

Biological value of proteins of cultivated mushrooms

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Abstract

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Introduction. The objectives of this research were to scientifically substantiate and experimentally prove the nutritional status of cultivated mushrooms as the probable source of easy-absorbed proteins, essential and dispensable amino acids, and other valuable biologically active components.

Materials and methods. Biochemical characteristics, such as the mass part of albumins, globulins, glutelins and prolamins, and the qualitative and quantitative composition of amino acids in free and constrained forms, of cultivated mushrooms, champignon (*Agaricus bisporus*) and oyster (*Pleurotus ostreatus*), and edible wild mushrooms, white mushrooms (*Boletus edulis*) and the brown-cap boletus (*Leccinum scabrum*), were determined.

Results and discussions. The biochemical composition of mushroom hats and legs is different in separate indices: the amount of dry substances in champignon hats is higher by 13–18%, the amount of proteins is higher by 14.6–23.5%, meanwhile, the amount of cellulose is lower by 17–19% in comparison with legs. This shows the substantial nutritional advantage of hats, and it must be taken into consideration in the industrial procession of mushrooms: hats should be separated from legs, following the optimal parameters of the process for each anatomic part. The champignon proteins contain all the indispensable amino acids and, therefore, can be the important source of lysine (4.95 mg%), phenylalanine (7.04 mg%), leucine (9 mg%), and threonine (7.6 mg%). About 7.6% of amino acids are in free form, half of which are essential. This would help the human body effectively use the amino acids to synthesize its own proteins.

The amount of proteins in fresh champignons is 6–9% of their mass, in oyster mushrooms it is 4–5%, in wild mushrooms, it is 6–8.5%, which outlines the priority of champignons particularly by their protein component. Easy-soluble fractions (albumins and globulins) at 70.3% present the champignon proteins; this index is slightly lower for oyster mushroom proteins (65%) and for brown-cap boletus, it decreased to 53.2%. Therefore, proteins of the cultivated mushrooms need the minimal amounts of energy to be dissociated to amino acids in the human body, and otherwise show the high grade of proteolysis (almost as milk proteins) under the influence of gut enzymes. These characteristics were achieved due to scientifically proven selection of raw materials, regarding their sensory characteristics that were estimated with the excellent grade. There were proposed criteria to select the cultivated mushrooms for culinary and industrial processing: the amount of proteins no less than 6–9%; cellulose 2–3.5%; carbohydrates 1–1.5%.

Conclusions. The cultivated mushrooms and the products of their procession with high content of proteins and other valuable components should become the essential constituent of diets in order to overcome the protein deficiency.

Introduction

Nutrient analysis and dietary quality for most people indicate a persistent protein deficiency, which should be exacerbated in the near future (Medek et al., 2017). Therefore, the search for potential new protein sources and production of high-protein foods are among the topical tasks for food technologies (Ivanov et al., 2021; Wu et al., 2014).

The National Center for Biotechnology Information (USA) highlighted that about 90 percent of adult people are aware of the advantages of high-protein foods (Chang & Wasser, 2012; Global Alternative, 2020). Due to ecological ideology and diffusion of vegetarianism, the production of proteinaceous foodstuffs from soybeans is the main stream in Asia, particularly in China, and, during the last years, in Europe (Elorinne & Kantola, 2016). The largest share belongs to champignons (*Agaricus bisporus*) and shiitake (*Lentinula edodes*) (Martinez-Medina et al., 2021; Stabnikova et al., 2010; Stojkovic et al., 2014). There is an array of scientific research on using the mushroom raw materials as the meat substitute (Pasichny et al., 2009). In fact, this became one of the main tendencies of the food industry through the latest period, which is believed to be increasing significantly (Batraksas et al., 2021; Ferdousi et al., 2020; Mubiana et al., 2012).

