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Chemical Composition of the Edible Mushroom *Pleurotus ostreatus* Produced by Fermentation

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***Pleurotus ostreatus* 'Florida' was grown in submerged liquid culture. The biomass yield of the fungus, grown for 3 days in 2-liter fermentors, where the mycelial pellets measuring 5 mm in diameter were formed, was 11.7 g (dry weight)/liter. Comparing the chemical constituents of fruiting bodies produced on cotton straw and mycelial pellets revealed several similarities in total nitrogen, protein, glycogen, fatty acids, RNA, and ash content. Differences were observed in the contents of six amino acids. Although the total fatty acid content was similar, there were more saturated fatty acids in the mycelium. Cell wall composition, typical for basidiomycetes, was observed in both mycelium and fruiting bodies, with laminarin as the main polymer.**

The growth of an edible mushroom is a lengthy and complex process involving the use of composts or lignocellulosic wastes such as straws followed by a long cultivation period (21, 22). The potential of mushroom as fungal protein, source of spawn, or flavoring agents makes the production of fungal mycelium in submerged liquid culture a most attractive prospect. Most researchers have concentrated on *Agaricus* spp. and *Morchella* spp. Humfeld and Sugihara (4, 5) were the first to grow *Agaricus campestris* under submerged, aerated, and agitated culture conditions. Development of mushroom flavor in this organism, grown in submerged culture, was studied by Moustafa (15). This subject has been reviewed in detail (10, 11). Morel mushrooms have recently been grown on cheese whey waste (8). Peat extract was used as a major substrate for submerged growth of both *A. campestris* and *Morchella esculantum* (13, 14).

Pleurotus ostreatus, the second most important edible mushroom in Europe (9), is a fast-growing, lignin-degrading fungus (17), which can therefore be grown for fruiting-body production on lignocellulosic waste such as cotton straw (16). In this study we have investigated the possibility of growing *P. ostreatus* in submerged fermentation, comparing the chemical compositions of mycelium and fruiting bodies.

***Pleurotus* fermentation.** *Pleurotus* spp. were grown in flasks containing 100 ml of medium, inoculated with 1 ml of a suspension containing 10 mg (dry weight) of mycelium, and incubated in a rotary shaker (New Brunswick Scientific Co., Edison, N.J.) at 28°C and 150 rpm. The medium used for all fermentation experiments, except when otherwise indicated, contained the following reagents (per liter): 20 g of glucose, 0.65 g of asparagine, 0.5 g of yeast extract (Difco Laboratories, Detroit, Mich.), 0.5 g of $MgSO_4 \cdot 7H_2O$, 1 g of KH_2PO_4, and 0.5 g of KCl. Of six *Pleurotus* strains grown in a glucose medium for 7 days, the one producing the most biomass, *P. ostreatus* 'Florida' F6, was chosen for a more detailed study. The optimal initial pH was found to be 5, and no growth was observed at pH levels lower than 3. Glucose, sucrose, fructose, galactose, and lactose, each at a concentration of 5 g/liter were tested as carbon source. Maximal growth was obtained with glucose. The optimal glucose concentration, 20 g/liter, yielded 11.5 g (dry weight) of biomass per liter. Sugar beet molasses was also found to be a suitable carbon source, yielding, at the optimal concentration of 25 g/liter, 7.5 g (dry weight) of biomass per liter. Nevertheless, molasses, because of its lower price, is the more attractive choice.

One flask of *Pleutorus* culture, 4 days after inoculation, was used to inoculate a 2-liter batch fermentor (Multigen; New Brunswick Scientific) with a 1.5-liter working volume. Cultures were agitated at 200 rpm during aeration. Air volume was 0.5 per medium volume per min during the first 24 h and then increased to 1.0 per median volume per min.

Since the size of the mycelial pellets (5 mm in diameter; Fig. 1) made sampling from the fermentor impossible, each fermentor was used as a replicate. Pellet formation was described for *Morchella* spp. (8, 12) and *A. campestris* (18) grown in liquid cultures. Schugerl et al. (18) discussed the problems involved in using fungi in pellet form. The concentration of dissolved oxygen within the pellet may drop below the critical limit, thus influencing the cell metabolism and causing autolysis of the center of the pellet (18). This may, in turn, be related to the formation of mushroom flavor in aged

