ELSEVIER

PII: S0308-8146(96)00310-X

Food Chemistry, Vol. 60, No. 1, pp. 61–65, 1997

0308-8146/97 $17.00 + 0.00

Chemical evaluation of some lesser known edible mushroom mycelia produced in submerged culture from soy milk waste

P. C.-K. Cheung

Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

(Received 5 July 1996; revised version received 9 September 1996; accepted 9 September 1996)

The feasibility of using food waste, such as soy milk residue, to produce nutritive fungal biomass was evaluated. Edible mushroom mycelia of *Volvariella bombycina*, *Lyophyllum ulmarius* and *Pleurotus citrinopileatus* were produced in liquid culture containing soy milk waste. The chemical composition of the mushroom mycelia is reported and compared with that of the mushroom fruiting bodies. There were great similarities in the crude protein, lipid, ash and nucleic acid contents between the mycelia and fruiting bodies. Differences were observed in the amount of total dietary fibre and amino acid composition. Lysine was the limiting amino acid found in all mushroom samples. In general, the nutritional quality of the proteins from the mushroom mycelia, as measured by their essential amino acid score, was comparable to those of the fruiting bodies and the FAO/WHO requirement pattern. © 1997 Elsevier Science Ltd

INTRODUCTION

The growth of an edible mushroom with its fruiting body as the most common edible form is a lengthy and complex process involving the use of solid compost or lignocellulosic waste, such as straw or cotton, followed by a long cultivation period (Vedder, 1978). Growing mushroom mycelium in liquid culture on a defined nutrient medium has long been a simple and fast alternative method to produce fungal biomass (Cirrillo *et al.*, 1960; Litchfield, 1967). Food by-products, such as soy bean residue generated from soy milk production, are rich in nutrients and found to be good substrates for cultivating mushroom mycelium (Cheung, 1995). The fruiting bodies of edible mushrooms are commonly used in human diets as a source of protein (Gray, 1973). However, there is little information on the potential use of mushroom mycelia in human or animal diets (El-Kattan *et al.*, 1991) despite the comparable nutritional values between mushroom mycelia and fruiting bodies (Hadar & Cohen-Arazi, 1986).

Three lesser known edible mushrooms, *Volvariella bombycina* (Silky Volvaria), *Lyophyllum ulmarius* (Elm Tree Mushroom) and *Pleurotus citrinopileatus* (Goldencap Mushroom), cultivated locally, were chosen for study (Chang & Mao, 1995). In this report the chemical composition of the mycelia from these three mushrooms, harvested from soy milk waste medium, was compared with their corresponding fruiting bodies harvested from regular composted materials. The potential nutritional values of these mushroom mycelia are discussed.

MATERIALS AND METHODS

Liquid culture of mycelium

The edible mushroom species used in this study (*Volvariella bombycina*, *Lyophyllum ulmarius* and *Pleurotus citrinopileatus*) were obtained from the Departmental Mushroom Culture Collection. Mycelia of these mushroom species were grown in flasks containing 300 ml of medium which were inoculated with 30 ml of homogenate of mycelia previously subcultured on potato dextrose agar plates. Cultures were incubated in a rotary shaker (New Brunswick Scientific, Edison, NJ) at 25°C, pH 7 and 200 rpm for 7 days. The medium used in all liquid culture experiments contained the following reagents (per litre): 20 g dried soy bean residue, 0.4 g potassium dihydrogen phosphate, 1 g potassium hydrogen phosphate, 0.5 g magnesium sulphate, 2 g asparagine. The mycelium was harvested by filtration through glass-fibre filter, washed with distilled water

and freeze-dried. Mature fruiting bodies of the mushroom species grown on regular lignocellulosic compost were cleaned to remove any residual compost and freeze-dried. All dried samples of mushroom mycelia and fruiting bodies were ground by a Cyclotech mill (Tecator, Hoganas, Sweden) to a particle size of less than 0.5 mm.

Proximate analysis

All determinations were carried out in triplicate. Moisture content, Kjeldahl nitrogen, total dietary fibre (TDF), fat and ash were determined on a dry weight basis of the mushroom samples according to AOAC (1990). Percent protein was calculated as N×4.38 (Crisan & Sands, 1978). Nucleic acid content was estimated by a modified method as described by Li and Chang (1982).

