

THE NUTRITIONAL VALUE OF *TRAMETES VERSICOLOR* MYCELIA IN SUBMERGED CULTIVATION

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ABSTRACT

Trametes versicolor (L.) Lloyd is a wood-decaying medicinal fungus belonging to the division *Basidiomycota*. Mycelial mass of *T. versicolor* IBK 353 was obtained after submerged cultivation on a liquid medium with waste bread (breadcrumb) and complex glucose-yeast medium with beer wort. The chemical composition and nutritional value of mycelia depended on the medium for cultivation. The ash, crude protein, and crude fat contents were 3.8, 1.6, and 1.4 higher correspondingly in mycelium cultivated on complex glucose-yeast medium with beer wort than on breadcrumb. The protein in *T. versicolor* IBK 353 mycelia contained 34-37 % of essential amino acids, and lipids comprised 60-76 % of unsaturated fatty acids. *T. versicolor* IBK 353 dry mycelia were enriched in folates, and 100 g contained a daily recommended dose of riboflavin and 81-100% of niacin. Mycelia of *T. versicolor* grown on two investigated media as a source of biologically active substances may serve to create functional foods and dietary substances.

Keywords: turkey tail mushroom, breadcrumb, complex glucose-yeast medium with beer wort, amino acid, fatty acid, vitamin composition

INTRODUCTION

Mushrooms have been known for their nutritional values for ages, being a good source of protein with a sufficient content of essential amino acids, dietary fiber and therapeutically active polysaccharides, vitamins and mineral elements (Cheung 2008). *Trametes versicolor* (L.) Lloyd, also referred to as turkey tail mushroom, is a widely distributed in nature wood-decaying medicinal mushroom belonging to the division *Basidiomycota*. The medicinal properties of *T. versicolor* are used to treat cough, asthma, bronchitis, cancer, hepatitis, and rheumatoid arthritis (Jin et al. 2018). Commercial preparations of polysaccharopeptide (PSP) and protein-bound polysaccharide Krestin (PSK) with anti-cancer effect have been obtained from batch-cultivated *T. versicolor* mycelia extracts (Tišma et al. 2021). In nature turkey tail mushrooms decompose dead, standing or fallen hardwood trees (Floudas et al. 2012). As a white rot fungus, *T. versicolor* possesses the biochemical capacity to utilize a wide range of chemicals and disposals, to bioremediate soils (Jokharidze et al. 2019) as well as to treat wastewater (Hu et al. 2021). Because of the limited resources of wild mushrooms, the submerged cultivation of fungal mycelia is a favorable and efficient process (Wang et al. 2017). Submerged fermentation has been defined as the process in which the growth and decomposition of substrates is accomplished by microorganisms in the excess of free water (liquid medium) (Kapoor et al. 2016; Behera and Ray 2019). Cultivation of *T. versicolor* mycelia in laboratories was performed on semi-synthetic media (Asatiani et al. 2007; Huang et al. 2021) and on agricultural by-products (Yemets et al. 2020; Jones et al. 2019). Wastes from food industries are among the most abundant solid and liquid wastes nevertheless are not always properly recycled (Tashyrev et al. 2022). Wastes of food for *T. versicolor* mycelia cultivation include milk whey (Cui et al. 2007), olive mill (Ntougias et al. 2015) and molasses wastewaters (Kahraman and Gurdal 2002), tomato (Freixo et al. 2008) and apple (Bosse et al. 2013) pomace, wastes of oil production (Krupodorova and Barshteyn 2015), residue of ethanol production from wheat grains (Songulashvili et al. 2007), mandarin, apple, and banana peels (Elisashvili et al. 2008). The biomass amounts collected from submerged cultivated *T. versicolor* on different complex media with salt supplementations were as follows: 4.77-5.50 g/L on glucose-peptone-yeast medium (Asatiani et al. 2007; Al-Maali 2017; Huang et al. 2021), 4.9 on glucose-yeast (Kobakhidze et al. 2016) and 6.63 g/L on glucose-peptone media (Duvnjak et al. 2016) as well as 8.9-10.6 g/L on glucose and yeast medium with addition of milk permeate (Cui et al. 2007) and 10.64 g/L on beet vinasse or molasses stillage (Ivanova et al. 2023).

