TECHNOLOGICAL PROPERTIES OF THE PROTEIN-POLYSACCHARIDE COMPLEX FROM THE MUSHROOM PLEUROTUS OSTREATUS

Mansky[1] O. A., Sayko[1] I. V., Sichkar[1] A. A., Kryklyva[1] I. O., Manska[2] K. G.

[1]National University of Pharmacy, Kharkiv, Ukraine

[2]SI "Institute of endocrine pathology named after V. Y. Danilevsky NAMS of Ukraine", Kharkiv, Ukraine

Introduction. One of the most important factors that determine the state of human health is a rational diet. It is necessary to maintain the normal functioning of a healthy body, creates conditions for physical and mental development, supports high performance, contributes to the prevention of diseases and increases the ability of the body to resist the effects of adverse environmental factors. The value of food is that it is a source of energy and plastic materials, as well as biologically active substances (BAS), which are necessary for the full functioning of the body.

In the recent decades, many countries around the world have experienced significant changes in the quality and quantity of food consumed, which has led to insufficient intake of necessary substances and violation of the principle of a balanced diet. Decreased intake of some BAS is related to their loss in the process of cooking food, using refined products and semi-finished products that are stored and contain a significant amount of preservatives and a widespread trend of self-restraint in nutrition to get rid of excess weight or constant eating fast food. In addition, natural quality products have become so expensive that many people are unable to afford to eat properly.

Rational nutrition is based on the principle of balanced food consumption. A balanced diet provides the best quantitative and qualitative relationships of the main food substances — proteins, carbohydrates, fats, vitamins and minerals. The balance of essential amino acids, fatty acids, complex and simple carbohydrates, the relationship between individual vitamins and other components of food, as well as the relationship and influence of mineral elements is of particular importance for the manifestation of biological properties of other food substances and their constituent parts in the body. A balanced intake of these substances creates conditions for physical and mental development, supports high productivity, increases the body's protective function, and helps prevent many diseases and stressful conditions [1].

One of the most common ways to solve the problem of food imbalance is the additional use of a sufficient number of necessary BAS in the form of special food additives — dietary supplements.

Recently, many dietary supplements contain edible mushrooms rich in vitamins, amino acids, minerals and antioxidants. They are relatively easy to cultivate productively, resistant to many pests and diseases, environmentally friendly, do not contain pesticides, nitrates, and heavy metal salts. In addition to the high nutritional value, a significant number of mushrooms show medicinal properties.

One such product is the oyster mushroom (OM, lat. Pleurotus ostreatus), that widespread in the world and now it is the third most cultivated after truffles and champignons [2].

OM is a valuable food product. Fruit bodies of OM contain water-soluble vitamins (B_1 (thiamine), B_2 (riboflavin), B_3 (niacin or vitamin PP), B_7 (biotin), C (ascorbic acid) and B_5 (pantothenic acid) and fat-soluble vitamins (D_2 (calciferol), a provitamin form of vitamin D_2 (ergosterol), tocopherol). The nitrogen content in the fruit bodies of OM is as in peas; phosphorus, as in fish; thiamine, as in cabbage; biotin, several times higher than in eggs and milk; B vitamins, 10 times more than in other foods; the amount of proteins and carbohydrates is the first among mushrooms. OM exceeds vegetables, fruits, meat and fish in the content of pantothenic acid; it is one of the richest products in terms of biotin content. By the amount of Vit. PP, which improves blood circulation, prevents the occurrence of blood clots in the blood vessels and improves the activity of the liver and stomach, OM has no equal among mushrooms.

In terms of protein content and amino acid composition, OM is closer to vegetables than to meat. The index of essential amino acids of OM exceeds the index of amino acids of vegetables, nuts, grains and is close to the index of milk. In the case of a simple diet, this mushroom can be an essential source of amino acids (tryptophan, cystine, aspartic acid, lysine, alanine, etc.) for the human body, since its protein contains all the essential amino acids, and the degree of digestibility of the mushroom protein reaches 90 %. Along with bound amino acids, the fruit bodies of OM contain free amino acids that take part in the synthesis of protein of a living cell, as well as in other parts of the metabolism, providing the synthesis of nucleic acids, enzymes, vitamins, etc.

