Functional meat and vegetable pate with spirulina

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Abstract. Fundamental research has extensively described the various biological and medical effects of consuming the microalgae spirulina or its constituents. A number of studies indicate an increase in the immune response when they are used for the manifestation of anti-inflammatory, antidiabetic and other important properties. The composition of spirulina and its biological effects make it a unique dietary supplement, the inclusion of which in food products will provide them with functional properties. It has been proven that spirulina has a number of important functional and technological properties for its use as a component in recipes for meat products. In the study of the functional and technological properties of spirulina, it was found that the water absorption capacity was 117.8%, fat absorption was 117.0% and the swelling degree was 177.6%. At the same time, spirulina had low emulsifying and gelling properties. The introduction of spirulina paste into the recipe in a dosage of 1 to 2.5% can increase the quality indicators of the finished product. Results of organoleptic and physicochemical indicators, the most optimal level of spirulina administration was established, which was 2%. Analysis of the results of changes in the peroxide value in the pate samples during storage showed the presence of a high antioxidant activity of spirulina, which will extend the shelf life of the finished product.

Introduction
Currently, a significant part of the market segment is occupied by food products of a functional orientation. Interest in them is shown not only by scientists, but also by a significant part of the population [1, 2].

As a unique ingredient in the formulations of functional products, it is proposed to consider microalgae, which have a number of beneficial properties. Microalgae can be sources of fairly valuable compounds that have a beneficial effect on the human body. In antioxidants, prebiotic polysaccharides, dietary fiber, etc. The richness of compounds in microalgae can contribute to the development of a food industry focused on the manufacture of innovative products in order to improve the diet of people.

Spirulina platensis (spirulina) is a type of green algae that contains a spectrum of biologically active substances with a wide range of medical applications. Spirulina contains powerful antioxidants—phycocyanin and beta-carotene—which are considered as active protective components against kidney failure, cancer and hypertension. In addition, these substances exhibit antiviral and antibacterial activity. Unlike other algae, spirulina is easy to digest and absorb. Has a stable green color even at high dilution, which is also important for the organoleptic characteristics of a number of products [3, 4].
Presently, in foreign literary, information on the use of spirulina in the food industry is insufficient for a full analysis of the situation [5]. Several characteristics of microalgae limit their use in food. Changes in the color and taste of food are usually perceived by consumers as undesirable. The green color of microalgae limits their applications in everyday products [6]. However, there are a number of studies showing the possibility of using spirulina as a functional ingredient in food [7, 8].

From the analysis of scientific and technical information, spirulina is a promising ingredient for innovative functional products. However, complex work of scientists and processors in all branches of the food industry is required [9, 10]. And currently, the study of the possibility of using spirulina is only at the beginning.

Material and methods

The object of the research is spirulina powder of "ORGTIUM" brand (Moscow), samples of meat and vegetable pate.

We used standard and published methods in the special literature:

- pH measurement was carried out on an IPL-201 pH meter/ionometer (MULTITEST "Semiko")
- the content of dry substances was measured by drying to constant weight at a temperature of 102–105 °C.
- the assessment of taste, consistency and consumer properties of the product was carried out by means of a tasting assessment of experts on a 5- and 10-point scale.
- the water-holding capacity was determined by centrifugation method. The method is based on the fact that from the object under study, which is in a fixed position in a laboratory centrifuge, due to the action of centrifugal force, a liquid phase is released, the amount of which depends on the degree of interaction of steps with the frame phase of the object. Samples weighing about 4 g were placed in a polyethylene tube with a perforated insert to drain the liquid. The samples were centrifuged for 20 minutes at a speed of 1000 rpm after centrifugation and then weighed. The amount of retained moisture was determined by the formula:

$$x = \frac{(a - (t_0 - t_1))}{t_0} \times 100$$

where: $x$ is the mass of retained moisture, %; $t_0$ is the sample mass before centrifugation, g; $t_1$ is the mass after centrifugation, g; $a$ is the total amount of moisture;

