UDC: 577.11+ 577.16: 582.284: 579.66: 664.66.002.68

COMPOSITIONAL STUDY OF *SCHIZOPHYLLUM COMMUNE* FR.: FR. GROWN ON THE NEW SUBSTRATE BREADCRUMB

T. IVANOVA, leading engineer

Institute of Food Biotechnology and Genomics NAS of Ukraine

L. TITOVA, PhD

National Technical University of Ukraine "KPI"

G. P. MEGALINSKA, PhD

National Pedagogical Dragomanov University

E-mail: ivanova_tatiana_wat2@bigmi.net

Abstract. *The investigation of crude protein, crude fat, carbohydrate, vitamin content as well as ash, gross energy values, amino acid and fatty acid composition of Schizophyllum commune mycelia was performed in submerged cultivation on the new substrate breadcrumb (waste of bread production). The improved content of crude protein, crude fat, essential amino acids, and vitamins in S. commune mycelia comparing to the substrate was established.*

Key words: *Schizophyllum commune, breadcrumb, submerged cultivation, amino acid, fatty acid, vitamin composition*

Schizophyllum commune Fr.: Fr. is widely distributed in nature wood-decaying medicinal mushroom belonging to phylum *Basidiomycota*. *S. commune* is also a model organism for genetic engineering in mushroom-forming fungi, and its genome is sequenced [1].

Medicinal properties of *S. commune* include antibacterial, antiparasitic, immunomodulating, antiinflamatory, antitumor, and hepatoprotective activities [2]. Schizophyllan is a β-glucan isolated from cultured broth of *S. commune* which have passed clinical trials and currently produced commercially by several Japanese pharmaceutical companies as anticancer agent [3].

Fruiting bodies of *S. commune* are consumed in tropical countries; consequently, there are some studies of fruiting bodies chemical composition [4, 5, 6]. In nature at least 150 genera of woody plants are substrates for *S. commune*, but it also colonizes soft-wood and grass silage [7]. Cultivation of *S. commune* mycelia in laboratories

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was performed on synthetic media [8, 9] as well as on natural substrates and wastes of food production, e.g., beer wort, molasses [9], hydrolysate of rice hull [10], coconut water [11], wastes of pasta, cacao [12], and oil production [13].

Waste bread (breadcrumb) is a new substrate for *S. commune* cultivation. Breadcrumb is made from unsold and off-test bread undergone inspection for visible mold, bacterial infection, and contaminations, followed by crumbling and desiccation. Breadcrumb can be assumed as a safe, cheap, and widely available substrate for cultivation of medicinal and edible mushrooms [14].

The aim of study was to investigate chemical composition of *S. commune* mycelia grown on breadcrumb in submerged conditions.

Material and methods. *S. commune* 1768 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 (IBK) [15]. The stock culture was maintained on wort agar slants at 4 ºC. The culture was inoculated in Petri dish containing glucose-peptone medium, g/l: 25 glucose, 3 peptone, 2 yeast extract, 1 KH_2PO_4, 1 K_2HPO_4, 0.25 $MgSO_4 \times 7 H_2O$, and 20 agar.

The substrate for submerged cultivation (breadcrumb) was donated by Bread Plant № 12 of the Open Joint-Stock Company “Kyivkhlib”, Kyiv, Ukraine. The liquid medium with breadcrumb in concentration 60 g/l was autoclaved for 40 min, with 1 atmosphere.

Fully colonized Petri dish with glucose-peptone medium was homogenized and used for 10% (v/v) inoculation of 250 ml flasks containing 50 ml liquid medium with breadcrumb. Submerged cultivated mycelium in active phase of growth (with average diameter of pellets about 0.5 mm) grown on breadcrumb was used for 10% (v/v) inoculation of medium for elimination of the lag phase. The flasks were incubated at 28 ± 2 ºC in a rotary shaker (120 rpm). After incubation for 4 days, the fungal mycelium was harvested by filtration, washed three times with water, desiccated at 60 ºC, and milled.

