Eco. Env. & Cons. 26 (2) : 2020; pp. (931-934)
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ISSN 0971–765X

Determination of amino acid and fatty acid composition of depth mycelium *Lentinus edodes*

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(Received 16 February, 2020; accepted 22 May, 2020)

ABSTRACT

The amino acid composition of the proteins of the deep mycelium *Lentinus edodes* strain 2541 was studied and it was found that the protein contains 17 types of various amino acids, 9 of which (47%) are essential amino acids. Among the essential amino acids, the predominant ones are leucine, lysine, valine and tyrosine. These data indicate the high nutritional value of the mushroom protein. The content of lipids and fatty acids in the cells of the deep mycelium was studied. It was revealed that a significant (75%) part of lipids in the cells is represented by unsaturated fatty acids, the degree of unsaturation of which is 1.46. The main component is the polyunsaturated diene linoleic (C18: 2) acid - 69% of the total fatty acids. Polyunsaturated fatty acids are indispensable components of nutrition, because, like essential amino acids, they cannot be synthesized in the human body.

Key words : *Lentinus edodes, Amino acids, Lipids, Fatty acids*

Introduction

Higher basidiomycetes are promising objects of modern biotechnology because they contain a unique complex of biologically active substances of carbohydrate, lipid and phenolic nature, vitamins, trace elements and other compounds vital for the human body (Xin *et al.*, 2016; Filipa S. Reis *et al*., 2017). One of the most widely cultivated species of edible xylotrophic fungi is currently Lentinus edodes (shiitake). The fruiting bodies of *L. edodes* contain proteins (26% of dry weight), lipids, including fatty acids (mainly linolenic acid); carbohydrates; minerals; vitamins B1, B2 and C; ergosterols (Badalyan *et al*., 2019; Himanshi *et al*., 2017).

Currently, the study of the biological properties of the biomass of *Lentinus edodes* fungi (shiitake) obtained under deep cultivation is attracting increasing interest. The results of these works can contribute to the development of new biotechnology for the production of biologically active additives with a wide range of therapeutic properties.

The scarcest component in human nutrition is complete protein. The nutritional value of proteins is determined mainly by the composition of amino acids. Great importance wherein is the content of essential amino acids. The proteins of the fruit bodies of *L. edodes* contain 18 amino acids that are part of the balanced nutrition formula, of which the irreplaceable are of particular value: lysine, threonine, valine, tryptophan, tyrosine, etc. In addition to proteins, these fungi are able to synthesize lipids and highly active lipid components in fairly large quantities. Lipids in fungi are represented by mono- and triglycerides, sterols and phospholipids (Bo¿ena Muszyñska *et al.*, 2018; Wen Li *et al.*, 2019). Informa-

tion on the physiological and biochemical characteristics, nutritional needs and conditions conducive to increased synthesis of the bioactive lipid components of most basidiomycetes grown by the deep method are very limited and sometimes contradictory. The purpose of this study was determination of the amino acid and fatty acid composition of the deep mycelium *L. edodes*.

Materials and Methods

The object of research was the pure culture of *L. edodes 2541* from the collection of hat mushroom cultures of the Institute of Botany named after N.G. Holodny of NAS of Ukraine (Kiev). For the cultivation of biomass, a nutrient medium of the following composition g/L was used: cellobiose –30; KH_2PO_4-1; K_2HPO_4-1; $MgSO_4$ 0.25; peptone - 3.5; yeast extract - 20 mL; pH is 6.

The experiments were performed on laboratory shakers (180 rpm) in 250 mL Erlenmeyer flasks with 50 mL of medium for 7 days. The intensity of aeration is 0.55 g O_2/l/h. Sterile media were inoculated with the homogenized biomass of L. edodes 2541 strain (10% volume). Cultivation temperature was 25ºC. After growing, the mycelium of the fungi was separated from the culture fluid through nylon tissue, washed repeatedly with water, dried at 60 °C to a constant weight, crushed and used for chemical analyzes.

