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Study of Nitrogen-Containing Substances of Powdered Semi-Finished Product from Cultivated Mushrooms *Pleurotus Ostreatus*

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***Abstract*—The technology of obtaining a powdered semi-finished product from *Pleurotus ostreatus* is proposed. Assessment of the quality of the semi-finished product was made according to the organoleptic and physico-chemical indicators. Found that the share of nitrogen of soluble substances, including non-protein nitrogen and nitrogen of water-and salt-soluble protein fractions, accounts for 77.06%, and the share of nitrogen of insolu-ble substances – 22.94%. Powdered semi-finished product from mushrooms *Pleurotus ostreatus* are the source of complete protein, which includes 18 amino acids, including 8 essential ones. Digestibility (in vitro) of proteins of the powdered semi-finished product is 1.3 times higher than the digestibility of fresh mushrooms.**

Keyword—mushroom, Pleurotus ostreatus, nitrogen-containing substances, powdered semi-finished product, amino acid

I. INTRODUCTION

Mushroom culture has moved toward diversification with the production of other mushrooms. According to Sánchez C. [1] *Pleurotus ostreatus* ranks second among edible mushrooms in the world after Agaricus bisporus. This is due to the fact that this mushroom has medicinal properties and ecological and economic values. *Pleurotus ostreatus* are able to colonize and degrade a large variety of lignocellulosic substrates and other wastes which are produced primarily through the activities of the agricultural, forest, and food-processing industries. Growing this type of mushroom is cheaper. This type of mushroom requires a shorter growth time in comparison to other edible mushrooms. The substrate does not require sterilization, only pasteurization, which is less expensive. Growing oyster mushrooms convert a high percentage of the substrate to fruiting bodies, increasing profitability. *Pleurotus ostreatus* demands few environmental controls, and their fruiting bodies are not often attacked by diseases and pests, and they can be cultivated in a simple and cheap way. All this makes *Pleurotus ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms.

Pleurotus ostreatus is a mushroom of pleasant flavour and possesses several proteins, minerals (Ca, P, Fe, Mg), and low carbohydrate quantities and fat, constituting excellent dietary food [2]. According to Croan [3] these mushrooms are a good source of nonstarchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. The protein content varies from 1.6 to 2.5%, and the niacin content is about ten times higher than that of any other vegetable. Moreover, Randive [4] reported that *Pleurotus ostreatus* mushrooms are rich in Vitamin C, B complex, and mineral salts required by the human body.

According to Patrick Ogwok et al [5] *Pleurotus ostreatus* is healthy food with regard to the low fat content and high amounts of LA.

«Fat content ranged between 0.24 and 5.23%. Variation was noted be-tween *P. ostreatus* grown in soil and on cotton seed husks ($p = 0.0090$). Poly-unsaturated fatty acids (PUFA) predominated over saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Linoleic acid (LA) was the most abundant fatty acid (FA) (30.91 to 54.35%). Ratios of PUFA/SFA, MUFA/SFA and PUFA/MUFA were within the desirable ranges. Omega-6 FA/ω-3 FA ratio was higher than that recommended for a healthy diet. However, the low amount of fat in mushrooms suppresses the negative effects of a high ω-6 FA/ω-3 FA ratio» [5].

Oyster mushroom powder rich in protein and low in fat contents can be incorporated into various recipes for improving the nutritional status of vulnerable population. Betaglucan has been shown to decrease blood cholesterol concentration in animal models and clinical intervention studies [6, 7].

The authors' research [8] suggests that 5% *Pleurotus ostreatus* supplementation provides health benefits, at least partially, by acting on the atherogenic lipid profile in the hypercholesterolaemic condition.