The chemical composition of breadcrumb and mycelium of *S. commune*, including moisture, ash, total carbohydrates, crude fat, and crude protein, was

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determined according to AOAC methods [16]. To obtain moisture content, samples were dried at 105 ºC until constant weight. The ash content was determined by incineration at 600 ºC until constant weight.

Nitrogen content was defined by Kjelhdal's method. For the calculation of crude protein, the Nitrogen content was multiplied by a factor of 5.7 for breadcrumb [17] and of 4.38 for mycelium of *S. commune* [18]. In present work we determined crude fat content by extracting a known weight of powdered samples, using a Soxhlet apparatus, with hexane as a solvent for breadcrumb and petroleum ether as solvent for mycelium of *S. commune*. The amount of total carbohydrates was calculated by difference: Total carbohydrates = 100 – (g moisture + g crude protein + g crude fat + g ash) [19]. The energy content was calculated with the following factors: protein 4.0 kcal/g; fat 8.37 kcal/g and carbohydrates 3.48 kcal/g [18].

The content of amino acids was estimated with a T-339 amino acid analyzer ("Mikrotechna", Prague, Czech Republic) [20]. 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 (whole-egg protein) multiplied by 100 [21].

To evaluate fatty acid composition, lipids were extracted according to described method [22], 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 method of vitamin B1 (thiamine) evaluation was based on oxidation of thiamine to thiochrome, extraction thiochrome into organic solvent, and measurement of fluorescence [23]. Vitamin B2 (riboflavin) content determined using riboflavin binding apoprotein from chicken eggs [24]. The method of vitamin B3 (PP) estimation was based on hydrolysis, quantitative obtaining of colored glutaconic aldehyde derivate, and further colorimetric determination [25]. Vitamin B9 (folic acid) content analyzed by the change in fluorescence intensity before and after oxidation of folates previously purifying them from interfering compounds [26].

Values are mean of three independent experiments done in triplicate and expressed as mean ± errors.

Results and discussion. The chemical composition of fungal mycelium depends on the substrate for cultivation. The content of ash was similar in *S. commune* mycelium and in breadcrumb (Table 1). At the same time, *S. commune* mycelium contained three times higher amount of ash (4.5 %) in cultivation on the liquid medium with waste of amaranth oil production (meal after CO_2 extraction), though such substrate had 1.7 times higher content of ash than breadcrumb [27]. Fruiting bodies of *S. commune* collected in Nigeria included 7.46 % of ash [4]; fruiting bodies found in India had similar content of ash – 8 % [5].

1. Proximate composition (g/100 g dry matter) and gross energy (kcal/g) values

Sample	Moisture	Ash	Crude protein	Crude fat	Total carbo-hydrates	Gross energy
Breadcrumb	9.38 ± 0.03	1.75 ± 0.04	10.94 ± 0.60	0.48 ± 0.01	77.45 ± 0.68	317.33
S. commune mycelium	10.24 ± 0.33	1.42 ± 0.02	18.83 ± 0.10	3.45 ± 0.10	66.06 ± 0.55	334.09

Crude fat and protein content was 7.2 and 1.7 times higher correspondingly in mycelium of fungus than in breadcrumb (Table 1). In cultivation of *S. commune* mycelia on the waste of amaranth oil production higher content of crude fat and protein was obtained (8.4 % and 45.4 % respectively), however such components content was higher in the substrate as well (1.5 % and 15.4 % accordingly) [27]. On the contrary, crude fat content was lower in fruiting bodies of *S. commune* than in mycelia grown on the breadcrumb (1.28% [4] and 2.0 % [5]). Crude protein content significantly altered in fruiting bodies of *S. commune*: from 9.63 % to 27.7 % [4–6].

Gross energy value of *S. commune* in our investigation was comparable to literature data (299–399 kcal/g) [4–6, 27] and similar to gross energy of breadcrumb (Table 1). Approximate calculation of *S. commune* energetic value shows that 100 g of mycelia cultivated on the breadcrumb satisfies 16.7 % of calorie need in average human daily diet 2000 kcal.

Essential amino acid content varies in mushroom protein from 34 % to 4 with predomination of Glutamate (12.6−24.0 %), Aspartate (9.1−12.1 %), and Arginine (3.7−13.9 %) [28]. Our results are in agreement with such pattern (Table 2).

