

The ability to form gels is not only a critical property and function of proteins. Still, it is also crucial for the targeted use of protein ingredients in the technology of certain food products **[51]**.

To determine the effect of the concentration of the chlorella preparation on the strength characteristics of thermoformable gels (Figure 7), the breaking stress of gels with a mixture content of 6 to 20% was studied.
According to the data obtained, the magnitude of the ultimate destruction stress linearly depends on the concentration of the protein mixture under study. Similar dependencies have been established for soy proteins **[36]**, whey **[42],** and gelatin **[52]**.

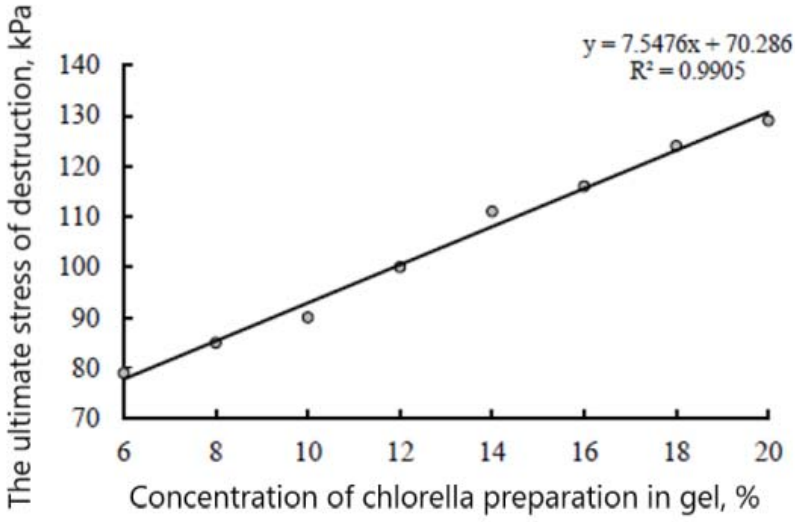

Figure 7 The effect of chlorella drug concentration on the strength of gels.

Observation of gels with different contents of the chlorella preparation formed during heating and subsequent cooling and stored for seven days at +8 °C showed that the gels do not emit synergistic fluid; that is, gels based on the chlorella preparation are characterized by high stability.

Thus, our experiments have shown that discharge-pulse treatment of chlorella suspension makes it possible to destroy the strong cellular structure of microalgae, thereby ensuring the transition of biologically active substances into suspension. The preparation obtained from destroyed chlorella cells showed a high protein content and physico-chemical properties that characterize the studied product as a good water-binding agent that can be used in food production.

CONCLUSION

Our experiments have shown that discharge pulse treatment contributes to the destruction of the strong cell wall of chlorella, which is confirmed by atomic force microscopy and the determination of the antioxidant activity of the suspension. Moreover, the antioxidant activity of the suspension grew along a steep trend line from 17.2 mg/l (1 pulse discharge) to 87.3 mg/l (100 pulse discharges), which indicates the nonlinearity of the effect of discharge-pulse treatment. From the treated suspension, we obtained it by holding the homogenate and centrifugate of the suspension in a heat-drying cabinet for 24 hours at a temperature of 105 °C. According to the data obtained, the dried biomass of chlorella contains about 56.8% protein with 12.6% fat. The high ash content is due to the high content of carbohydrates included in the structure of the cell wall of chlorella in the form of composite glycoproteins. It was found that in the temperature range at which the main technological processes of meat processing take place – from +4 °C to +20 °C, from 1.11 to 1.24 mg/ml of protein passes into the solution, which is 42.9 and 48%, respectively, to their total content determined at +20 °C.

We found that in the presence of sodium chloride, the solubility of the proteins of the studied mixture increases significantly. The most noticeable changes were noted when the NaCl content was up to 1% by weight of the solution. With an increase in the NaCl content in the system, the solubility of proteins also increases, but less intensively. Comparison of data on the solubility of chlorella proteins at +4 °C without the addition of NaCl and in the presence of 0.5; 1.0; 1.5; 2.0, and 2.5% NaCl showed that the values of the indicator increased by 1.23; 1.59; 1.66; 1.70 and 1.75 times, respectively. It was found that the moisture absorption capacity of chlorella proteins was 642%. Observation of gels with different contents of the chlorella preparation formed during heating and subsequent cooling and stored for seven days at +8°C showed that the gels do not emit synergistic fluid; that is, gels based on the chlorella preparation are characterized by high stability. Thus, it can be concluded that the preparation based on disintegrated chlorella has a high potential for functional and technological application in food technologies.

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