The amino acid composition of the proteins was studied using an "AAA-881" amino acid analyzer ("Mirotechna", Czech Republic). Lipids were extracted using the Folch method (Folch *et al.,* 1957), the fatty acid composition of lipids was analyzed as methyl esters of fatty acids on a "Chrom-5" gas-liquid chromatograph (Czech Republic) with a flame ionization detector using a stainless steel column 3,7 m in length, filled with N-AW chromaton with 15% polyethyleneglycol succinate in isothermal mode at an evaporator temperature of 210 °C and a column temperature of 160 °C. The identification of fatty acids was carried out by the relative volumes of retention, as well as in comparison with the indicators of the control methyl esters of fatty acids (Vereshchagin *et al.,* 1963; Cates M., 1975).

Results and Discussion

Determination of the amino acid composition of the proteins of the deep *mycelium* L. edodes 2541

As a result of the experiments, it was found that the protein of the deep mycelium *L. edodes 2541* contains all of the essential amino acids that are included in the formula of a balanced human diet. The high nutritional value of the L. edodes 2541 deep mycelium protein is evidenced by the significant content of essential amino acids in it, which make 47% of the sum of all amino acids (Table 1).

Table 1. Amino acid composition of the proteins of the deep mycelium *L. edodes 2541*

Amino acid	Amino acid,% in protein
Leucine	7.5 ± 0.18
Lysine	6.9 ± 0.2
Valine	6.9 ± 0.15
Tyrosine	6.5 ± 0.18
Threonine	4.5 ± 0.11
Cystine	3.2 ± 0.08
Methionine	2.7 ± 0.07
Isoleucine	5.2 ± 0.11
Phenylalanine	4.0 ± 0.12
Amount of Essential Amino Acids	47.4 ± 1.2
Glutamic acid	17.0 ± 0.32

Among the essential amino acids in the biomass of the studied strain, the highest content of the following amino acids was detected: leucine, lysine, valine and tyrosine. It should be noted that the composition of plant proteins (cereals in particular) includes a very small amount of lysine and leucine. Among the essential amino acids in the protein of the studied strain of L. edodes 2541, the largest proportion is glutamic acid.

Determination of the fatty acid composition of the lipids of the deep *mycelium* L. edodes 2541

Lipids play a special role in a number of vital cell processes (information transfer, secretion of metabolites, etc.). That is why it is of particular interest to determine the amount of lipids of the deep mycelium of the studied L. edodes 2541 strain and to study their fatty acid composition. In the initial experiments it was found that the lipid content in the biomass of the studied strain is 7.0-0.2%. For more accurate characteristic of the nutritional value, an analysis of the fatty acid content was carried out (Table 2).

Data presented in Table 2 demonstrate that the polyunsaturated diene linoleic (C18: 2) acid prevails in the lipids of the studied L. edodes strain - 69% of the total fatty acids. It should be noted that the main fatty acid of many vegetable oils (sunflower, soy-

Table 2. Analysis of the fatty acid content

Indicators	Total Fatty Acids, %
Trivial Fatty Acid Name	Systematic Name (IUPAC) of Fatty Acid
	Saturated Fatty Acids
Myristic ($C_{14:0}$)	Tetradecane	0.73 ± 0.01
Pentadecyl ($C_{15:0}$)	Pentadecane	1.58 ± 0.04
Palmitic ($C_{16:0}$)	Hexadecane	19.23 ± 0.50
Margarine ($C_{17:0}$)	Heptadecane	0.45 ± 0.01
Stearic ($C_{18:0}$)	Octadecane	2.30 ± 0.05
	Unsaturated Fatty Acids	
Palmitoleic($C_{16:1}$)	Cis-9-hexadecene	0.85 ± 0.01
Heptadecene($C_{17:1}$)	Cis-9-heptadecene	0.45 ± 0.01
Oleic($C_{18:1}$)	Cis-9-octadecene	3.79 ± 0.03
	Polyunsaturated Fatty Acids	
Linoleic ($C_{18:2}$)	Cis, cis-9,12-octadecadiene	69.42 ± 1.20
HF (degree of lipid unsaturation)		1.46 ± 0.01
Sum of Saturated Fatty Acids		24.29 ± 0.61
Sum of Unsaturated Fatty Acids		75.41 ± 2.00

bean, corn, cotton) is also linoleic acid, the content of which is 50–70%.