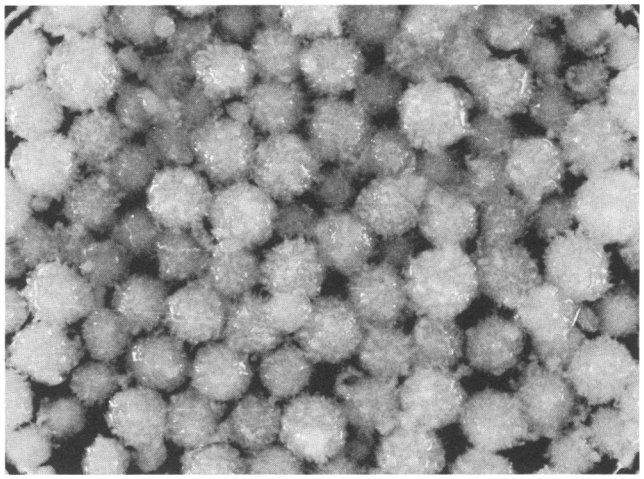

FIG. 1. *P. ostreatus* 'Florida' F6 mycelial pellets produced during growth in a 2-liter fermentor.

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TABLE 1. Biomass accumulation of *P. ostreatus* 'Florida' grown in a fermentor[a]

Carbon source (%)	Biomass accumulation (g[dry wt]/liter) at time from inoculation:		
	24 h	48 h	72 h
Glucose (2)	2.0 ± 0.24	3.80 ± 0.08	6.45 ± 1.44
Mollasses (2.5)		4.17 ± 0.2	6.74 ± 1.96
Glucose (1.5) + Molasses (2.5)	3.15 ± 0.35	5.85 ± 0.85	11.71 ± 2.63

[a] Each value represents the average of three different fermentor experiments.

pellets (21). Three growth media, containing different carbon sources, were compared: glucose (20 g/liter), molasses (25 g/liter), and glucose (15 g/liter) plus molasses (25 g/liter). The highest growth rate and biomass accumulation were observed with the medium containing glucose plus molasses (Table 1). In the fermentor growth was faster than in shaken flasks, and maximal biomass was obtained 3 rather than 6 to 7 days after inoculation. *Pleurotus* growth rate (11.7 g/liter in 3 days) was superior to that of both *A. campestris* (5 g/liter in 8 days) (14) and *M. crassipes* (22 g/liter in 15 days) (8).

Chemical constituents of *Pleurotus* mycelium produced in submerged culture in comparison to fruiting bodies. Fruiting bodies were produced on sterilized chopped cotton straw (16). Mycelial pellets and fruiting bodies were washed with distilled water, lyophilized, and ground. The following were then determined: nitrogen content, by a Kjelterc Auto Analyzer 1030 (Tector, Hogenes, Sweden); protein content, using Coomassie blue (19); amino acid composition, after acid hydrolysis (6 N HCl), by an automatic amino acid analyzer, LC700 (Biotronic, Munich, Federal Republic of Germany); glycogen, after precipitation with ethanol with enzymatic hydrolysis by amyloglucosidase (EC 3.2.1.3.), according to Johnson and Fusaro (6); and liberated glucose, by the glucose oxidase reagent (Sigma Chemical Co., St. Louis, Mo.). Lipids were extracted with chloroform–methanol (2:1, vol/vol) according to Folch et al. (3). Samples of the lipid fraction, transmethylated with 5% (vol/vol) sulfuric acid in methanol, were analyzed in a gas-liquid chromatograph, Packard 419 (Downers Grove, Ill.) (20). RNA content was determined by using the orcinol reagent (1) and ash content in crucibles at 550°C for 2 h.

Analysis of some chemical constituents shows a high similarity in the content (percent, dry weight) of total nitrogen (4.0 ± 0.05 and 3.8 ± 0.13), protein (24.3 ± 0.06 and 23.3 ± 0.08), glycogen (13.4 ± 0.3 and 13.3 ± 0.2), fatty acids (1.6 ± 0.19 and 1.5 ± 0.05), RNA (1.0 ± 0.03 and 1.2 ± 0.06), and ash (6.1 ± 0.08 and 5.8 ± 0.14), of fruiting bodies, produced on cotton straw, and mycelial pellets, produced in a submerged culture containing glucose. These results are in agreement with those described by Litchfield (10) for some mushrooms, including *Morchella* spp. and *A. campestris*.

Amino acid analysis (Table 2) shows similarities in most amino acids: the mycelium contained higher levels of aspartic acid, cystein, phenylalanine, and leucine, while fruiting bodies contained more valine and isoleucine. However, though the total content of fatty acids was similar, their composition differed: the mycelium contained more saturated fatty acids than the fruiting bodies (Table 3).