In the current study, compositions of the liquid nutrient media for *T. versicolor* submerged cultivation included food by-products, namely, waste bread (breadcrumb) and beer wort. Breadcrumbs are made from unsold and off-test bread that undergone inspection for visible mold, bacterial infection, and contamination, followed by crumbling and desiccation (Ivanova et al. 2014). Breadcrumbs can be considered a safe, cheap, and widely available substrate for the cultivation of medicinal and edible mushrooms (Ivanova et al. 2014). Beer wort was introduced into the glucose-yeast medium as an additional source of carbon in the form of maltose. The composition of the complex glucose-yeast medium with beer wort (CGYB) was previously optimized (Antonenko 2013).

Although the medicinal properties of *T. versicolor* are extensively investigated (Habtemariam 2020) the information about the nutritional value of turkey tail biomass is limited because of the hard fruiting body. The biotechnological approach of mycelium submerged cultivation allows to produce of food ingredients more fast and taking less space compared to the production of fruiting bodies. Study aimed to investigate the nutritional value of *T. versicolor* mycelia grown on liquid media with breadcrumbs compared to CGYB in submerged conditions for the possible production of functional foods and dietary substances.

MATERIAL AND METHODS

Fungal strain and cultivation

T. versicolor IBK 353 was kindly supplied by the Culture Collection of Mushrooms from the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (Bisko et al. 2016). The stock culture was maintained on wort agar slants at 4 °C (Ivanova et al. 2015). The culture was inoculated in Petri dish containing glucose-peptone medium, g/L: 25 glucose, 3 peptone, 2 yeast extract, 1 KH₂PO₄, 1 K₂HPO₄, 0.25 MgSO₄ × 7 H₂O, and 20 agar (Ivanova et al. 2015).

Same as for our previous works (Ivanova et al. 2014; Ivanova et al. 2015), the substrate for submerged cultivation of fungal mycelium (breadcrumbs) was donated by Bread Plant № 12 of the Open Joint-Stock Company "Kyivkhlіb", Kyiv, Ukraine. The chemical composition of breadcrumb was previously reported (Ivanova et al. 2014; Ivanova et al. 2015). The liquid medium CGYB comprised the following components, g/L: 10 glucose, 3 yeast extract, 2.5 (NH₄)₂SO₄, 1 K₂HPO₄, 1 KH₂PO₄, 0.25 MgSO₄ × 7 H₂O, and 20 beer wort, pH 5.0. The liquid media sterilization was carried out by processing with 1 atmosphere pressure for 40 min in an autoclave.

Inoculate preparation and submerged cultivation of fungal mycelia repeated the procedures of our described investigation (Ivanova et al. 2015) in order to compare submerged cultivation of two species (*T. versicolor* and *S. commune*) on the same substrate (breadcrumb). A fully colonized Petri dish with glucose-peptone medium was homogenized and used for 10% (v/v) inoculation of 250 ml Erlenmeyer flasks containing 50 mL liquid medium with breadcrumbs (concentration 50 g/L) or CGYB. Submerged cultivated mycelium was used for 10% (v/v) inoculation of the medium. The flasks were incubated at 28 ± 2 °C in a rotary shaker (120 rpm). After incubation for 5 days, the fungal mycelium was harvested by filtration, washed three times with water, desiccated at 60 °C, and milled.

The chemical composition and nutritional value of *T. versicolor* mycelia

The chemical composition of *T. versicolor* mycelia, including moisture, ash, total carbohydrates, crude fat, and crude protein, was determined according to AOAC methods (Cunniff 1995). The ash content was determined by burning off the organic part via incineration at 600 °C until the constant weight (Ivanova et al. 2015).