- the solubility index was determined in the following way: a sample weight of 1.25 g was quantitatively transferred into a centrifuge tube, 4-5 ml of hot water was added, thoroughly rubbed with a glass rod until a homogeneous mass is obtained. The stick was rinsed with a small amount of water into the same test tube and warm water was added up to 10 cm$^3$. 2-3 drops of paint were added to each test tube (0.1 g of naphthol red or neutral red, or methyl green, dissolved in 100 cm$^3$ of distilled water). Then the tube was closed with a stopper and shaken several times. The tubes were placed in centrifuge cartridges, placing them symmetrically against one another, stoppers towards the center. The tubes were centrifuged for 5 minutes at 1000 rpm. The volume of the sediment was counted with the stoppers up. With an uneven level of the sediment, it was counted along the centerline between the upper and lower positions. The solubility index was expressed in cm$^3$ of the crude precipitate (0.1 cm$^3$ of the crude precipitate corresponds to 1% of the insoluble precipitate of the protein product);

- the emulsifying capability was determined as follows: for the preparation of the emulsion, a 1% protein solution of spirulina and deodorized refined vegetable sunflower oil were used. A series of emulsions with a fat content of 10 to 80% were prepared. Then the emulsion was poured with a syringe into test tubes with a diameter of 5 mm and a height of 100 mm and thermostated at a temperature of 85 °C within 15 minutes and centrifuged at 6000 rpm for 20 minutes. The criterion for the stability of the emulsion at the initial ratio of the fat and water phases was the average for test tubes the ratio of the height of the emulsion layer separated after testing to the total layer height (in%
by volume). Based on the results obtained, the following diagrams of the stability of emulsions in the axes were plotted: the initial volume fraction of the fat phase vs. the ratio of the volumes of the phases in %.

– the water absorption capacity was studied using a stainless steel mesh glass (80 mm in height, mesh hole diameter of 1.5 mm, the number of holes per 1 cm$^2$ was 10-20). The bottom and walls of the glass were covered with filter paper to avoid loss of small particles. The glass with a filter paper was moistened with water. After 20 minutes, to let the water flow down, the beaker was weighed. 2±1 g of the sample was placed in it, after which the beaker with a sample was placed for 20 minutes into room-temperature water. After draining for 20 minutes, the outer walls and the bottom of the beaker was wiped with filter paper. The weighing was carried out and the water absorption capacity (%) was calculated as the ratio of the mass of the product after soaking to the mass of the product before soaking.

– fat-absorbing ability was determined similarly to the water absorbing ability, by immersing an empty glass, and then a glass with a sample in sunflower oil. Fat absorption capacity was determined in percent as the ratio of the mass of the product after immersion in oil to the mass of the product before immersion in oil:

$$g = \frac{m_2}{m_1} \times 100$$  \hspace{1cm} (2)

$m_1$ is the product mass before immersion in oil, g;
$m_2$ is the product mass after immersion in oil, g.

– determination of the degree of swelling: a weighed portion of 3 g of spirulina powder was placed in a 50–100 ml centrifuge tube, 15 ml of distilled water was added at room temperature, mixed thoroughly and kept for 1 hour. Then centrifugation was carried out for 5 minutes at 3000 rpm. The centrifugate was decanted and the moisture content in the sediment was determined.

The degree of swelling was determined as

$$a = \frac{m - m_0}{m_0 \cdot 100}$$  \hspace{1cm} (3)

$m$ is the mass of the protein after swelling, g:
$m_0$ is the sample mass of dry spirulina, g;
$B$ is the moisture content in dry spirulina, %;
$B_1$ is the moisture content in swollen spirulina, %;

– determination of the degree of hydration: a sample of spirulina powder was mixed with water in the ratio of spirulina to water from 1:1 to 1:20. The suspension was thoroughly mixed until a homogeneous consistency was obtained. Lumps were unacceptable. The resulting suspension was kept for 17 hours at room temperature, after which the percentage of released moisture was determined.

– determination of the gelling ability: a weighed portion of spirulina powder was mixed with water in a ratio of spirulina to water from 1:1 to 1:20. The suspension was thoroughly mixed until a homogeneous consistency was obtained. Lumps were unacceptable. The resulting suspension was kept at a temperature of 80 °C to a temperature in the center of the sample of 70–72 °C and then cooled to a temperature of 5–10 °C. Then the presence of the gel was visualized.