This mushroom contains a lot of minerals (iron, phosphorus, potassium), polysaccharides, phytosterols and a number of unsaturated fatty acids, which are well absorbed and necessary for humans. These acids ensure normal tissue growth and metabolism, and prevent the deposition of cholesterol. In terms of the ratio of unsaturated and saturated fatty acids, lipids of OM are close to vegetable oils. Among unsaturated fatty acids, 70-80 % is linolenic acid. Fruit bodies are also rich in enzymes that help break down fat and glycogen and better digest food.

The fruit bodies of cultivated OM contain a large number of different BAS that can prevent and treat a wide range of diseases. Experiments show that a high content of pure protein (up to 47.7 %) contributes to the prevention and treatment of hepatitis, stomach ulcers, atherosclerosis, helps to normalize blood pressure, both in hypertensive and hypotonic patients, has an antitumor effect, increases the immune resistance of the body. OM has a bactericidal effect, promotes the elimination of radioactive elements and cholesterol from the body [2].

The highest pharmacological activity is found in BAS with a high molecular weight and well-soluble in water. It was found that polysaccharides and protein-polysaccharide complexes of the fungus are able to activate

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the immune system, increasing the potential for protective activity of the body. There is reliable evidence that the water-soluble protein-polysaccharide complex (WSPPC) from the fruit body of the OM has hypolipidemic properties, so nutritionists recommend this type of mushroom for use in dietary nutrition for those who want to lose weight [2]. Recent studies confirm the ability of OM not only to reduce cholesterol levels in the blood, but also to bind free radicals [1]. This mushroom is a natural source of lovastatin (which is widely used in clinical practice for the treatment of dyslipidemia) and contains a significant amount of β-glucans, which cause significant antioxidant potential. Since lipid peroxidation and accumulation of low-density lipoproteins (LDL) are the main components of the pathogenesis of atherosclerosis, OM have a high value in dietary nutrition to provide an anti-atherogenic effect [1, 2]. The listed properties of the fungus P. ostreatus make it very promising for use in the treatment of many diseases.

In our opinion, the use of P. ostreatus as a dietary supplement will provide significant antioxidant and hypolipidemic potential, which can be used to correct the diet of patients with dyslipidemia and atherosclerosis.

The purpose of this work was to obtain and study the technological properties of WSPPC from the mushroom Pleurotus ostreatus for further development of a dietary supplement based on it.

Materials and methods

When conducting research, we used generally accepted research methods according to the State Pharmacopoeia of Ukraine [3]. The final conclusions were made based on five repeated measurements that provide reliable data.

The object of research was the fruit bodies of the OM, dried in air at room temperature, crushed and sifted through a sieve with holes of 5-7 mm. WSPPC was obtained by extracting the prepared fruit bodies of OM according to the parameters defined and described earlier by other authors [4, 5]. Purified water with a temperature of 50 ºC was used as an extractant; the ratio of raw materials to extractant was 1 : 15; the extraction method was a three-stage remaceration, which lasted 90 minutes. The resulting extract was partially evaporated and WSPPC was precipitated with three times the amount of 96% ethanol. Ethanol was added gradually and the mixture was mixed thoroughly. The precipitate was settled for 2 hours, and the supernatant was separated. The wet precipitate was dried at a temperature of 50 °C to a residual humidity of 4-5 %.

The dry powder containing WSPPC was crushed and physical and chemical and technological properties were studied: microscopic characteristics, fractional composition, particle size, moisture content, flawability, porosity, bulk density before and tapped density after shrinkage [3, 6].

In order to determine the ability of WSPPC to absorb moisture, the tests were performed as follows. The 7.5 g samples of the substance were weighed in two containers. One container was placed in a desiccator with a saturated solution of sodium chloride and a relative humidity of 75 %. The other container was placed in an average relative humidity of 34 %. Moisture absorption analysis was performed every two days. The “Sartorius MA-150” express analyzer was used to determine the moisture content in the substance.