Nitrogen content was analyzed by Kjeldhal’s method as described previously (Volodko et al. 2020). For the purpose of crude protein calculation, the Nitrogen content was multiplied by a factor of 4.38 for the mycelium of *T. versicolor* (Kalač 2016). In the present work we defined crude fat content by extracting a known weight of powdered samples, using a Soxhlet apparatus, with petroleum ether as solvent (Liu et al. 2012) for mycelium of *T. versicolor*. The quantity of total carbohydrates (g/100 g dry matter) was calculated by difference: total carbohydrates = 100 – (crude protein + crude fat + ash) (Kalač 2016). The energy content (gross energy) was calculated from the mean values of energy-containing compounds with the following factors: protein 4.0 kcal/g; fat 8.37 kcal/g and carbohydrates 3.48 kcal/g (Crisan and Sands 1978).

The amino acid composition was estimated with a T-339 amino acid analyzer (“Mikrotechna”, Prague, Czech Republic). The chemical score was calculated as the ratio of a gram of the limiting amino acid in a test protein to the same amount of the corresponding amino acid in a reference diet protein multiplied by 100 (FAO/WHO/UNU 2007).

To evaluate fatty acid composition, lipids were extracted according to the described method by Christie (1989), and fatty acid content was estimated using gas chromatograph/mass spectrometer system Agilent 6890N/5973 inert (Agilent technologies, USA) on the capillary column HP-5MS (Agilent technologies, USA).

The vitamin contents were analyzed similarly to the previous study (Ivanova et al. 2015).

Statistical analysis

Values are the mean of three independent experiments done in triplicate and are expressed as mean ± error of the mean. Data were statistically analyzed similarly to the article (Ivanova et al. 2014) by *t*-test employing OriginPro 8.5.1, OriginLab Corporation, USA. Differences between means at the level of 5% (*p* < 0.05) were assumed to be significant.

RESULTS AND DISCUSSION

The cost of the fermentative biological process depends on the nutrient medium and optimization parameters (Atilano-Camino et al. 2020). The optimal duration of *T. versicolor* IBK 353 submerged cultivation on CGYB and breadcrumb (5 days) was determined in the previous investigations (Antonenko 2013; Ivanova et al. 2014). After 5 days of submerged cultivation on two investigated media, we obtained about the same amounts of *T. versicolor* IBK 353 biomass based on dry weight (16.0 ± 0.5 g/L on CGYB and 15.8 ± 0.5 g/L on breadcrumb), and such biomass concentrations were 1.5-3 times higher comparing to submerged cultivation on other substrates (Al-Maali 2017; Asatiani et al. 2007; Cui et al. 2007; Duvnjak et al. 2016; Huang et al. 2021; Ivanova et al. 2023; Kobakhidze et al. 2016).

Nutrient medium composition may significantly influence mushroom growth and the production of biologically active substances by fungal cultures (Elisashvili et al. 2022; Ivanova et al. 2023). A compositional study revealed that *T. versicolor* IBK 353 mycelia cultivated on investigated media were rich in carbohydrates and protein, but low in crude fat which is generally characteristic to medicinal mushroom mycelia (Huang et al. 2021). According to the recommendations of the World Health Organization (2020), total fat intake for humans should be less than 30% of the total energy intake to prevent unhealthy weight gain in adults. Calculated crude fat contributions in gross energy content of *T. versicolor* IBK 353 mycelia are only 5.60 % after cultivation on the breadcrumb and 8.20 % after cultivation on CGYB. *T. versicolor* mycelium contained 3.8, 1.6, and 1.4 times higher amounts of ash, crude protein, and crude fat accordingly in cultivation on the CGYB than in cultivation on breadcrumb (Table 1). At the same time, the content of ash, crude protein, and crude fat in *T. versicolor* IBK 353 mycelium cultivated on the breadcrumb was 1.9, 1.8, and 4.9 times higher correspondingly than in substrate breadcrumb (Ivanova et al. 2014). Fruiting bodies of *T. versicolor* from Taiwan (Mau et al. 2001) included much lower amounts of crude protein (4.20 %) and crude fat (1.10 %) than in *T. versicolor* mycelia. However, ash content (6.37 %) was in values between *T. versicolor* mycelia cultivated on two media under study. It was shown (Elisashvili et al. 2008) that protein content in *T. versicolor* mycelia depends on the nitrogen source nature and amounts as well as can be increased up to 1.7 times by the supplementation of media with additional nitrogen source.