The artificial cultivation of mushrooms becomes very important because the fruit bodies of forest mushrooms have the ability to accumulate heavy metals and radionuclides, thus becoming perilous for consumers' health and life (Struminska-Parulska et al., 2021). There is a point of view that in the nearest future about two thirds of protein needs for humans will be met through the consumption of mushrooms grown in industrial conditions. (Bolotskikh & Volfovsky, 2007). These mushrooms are ecologically clean, and their taste could be improved by addition of sodium glutamate (Chang, 2006). Mushrooms are widely used in production of therapeutic and preventive remedies with hepatoprotection, radioprotection, antidiabetic, anticancer, and immunoregulatory activities (Martinez-Medina et al., 2021; Sanket & Pravin, 2021; Valverde et al., 2015; Yaschenko, 2012). It was shown that consumption of mushrooms increased the immunity to inflectional and oncologic diseases (Krasnopolskaya et al., 2007; Meera et al., 2009; Wasser & Weis, 1999; Wasser et al., 2000); they get involved into metabolic processes and do not have cumulative ability (Cultivation, 2021; Yaschenko, 2012). Regular consumption of cultivated mushrooms can significantly increase the content of antioxidant markers and decrease the level of oxidative stress (Calvo et al., 2016; Glamočlija et al., 2015). Mushrooms can also become the only plentiful sources of vitamin D of non-animal origin (Bernas & Jaworska, 2017; Cardwell et al., 2018; Simon et al., 2013).

Therefore, the problem of increasing the volumes of consumption of cultivated mushrooms is scientifically proven and is actual for the population of over the world.

The protein content of mushrooms determines their biological value. In this case, the content of amino acids in the protein must meet the needs of the human body for the synthesis of its own proteins (Tagkouli et al., 2020). Moreover, proteins, upon being the most essential component of food, are responsible for growth, creation of the new tissues and restoration of those damaged (Malecki et al., 2021). Besides, all enzymes and certain hormones are proteins too. Decidedly, only the plentiful proteins provide the correlations of amino acids, which are compatible with human body needs.

Unfortunately, these problems are now studied sporadically. The majority of updated research are dedicated to the principles of mushroom cultivation (Royse, 2003; Zhang et al., 2014), their industrial production (Simakhina et al., 2014); elaboration of eco-friendly and wasteless cultivation technologies (Guan et al., 2016; Simon et al., 2011); improvement of the methods to process mushroom raw materials, including drying, fermentation and

freezing; mycelium preparation and so on. Only some studies deal with the general amount of proteins in oyster mushrooms, touching upon their amino acid content and the proportions between dispensable and indispensable amino acids, practically leaving aside the ways to increase the mushroom biological value and other related issues (Tolera & Abera, 2017).

Issues that have so far received insufficient attention include study of the fractional composition of proteins of cultivated mushrooms which is an essential index to predict the level of their absorption in human body; effectiveness of protein digestibility by proteolytic gut enzymes; elaboration of the criteria to select the sorts of cultivated mushrooms (starting from their sensory evaluation), compliance with which would guarantee obtaining the high-quality half and final products with increased biological and nutritional value.

The aim of the present research was scientifically substantiality and experimentally proven of the nutritional status of cultivated mushrooms as the probable source of easily-absorbed proteins, essential and dispensable amino acids and other valuable biologically active components for their use in the food industry. To achieve this goal it was necessary to examine the quantitative and qualitative content of the main nutrients in cultivated mushrooms, particularly, the fractional composition of proteins; to estimate the ratio between dispensable and indispensable amino acids; the grade of their digestibility by proteolytic gut enzymes; their sensory indices, and to formulate the criteria to select the champignons for both direct consumption and industrial procession.

Materials and methods

Mushrooms

Champignons (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus ostreatus*) became the object for the main part of research. For a comparative study, some experiments were conducted in parallel with wild white mushrooms (*Boletus edulis*) and brown-cap boletus (*Leccinum scabrum*). After having selection, washing, and removing the waste from the raw materials, the biochemical characteristics of mushrooms were evaluated, namely, fractional distribution of protein, content of amino acids, sensory characteristics, and the ratio of free and constrained amino acids, both dispensable and indispensable.

Determination of dry matter

The dry matter was determined using differential refractometry (Hernandez et al., 1998) using of IRF-454 B2M refractometer (Laboratorna tekhnika Ltd., Kharkiv).