Results and discussion

The shape, the contact surface character and the approximate size of the particles of the WSPPC powder were determined using a luminescent microscope of the "Lumam R1" type, which allows observing and photographing the image of an object in passing light. The shape of the particles was determined in relation to the average length to the average width of the particles. Microscopy of WSPPC particles from the OM is shown in Fig. 1.

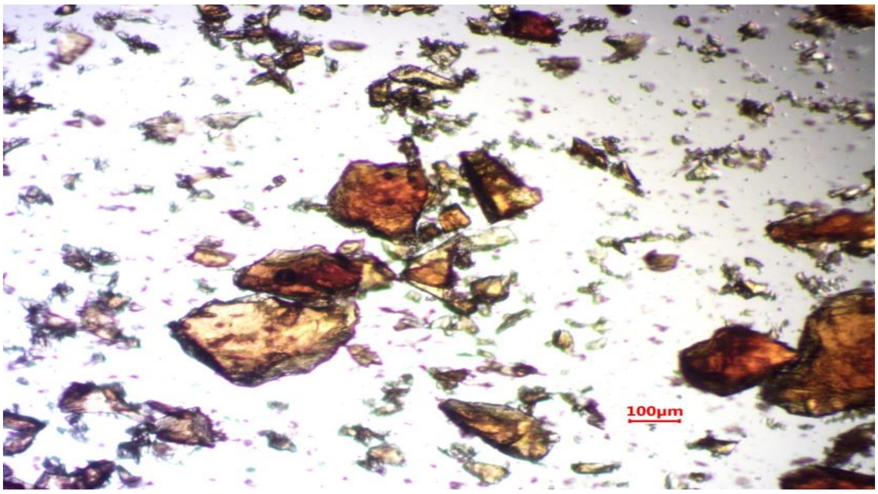

Fig. 1. Microscopy of WSPPC particles from the fungus *P. ostreatus*.

The resulting images show that the powder particles WSPPC from fruit bodies of mushroom Pleurotus ostreatus are anisodiametric (asymmetric and multiaxial), have a smooth surface and a plate-like shape, in which length exceeds width and thickness of not more than 3 times.

Fractional composition or distribution of powder particles by size, affects its flowability, and, accordingly, the correct operation of devices for mixing and dosing, as well as the quality characteristics of the finished form. The

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particle size and fractional composition of the dry WSPPC extract were determined by sieve analysis in accordance with the requirements of SFU through sieves with certain numbers [3, 6]. The research results are shown in table 1.

Table 1. Fractional composition of the WSPPC powder

	Distribution of powder particles by size, mm			
	less 0.1	0.1 – 0.3	0.3 – 0.5	over 0.5
Fractional composition	11.23 %	28.23 %	53.98 %	6.56 %

According to table 1, WSPPC powder has the largest fraction with particles of 0.3-0.5 mm, which is usually well dosed by volume.

The next parameter being studied is the flawability of the powdery mass, which is a complex characteristic determined by the shape and character of the particle surface, humidity, and fractional composition. This technological characteristic can be used for the correct selection of production technology, excipients and equipment. If powdered mixtures containing 80-100 % of the fine fraction (particle size less than 0.2 mm) are poorly dosed, it is necessary to conduct a directed increase in the particles of such masses, i.e. granulation [6]. In other cases, the flawability can be improved by adding excipients. The material that characterized by poor flowability may adheres to the walls of the loading funnel, which disrupts the dosing process. An indirect characteristic of the flow is the angle of repose, which varies widely – from 25–30° for loose powders and 60–70° for bound materials.

It is important to determine such volumetric indicators as bulk density before shrinkage, shrinkability, tapped volume and density after shrinkage during mechanical shaking, porosity. Bulk density (density before shrinkage) depends on the shape, size, density of the powder particles, humidity of the mass. The difference between the bulk volume of the bulk material and the volume after shrinkage shows the ability to shrink. With the knowledge of these indicators, it is possible to predict the required volume of packaging of powdery mass [6].

The results of the research of technological parameters WSPPC with mushroom P. ostreatus are given in table 2.