2. Amino acid composition of *S. commune* and substrate breadcrumb, %

Amino acids	*S. commune*	Breadcrumb
Lysine	5.38±0.12	1.32±0.11
Threonine	5.23±0.12	2.73±0.11
Valine	6.57±0.12	4.12±0.12
Methionine	1.39±0.11	1.24±0.11
Cystine	3.37±0.11	1.49±0.11
Isoleucine	3.90±0.11	2.42±0.11
Leucine	7.24±0.12	6.06±0.12
Phenylalanine	4.16±0.12	4.23±0.12
Tyrosine	4.16±0.11	3.02±0.11
Histidine	2.18±0.11	1.54±0.11
Arginine	5.98±0.12	2.35±0.12
Aspartate	9.69±0.30	4.59±0.12
Serine	6.25±0.12	5.41±0.12
Glutamate	18.89±0.32	40.35±0.50
Proline	3.87±0.11	12.87±0.21
Glycine	4.82±0.11	3.26±0.11
Alanine	6.91±0.12	3.00±0.11
Total essential amino acids	41.40	26.63
Chemical score	98	24

The percent of essential amino acids in *S. commune* mycelium was 1.6 times higher than in the substrate breadcrumb (Table 2). Accoding to calculations, 100 g of *S. commune* mycelium after desiccation at 60 °C contained 8.7 g of essential amino acids and chemical score 98. Compared to reference whole egg protein, mycelium of *S. commune* is limited in Lysine and Isoleucine; the percent of other essential amino acids was higher in protein of *S. commune* mycelia than in reference protein of whole eggs [21]. According to limiting amino acid Lysine in breadcrumb, the chemical

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score of breadcrumb was four times lower than the chemical score of *S. commune* grown on this substrate (Table 2).

The waste of amaranth oil production had better amino acid composition than breadcrumb with 31.4 % essential amino acids and chemical score 53 according to limiting amino acid Isoleucine, though protein in mycelium of *S. commune* didn’d have significantly improved amino acid composition with 33.7 % of essential amino acids and chemical score 56 according to limiting amino acid Valine [27]. The protein in fruiting bodies of *S. commune* had 34 % of essential amino acids with chemical score 28 according to limiting amino acid Methionine [5].

Fatty acid composition of lipids was similar in *S. commune* mycelia and in the substrate breadcrumb (Table 3) with predomination of unsaturated fatty acids. At the same time, in literature data [5, 27] lipids of *S. commune* contained about 10 % higher ratio of unsaturated fatty acids. Palmitic (16:0), Oleic (18:1), and Linoleic (18:2) fatty acids were the main components of *S. commune* lipids and accounted for almost the whole of the fatty acids determined in our investigation (90.5 %) and in references (92.5 % [5], 94.5 % [27]).

3. Fatty acid composition of *S. commune* mycelia and breadcrumb, %

Fatty acid (Carbon : double bond ratio)	*S. commune*	Breadcrumb
16:1	0.34 ± 0.02	0.27 ± 0.02
18:1	27.28 ± 0.30	31.10 ± 0.30
18:2	34.84 ± 0.35	33.79 ± 0.31
22:1	0.32 ± 0.01	-*
15:0	0.36 ± 0.02	-
16:0	21.08 ± 0.25	24.84 ± 0.18
17:0	0.31 ± 0.02	-
18:0	5.97 ± 0.07	5.86 ± 0.10
20:0	0.36 ± 0.02	0.29 ± 0.02
22:0	0.70 ± 0.04	0.67 ± 0.03
24:0	0.38 ± 0.02	0.17 ± 0.01
Total unsaturated fatty acids	62.78	65.16
Total fatty acids	91.94	96.99
% Unsaturated fatty acids	**68.28**	**67.18**

Note: * – fatty acid was not detected in the sample.

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The analysis has shown that the amount of vitamins in the mycelia *S. commune* multiplied 3–23 times if compared to the substrate (Table 4). Among investigated vitamins, the highest amounts were observed for niacin in fungal mycelium and in the substrate. In fruiting bodies of *S. commune* niacin also prevailed among investigated vitamins [4], but its values were 6.4 times lower than in mycelium cultivated on breadcrumb. The level of thiamine was similar to our results in fruiting bodies of *S. commune* [4], although riboflavin amount was 4 times higher in *S. commune* mycelia grown on the breadcrumb.