Amino acid analysis

Amino acids in mushroom samples (in duplicate) were determined with a Beckman 6300 automated amino acid analyser (Beckman Instruments, Fullerton, CA) using an acid hydrolysis and ninhydrin procedure (Danell & Eaker, 1992). The hydrolysis of a mushroom sample (5 mg) was performed in sealed ampoule for 24 h at 110°C using 1 ml of 6 M HCl (Sigma H0636) containing 5 mg phenol ml^{-1} (for protection of tyrosine) and 5 μmol of norleucine as an internal standard. The hydrolysate was evaporated using a Speedvac concentrator (Savant Instrument, Farmingdale, NY) and the dried residue was redissolved in 0.5 ml of citrate buffer. The sample was filtered through a 0.45 μm nylon filter before being injected into the amino acid analyser. A performic acid oxidation procedure (Moore, 1963) was performed on a separate sample prior to the acid hydrolysis to quantify cystine and methionine. The contents of the different amino acids recovered are presented as mg g^{-1} protein and are compared with the FAO/WHO (1991) reference pattern. The essential amino acid (EAA) score was calculated by the method of FAO/WHO (1991) as shown below:

$$\text{Essential amino acid score} = \frac{\text{mg of EAA in 1 g of test protein}}{\text{mg of EAA in 1 g of reference protein}} \times 100$$

RESULTS AND DISCUSSION

Yields of mushroom mycelia

The maximum yield was obtained at the seventh day, after which the yield decreased due to autolysis of the mycelium. The biomass accumulations (g dry weight $litre^{-1}$ of culture medium, mean ± standard error of the mean [SEM] with $n = 3$) of the three mushroom mycelia grown in submerged culture were comparable to each other: *V. bombycina* 12.7 ± 0.34, *L. ulmarius* 13.0 ± 0.51, *P. citrinopileatus* 13.5 ± 0.47.

Proximate composition

Proximate compositions of the mycelia and fruiting bodies of edible mushrooms are shown in Table 1. Generally, the crude protein content of the mushroom mycelia was very similar to that of the fruiting bodies. The values for crude protein content of the mushroom fruiting bodies were comparable to other mushroom species of the same genus (Crisan & Sands, 1978). Earlier estimates of crude protein value in mushrooms were based on the total nitrogen value (mainly Kjeldahl N) multiplied by a factor of 6.25. However, based on studies on the non-protein nitrogen content of mushrooms (Kurkela *et al.*, 1980), a conversion factor of 4.38, which is based on the assumption of a 60–70% digestibility of crude mushroom protein, is more appropriate (Crisan & Sands, 1978; Haytowitz & Matthews, 1984).

As shown in Table 1, the TDF content of all mushroom mycelia was higher than that of the fruiting bodies, which was consistent with previous results (Cheung, 1996). The mycelium of *L. ulmarius* had the highest TDF content of 48.3%. The TDF content was much higher than that reported by Crisan and Sands (1978), who probably underestimated the actual fibre content due to the use of the crude fibre method. The mushroom dietary fibre is mainly composed of chitin and β-glucan cell-wall material (Bartnicki-Garcia, 1968).

Mushroom mycelia had a similar amount of ash as the fruiting bodies (Table 1). *V. bombycina* contained almost double the amount of ash found in *P. citrinopileatus*. The ash content of the mushroom samples was in agreement with previous results (Crisan & Sands, 1978; Haytowitz & Matthews, 1984).

Table 1. Chemical composition (g per 100 g dry matter) of the fruiting bodies and mycelia of edible mushrooms

Mushroom species		Moisture	Crude protein (N×4.38)	Crude lipid	Total dietary fibre	Ash	Nitrogen-free extract
V. bombycina	Fruiting bodies	9.90 ± 0.12	22.1 ± 0.15	2.75 ± 0.15	25.7 ± 0.55	11.2 ± 0.21	38.3
	Mycelia	8.85 ± 0.07	24.3 ± 0.14	2.56 ± 0.11	43.4 ± 0.83	10.9 ± 0.12	18.8
L. ulmarius	Fruiting bodies	9.34 ± 0.09	19.5 ± 0.12	2.09 ± 0.08	33.2 ± 0.71	8.85 ± 0.11	36.4
	Mycelia	8.33 ± 0.06	17.2 ± 0.09	2.01 ± 0.06	48.3 ± 0.79	8.66 ± 0.16	23.8
P. citrinopileatus	Fruiting bodies	10.3 ± 0.11	24.7 ± 0.08	1.85 ± 0.19	35.6 ± 0.59	6.43 ± 0.10	31.4
	Mycelia	8.94 ± 0.09	24.3 ± 0.05	1.76 ± 0.10	38.5 ± 0.66	6.23 ± 0.07	29.2

All values are means ± SEM of triplicate determinations.