Determination of protein and amino acid content

The general amount of proteins and the qualitative and quantitative content of amino acids were determined by the method described in (Redweik et al., 2012) with a usage of capillary electrophoresis. The ratio between dispensable and indispensable amino acids in free and constrained forms was determined by the method of Moore – Stein (Moore & Stein, 1972).

Determination of sugars content

The general amount of sugars was determined by ion analysis method using Bioscan 817 chromatographer (Metrohm IC). To prepare the sample for the analysis, mushrooms were powdered to homogenous mass and put into the automatic sample taker of the chromatograph.

Determination of cellulose content

Content of cellulose was determined by the method of direct weighing analysis, which combines oxidation, destruction, and solution of various chemicals, except for cellulose that, in process, was removed, dried and weighed (Kumar & Turner, 2015).

Fractionation of mushroom proteins

Fractionation of mushroom proteins was carried out according to (Table 1). The disintegrated samples of mushrooms (particle size 2-3 mm) were extracted, and then centrifuged for 15 min at 6000 rpm. The sediments were washed, and the volume of every extract was replenished to 150 ml by washing waters. The content of protein was determined in the extracts and sediments by the method (Redweik et al., 2012).

Table 1

Fractionation of mushroom proteins

Method	Fractions of mushroom proteins			
	Albumins	Globulins	Glutelins	Prolamins
Solvents	Water	1 M NaCl in 0.1 M phosphate buffer (pH 6.8)	0.1 N NaOH	70% ethyl alcohol
Weight ratio between mushroom mass and solvent	1 : 3	1 : 3	1 : 2.5	1 : 2.5

Evaluation of sensory characteristics of cultivated mushrooms

The selection of raw materials, primarily by the sensory characteristics, is the essential step to use the fresh mushrooms and, subsequently, obtaining the mushroom semi-finished products with suitable consumer properties and high biological value (Phat et al., 2016). Thus, high quality of mushroom semi-finished products and foods with their usage is guaranteed (Table 2).

Table 2

Methods to evaluate the main sensory characteristics of fresh mushrooms (champignons)

Characteristics	Description
Appearance	Mushrooms are clean, undamaged, elastic, fresh looking, without excessive external humidity, not frozen, not injured by harmful insects; legs are either cut or uncut. In the first case, the cut should be clean; in the second, the traces of greenhouse material are accessible. Insignificant surface damages are allowed if they do not affect the quality, storage terms and commercial appearance of the packed items.
Taste and smell	Typical for fresh champignons, without strange smells and smacks.
Color	The hat surface is white, cream-colored or brown with various hues typical to the cultivated sorts; the fresh cut of the hat is white with rosy hue.
Maturity grade	Mushrooms are of forms and colors typical for the certain botanical sort, homogenous in maturity grade, well shaped. The hats are open or closed but not flat. The plate color from the bottom side of the hat is pale rosy.

The sensory characteristics of mushrooms were evaluated according the 5-point scale proposed by the authors (Table 3).

Table 3

Scoring of sensory characteristics of fresh champignons

Index	Score points	Estimation of fresh champignons quality by score points
Appearance	5	Fresh, whole, without defects and microbial damages, homogenous.
	4	Fresh, whole, practically without defects.
	3	Whole, partly withered, slightly damaged.
	2	The significant share of withered and damaged mushrooms.
	1	Inhomogeneous, with defects and microbial damages.
Taste and smell	5	Typical for fresh champignons, without strange taste and smell.
	4	Slight strange taste and smell.
	3	Stable and obvious strange taste and / or smell.
	2	Stable and expressed, atypical strange taste and / or smell.
	1	Strong rotting stench and atypical taste.
Color	5	The hat surface is white or cream-colored; the fresh cut of the hat is white with rosy hue.
	4	The hat surface is white or cream-colored; the fresh cut of the hat is white.
	3	The hat surface is grayish as well as the fresh cut.
	2	The hat surface is grey with dark blots; the cut is grey.
	1	The hat surface is dark; the cut is rotten.
Maturity grade	5	Mushrooms are homogenous in maturity grade, well shaped. The hats are not flat. The plate color from the bottom side of the hat is pale pink.
	4	Mushrooms are sometimes inhomogeneous in maturity grade, well shaped. The hats are not flat. The plate color from the bottom side of the hat is pale.
	3	Mushrooms are slightly inhomogeneous in maturity grade, mostly well shaped. The hats are not flat. The color of the hat plates is grayish.
	2	Mushrooms are practically inhomogeneous in maturity grade, different in shape. The hats are mostly flat. The color of the hat plates is grey.
	1	Mushrooms are different in maturity grade, non-calibrated. The plate color from the bottom side of the hat is rotten brown.