Table 1 Proximate composition (g/100 g dry matter) and gross energy (kcal/g dry matter) values of *T. versicolor* IBK 353 mycelia on different media*

Medium for submerged cultivation	Ash	Crude protein	Crude fat	Total carbohydrates	Gross energy**
Breadcrumbs	3.3±0.0	19.9±0.1	2.4±0.1	74.4±0.2	358.6
CGYB	12.6±0.4	32.0±0.1	3.3±0.4	52.1±0.9	336.9

*All means within the same column are significantly different (*p* < 0.05).

The gross energy value of *T. versicolor* mycelium cultivated on breadcrumb was similar to the gross energy of *T. versicolor* mycelium cultivated on CGYB (Table 1) and gross energy of breadcrumbs (Ivanova et al. 2015). An approximate calculation of *T. versicolor* energetic value shows that 100 g of mycelia cultivated on CGYB and breadcrumbs satisfies 17-18 % of the calorie need in the average human daily diet of 2000 kcal. However, the gross energy of *T. versicolor* fruiting bodies (Mau et al. 2001) was 25-30 % lower than the gross energy of *T. versicolor* mycelia cultivated on CGYB and breadcrumbs.

Amino acid composition

It was previously observed (Cheung 2008) that essential amino acid content generally ranges in mushroom protein from 34 % to 47 % with predomination of glutamate (12.6–24.0 %) and aspartate (9.1–12.1 %). The amino acid profile of *T. versicolor* IBK 353 mycelia was in agreement with such a pattern (Table 2). *T. versicolor* protein in mycelia comprised 37.38 % and 34.39 % of essential amino acids after cultivation on breadcrumbs and CGYB correspondingly, wherein the highest percent fell to the share of nonessential amino acids glutamate (15.12-18.67 %) and aspartate (10.03-10.21 %).

Table 2 Amino acid composition of *T. versicolor* IBK 353 mycelia on different media***, % sum of amino acids

Amino acids	Medium for submerged cultivation	
	Breadcrumbs	CGYB
Lysine	4.84±0.12*	6.72±0.12*
Threonine	5.48±0.12	5.59±0.12
Valine	7.09±0.12*	4.56±0.11*
Methionine	1.27±0.11*	1.91±0.11*
Cystine**	2.73±0.11*	0.75±0.11*
Isoleucine	2.84±0.11	2.91±0.11
Leucine	6.84±0.12*	7.74±0.11*
Phenylalanine	3.53±0.11*	4.96±0.12*
Tyrosine	2.76±0.11*	3.19±0.11*
Histidine	1.41±0.11*	2.46±0.11*
Arginine	5.54±0.12*	7.01±0.11*
Aspartate	10.03±0.31	10.21±0.31
Serine	6.60±0.12*	6.33±0.11*
Glutamate	18.67±0.31*	15.12±0.31*
Proline	7.32±0.12*	5.86±0.11*
Glycine	5.51±0.12*	6.19±0.12*
Alanine	7.54±0.12*	8.51±0.12*
Total essential amino acids	37.38*	34.39*

*Means within the same raw are significantly different (*p* < 0.05);

