In vivo experimental evaluation of functional food ingredient being powdered prefabricated acon

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Abstract. People’s health and performance directly depends on their diet, the composition of which is of great importance. Dietary fiber has recently been included in the group of factors determining the balanced diet. The use of dietary fiber as functional elements is currently an urgent task for the confectionery industry, since their use can improve health and reduce the risk of a number of chronic diseases. It is proposed to use yacon and its derived products as functional food ingredients. Prefabricated yacon is a powdery product of a light cream color with a fruity aftertaste and smell. It has low humidity and contains inulin, mono- and disaccharides, fiber. It is rich in calcium. The functional food ingredient was evaluated by means of an in vivo experiment, which implied the introduction of dried yacon into the diet of female rats Wistar. A comparative analysis of the parameters of a general blood test, histological studies of the liver, kidneys and stomach of laboratory animals of the control and experimental groups were performed. The prospects of using yacon and its processing products as an enriching ingredient in food products have been established.

1. Introduction
Diet serving as a key factor that activates working capacity and increases life expectancy, thereby preserving the nation’s gene pool is of crucial importance for human health. The issues of healthy nutrition are gaining more urgency and are being increasingly addressed at the state level [1].

The policy of our state in terms of nutrition is aimed to preserve and strengthen the health of the population as well as to prevent diseases caused by malnutrition and unbalanced nutrition. Despite all the measures taken by the state with this regards, the percentage of chronic diseases, according to statistical studies, remains at a rather high level. The diet of the majority of the population does not meet the requirements of a healthy diet. At the same time, a healthy diet should ensure the normal development and vital activity of a person as well as help to strengthen health and prevent diseases [2].

Currently, there are many studies in the field of enriching food products with functional ingredients. However, the market of enriched and functional foods is still not filled [3].

The food industry (including the confectionery sphere) is currently facing the task of developing new types of products, improving the assortment structure, saving scarce raw materials, reducing sugar
content as well as creating therapeutic and preventive products with an extended storage period. At the same time, the confectionery industry shows great interest in the use of non-traditional plant materials in manufacturing. The developed formulations of functional confectionery products for being used with prophylactic and therapeutic objectives use raw materials of plant origin as a source of protein, minerals, vitamins, digestible and non-digestible carbohydrates as well as fats. One of the functional food ingredients is dietary fibers [4].

On the one hand, dietary fibers adsorb toxic elements and other substances with a toxic effect and, on the other hand, they are prebiotics that enable the normal growth and development of beneficial microflora for the human gastrointestinal tract. The development and implementation of rational nutrition from the standpoint of satisfying physiological norms for dietary fiber contributes not only to the prevention of cardiovascular diseases and type 2 diabetes but also to a number of oncological diseases [5].

The aim of our study is to substantiate the effectiveness of yacon fiber as a functional ingredient in flour confectionery goods.

2. Objects and research methods
Yacon, dried yacon tubers and a powdery prefabricated product made from yacon are the objects of study [6,7,8,9].

The rich chemical composition of yacon has a positive effect on human health and life. The effect of inulin on human body functioning and its various organs has been studied and is confirmed by many sources [10,11,12,13,14,15].

Experimental studies were carried out by the departments of Commodity science and goods examination, General livestock production, the laboratory of mass analysis of the FSBEI HE Voronezh State Agrarian University.

In the work, dried yacon root tubers grown in the Federal State Budgetary Institution “Federal Scientific Center for Vegetable Production” (professors Gins VK, Gins MS, Kononkov P.F.) were used as an enriching additive.

The standardized methods were applied for studying powdered prefabricated yacon concerning organoleptic, physicochemical and safety indicators, including microbiological characteristics [16].

At the preliminary stage of the research, the organoleptic and physicochemical parameters of the crushed dried prefabricated yacon were evaluated according to organoleptic indicators. The results of organoleptic quality assessment are presented in Table 1.

| Table 1. Organoleptic characteristics of a powdered prefabricated yacon |
|-----------------|-----------------|-----------------|
| Product name    | Taste           | Smell           | Color            |
| Powdered prefabricated yacon | Sweet, without off-flavors, not sour, not bitter | Mild fruity, without off-flavors and off-smells | Cream white |

Yacon physical and chemical characteristics were studied and the most important indicators of the chemical composition were established in order to assess the spheres of applying this powdered prefabricated product as an enriching ingredient in food products.