It was found that among saturated fatty acids in the lipids of the strain L. edodes 2541, monoenic palmitic (C16: 0) acid prevails, the content of which is 19% of the total fatty acids. In the composition of lipids of the strain L. edodes 2541, the amount of unsaturated fatty acids (75%) significantly exceeds the amount of saturated fatty acids (24%). In this regard, the degree of unsaturation of lipids is 1.46.

In Shiitake fruiting bodies, fatty acids comprise 3-3.4% of the lipid content. They include the following acids: linoleic (C18: 2) - 72.8%, palmitic (C16: 0) - 14.7%, oleic (C18: 1) - 3.0%, tetradecene (C14: 1) - 1, 6%, stearic (C18: 0) - 0.9% and myristic (C14: 0) - 0.1%.

Conclusion

The deep mycelium *L. edodes 2541* contains all the essential amino acids that are part of the balanced human nutritional formula. Among the essential amino acids in the biomass of the studied strain, the highest content was found for leucine, lysine, valine and tyrosine. Also it was found that in the fruit bodies of *L. edodes*, as in a number of other basidiomycetes, lipids do not exceed 5-6% by weight, and, as a rule, their content is higher in the cap than in the stem. This is due to the fact that the basidiospores in the hat are very rich in lipids.

Comparison of the lipid composition of the fruiting bodies of L. edodes with the fruiting bodies of the widely cultivated edible fungi A. bisporus (champignon) and P. ostreatus (oyster mushroom) showed that the main features inherent in lipids of the fungi class Basidiomycetes (predominance C18: 2, high values of the degree of unsaturation (CH) lipids (1.4-1.5)) are also found in shiitake. However, this fungus also has a number of differences, for example, a high level of palmitic acid (C16: 0) and presence of palmitoleic acid (C16: 1).

Acknowledgements

This study was conducted with financial support from Ministry of Education and Sciences of the Republic of Kazakhstan (Grant # AP05130493).

References

Badalyan, S.M., Barkhudaryan, A. and Rapior, S. 2019. Recent Progress in Research on the Pharmacological Potential of Mushrooms and Prospects for Their Clinical Application. *Medicinal Mushrooms Recent Progress in Research and Development*. Springer Nature Singapore Pte Ltd.

Bo¿ena Muszyñska, Agata Grzywacz-Kisielewska, Katarzyna Ka³a. Joanna Gdula-Argasiñska. 2018. Anti-inflammatory properties of edible mushrooms: A review. *Food Chemistry*. 243 : 373-381.

Cates M. Technique of Lipidology (1975). M.: Mir.

Filipa S. Reis, Anabela Martins, M. Helena Vasconcelos and Patricia Morales, 2017. Functional foods based on extracts or compounds derived from mushrooms. *Trends in Food Science & Technology*. 66 : 48-62.

Folch, I., Lees, M. and Sloan-Staulet, G.H.S. 1957. A simple method for isolation and purification of total lipids from animal tissues.*J. Biol. Chem*. 226 (1) : 491-509.

Himanshi Rathore and Shalinee 2017. Prasad Mushroom nutraceuticals for improved nutrition and better human health: A review. *Pharma Nutrition*. 5 (2) : 35-46.

Sandrina A. Heleno, Raíssa Carolina Ferreir 2015. Nutritional value, bioactive compounds and antioxidant properties of three edible mushrooms from Poland. *Food Biosc*. 11 : 48-55.

Vereshchagin, A.G., Skvortsov, S.V. and Iskhakov, N.I. 1963. The composition of triglycerides of cottonseed oil. *Biochemistry*. 28(5) : 868-878.

Wen Li, Jinbin Wang, Wanchao Chen and Yan Yang 2019. Analysis of volatile compounds of Lentinula edodes grown in different culture substrate formulations. *Food Research International.* 125: 108517.

Xin Meng 2016. Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. *Carbohydrate Research*. 424: 30-41.