**In sample pretreatment procedure cysteine oxidized to cystine, therefore both amino acids were analyzed together;

***Tryptophan was not determined in this study.

The essential amino acid profiles of *T. versicolor* mycelia compared to Food and Agricultural Organization/World Health Organization requirement patterns (FAO/WHO/UNU 2007) revealed that the proteins are especially rich in threonine, which was reported also for fruiting bodies of other mushrooms (Cheung 2008). The protein of *T. versicolor* mycelium cultivated on the breadcrumbs was also rich in sulfur-containing amino acid cystine, comprising 47 % higher levels of aforementioned amino acids than in the protein of the substrate breadcrumbs. Also, the most significant difference was in *T. versicolor* cystine content, which was 3.64 times higher in mycelium cultivated on the breadcrumb compared to mycelium cultivated on CGYB. According to FAO/WHO/UNU (2007) requirement pattern, *T. versicolor* proteins had limiting amino acids with chemical scores, %: methionine 79, histidine 94, and isoleucine 95 after cultivation on breadcrumbs as well as isoleucine 97 after cultivation on CGYB.

Fatty acid composition

Mushrooms generally have a low lipid level of less than 10 % on the dry weight basis but nevertheless are a source of unsaturated fatty acids, especially oleic (18:1 *cis*-9) and linoleic (18:2 *cis,cis*-9,12) acids (Krupodorova et al. 2012; Cheung 2008). In our investigation, *T. versicolor* IBK 353 mycelia had 2.4-3.3 % of crude fat with the sum of fatty acids 18:1 and 18:2 comprising more than 50 % of total fatty acids (Table 1, 3). Herewith the level of unsaturated fatty acids in *T. versicolor* IBK 353 mycelia was 60.45 % after cultivation on breadcrumbs and 75.90 % after cultivation on CGYB (Table 3). Interesting that unsaturated fatty acid 16:1 was determined in significant amounts (16.1 % of total fatty acids) in *T. versicolor* mycelia obtained after cultivation on CGYB and was absent in *T. versicolor* mycelia obtained after cultivation on breadcrumbs.

Table 3 Fatty acid composition of *T. versicolor* IBK 353 mycelia on different media, % sum of fatty acids

Fatty acid (Carbon : double bond ratio)	Medium for submerged cultivation	
	Breadcrumbs	CGYB
16:1	-**	16.1 ± 0.12
17:1	-	0.86 ± 0.04
18:1	22.45±0.20*	25.94 ± 0.30*
18:2	35.03±0.32*	48.11 ± 0.46*
18:3	-	0.39 ± 0.12
20:1	0.39±0.02	-
14:0	-	0.31 ± 0.02
15:0	0.58±0.03*	0.11 ± 0.04*
16:0	24.81±0.15*	22.73 ± 0.30*
17:0	0.48±0.03	0.45 ± 0.02
18:0	7.92±0.08*	5.48 ± 0.54*
20:0	0.62±0.03*	0.31 ± 0.16*
22:0	1.83±0.01*	0.43 ± 0.01*
24:0	1.25±0.01	-
26:0	0.37±0.02	-
Other fatty acids	4.27	-
Total determined fatty acids	95.73	100
% Unsaturated fatty acids from determined fatty acids	60.45*	75.90*

*Means within the same row are significantly different ($p < 0.05$);

**Fatty acid was not detected in the sample.

In fruiting bodies of turkey tail mushroom Yokokawa (1980) found only 0.5 % of crude fat based on the dry weight but *T. versicolor* contained 81.5 % of unsaturated fatty acids with fatty acids, of total: 2.4 % of 16:1, 9.1 % of 18:1, 67.1 % of 18:2, and 2.9 % of 18:3. Thereby, unsaturated fatty acids 18:1 and 18:2 in lipids of turkey tail mushroom predominated like in *T. versicolor* mycelia of the present study. The highest percentage among saturated fatty acids accounted for 16:0 (palmitic) fatty acid in fruiting bodies *T. versicolor* (Yokokawa 1980) and in mycelia (Table 3). It should be noted that fatty acids 18:1, 18:2, and 16:0 prevailed also in breadcrumb (31.10 %, 33.79 %, and 24.84 % correspondingly) as well as in mycelium of *Schizophyllum commune* Fr.:Fr. IBK 1768 (Ivanova et al. 2015) cultivated on breadcrumb (27.28 %, 34.84 %, and 21.08 % accordingly).