Note: mushrooms with quality estimated as 1 or 2 points are not recommended for further procession.

Upon selection of mushroom raw material for technological purposes, the sensory evaluation should be complemented with the characteristics of mushroom biochemical compounds.

Results and discussion

Biochemical characteristics of mushroom fruit bodies

For the certain species of mushrooms, some essential biochemical characteristics of hats and legs were determined. The results are shown in Table 4.

Table 4

Biochemical characteristics of mushroom fruit bodies

Content, % to dry matter	Mushrooms species and anatomic parts					
	White mushrooms		Champignons		Oyster mushrooms	
	Legs	Hats	Legs	Hats	Legs	Hats
Water	87.0	85.5	86.0	84.2	91.0	90.5
Dry matter	13.0	14.5	14.0	15.8	9.0	9.5
Proteins	6.8	8.4	7.5	8.6	4.0	5.1
Carbohydrates	1.22	1.53	2.4	1.9	1.2	1.6
Cellulose	4.23	3.5	3.0	1.6	3.1	1.9

Analysis of the biochemical composition of separate fruit body parts of cultivated mushrooms showed the quantitative difference between hats and legs. The results show that hats and legs of champignons contain more proteins than any other studied mushroom species. The protein content in hats of champignons is 8.6%; in white mushrooms 8.4%; in oyster mushrooms 5.1%. The content of dry substances was slightly higher in hats, and the protein content was by 14.6-23.5% higher in hats than in legs, which is according to the results of other researchers (23, 24).

There is a problem of cellulose impact on nutritive value of cultivated mushrooms (Dubinina, 2009; Synytsia, 2009). Cellular membrane of mushrooms, due to the content of chitin (about 60% to dry matter), is able to reveal the antiviral and antibacterial action and absorb the heavy metals and radionuclides (Meera, 2009; Wasser, 2000). The large amount of cellulose represented by indigestible food fibers would retard the process of protein dissociation in the gut and their further absorption by the organs and tissues; it would mean that mushrooms are unsuitable for dietetic nutrition. Therefore, from our viewpoint, the amount of cellulose in cultivated mushrooms destined for obtaining the food products with increased nutritional and biological value should be within 3-3.5%.

Taking into account the difference between biochemical characteristics of hats and legs, we propose the notion of heterogeneity grade of anatomic parts of mushrooms by two main constituents – proteins and cellulose. This index should be evaluated with a coefficient:

$CP = P / Cel$, in which P is the protein content, % ;

Cel – cellulose amount, % .

For the studied types of mushrooms, the CP coefficient counts:

- White mushrooms: legs – 1.62; hats – 2.4;
- Champignons: legs – 1.87; hats – 2.15;
- Oyster mushrooms: legs – 1.29; hats – 2.6.

This discrepancy between the heterogeneity grades of different anatomic parts of mushrooms is evidence of their structural, mechanical properties and tissue firmness. Because mushroom hats and legs have different content of essential nutrients, we conclude that, upon elaboration of technology to produce mushroom semi-finished products, independently on the species, the hats should be separated from legs prior to procession, and then the optimal procession parameters should be determined for each of the anatomic parts.

Amino acid contents in fresh champignon proteins

Amino acids are the main structural elements of proteins. Twenty-six amino acids were observed in proteins, and the typical constituents of proteins are considered twenty of them. The latter are categorized into dispensable (total amount of twelve) and indispensable, or essential (total amount of eight) obtained only from foodstuffs. The results on the qualitative and quantitative content of fresh champignons to be later estimated as the ratio between dispensable and indispensable amino acids in free and constrained forms (as the characteristics of protein biological value) are shown in Table 5.