Khanna and Garcha (7), studing the nutritive value of *P. florida* grown on paddy straw, found higher protein and lipid levels, 37 and 6%, respectively. These differences could stem from either differences in fungal strain or growth conditions and substrates.

Cell walls of both mycelium and fruiting bodies were prepared, hydrolyzed, and analyzed according to Chet and Huttermann (2). Cell wall constituent content (percent, dry weight) was as follows: nitrogen, 1.3 ± 0.08 and 3.1 ± 0.1; ash, 1.9 ± 0.11 and 5.4 ± 0.26; total sugars, 64.8 ± 2.3 and 50.4 ± 1.93; glucoseamines, 4.5 ± 0.35 and 3.5 ± 0.47; and glucose, 49 ± 0.28 and 41 ± 0.28, in fruiting bodies and mycelial pellets, respectively. The infrared spectra of cell walls were determined in a Nicolet MX-S Fourier Transform infrared spectrophotometer (Madison, Wis.), using pellets containing 10 mg of cell wall and 90 mg of KBr. The infrared spectroscopy revealed a close overlap in the spectra of the two cell walls (Fig. 2). All peaks found in the commercial laminarin analysis coincided with those found in the cell wall, suggesting that laminarin is indeed a major component of the *Pleurotus* cell wall.

The comparison of the chemical compositions of fruiting bodies grown on cotton straw and mycelial pellets produced in submerged culture revealed a great similarity between the two fungal structures. Thus, it is possible to produce *P. ostreatus* mycelial pellets in a short time by fermentation.

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TABLE 2. Amino acid composition of mycelium and fruiting bodies of *P. ostreatus* 'Florida' F6

Amino acid	mg/g (dry wt)	
	Mycelium	Fruiting bodies
Aspartic acid	27.6	17.9
Threonine	7.8	8.5
Serine	8.0	9.7
Glutamic acid	18.1	21.7
Proline	6.5	6.0
Glycine	7.7	9.0
Alanine	12.3	12.8
Cysteine	13.4	2.8
Valine	2.8	10.7
Methionine	5.9	4.6
Isoleucine	11.0	6.6
Leucine	3.0	12.2
Tyrosine	6.4	6.0
Phenylalanine	10.4	7.2
Histidine	19.0	15.0
Lysine	8.9	9.7
Arginine	9.9	12.1

TABLE 3. Fatty acid (FA) composition of mycelium and fruiting bodies of *P. ostreatus* 'Florida' F6

Fatty acid	Mycelium		Fruiting bodies	
	mg of FA/g (dry wt)	% FA[a]	mg of FA/g (dry wt)	% FA
Palmitic acid ($C_{16:0}$)	5.3	35.0	2.2	14.0
Stearic acid ($C_{18:0}$)	0.8	5.5	0.5	3.0
Oleic acid ($C_{16:1}$)	3.3	22.0	2.9	18.0
Linoleic acid ($C_{18:2}$)	5.7	37.5	10.3	65.0
Unsaturated/saturated	1.47		4.85	

[a] Percentage of total fatty acids.

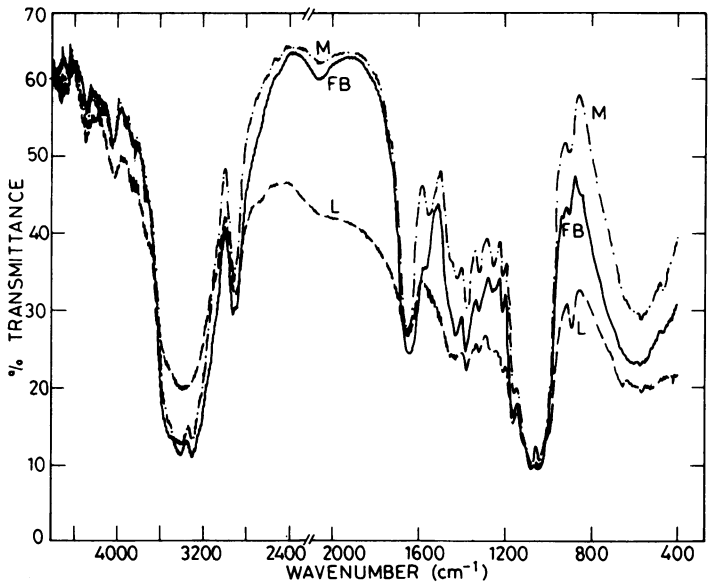

FIG. 2. Infrared spectrum of laminarin (L) as compared with cell wall of *P. ostreatus* 'Florida' F6 mycelial pellets (M) and fruiting bodies (FB).

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