II. EXPERIMENTAL

Processing *Pleurotus ostreatus* according to traditional technologies, which guarantees the production of the finished product of high quality, is quite a time-consuming process. This is due to the fact that the features of the morphological structure of the fruit bodies *Pleurotus ostreatus* do not allow to mechanize the stage of mechanical cooking. Therefore, all operations (cleaning, division of «families» into separate instances, washing, sorting, division of fruit bodies into anatomical parts, cutting) at this stage must be done manually, which leads to an increase in the cost of the product. Less time-consuming and more effective is the technology that provides for mechanical grinding without preliminary separation of «families» into separate specimens and fruit bodies into anatomical parts, which is proposed to be implemented in the technology of powdered semi-finished products from mushrooms.

In the production of semi-finished powdered main prescription components are mushrooms *Pleurotus ostreatus* and potato starch. Semi-finished product is a powdered product obtained by drying followed by grinding. Drying is one of the most effective ways to extend the shelf life of food, does not change the natural taste, aroma.

More detailed study of the protein substances of the developed product was carried out in the following areas:

- study of the fractional composition;
- determination of the degree of the fungal proteins usefulness in amino acid composition.

The study of the fractional composition was carried out by determining the nitrogen of non-protein and nitrogen of protein fractions extracted with an aqueous solvent, alcohol and a dilute solution of caustic soda by the conventional method.

The amino acid composition of semi-finished proteins was studied on an amino acid analyzer LKB 41511 “Alfa Plus”.

To assess the enzymatic attackability of protein substances, enzymatic hydrolysis of products was carried out by the main proteolytic enzymes – pepsin, trypsin and chymotrypsin. The degree of enzymatic hydrolysis was expressed in μm/mg of tyrosine and it was determined using a calibration curve by the optical density of the solutions subjected to photocolorimetry. As the control, fresh mushrooms Pleurotus ostreatus were used.

III. RESULTS AND DISCUSSION

Assessment of the quality of the semi-finished product was made according to the organoleptic and physico-chemical indicators.

Characteristics of the total chemical composition of the developed products are shown in Table I.

Organoleptic indicators of semi-finished products are presented in Table II.

The nutritional value of ready products is largely determined by the composition of the components that make up the feedstock. In this regard, the more detailed study of the physical and chemical characteristics of the semi-finished product is of great interest.

TABLE I. THE CHEMICAL COMPOSITION OF THE POWDERED SEMI-FINISHED PRODUCT FROM MUSHROOMS *PLEUROTUS OSTREATUS*

Indicator	Content,%	
	product	***dry residue***
Protein	11.6±0.6	12.6±0.7
Fat	-	-
Carbohydrates,	76.4±3.7	83.0±3.9
including mono - and disaccharides,	4.6±0.2	5.0±0.2
including reducing	3.1±0.1	3.4±0.1
starch	40.5±0.6	44.0±0.6
cellulose	6.4±0.3	6.9±0.3
undetectable	≤24.9	≤27.1
Ash	4.0±0.2	4.4±0.2
Water	8.0±0.3	-

TABLE II. ORGANOLEPTIC CHARACTERISTICS OF SEMI-FINISHED PRODUCT FROM THE MUSHROOMS *PLEUROTUS OSTREATUS*

Indicator	Characteristic
Appearance	Fine dry powder, the presence of easily crumbling lumps is allowed
Color	From light cream to light brown
Taste and smell	Mushroom. Outsider taste and smell are not allowed
Consistency	Homogeneous friable mass, easy crumbling lumps are allowed

Nitrogen-containing substances are one of the main components of the chemical composition of mushrooms, and, consequently, the products of their processing. As shown by the research, the content of crude protein in the powdered semi-finished product of mushrooms *Pleurotus ostreatus* is 12.60% (dry matter). Thus, the proposed products can be considered an additional source of protein substances in human nutrition. Therefore, it is important to know not only the amount of protein, but also its quality characteristics.

The results of the study of the fractional composition of the protein of the powdered semi-finished product from the mushrooms *Pleurotus ostreatus* are shown in Fig. 1.