– determination of the peroxide number: a titrimetric method based on the ability of peroxides to oxidize hydroiodic acid with the release of free iodine. 10 ml of chloroform and 15 ml of glacial acetic acid were added to a weighed portion of the test sample. Then 1 ml of a freshly prepared saturated solution of potassium iodide was added. After 3 minutes, 75 ml of distilled water was poured into the flask, into which 5 drops of a 1% starch solution were added in advance until a violet-blue color appeared. The released iodine was titrated with 0.01N sodium thiosulfate solution to a milky white color stable for 5 seconds (experiment). The control experiment was carried out in parallel: instead of the weighed portion of the product, 1 ml of water was taken.

The peroxide number (IF, g / 100 g) of the analyzed fat was calculated using the following formula:
\[
P_N = \frac{(V_0 - V_k) \times 0.001269}{m} \times 100 \quad \text{(5)}
\]

where \(V_0\) is the volume of 0.01N sodium thiosulfate solution consumed for titration of the test sample, ml;

\(V_k\) is the volume of 0.01 N sodium thiosulfate solution consumed for titration of the control sample, ml;

0.001269 is the titer of 0.01 N sodium thiosulfate solution, g/ml;

100 is the conversion factor per 100 g of the analyzed sample;

\(m\) is the mass of the test sample, g.

**Results**

The properties are understood as the physicochemical characteristics that determine its behavior during processing into food products, as well as provide the desired structure, technological and consumer properties of finished food products.

**Table 1. The properties of spirulina powder (n = 3, V < 5%)**

| Name                        | Spirulina     |
|-----------------------------|---------------|
| Water absorption capacity, %| 117.8±0.5     |
| Fat absorption capacity, %  | 117.0±0.3     |
| Swelling degree, %          | 177.6±1.2     |
| Solubility index, cm³       | 1.85±0.03     |

![Figure 1. Diagram of spirulina emulsion](image-url)

The behavior of protein in real food systems is always considered in conjunction with both other components (water, fat, minerals, etc.), and with changing environmental conditions (pH, t) during the technological processing of raw materials. Knowledge of the functional properties of protein preparations, the nature of the change in these properties under the influence of various technological factors is necessary for the correct selection of formulation components, the formation of the required characteristics of the finished product.

Some properties of the spirulina protein are presented in Table 1.

The results of studies of the emulsifying ability of spirulina are shown in Figure 1.

From the data obtained, it follows that spirulina in the form of a dried powder does not have sufficient emulsifying ability. With an increase in the concentration of oil, emulsifying properties gradually appear, but are not sufficiently substantiated. A slight emulsifying ability begins to form when 60% of the fat fraction is added (Table 2).
Table 2. Gelling capacity of spirulina powder (n = 3, V <5%)

| Hydration degree | Glass mass after heat treatment [g] | Empty glass mass [g] | Separated moisture [%] | Water-holding capacity, % of the sample weight |
|------------------|-------------------------------------|----------------------|------------------------|---------------------------------------------|
| 40 g of spirulina – 10 ml of water | 133.84                          | 94.20                | -                      | 100                                         |
| 30 g of spirulina – 20 ml of water  | 120.12                          | 96.54                | -                      | 100                                         |
| 20 g of spirulina – 30 ml of water  | 114.74                          | 96.76                | -                      | 100                                         |
| 10 g of spirulina – 40 ml of water  | 109.45                          | 95.02                | -                      | 84.6                                        |

Table 3. Recipes of test samples of paste masses

| Raw material name                          | Unit of measurement | Control Sample 1 (1%) | Sample 2 (1.5%) | Sample 3 (2%) | Sample 4 (2.5%) |
|--------------------------------------------|---------------------|-----------------------|-----------------|---------------|-----------------|
| Pork liver blanched                        | kg                  | 35.0                  | 35.0            | 35.0          | 35.0            |
| Pork fat                                   | kg                  | 12.5                  | 12.5            | 12.5          | 12.5            |
| Milk protein-carbohydrate concentrate "Lactobel" | kg          | 3.0                   | 3.0             | 3.0           | 3.0             |
| Millet groats, cooked                      | kg                  | 5.0                   | 5.0             | 5.0           | 5.0             |
| Boiled red carrots                         | kg                  | 8.0                   | 8.0             | 8.0           | 8.0             |
| Boiled chickpeas                           | kg                  | 12.5                  | 11.5            | 11.0          | 10.5            |
| Spirulina powder                           | kg                  | -                     | 1.0             | 1.5           | 2.0             |
| Fried onions                               | kg                  | 4.0                   | 4.0             | 4.0           | 4.0             |
| Broth from cooking offal                   | l                   | 20.0                  | 20.0            | 20.0          | 20.0            |
| TOTAL:                                     |                     | 100.0                 | 100.0           | 100.0         | 100.0           |
| Water above the recipe                     | l                   | -                     | 3               | 6             | 9               |
| Spices and materials                       |                      |                       |                 |               |                 |
| Edible table salt                          | kg                  | 1.25                  | 1.25            | 1.25          | 1.25            |
| Ground black pepper                        | kg                  | 0.085                 | 0.085           | 0.085         | 0.085           |
| Nutmeg                                     | kg                  | 0.1                   | 0.1             | 0.1           | 0.1             |
| Artificial casing Ø = 35 mm                |                     |                       |                 |               |                 |

