Biomedical and biotechnological aspects of the production of functional ingredients based on yeast biomass

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Abstract. The results obtained in recent studies shown that use of the biologically active substances of microbial origin is prospect as natural components of nutrition, including the non-pathogenic yeast Saccharomyces cerevisiae, applying in the food industry. The aim of this work was to study the peptide and amino acid composition of yeast fermentolysates as factors that form their functional properties and biomedical activity. As a result of biocatalytic destruction during 6 and 12 hours, yeast biomass fermentolysates were produced, that mainly contained low molecular weight peptides (57.3% and 85.0% of the protein substances total mass) and free form amino acids (15.7% and 50.2% of the total amino acids). Spectral composition analysis of low molecular weight peptides confirmed a high degree of the protein hydrolysis. It was established that after 12 h of the hydrolysis, fermentolysate mainly contained peptides with a molecular weight of 80 to 300 Da, as well as free amino acids. The efficiency and safety of yeast fermentolysates that are sources of low molecular weight peptides and free amino acids, that determine their functional properties, was proven using in diets of experimental animals. Biomedical research and clinical trials confirmed that yeast fermentolysates had a high digestibility, promoted the normalization of metabolism, improved the functional state of the body, the recovery of patients and the restoration of their operability, had a general fortifying and immunomodulatory effect.

1. Introduction
Nutrition is a significant factor of supplying the normal functioning of the body and preventing the development of alimentary-dependent diseases. The modern concept of therapeutic and prophylactic diet assumes, along with nutritional value, to pay special attention to the physiological effect of the ingredients that compounding specialized products [1-4]. When designing balanced products with target functional properties, it is necessary to take into consideration the biomedical effectiveness of components that help maintain health and reduce the risk of diseases. The works of recent years shown that use of microbial origin functional ingredients that are sources of biologically active substances is prospect as natural food ingredients [5-7].

The genetic resources of microorganisms are recognized as the most numerous of biological resources of our planet and have a number of significant advantages over high-yielding plants and productive livestock. Primarily, this is a high growth rate of microorganisms, the possibility of regulating biosynthesis processes for generation the necessary target metabolites, the prospects of using...
residual microbial biomass as a substrate for production various biologically active additives for food and feed purposes [6, 8-12].

The biomass of non-pathogenic Saccharomyces cerevisiae yeast, that widely used in the food industry, contains essential amino acids, vitamins, valuable polysaccharides and trace elements [5, 8, 13, 14]. The presence of biologically significant elements makes it possible to consider this kind of raw as a promising source of functional ingredients. Despite of all the advantages, the nutritional value of yeast biomass is limited by the low availability of cell contents for the digestive enzymes action. For increase in the bioavailability of the yeast cellular content, enzyme systems was selected that implement the destruction of biomass polymers to convert the biologically valuable substances (BAS) contained in them into free forms [5, 14]. A particularly important role belongs to proteases, as a result of the their catalytic action physiologically active low molecular weight peptides and free amino acids are formed.

It is known that protein substances (polypeptides, low molecular weight peptides and amino acids) are an important component in a balanced diet. Proteins and amino acids, that are the constructive basis of all forming tissues in the human body, also play a regulatory role in metabolic processes, wherein the composition and amount of amino acids, including essential ones, are especially important. Therefore, studies aimed at producing dietary supplements based on microbial biomass that is the source of biologically complete protein, are relevant.

The aim of this work was to study the peptide and amino acid composition of Saccharomyces cerevisiae yeast fermentolysates as factors that form their functional properties and biomedical activity.

2. Research materials and methods

The objects of the study were fermentolysates of the baker's yeast Saccharomyces cerevisiae RCAM 01137 biomass, with different degree of enzymatic destruction [8].

The biocatalytic conversion process of yeast biomass intracellular polymers was carried out according to the developed method using enzyme preparations that are sources of proteases and β-glucanase, at a temperature of 48-50 °C (for 6 h) and at 40 °C (ditto) [14].

Spectral analysis of the low molecular weight peptides composition was determined by HPLC on a quadrupole mass spectrometric system Agilent 6120 [15]. The amino acids content of the microbial biomass and fermentolysates was determined via KNAUER EUROCHROM 2000 chromatograph followed by detection of the components using Smartline UV Detector 2500 (Germany) spectrophotometric detector at λ = 570 nm.

Studies of the toxicity and physiological effects of yeast fermentolysates were carried out according to the "Guidelines for the experimental (preclinical) study of new pharmacological substances" [16].

3. Results and discussion

As a result of biocatalytic conversion of yeast subcellular structures using β-glucanase and proteolytic enzymes after 6 h and 12 h of hydrolysis, two samples of biomass fermentolysates with different degrees of polypeptide destruction were produced. The fractional composition analysis of fermentolysates showed the presence of low molecular weight peptides and free amino acids (AA) (figure 1). In fermentolysate-1 (6 h of hydrolysis), the fraction of peptides with a molecular weight (MW) of more than 1000 Da decreased to 25% compared to the initial biomass, and low molecular weight peptides (LMP) and free amino acids were formed. In the fermentolysate-2, after 12 h of hydrolysis, high molecular weight peptides (more than 1000 Da) were absent, and 85% of the total mass of protein nature substances were peptides with MW less than 500 Da. Simultaneously, 50.2% of the total amino acids amount was released into a free state (figure 1).
Figure 1. The free amino acids and peptides content of the yeast Saccharomyces cerevisiae RCAM 01137 fermentolysates.

The dependence of the spectral composition of LMP in the studied yeast fermentolysates on the duration of the hydrolysis process was established. In the fermentolysate produced after 12 h, the intensity of the peaks corresponding to peptides with a molecular weight (MW) below 300 Da was higher (figure 2).