Studies show that the share of nitrogen of soluble substances, including non-protein nitrogen and nitrogen of water-and salt-soluble protein fractions (albumin and globulin), accounts for 77.06%, and the share of nitrogen of insoluble substances, consisting of nitrogen of alcohol-

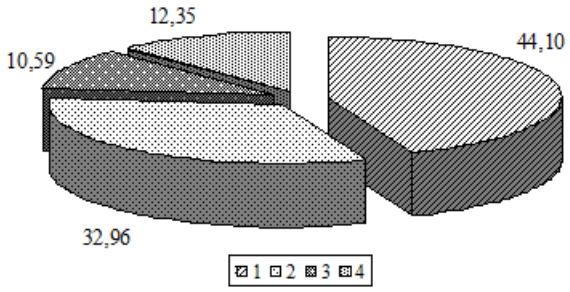

Fig. 1. Fractional protein composition of powdered semi-finished product from mushrooms Pleurotus ostreatus (% of total nitrogen extracted): 1 – non-protein nitrogen; 2 – nitrogen of water-and salt-soluble proteins; 3 – nitrogen of alcohol-soluble proteins; 4 – nitrogen of alkali-soluble proteins.

soluble and alkali-soluble protein fraction – 22.94%. This is consistent with the data of other authors [9], whose studies show that 69...85% of the nitrogen in the mushrooms is in the form of digestible nitrogen.

The share of nitrogen insoluble fractions, which include alcohol-soluble (prolamins) and alkali-soluble (gluten) fraction, accounts for a smaller part. At the same time, several more glutelins than prolamines were found in the studied products.

Albumins and globulins are the most balanced in amino acid composition, they are also the most easily absorbed by the body [10]. Prolamines are the least balanced by amino acid composition. Their characteristic feature – a low content of lysine, and they have virtually no tryptophan. Glutelins occupy an intermediate position between albumins, globulins and prolamins in the balance of amino acids.

It should also be noted that the total nitrogen extraction of protein fractions by all solvents is 46.7%. This leads to the conclusion that a significant part of proteins is represented by complex, insoluble compounds.

In addition to protein substances in the composition of the powdered semi-finished product from the mushrooms *Pleurotus ostreatus*, non-protein nitrogenous substances were found in significant amounts, the share of nitrogen of which accounts for up to 44.10%. The composition of non-protein nitrogenous substances of mushrooms, and therefore their products include free amino acids, peptides, peptons, amines, amides, ammonia compounds, pyrimidine and purine bases (guanine, adenine, xanthine, hypoxanthine), nucleic acids, urea [11].

One of the most important indicators of nutritional value of any product is the balance of amino acid composition and, in particular, the content and quantitative ratio of essential amino acids. To this end, the amino acid composition of semi-finished protein (Table III).

TABLE III. AMINO ACID COMPOSITION OF THE POWDERED SEMI-FINISHED PRODUCT FROM THE MUSHROOMS *PLEUROTUS OSTREATUS*

Amino acids	***Content***		
	mcg /100 g of dry matter	***mcg/100 g of product***	***% of total amino acids***
Essential amino acids	3628	3338	32.7
Among them:			
Isoleucine	296	272	2.7
Leucine	490	451	4.4
Lysine	646	594	5.8
Methionine + cystine	527	485	4.8
Phenylalanine + tyrosine	690	635	6.2
Threonine	325	299	2.9
Tryptophan	181	167	1.6
Valine	473	435	4.3
Nonessential amino acid	7471	6873	67.3
Among them:			
Aspartic acid	1027	945	9.2
Serine	364	334	3.3
Glutamic acid	1358	1249	12.2
Proline	2166	1993	19.5
Glycine	390	359	3.5
Alanine	1387	1277	12.5
Arginine	573	527	5.2
Histidine	206	190	1.9
Total amino acids	11099	10211	100.0

In the study of protein hydrolysates, 18 proteinogenic amino acids, including 8 essential ones, were found in them. This indicates that the protein of the product under study is complete.