When conducting a visual inspection for the presence of gelling of the test samples of spirulina, it was revealed that there was no gelling ability. In the course of the experiment, it was found that the water-holding capacity of spirulina powder was manifested in all ratios of powder and water, however, with a greater degree of hydration, this ability decreases.

To study the effect of spirulina on the quality indicators of prototypes of pates, a biologically active additive was added in the amount of 1, 1.5, 2 and 2.5%. The control sample contained no spirulina (Table 3).
The green color of microalgae limits its use in everyday products as it negatively affects consumer perception of taste and quality. However, in the case of pate, which has a pronounced taste and smell of pork liver; all samples received a high score (Figures 2 and 3).

It was noted that with an increase in the dose of spirulina, the manifestation of a specific taste and smell, as well as color on raw minced meat, and an increase in the intensity and depth of coloring of the product are characteristic. At the same time, after heat treatment, no pronounced changes in organoleptic assessments were established by experts.

The most preferred dosage was 2% spirulina. At this dosage, expert tasters noted the absence of extraneous tastes and odors. And the color most associatively matched the color of traditional pates (Figure 4).

Samples of raw minced meat and finished products were examined for physical and chemical indicators (Table 4).

**Figure 2.** Organoleptic evaluation of pate samples with different levels of spirulina administration

**Figure 3.** Organoleptic evaluation of pate samples with different levels of spirulina administration
Figure 4. Appearance of mixtures and ready-made pates with different doses of spirulina

Table 4. Physicochemical indicators of pate samples with varying degrees of spirulina administration (n = 3, V <5%)

| Investigated indicators         | Samples of pates                  |
|---------------------------------|-----------------------------------|
|                                 | control 1 (1%) 2 (1.5%) 3 (2%) 4 (2.5%) |
| Raw minced meat                 |                                    |
| Moisture content, %             | 56.6±0.1 57.6±0.2 59.2±0.3 61.1±0.2 63.4±0.1 |
| pH, units                       | 6.47±0.02 6.51±0.03 6.53±0.04 6.55±0.01 6.52±0.02 |
| Water-binding capacity, % of total moisture | 47.84±0.5 50.7±0.4 53.4±0.8 53.7±0.9 54.3±0.5 |
| Finished product                |                                    |
| Moisture content, %             | 50.4±0.05 53.2±0.02 53.4±0.03 51.7±0.05 55.8±0.02 |
| pH, units                       | 6.58±0.01 6.52±0.02 6.51±0.03 6.57±0.02 6.58±0.02 |
| Water-holding capacity, % of total moisture | 33.1±0.1 39.8±0.3 48.4±0.6 51.0±0.4 51.3±0.3 |
| Plasticity, cm³                 | 6.1±0.1 6.5±0.2 6.9±0.2 7.7±0.3 7.8±0.1 |
| Output, %                       | 117±2 120±1 123±3 126±1 128±2 |

It was revealed that the mass fraction of moisture in the test samples, both in raw minced meat and in finished products, changes. As the level of spirulina administration increases, the mass fraction of moisture increases and its maximum amount was 63.4% in sample No. 4. This fact indicates that the addition of spirulina powder has a positive effect on the binding of moisture in the minced meat.

In addition, the peroxide number was determined during storage of pate samples at a temperature of 0-4 °C (Table 5).