Figure 2. Spectral composition of low molecular weight peptides of the fermentolysate-2 after 12 hours of the yeast biomass hydrolysis.
Basically, these are substances with a mass of 80 to 500 Da. Apparently, they include biologically active peptides with a content of no more than 2-5 amino acid residues, as well as essential and neurotransmitter amino acids that are necessary for the normal human body functioning.

The study of the essential amino acids (EAA) composition, that released into a free state as a result of proteolysis, confirmed their complete presence in fermentolysates. It was established that the composition of yeast fermentolysates contained 20.22% of EAA in bound and free form, that is, about 40% of the total amount of protein substances of the yeast biomass (53.15%), that is consistent with generally known data about a biological value of the yeast protein (table 1). The biocatalytic conversion for 12 hours made it possible to increase the depth of protein hydrolysis and released more than 50% of essential amino acids into free.

Table 1. The essential amino acids content in free form in yeast biomass fermentolysates.

| Amino acid       | Essential amino acids content, % |
|------------------|----------------------------------|
|                  | Total   | Free     | Fermentolysate-1 | Fermentolysate-2 |
| Threonine        | 2.61    | 0.49     | 1.32             |
| Valine           | 2.34    | 0.39     | 1.15             |
| Methionine       | 0.76    | 0.17     | 0.40             |
| Isoleucine       | 2.12    | 0.35     | 1.05             |
| Leucine          | 3.01    | 0.48     | 1.50             |
| Phenylalanine    | 2.36    | 0.39     | 1.17             |
| Lysine           | 2.97    | 0.50     | 1.53             |
| Tryptophan       | 4.05    | 0.61     | 2.05             |
| Total essential amino acids | 20.22 | 3.38 | 10.17 |
| Total amino acids | 53.15 | 8.34 | 26.68 |

Thus, it was shown that fermentolysates of the yeast Saccharomyces cerevisiae biomass are sources of low molecular weight peptides and free amino acids, including essential ones, which determine their functional properties.

The conducted tests of acute toxicity and biological effectiveness of yeast fermentolysate confirmed that a single oral administration of a wide range of doses (200-2000 mg/kg) of the study drug to experimental animals (outbred white male mice) did not cause negative side effects and provided a rapid increase in their body weight when stable physiological and dynamic state of mice. The use of the biological product in the diets of animals for two weeks at a dose of 1100 mg / kg of body weight increased their appetite, activity and stamina. The biological activity of yeast fermentolysate manifested itself in the form of a tonic effect. Evidence of this is the activity increase of experimental animals in the "open field".

Biomedical studies shown the presence of a number of antioxidant effects in S. cerevisiae yeast fermentolysates, that expressed in the normalization of the glutathione-dependent link activity level [17]. Apparently, the soluble biologically active substances that contained in the preparation, quickly enough overcame the blood-brain barrier and were easily absorbed into the blood, and then entered into biochemical processes in the body, normalizing the system of cellular and humoral immunity [18]. According to Chang and Kao, biological preparations based on yeast biomass can fight obesity, reduce the level of triglycerides in the liver and blood serum, and increase the antioxidant activity of first one [7].

Moreover, the cytotoxic effect of yeast biomass fermentolysates on the cells of transplanted tumors was revealed. It was found that fermentolysate-2 with a deeper degree of destruction of subcellular structures of yeast biomass had the ability to activate caspase-3 on the lines HeLa, U937, MCF7, L929,
and influenced their cell cycle, causing an increase in the number of apoptotic cells, and reducing the ability of cells to mitosis. In this case, the effect of antitumor activity was the ability to induce selective apoptosis only on cancer cells, without suppressing normal ones [19-23].

As you know, balanced nutrition plays a special role in professional sports [24-28]. The use in sports nutrition of biologically active additives and specialized products with targeted functional properties, capable of selectively affecting metabolic processes, holds key positions in ensuring sports results, insofar as professional sports are characterized by extremely high emotional stress and a large volume of physical activity [29]. The studies results of the sports and consumer properties of yeast fermentolysates, that was analyzed at the Russian State University of Physical Culture, Sports and Tourism, was shown that their use in sports and physical practice contributed to an increase in the effectiveness of training and competitive activity of athletes due to the revealed effects of stimulating the operability and health improvement of athletes. The data of nonspecific anti-infectious resistance, obtained as a result of the experiment, confirmed the improvement of the body's immune defense under the conditions of the athletes efficiency stimulation cycle.

The assessment of the immunomodulatory effect of yeast fermentolysates in children with impaired anti-infectious protection, with reduced resistance to viral and bacterial infections was carried out. During research, the drug was well tolerated, there were no side effects and a pronounced therapeutic effect, that confirmed the high biological value of biopreparations for increasing the immune status and functional endurance of the body, improving metabolic processes [30-35].

The results of clinical studies of the use of yeast biomass fermentolysates in the rehabilitation of patients with craniocerebral trauma showed that the tested preparations influenced the correction of health, improving the functional state of the body, increasing the effectiveness of treatment procedures in patients, and contributed to a decrease in the tension of the cardiovascular system under stress during healthy-physical exercises training [36-39]. All patients tolerated nootropic and antibiotic therapy well, no complications were observed, symptoms of intoxication decreased faster than the control group, and there was no exacerbation of concomitant pathology.

4. Conclusion
Thus, the conducted biochemical and biomedical studies was shown that due to the deep hydrolysis of cell wall polysaccharides and protoplasmic proteins of _S. cerevisiae_, biomass fermentolysates are sources of free amino acids and biologically active low molecular weight peptides, have high digestibility, and contribute to the normalization of human protein metabolism, regulation of body weight, restoration of operability, have a general fortifying and immunomodulatory effect, that makes it possible to use them as biologically active additives to food and to form the functional properties of specialized products.

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