In accordance with the science-based concept of nutrition, the biological value of products is determined not only by the presence and mass fraction of individual amino acids, but mainly by their balance. The biological value of a protein by its amino acid composition can be estimated by comparing it with the amino acid composition of the reference protein proposed by FAO/WHO [12]. Calculations of amino acid scores of essential amino acids of the investigated product were carried out (Table IV).

It is known [13] that plant proteins are characterized by a low content of lysine, leucine, threonine, methionine and tryptophan. A comparative analysis of amino acid score showed that the powdered semi-finished product from mushrooms *Pleurotus ostreatus* exceeds the reference protein by 114% in terms of tryptophan content, by 78% in terms the total content of methionine and cystine and by 31% in terms of the total content of phenylalanine and tyrosine. The semi-finished product is content of lysine (105%), threonine (102%) and valine (92%) is close to the standard.

The protein of the studied semi-finished product is limited in leucine and isoleucine, which is generally characteristic for the proteins of the mushrooms *Pleurotus ostreatus*. At the same time, it is noteworthy the high content of lysine, i.e. that amino acid that is deficient in proteins of legumes and cereals.

For the more complete assessment of the biological value of protein such indicators as the coefficient of utility of essential amino acids in comparison with the standard, the coefficient of rationality (utility) of amino acid composition, numerically describing the balance the essential amino acids in relation to the necessary physiologically norm (standard), the index of «comparable redundancy» were used [14].

The results of the studies are presented in Tables V-VI.

TABLE IV. THE CONTENT OF ESSENTIAL AMINO ACIDS IN THE PROTEIN OF THE SEMI-FINISHED PRODUCT FROM THE MUSHROOMS *PLEUROTUS OSTREATUS* IN COMPARISON WITH THE REFERENCE PROTEIN OF FAO/WHO

Amino acids	**In g amino acid per 100 g of crude protein**		**Score of amino acids, % to the protein FAO/WHO**
	Semi-finished product from mushrooms Pleurotus ostreatus	***Reference protein FAO/WHO***	
Isoleucine	2.30	3.00	77
Leucine	3.81	6.10	62
Lysine	5.02	4.80	105
Methionine + cystine	4.09	2.30	178
Threonine	2.53	2.50	101
Phenylalanine + tyrosine	5.36	4.10	131
Tryptophan	1.41	0.66	214
Valine	3.68	4.00	92

TABLE V. COEFFICIENT OF UTILITY OF ESSENTIAL AMINO ACIDS OF SEMI-FINISHED PRODUCT FROM MUSHROOMS *PLEUROTUS OSTREATUS*

Essential amino acids	The coefficient of utility, units
Isoleucine	0.80
Leucine	1.00
Lysine	0.59
Methionine + cystine	0.34
Phenylalanine + tyrosine	0.61
Threonine	0.47
Tryptophan	0.29
Valine	0.67

TABLE VI. BIOLOGICAL VALUE OF PROTEIN OF POWDERED SEMI-FINISHED PRODUCT FROM MUSHROOMS *PLEUROTUS OSTREATUS*

Product	Essential amino acids		Coefficients	
	prevailing	***limiting***	***rationality (utility) of amino acid composition, units***	***comparable redundancy, %***
Powdered semi-finished product	Tryptophan, methionine + cystine	Isoleucine, leucine	0.61	18.02

As can be seen from the table V, despite the fact that the content of tryptophan test product exceeds the reference protein, the degree of utility of its is lowest, and is 0.29 units. This is due to the fact that the protein has a low amino acid score on the limiting amino acid leucine.

Indicators characterizing the biological value of powdered semi-finished products from mushrooms *Pleurotus ostreatus* are presented in table VI.

To characterize the nutritional value of the studied product, the index of essential amino acids (ΣAA-index) and the nutritional index were calculated (Table VII).