The peroxide number serves as a quantitative indicator of the presence of primary oxidation products of peroxides and hydroperoxides, that is, oxidative changes occurring in fats. By the value of the peroxide number, one can judge only about the initial stage of lipid oxidation, at which peroxides and hydroperoxides are formed, which do not significantly affect the organoleptic properties of fat. By the magnitude of the peroxide number, one can judge the freshness of the fat long before the appearance of an unpleasant taste and smell.

During the storage of the pate, the peroxide value of the control sample exceeded the permissible norm (0.10% iodine) already on the third day of storage. The peroxide number of the test samples remained below the norm even after 5 days of storing the pate. Moreover, the higher the dose of spirulina powder, the lower the peroxide number of the pate.
### Table 5. The results of the study of the peroxide number in the pate samples

| Test samples | Peroxide number during storage |
|--------------|--------------------------------|
|              | 3 days | 5 days | 7 days |
| control      | 0.117  | 0.127  | 0.178  |
| 1%           | 0.046  | 0.089  | 0.175  |
| 1.5%         | 0.044  | 0.077  | 0.145  |
| 2%           | 0.038  | 0.052  | 0.137  |
| 2.5%         | 0.032  | 0.043  | 0.129  |

### Discussion

Spirulina consists of proteins biologically valuable for humans, the content of which reaches 60-70 g per 100 grams of dry product. They have a balanced amino acid profile in accordance with the WHO/FAO recommendations regarding human needs for vital amino acids [11].

Žugčić T. believes that spirulina protein is able to compete successfully with animal protein. The use of spirulina for partial replacement of meat protein can be extremely beneficial for human health due to its amino acid composition, lack of cholesterol, high content of vitamins, minerals, essential fatty acids, polyphenols and pigments [12].

An important property of protein for meat-containing products is water absorption—the result of spontaneous “taking” of water by the protein matrix. If the water absorption is infinite, then the protein will dissolve, if not, water absorption will take place until the moment limited by some molecular forces in the swollen sample [13]. The water absorption capacity of spirulina is 117.8%, which suggests a positive effect on the structure of the product after heat treatment. Fat absorption characterizes the ability of proteins to absorb a certain amount of fat. A high level of fat absorption leads to the formation of stable emulsions, which has a positive effect on the quality of emulsified food products. However, according to the analysis of the emulsifying ability of spirulina does not form stable emulsions. At the same time, in the manufacture of pates, these indicators are not significant.

Ageev I.A. and others [14] showed that the introduction of algae in the form of a dry preparation in the formulation of meat pates (10%) improves their functional and technological properties.

Our studies also show that the addition of spirulina from 1 to 2.5% leads to an increase in water binding and water retention capacity, which is associated with the redistribution of moisture in the structure of muscle protein and spirulina protein, which is able to bind and retain water. It has been proven that an increase in the level of spirulina administration has a positive effect on the binding of moisture and its retention by the protein matrix after heat treatment. The obtained data on the hydrogen index show that both in the samples of raw minced meat and in finished products, the indicator changes insignificantly, which also has a positive effect on the water-binding and water-holding capacity and yield.

It has been established that an increase in the level of spirulina administration contributes to a slight increase in the yield of finished products, which is probably associated with an increase in the water-binding and water-retaining abilities and the ability of spirulina to retain moisture in the finished product. The maximum rate was found in samples with a spirulina injection level of 2 and 2.5%.

The index of plasticity of samples of pates characterizes the structural and mechanical indicators of minced meat and finished products. The data obtained indicate the liquefaction of the meat system with an increase in the level of spirulina administration. The maximum indicator was set in the sample with the introduction of spirulina of 2.5%, as evidenced by the more delicate texture of the finished product.

### Conclusion

It was found that the addition of spirulina powder has a positive effect on the stability of the lipid fraction of the pate. In the samples of pates with spirulina, with an increase in the dose of spirulina, the process of oxidative spoilage slowed down, as evidenced by the dynamics of the increase in the peroxide value during storage. Moreover, after storage for 7 days in a refrigerator, in all samples, except for samples containing 2 and 2.5% spirulina, mold appeared on the surface of the pate.
Thus, the introduction of spirulina into paste masses has a positive effect on the quality of finished products, is expedient and meets the goals and objectives of the state policy in the field of healthy nutrition in the Russian Federation. The addition of spirulina in the form of a dry preparation to the formulation of meat and vegetable pate improves their organoleptic, functional and technological properties, and the most optimal in this regard is the introduction of 2% spirulina instead of chickpea.

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