Table 5

Amino acid contents in fresh champignon proteins

Amino acid	Total amount, %	Free		Constrained	
		mg%	% to the total amino acid amount	mg%	% to the total amino acid amount
Lysine	4.98	0.38	0.26	4.60	3.21
Histidine	8.98	0.78	0.54	7.70	5.38
Phenylalanine	7.036	0.136	0.10	6.90	4.82
Tyrosine	2.51	0.05	0.03	2.46	1.72
Leucine	9.0	0.5	0.34	8.50	5.94
Isoleucine	2.94	0.64	0	2.30	1.60
Valine	5.08	0.7	0.48	4.38	3.06
Methionine	1.71	0.01	0.01	1.71	1.18
Alanine	7.4	1.3	0.90	6.10	4.26
Glycine	17.17	0.27	0.18	16.91	11.81
Proline	2.31	0.01	0.01	2.32	1.60
Serine	9.00	0.40	0.27	8.60	6.01
Threonine	7.63	0.53	0.37	7.11	4.96
Asparagine acid	21.72	0.38	0.26	21.34	14.92
Cystine	0.31	0.02	0.01	0.29	0.20
Arginine	-	-	-	-	-
Tryptophan	1.05	-	-	1.05	0.73
Glutamine acid	34.7	1.5	1.04	33.2	33.2
Total	143.021	7.6	-	135.4	-

Fractional composition of fresh champignon proteins

Biological value of proteins in any foodstuffs determines not only by the total amount or the amino acid content, but also by fractional composition. The proteins are classified into four classes, namely, albumins, globulins, prolamins, and glutelins (Garidel, 2013). Albumins, water-soluble proteins, are characterized by the highest biological and nutritional value; globulins, salt-soluble proteins, have also high biological value but are poor in sulfur-containing amino acids. The last two, prolamins, alcohol-soluble, and glutelins, alkali-

soluble, have no some indispensable amino acids in their compositions, harder digested by proteolytic enzymes and thus have lower the biological value.

The literary data about fractional composition of proteins of cultivated mushroom are still limited. Therefore, fractional composition of mushrooms proteins were studied in the present research, and compared (Table 6).

Table 6

Fractional composition of mushroom proteins

Protein fractions	Ratio of fractioned proteins,% of total protein amount		
	Brown-cap boletus	Champignons	Oyster mushrooms
Water-soluble (albumins and easy-soluble globulins)	30.8	46.4	39.8
Salt-soluble (hard-soluble globulins)	22.4	23.9	25.2
Alkali-soluble (glutelins)	12.6	8.06	10.6
Alcohol-soluble (prolamins)	11.5	5.6	3.6
Unsolved remnant	22.7	16.24	20.8

According to results, cultivated mushrooms have higher biological value, because protein substances are mostly presented by easy-soluble factions – 70.3% in champignons and 65% in oyster mushrooms. These proteins are alleged to dissociate in human body to amino acids, which are necessary for synthesis of the native proteins, with minimal energy losses.

Fractional composition of champignon proteins is slightly better than of oyster mushrooms, however, both kinds of mushrooms are suitable for direct usage and industrial procession into proteinaceous semi-finished products as ecologically clean, useful and safe enough raw materials.

Wild mushroom proteins contain less albumins and globulins (53.2%), therefore, they are worse soluble in water and neutral salt solutions. Human body worse absorbs such proteins, and their biological availability and value are lower in comparison to cultivated mushrooms.

Because of high content of albumins and globulins, proteins of cultivated mushrooms will be far easily hydrolyzed in the gut by proteolytic enzymes, and proteins of wild mushroom have lower proteolysis degree because they contain much more cellulose, which may block the enzyme access to protein substances.

Sensory characteristics of champignons

Therefore, conducted research and obtained results showed the perspectives for using of cultivated mushrooms, particularly hats of champignons, as the reserve of native proteins, well-balanced proportion of essential and dispensable amino acids, prevalent content of easy-soluble factions, and higher grade of digestibility by proteolytic enzymes. Regarding to the fact that appearance is considered the complex index to include the shape, size, maturity grade, freshness, and color, the maximal figure of quality coefficient will be 0.35 (Table 7).