The mass fraction of moisture determines the manufacturability of the product, including its portability and ability to maintain properties for a sufficiently long time period. Acidity determines the possible limits of its use in the formulation. The mass fraction of reducing sugars is one of the main functional and technological properties that determine the structure and color of the end products as well as their hygroscopicity during storage.

The concentration of protein, fat, total sugars, fiber and macrocells, specifically, phosphorus and calcium, were determined basing on the chemical composition.
The outcomes of studying the selected samples of dried prefabricated yacon are shown in Table 2.

**Table 2. Physico-chemical characteristics of the powdered prefabricated yacon**

| Indicator               | Characteristics of powdered prefabricated yacon |
|-------------------------|-----------------------------------------------|
| Moisture content, %     | 6.35±0.19                                     |
| Total acidity, degrees  | 9.3±0.3                                       |
| Content, %              |                                               |
| protein                 | 6.88±0.21                                     |
| fat                     | 0.66±0.04                                     |
| total sugar             | 5.63±0.17                                     |
| reducing sugars         | 4.94±0.03                                     |
| phosphorus              | 0.10±0.01                                     |
| calcium                 | 0.12±0.01                                     |
| fiber                   | 5.68±0.23                                     |

The powdery prefabricated product has a low humidity, which implies a fine preservation of its properties over a long period of time. The low fat content and, consequently, the poor ability of the powdery prefabricated product to be subject to oxidize spoilage contribute to this as well.

The powdery prefabricated yacon has a rather high acidity of 9.3 degrees. On the one hand, non-volatile organic acids enable to form a delicate sourish shade. On the other hand, it helps to limit microbiological damage, which is also a factor of prefabricated yacon preservation.

The reducing sugars concentration at the level of about 5% is of technological importance. It is possible to use this prefabricated product in the technology of products based on microbiological processes. Reducing sugars are digestible and can be used as substrates for yeast cells, lactic acid bacteria and other microflora, which determines the maturation of prefabricated products and finished products. At the same time, the technological function of reducing sugars is associated with their role in the process of melanoidin formation and, accordingly, the formation of end products color.

It was found that protein concentration in the powdered prefabricated yacon is sufficiently high, specifically, it is more than 6% and the fiber content is about 6%, which was expected. In the future, it is advisable to study the powdered prefabricated product with regards to the content of inulin being the main dietary fiber of the icon. A relatively high calcium content of 1200 mg/kg is an important factor ensuring the product nutritional status [17].

The raw materials used in the research met the requirements of the corresponding Technical Regulations of the Customs Union, national and interstate standards as well as other regulatory and technical documents.

In general, preliminary studies have indicated rather high technological properties and content of physiologically valuable nutrients in the powdered prefabricated product.

At the same time, the prospects for applying powdered prefabricated product in the technology of enriched diets set the main task of the work, specifically, to study its effectiveness in laboratory animals.

A chopped prefabricated yacon pre-dried by convective method was introduced into the diet of female rats with the objective to study the yacon influence on the physiological state of laboratory animals.

We used 9 individuals of female rats. Prior to the experiment rats were fed with oats and barley during 2 weeks. They could drink water without any restrictions. Three individuals of control group were kept in the first cage, while the second and the third cages kept three individuals of the experimental groups each. The temperature was equal to 21 ± 1°C with the lighting mode of 12/12 hours. The cage was cleaned 2 times a week. They were fed only in the morning. Animals of the first (control) group received 190 g of the main diet (oats and barley). Animals of the second group...
received 90 g of the main diet and 15 g of yacon of the first lot (for each individual), the third group received 90 g of the main diet and 15 g of yacon of the second lot.