Table 2. Technological properties of WSPPC obtained from the fungus P. ostreatus

Indicator studied	The acceptable criteria	Result
Average particle size, mm	0.3-0.5	0.4 ± 0.09
Moisture content, %	3.0-5.0	4.0±1.0
Flawability, g/sec	More 4.0	7.1± 0.3
Angle of repose, deg.	25-35	35.8±1.5
Bulk density (density before shrinkage), g / ml	0.5-0.6	0.632±0.003
Tapped density (density after shrinkage, g / ml	0.55-0.75	0.728±0.002
Porosity	0.5-0.65	0.6325± 0.0023
Carr index	1-15	13
Hausner ratio	1-1.18	1.15

According to table 2, the WSPPC powder has a good flawability, which is also confirmed by the determination of the angle of repose and the calculation of the Carr index and the Hausner ratio. Such indicators are explained by the smooth surface of the particles and a sufficiently large particle size of the main fraction.

The results of determining the moisture absorption of WSPPC at a relative humidity of 34 % and 75 % are shown in figure 2.

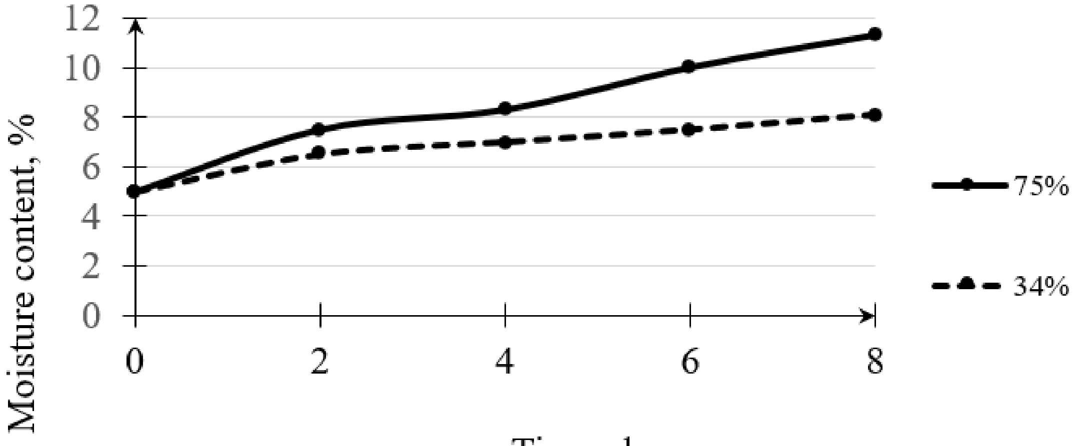

Fig. 2. WSPPC moisture absorption

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Figure 2 shows that the substance tends to absorb moisture and can be classified as hygroscopic. Therefore, when developing the composition of a dietary supplement, it is necessary to provide the introduction of such excipients as moisture regulators for the stability of WSPPC during storage.

Conclusions

The obtained indicators of technological parameters of WSPPC from the P. ostreatus mushroom allow us to conclude that WSPPC can be further used as a component in the development of a dietary supplement of hypolipidemic and antioxidant action in the form of a solid dosage form (tablets, capsules, etc.).