As it is evidenced by the obtained data, ΣAA-index of semi-finished from mushrooms *Pleurotus ostreatus* is higher than that for fresh mushrooms. The nutritional index of the studied products is comparable with the minimum value of this indicator for fresh mushrooms. This can be explained by the fact that the product, in addition to mushrooms, includes other raw materials that do not contain protein. And, as a result, the proportion of protein in the total chemical composition of the products is lower than in the original mushrooms.

Simultaneously with the studying the nitrogen-containing substances of the developed products the evaluation of enzymatic attachments of protein substances was conducted. Based on the results of the experiments, graphs showing the degree of hydrolysis of the samples under the influence of various enzymes are constructed (Fig. 2).

TABLE VII. NUTRITIONAL VALUE OF POWDERED SEMI-FINISHED PRODUCT FROM MUSHROOMS *PLEUROTUS OSTREATUS*

Indicator	Fresh mushrooms Pleurotus ostreatus	Powdered semi-finished product
Index of essential amino acids (ΣAA-index)	55…89	102.7
The index of nutritional value	13…22	12.9

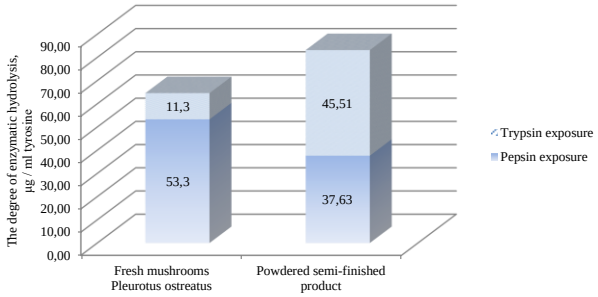

Fig. 2. The degree of enzymatic hydrolysis of protein by proteolytic enzymes.

As can be seen, the depth and nature of enzymatic hydrolysis of proteins in the samples for the entire period of exposure have significant differences. Thus, in the process of pepsin exposure the depth of hydrolysis in the control was of 53.30 mcg of tyrosine in the powdered mix – it is 1.4 times less (37.63 mcg /ml tyrosine). A completely opposite pattern is observed with further trypsin exposure: for mushrooms *Pleurotus ostreatus* the depth of hydrolysis amounted to 11.30 mcg /ml of tyrosine, for powdered semi-finished – 45.51 mcg/ml of tyrosine (4.0 times more control).

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Ultimately, the results of enzymatic hydrolysis are the following: proteins of the control sample are worse exposed by hydrolysis (64.60 mcg/ml tyrosine), the proteins of powdered semi-finished product are better exposed by hydrolysis (83.14 mcg/ml tyrosine, which is 1.3 times more than in control).

Protein attack by enzymes depends on a number of factors:

- molecular structure and denaturation degree;
- presence of cell membranes;
- the ratio and degree of contact of enzymes and substrate;
- the presence of inhibitors in the system, etc.

In the case of enzymatic hydrolysis of the powdered semi-finished product, the presence of starch may have prevented the hydrolysis of proteins at the pepsin exposure stage. At the same time, heat treatment, as well as finer grinding, which leads to an increase in the surface area of the product, provide the availability of protein substances to the action of proteolytic enzymes, which leads to the intensification of enzymatic hydrolysis of protein by proteolytic enzymes at the stage trypsin exposure.

IV. CONCLUSION

During the conducted studies it was found that the heat treatment and the degree of grinding have the significant impact on the degree of digestibility of proteins of mushrooms. Also, the results confirm the information contained in the literature that to improve the digestibility of fungal proteins, mushrooms in the preparation of dishes must be subjected to grinding.

Thus, the study of nitrogen-containing, including protein, substances of powdered semi-finished product from mushrooms *Pleurotus ostreatus* reveals their qualitative diversity and gives encouraging results with regard to their nutritional and biological value. The analyzed products are the source of complete protein, which includes 18 amino acids, including 8 essential ones. Digestibility (in vitro) of proteins of the powdered semi-finished product is 1.3 times higher than the digestibility of fresh mushrooms.

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