Histological experiments were performed on healthy sexually mature female rats Wistar weighing 210–240 g. They were kept in a vivarium [18]. White rats were placed in standard plastic cages on a bed of fine wood shavings. The microclimatic conditions of rats in the vivarium corresponded to the following required standards: $t = 20-24^\circ$С, with humidity of $50 \pm 20\%$, air exchange volume (exhaust/inflow) of 8:10, light mode (day/night) of 1:1.

### 3. Results and discussions

| Table 3. General blood test results in laboratory animals |
|---------------------------------------------------------|
| **Indicators**                                           | **Results – laboratory animals** |
|                                                         | **Reference range** | **№1 (Control)** | **№2** | **№3** |
| White blood cells (WBC) (qty x 10^9 /l)                  | 5-23                | 5.0 ±0.10 ✓        | 3.4±0.07 ✓        | 5.5±0.20 ✓        |
| Lymphocytes # (qty x 10^9/l)                             | 5-7                 | 2.6±0.07% ✓        | 1.8±0.07% ✓        | 3.1±0.20% ↓       |
| Monocytes # (qty x 10^9/l)                               | 0-10                | 0.3±0.04 ✓         | 0.2 ± 0.02 ✓       | 0.2 ± 0.01 ✓       |
| Granulocytes # (qty x 10^9/l)                            | 2.1±0.04            | 1.4 ± 0.05         | 2.2 ± 0.09         |
| Lymphocytes,%                                           | 52.1 ± 1.1          | 54.2 ± 1.5         | 55.8 ± 2.6         |
| Monocytes,%                                             | 6.9 ± 0.34          | 5.1 ± 0.32         | 4.4 ± 0.32         |
| Granulocytes,%                                           | 41.0 ± 1.1          | 40.7 ± 1.9         | 39.8 ± 1.8         |
| Hemoglobin (HGB) (g/l)                                  | 120-180             | 125 ± 0.58 ✓        | 123 ± 1.08 ✓       | 127 ± 1.19 ✓       |
| Red blood cells (RBC) (qty x 10^12/l)                   | 7-10                | 7.0 ± 0.11 ✓        | 7.2 ± 0.10 ✓       | 7.5 ± 1.13 ✓       |
| Hematocrit (HCT)                                        | 35-45               | 45.9 ± 2.6          | 44.8 ± 1.4         | 46.7 ± 2.3         |
| Red blood cell mean volume (MCV) (FL)                   | 65.6 ± 1.9          | 62.6 ± 2.1          | 62.8 ± 1.8         |
| The average hemoglobin content in erythritol (MCH) (pg)  | 17.8 ± 0.95         | 17.1 ± 0.68         | 17.0 ± 0.98         |
| The average concentration of hemoglobin in erythritol (MCHC) (g/l) | 27.2 ± 0.68        | 27.4 ± 1.3          | 27.1 ± 1.18         |
| The width of the distribution of red blood cells (RDW)   | 12.4 ± 0.69         | 10.8 ± 0.58         | 11.3 ± 0.63         |
| Platelets (PLT) (qty x10^9/L)                           | 150-326             | 747.0 ± 4.0 ✓       | 782.0 ± 1.93 ✓     | 787.0 ± 2.6 ↑      |
| Platelet Volume (MPV) (FL)                              | 6.3 ± 0.35          | 6.1 ± 0.21          | 6.5 ± 0.14         |
| ESR                                                    | 0.5-4               | 7.0 ± 0.41 ✓        | 7.0 ± 0.32 ✓       | 7.0 ± 0.27 ✓       |
| White blood cell count                                   |                      |                      |                    |
| Stab                                                   | 2.0 ± 0.19          | 2.0 ± 0.19          | 2.0 ± 0.0          |
| Segmented                                              | 32.0 ± 0.68         | 32.0 ± 1.16         | 31.0 ± 1.73        |
| Lymphocytes                                            | 65-77               | 52.2 ± 1.73 ✓       | 54.3 ± 1.49 ✓      | 56.4 ± 1.65 ↓     |
| Monocytes                                              | 0-4                 | 7.0 ± 0.32 ✓        | 5.0 ± 0.41 ✓       | 4.0 ± 0.32 ✓       |
| Eosinophils                                            | 0-1                 | 7.0 ± 0.0 ✓         | 7.0 ± 0.32 ✓       | 7.0 ± 0.27 ✓       |
| Basophils                                              | 0-1                 | 0 ✓                 | 0 ✓               | 0                 |

**Identifiers:**

✓ - The indicator is normal (norm)

↑ - The indicator is very high

馗 - The indicator is critically high

↓ - The indicator is critically low

! - The indicator is high (pathology!)