4. Vitamin content, mg / 100 g

Sample	Thiamine	Riboflavin	Niacin	Folic acid
Breadcrumb	0.095 ± 0.005	0.038 ± 0.003	1.13 ± 0.07	0.0130 ± 0.0002
S. commune	0.290 ± 0.025	0.890 ± 0.024	8.29 ± 0.29	0.27 ± 0.02

It should be noted that addition of vitamins to nutrient medium can increase the growth of fungal mycelia. Thus, addition of thiamine and folic acid resulted in 1.8 and 2.6 times higher biomass accumulation of *S. commune* accordingly, and addition of riboflavin and niacin leaded to lower biomass increase (15 % and 42 % correspondingly) [29]. Despite that fact, the presence of vitamins in the nutrient medium is not essential for *S. commune* growth [30], since this fungus is able to synthesis the vitamins.

Conclusions

1. The protein of *S. commune* 1768 mycelium grown on the new substrate breadcrumb can be considered a protein of high value according to ratio of essential amino acids 41.4 % (8.7 g in 100 g of mycelium desiccated at 60 °C) and chemical score 98. Essential amino acids, crude fat, and crude protein content in mycelium of *S. commune* 1768 cultivated on the liquid medium with breadcrumb was 1.6, 7.2, 1.7 times higher correspondingly comparing to substrate breadcrumb.

2. In lipids of *S. commune* 1768 mycelium grown on the breadcrumb preva unsaturated fatty acids (68.28 %).

3. Mycelium of *S. commune* 1768 grown on the breadcrumb contained vitamins: thiamine, riboflavin, folic acid, and niacin, wherein their contents in mycelium are 3.1, 7.3, 20.8, and 23.4 times accordingly higher than in the substrate.

4. On the basis of aforesaid can be summarized that mycelium of *S. commune* grown on the breadcrumb is a source of biologically active substances and may serve as a basis creation of dietary substances and functional foods.

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ДОСЛІДЖЕННЯ СКЛАДУ *SCHIZOPHYLLUM COMMUNE* FR.: FR., ОТРИМАНОГО ПРИ КУЛЬТИВУВАННІ НА НОВОМУ СУБСТРАТІ СУХАРНІЙ КРИХТІ

Т. С. Іванова, Л. О. Тітова, Г. П. Мегалінська

Анотація. *Досліджений вміст білків, жирів, вуглеводів, вітамінів, а також зольність, енергетична цінність, амінокислотний та жирнокислотний профіль міцелію* Schizophyllum commune, *отриманого за глибинного культивуванні на новому субстраті сухарна крихта (відходи виробництва хліба). Встановлений покращений вміст білків, жирів, незамінних амінокислот та вітамінів у міцелії* S. commune *в порівнянні із субстратом.*

Ключові слова: Schizophyllum commune, *сухарна крихта, глибиннекультивування, амінокислотний, жирнокислотний, вітамінний склад*

ИССЛЕДОВАНИЕ СОСТАВА *SCHIZOPHYLLUM COMMUNE* FR.: FR., ПОЛУЧЕННОГО ПРИ КУЛЬТИВИРОВАНИИ НА НОВОМ СУБСТРАТЕ СУХАРНОЙ КРОШКЕ

Т. С. Иванова, Л. А. Титова, А. П. Мегалинская

Аннотация. *Исследовано содержание белков, жиров, углеводов, витаминов, а также зольность, энергетическая ценность, аминокислотный и* *жирнокислотный профиль мицелия Schizophyllum commune, полученного при глубинном культивировании на новом субстрате сухарная крошка (отходы производства хлеба). Установлено улучшенное содержание белка, жиров, незаменимых аминокислот и витаминов в мицелии S. commune по сравнению с субстратом.*

Ключевые слова: *Schizophyllum commune, сухарная крошка, глубинное культивирование, аминокислотный, жирнокислотный, витаминный состав*