Technological properties of the protein-polysaccharide complex from the mushroom Pleurotus ostreatus
Mansky O. A., Sayko I. V., Sichkar A. A., Kryklyva I. O., Manska K. G.
Introduction. A balanced intake of the main food substances — proteins, carbohydrates, fats, vitamins and minerals — creates conditions for physical and mental development, supports high productivity, increases the body's protective function, and helps prevent stressful conditions and many diseases. The valuable product is the oyster mushroom (OM, lat. Pleurotus ostreatus), fruit bodies of which contain vitamins (thiamine, riboflavin, niacin, biotin, ascorbic acid, pantothenic acid, calciferol, tocopherol, ergosterol), amino acids (tryptophan, cystine, aspartic acid, lysine, alanine, etc.), minerals (iron, phosphorus, potassium), polysaccharides and phytosterols. One of the most common ways to solve the problem of food imbalance is the additional use of a sufficient number of necessary biologically active substances in the form of special food additives as dietary supplements. The article is devoted to the study of the technological properties of the water-soluble protein-polysaccharide complex (WSPPC) from the fungus Pleurotus ostreatus to create a dietary supplement based on it to correct the diet of patients with dyslipidemia and atherosclerosis. **The purpose of this work** was obtaining WSPPC from the mushroom Pleurotus ostreatus and studying technological properties of WSPPC for further development of a dietary supplement with it. **Materials and methods.** When conducting research, we used generally accepted research methods according to the State Pharmacopoeia of Ukraine. The object of research was the dried, crushed and sifted fruit bodies of the oyster mushroom. WSPPC has been obtained by extracting the prepared fruit bodies with purified water at a temperature of 50 ^{o}C. The ratio of raw materials to extractant was 1 : 15. The extraction method was a three-stage remaceration, which lasted 90 minutes. The obtained extract has been partially evaporated and precipitated with three times the amount of 96 % ethanol. The supernatant has been separated after two hours of extract settling. The wet precipitate has been dried at a temperature of 50 °C to a residual humidity of 4-5 %. The resulting dry powder of WSPPC has been crushed. The final conclusions were made based on five repeated measurements that provide reliable data. **Results and discussion.** According to the microscopic analysis it was established that the powder particles WSPPC from fruit bodies of mushroom Pleurotus ostreatus have a smooth surface and a plate-like shape. WSPPC powder has the largest fraction with particles of 0.3-0.5 mm, which is usually well dosed by volume. According to the technological studies, the samples of WSPPC have a good flowability. The values of the Carr index, the Hausner ratio and angle of repose make it possible to conclude that there is not a large force of cohesion between the particles. The difference between the bulk density of the substance and tapped density after shrinkage showed the ability to shrink. The kinetics of moisture absorption was investigated. The WSPPC powder may absorb moisture and can be classified as hygroscopic. Therefore, when developing of a dietary supplement, it is necessary to introduce moisture regulators to the composition for the stability of WSPPC during storage. **Conclusion.** The results of the studies indicate that the WSPPC powder have satisfactory parameters of the technological properties and such results can be used in the development of technology of a dietary supplement with lipid-lowering and antioxidant action in the form of a solid dosage form.

Key words: water-soluble protein-polysaccharide complex, oyster mushroom, technological properties, dyslipidemia, atherosclerosis, dietary supplement.

References

1. Grundy S. M., Stone N. J., Bailey A. L. et al. (2019) 2018 AHA/ ACC/ AACVPR/ AAPA/ ABC/ ACPM/ ADA/ AGS/ APhA/ ASPC/ NLA/ PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on clinical practice guidelines, 22 p. https://www.acc.org/~/media/Non-Clinical/Files-PDFs-Excel-MS-Word-etc/Guidelines/2018/Guidelines-Made-Simple-Tool-2018-Cholesterol.pdf
2. Alekseenko O. M, Polishko T. M, Vinnikov A. I.. Food, medicinal and environmental values of mushrooms Pleurotus ostreatus. Visnyk of Dnipropetrovsk University Biology ecology. 2010.18 (1). 3-9
3. Derzhavna Farmakopeia Ukrainy. 2 vyd. T 1. State Pharmacopoeia of Ukraine. 2nd edition. Vol. 1. Kharkiv: Derzhavne pidpryiemstvo .Naukovo-ekspertnyi farmakopeynyi tsentr. 2015.1128 p.
4. Kucherenko N. V., Dem'yanenko V. G. Study of the dynamics of extraction of the active substances from raw Oyster mushroom Pleurotus ostreatus. Farmatsevtychnyi zhurnal. 2006. 4. 74-77. https://pharmj.org.ua/archives/2006/2006-4.pdf
5. Kucherenko N. V., Dem'yanenko V. G., Stoletov Yu. V., (2008). Patent of Ukraine for invention No. 83530 from 25 July 2008, The method of obtaining a protein-polysaccharide complex with hypolipidemic action.
6. Gladukh Ie.V., Ruban O.A., Sayko I.B. et al. Industrial technology of drugs: a basic textbook for students of higher education (pharmaceutical faculties). Kh . NPhU. Original.2016. 632 p. https://dspace.nuph.edu.ua/handle/123456789/23539?locale=en