→ - The indicator is normal (norm)
The study material was the stomach, liver, and kidneys of sexually mature female white rats. For histological examination, tissue samples were fixed in a 10% solution of neutral formalin. Having been washed in flowing water, the fixed samples were subjected to dehydration by placing the test material in alcohols with increasing concentration and poured into paraffin according to the standard method. Histological 4–5 μm thick cross sections were stained with hematoxylin-eosin [19].

Studies were conducted on laboratory animals to identify the effect of the dried prefabricated yacon on the body of rodents. Since white rats are considered the test organisms, the results of the studies can subsequently accept or reject the introduction of a powdered prefabricated product in the diet of humans. Studies of standard biochemistry indicators/blood tests in rats were conducted earlier and are reflected in a number of scientific papers [20].

The results of studying the indicators of the general blood analysis in laboratory animals are presented in Table 3.

According to the study outcomes, the diet of the control rat (group 1), which received 190 g of the main diet (oats and barley), was not balanced. This led to disruption of the processes in the liver and a decrease in the hormone insulin, which increases albumin and globulins, and lowers blood sugar. In the animals of the second group, fed by 90 g of the main diet and 15 g of the first lot yacon, and of the third group, receiving 90 g of the main diet and 15 g of the second lot yacon, there was an improvement in ALT (alanine aminotransferase), AST (aspartate aminotransferase), urea, total protein, normal lymphocytes. The immunity increased due to monocytes, as well, the hemoglobin index was improved and glucose was normalized, which confirms the improvement of their physiological state.

Thus, we can conclude that the introduction of dried prefabricated yacon into the diet of laboratory animals has a favorable effect on the physiological state of their body.

During histological examination of the control group of rats, the structure of the stomach was represented by the mucosa, submucosa, muscle and serous membrane. There is a close contact of the mucous membrane with muscle. The surface of the gastric mucosa is covered with a single-layer prismatic epithelium over the entire surface, including the fossa. The mucous membrane had multiple folds and was lined with a single-layer columnar epithelium. Histological examination of the gastric mucosa revealed a moderate amount of mucus produced by columnar epithelium. The cells of the body and the bottom of the glands are colored basophilically more strongly than the excretory ducts. The cells of the glands are placed in the form of continuous cords, tightly adjacent to each other. The central arrangement of nuclei in the cells was revealed. The nuclei had a spherical shape. Expansion of blood vessels and stasis is indicated. Destructive changes in the glandular epithelium of the gastric mucosa are diffusely observed. The glands lumens are expanded.

![Figure 1](a) (b) (c)

**Figure 1.** The architectonics of rats (control group) stomach. (a) Hematoxylin-eosin stain about 10 × vol. 10; (b) Hematoxylin-eosin stain about 10 × vol. 10; (c) Hematoxylin-eosin stain about 10 × vol. 10

Morphological examination of the liver of the control group revealed a radial arrangement of beams. Hepatocytes form cords tightly adjacent to each other. A focal arrangement of hepatocytes with foamy cytoplasm was revealed under the capsule. Individual hepatocytes merge into single
enlightened field. Accumulation of liver cells with foamy cytoplasm was also detected in the central part of the lobule. The hepatocyte cytoplasm ruptured diffusely and the cell contents entered the organ parenchyma. The nuclear apparatus of the liver cells was hardened, in some places karyolysis and karyorexis were observed.

![Image](a)
 ![Image](b)
 ![Image](c)

**Figure 2.** The architectonics of rat liver (control group). (a) Hematoxylin-eosin stain about 10 × vol. 10; (b) Hematoxylin-eosin stain about 10 × vol. 10; (c) Hematoxylin-eosin stain about 10 × about 40.