Table 7

Scoring the sensory characteristics of fresh champignons

Properties	Coefficient	Score points	Characteristics
Appearance	0.35	5	Mushrooms are whole, clean, elastic, fresh, without excessive external humidity, non-frozen and non-damaged by agricultural pests. Cut champignons should have their cuts clean; surface damages are not allowed.
Taste and smell	0.25	5	Typical for fresh champignons, without strange smell and smack.
Color	0.15	5	Hat surface is white or cream-colored, with different hues typical for certain sorts; hat pulp on the cut is white with pink hues; leg pulp is slightly darker due to higher cellulose content.
Maturity grade	0.25	5	Mushrooms are typical in appearance and color for the certain botanical species, homogenous in maturity, well shaped. Hats are closed or opened but not flat. The color of under-cup plates is pale pink. Legs of non-cut mushrooms may carry the traces of greenhouse soil materials.

Table 8

Criteria for selection of champignons for direct consumption and processing in semi-finished products

No.	Criterion	Criterion characteristics
1	High protein content (6-9% and more)	The significant reserve of food proteins; their validity in terms of correlation between dispensable and indispensable amino acids; the important additional source of lysine, phenylalanine, asparagine and glutamine amino acids
2	High biological value	The presence of all the indispensable amino acids; correspondence of amino acid composition to human needs for synthesis of the native proteins; protein digestibility equal to the one of milk proteins
3	Optimal cellulose content (2-3.5%)	Positive impact on gut functions; adsorption of heavy metals and radionuclides; prebiotic properties
4	Sufficient carbohydrate content (1-1.5%)	The ability to stimulate anti-body synthesis and thus to increase the immune protection; cancer-protecting properties due to presence of polysaccharides
5	Relative initial humidity (no more than 80-84%)	Quite an intensive drying process should be maintained; circa 90% of moisture are represented by free faction to be removed easily
6	The absence of toxic substances, heavy metals and carcinogens	Environmental friendliness of production and procession; safety for consumers in both fresh and processed forms
7	Sensory characteristics	Appearance, taste and smell, color and maturity grade

Moreover, since mushrooms are discrepant in appearance to the requirements proposed, the usage of all the other criteria appears to be inexpedient. In case when mushrooms are discrepant in appearance to the requirements proposed, the usage of all the other criteria appears to be inexpedient. The studied champignons by all the sensory characteristics scored the maximal five points, confirming their status of a reliable source of proteins, amino acids and food cellulose that are the main nutrients in human diets.

Upon taking into account, the results of studying the biochemical composition of champignons and scoring their sensory characteristics, the criteria to select mushrooms for either cookery or industrial procession were established (Table 8).

Conclusions

1. Proteins as macronutrients are essential in growth, creation of the new tissues and recovery of the damaged ones. They take part in regulation of the majority of vital processes in human body, enhance the biological influence of other nutrients, and provides the transport of oxygen, hormones and trace elements. Insufficient supply of proteins or separate amino acids with foodstuffs would lead to protein deficiency, causing serious damages in the body due to misbalance between protein anabolism and catabolism. This is why the searches for new untraditional sources of proteins are relevant today. One of the ways to solve this problem is the usage of cultivated mushrooms that contain about 50% of proteins (in terms of dry matter) and other value biocomponents.
2. It was shown that cultivated champignons and oyster mushrooms have in their fractional composition a high content of easily digestible proteins (more than 70%), which facilitates their digestibility by proteolytic enzymes. At the same time, they contain all the essential amino acids that confirms their nutritional value. Organoleptic criteria have been proposed for the selection of cultivated mushrooms in order to use them in obtaining high-quality food products.
3. The advantages of cultivated mushrooms over the wild ones in terms of ecological friendliness and safety were demonstrated.
4. The optimal ration between proteins and cellulose in champignons (3 : 1) will provide the high grade of protein digestion by proteolytic enzymes and allow using the detoxifying properties of cellulose as the natural sorbent. Therefore, the further studies on cultivated mushrooms, the search of new high-protein species, and the design of effective methods to process the mushroom raw materials into semi-finished and final products are tasks targeted at overcoming the protein deficiency and ameliorating the human health.

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