Vitamin content

Mushrooms are believed to be a valuable source of several vitamins, such as folates, niacin, and riboflavin (Cheung 2008). *T. versicolor* IBK 353 mycelia cultivated on breadcrumb and CGYB had the highest level of niacin among investigated vitamins (Table 4). The content of riboflavin and niacin was similar in mycelia from different media, but the content of thiamine was 1.7 times higher after cultivation on breadcrumb, and the content of folic acid was 4.7 times higher after cultivation on CGYB.

Table 4 Vitamin content of *T. versicolor* IBK 353 mycelia on different media, mg / 100 g dry matter

Medium for submerged cultivation	Thiamine	Riboflavin	Niacin	Folates
Breadcrumbs	0.44±0.01*	1.23±0.02	12.17±0.88*	0.64±0.02*
CGYB	0.26±0.03*	1.27±0.12	14.90±0.50*	3.03±0.20*

*Means within the same column are significantly different ($p < 0.05$).

According to the Institute of Medicine (U.S.) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline (1998), the Recommended Dietary Allowance for adults is as follows: thiamine 1.2 mg/day for men and 1.1 mg/day for women, riboflavin 1.3 mg/day for men and 1.1 mg/day for women, niacin 16 mg/day for men and 14 mg/day for women, and dietary folate equivalence 0.40 mg/day. At the same time, the Tolerable Upper Intake Levels were set only for niacin (35 mg/day) and folates (1.00 mg/day) which should be taken into account. Comparing to the obtained results, 100 g of dried *T. versicolor* IBK 353 mycelia contain about the daily dose of riboflavin (after cultivation on both media), 81-100 % of niacin daily requirements, and only 23-38 % of thiamine. Obtained mycelia were especially rich in folates, from 10 to 50 g was enough to satisfy the daily requirements.

It should be mentioned that both investigated media contained thiamine, riboflavin, niacin, and folic acid: CGYB in the composition of yeast extract as well as breadcrumbs according to previous investigations (Ivanova et al. 2015). The addition of vitamins to the nutrient medium can stimulate lignolytic enzyme production (Levin et al. 2010) and increase the growth of fungal mycelia (Jonathan and Fasidi 2001). Thus, the addition of thiamine resulted in 1.8-1.9 times higher biomass accumulation of *S. commune* and *Lentinus subnudus* Berk mycelia, and folic acid addition caused 1.7 and 2.6 times higher biomass accumulation of *L. subnudus* and *S. commune* accordingly (Jonathan and Fasidi 2001).

CONCLUSION

Comparing the data of *T. versicolor* IBK 353 mycelia submerged cultivation on breadcrumbs and CGYB it can be concluded that significantly different chemical composition results were obtained. Particularly, the ash, crude protein, and crude fat contents were 3.8, 1.6, and 1.4 higher correspondingly in mycelium cultivated on CGYB than on breadcrumbs. The protein in *T. versicolor* mycelium had 37.38 % of essential amino acids after cultivation on breadcrumb, and 34.39 % after cultivation on CGYB. Lipids of *T. versicolor* mycelium comprised 60.45 % of unsaturated fatty acids after cultivation on breadcrumbs and 75.90 % of unsaturated fatty acids after cultivation on CGYB. *T. versicolor* IBK 353 dry mycelia were enriched in folates, and 100 g contained daily recommended doses of riboflavin and 81-100% of niacin.

Based on the aforesaid it can be concluded that mycelium of *T. versicolor* grown on two investigated media is a source of biologically active substances and may provide a basis for the creation of functional foods and dietary substances. The selection of nutrient medium using affordable and safe food by-products or wastes is a tool for efficiently obtaining turkey tail mushroom biomass with the desired composition of essential nutrients.

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