The architectonics of the kidneys is as follows: organ’s plethora as well as the split into the cortical and brain layers was observed in the control group of white rats. The renal corpuscles of regular round shape were in the cortical layer. The relative safety of glomerular tissues was observed. The convoluted tubules of both the cortical and the medulla are moderately enlarged, the cellular composition of the lining epithelium is cylindrical, and the nuclei usually occupied a central position. In some places, the expansion of the microvasculature of the kidneys cortical layer and foci of hemorrhage in the kidney parenchyma were revealed. Protein masses in the tubules gaps and slight expansion of the cavity of the Bowman-Shumlyansky capsule were found (Figure 3).

![Image](a)
 ![Image](b)
 ![Image](c)

**Figure 3.** Rat kidney architectonics (control group). (a) Hematoxylin-eosin stain about 10 × vol. 10; (b) Hematoxylin-eosin stain about 10 × vol. 10; (c) Hematoxylin-eosin stain about 10 × vol. 40.

During histological examination of rats belonging to experimental groups, the architectonics of the stomach is preserved. There is a clear division into the mucous, submucous, muscle and serous membranes. The surface of the mucous membrane is uneven. Folds are formed. The epithelium of the stomach, plunging into its own plate of the mucous membrane forms the glands of the stomach.
opening in the bottom of the gastric fossa being the deepening of the integumentary epithelium. The gastric mucosa represents a cylindrical-cubic epithelium with moderate mucus formation and its own plate of the mucosa with moderate full-blooded vessels. In this case, the normal structure of the main cells was noted. Mitosis figures are often found in cervical cells in contrast to other cells of the fundus glands (Figure 4).

![Figure 4](image)

**Figure 4.** Architectonics of the stomach of rats (experimental groups). (a) Hematoxylin-eosin stain about 10 × vol. 10; (b) Hematoxylin-eosin stain about 10 × vol. 10; (c) Hematoxylin-eosin stain about 10 × × vol. 40.

In the experimental groups, it was found that the structural organization of the rats’ liver was not disturbed, the location of hepatocytes was radial, and the hepatic sinuses were moderately blood-filled. The liver was represented by lobules consisting of radially located liver beams, sinusoidal capillaries were located between the beams, the central veins of the lobules, which launched the border between the lobules, are not clearly visible. Hepatocytes are mononuclear, the nucleus is uniformly colored and the cytoplasm is homogeneous. Hepatocyte nuclei are without pathological changes (Figure 5).

![Figure 5](image)

**Figure 5.** The architectonics of the liver of rats (experimental groups). (a) Hematoxylin-eosin stain about10 × vol. 10; (b); (c) Hematoxylin-eosin stain about 10 × vol. 40.

A histological examination of the kidney in the experimental group revealed a clear border between the cortical and brain layers. The renal corpuscles have regular spherical shape. The glomerular tissue was preserved. The capsule lumen had no content. The moderate blood filling of the glomerular capillaries is indicated. The convoluted tubules are expanded. Decorated convoluted tubules have no
pathological changes, the nuclei had a central location in the epithelial cells and the distal and proximal sections have no dystrophic changes (Figure 6).

Figure 6. The architectonics of the kidney of rats (experimental groups). (a) Hematoxylin-eosin stain about 10 × vol. 10; (b) Hematoxylin-eosin stain about 10 × vol. 10; (c) Hematoxylin-eosin stain about 10 × vol. 40).

Histological studies revealed pathological processes in the liver of control rats. Vacuole dystrophy was found under the capsule and in the central part of the organ. Significant changes in the stomach and kidneys in control animals were not detected. They were within the permissible norm for a given animal species.

Pathological processes were not observed in the structure of liver, kidneys and stomach when feeding rats of the experimental group with yacon. No dystrophic processes were observed in the liver cells of the experimental groups in comparison with the control one.

4. Conclusion
A yacon in vivo study on female rats Wistar showed its beneficial effect on the physiological state of animals and absence of pathological changes in the structure of liver, kidneys and stomach. On this basis, it is possible to recommend the powdered prefabricated product as a food-enriching ingredient. This is proved by the results of previous studies of the composition and properties as well.

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