Table 2. Fatty acid composition (%) of the fruiting bodies and mycelia of edible mushrooms

Mushroom species		$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Unknown	P:S
V. bombycina	Fruiting bodies	0.95±0.04	0.53±0.02	10.5±0.21	1.03±0.04	1.59±0.01	84.0±1.02	0.28±0.02	1.13±0.03	6.09
	Mycelia	0.65±0.02	0.34±0.01	11.4±0.33	1.22±0.02	2.01±0.04	83.1±1.33	0.33±0.01	0.97±0.02	5.85
L. ulmarius	Fruiting bodies	n.d.	1.12±0.09	15.4±0.24	2.54±0.08	11.2±0.36	67.4±1.12	0.22±0.02	2.09±0.10	3.72
	Mycelia	n.d.	1.33±0.10	14.0±0.30	3.11±0.06	10.5±0.33	69.1±1.09	0.28±0.04	1.66±0.03	3.97
P. citrinopileatus	Fruiting bodies	n.d.	1.10±0.06	10.2±0.18	2.31±0.05	13.5±0.25	71.3±1.55	0.54±0.05	1.05±0.02	6.35
	Mycelia	0.53±0.03	1.23±0.11	22.8±0.12	5.45±0.08	12.7±0.22	56.6±1.31	0.12±0.01	0.59±0.01	2.27

All values are means ± SEM of triplicate determinations.
n.d., not detected.

The crude lipid content of samples (Table 1) varied only within a narrow range, as was found by Crisan and Sands (1978). The fatty acid profiles of the mushroom mycelia were similar to those of the fruiting bodies (Table 2). The only exception was *P. citrinopileatus*, in which the mycelium had a lower unsaturation:saturation (P:S) ratio (more palmitic acid and less linoleic acid) than the fruiting body (Table 2). In general, the mushrooms examined contained high levels of unsaturated fatty acids of which linoleic is the most prominent; these results are in agreement with previous data (Senatore *et al.*, 1988).

Nucleic acid content

There was great similarity in the total nucleic acid content between the mushroom mycelia and fruiting bodies (Table 3). The fact that the mushroom DNA levels were much lower than those of the RNA was consistent with previous findings (Li & Chang, 1982). The range of the total nucleic acid content of the mushroom samples was 3.51–4.15%, which was comparable to that of general filamentous fungi (Kihlberg, 1972). In reference to the maximum daily dietary intake of 4 g of nucleic acid suggested by the Protein Advisory Group of the United Nations Systems (PAG, 1970), the amounts of nucleic acids found in the mushroom samples do not limit their use as a daily vegetable.

Amino acid composition

The amino acid profiles and the essential amino acid scores of mushroom samples are presented in Table 4. The amino acids analysed represented both the free and combined amino acids. The mushroom mycelia and fruiting bodies contained all the essential amino acids (in different proportions, except tryptophan which was not measured) which comprised 37–56% of the total amino acid content. The total EAA content of the mushroom mycelia was similar to that of the fruiting bodies, except in *L. ulmarius* which had a much higher EAA content than its fruiting bodies. The limiting amino acids are as follows: in *V. bombycina*, lysine (only in fruiting bodies), leucine and sulphur-containing amino acids (in both mycelia and fruiting bodies); in *L. ulmarius*, lysine (in both fruiting bodies and mycelia); in *P. citrinopileatus*, lysine (in both fruiting bodies and mycelia), valine and leucine (only in mycelia). While all mushroom samples contained higher levels of the other EAAs, the mycelia of *L. ulmarius* and *P. citrinopileatus* contained more than four times the levels of isoleucine and sulphur-containing amino acids, respectively, when compared with the FAO/WHO requirement pattern (FAO/WHO, 1991).

In *V. bombycina*, there were no major differences in the amino acid composition between the mycelia and the fruiting bodies except for histidine, which was higher in the mycelia (Table 4). The amino acid profile of *V. bombycina* (low levels of serine and glycine accompanying a high levels of aspartic acid and glutamic acid) was common to other *Volvariella* species (Crisan & Sands, 1978; Zakhary *et al.*, 1984). The mycelia of *L. ulmarius* had much more tyrosine and isoleucine but much less alanine and glutamic acid than the fruiting bodies. The results also showed that the mycelia of *P. citrinopileatus* had higher levels of aspartic acid and cystine but lower levels of valine and leucine than the fruiting bodies, which was consistent with previous results (Hadar & Cohen-Arazi, 1986).

It is not surprising to find that the relative amounts of amino acids in the mushroom samples were somewhat

Table 3. Nucleic acid content (g 100 g^{-1} dry matter) of the fruiting bodies and mycelia of edible mushrooms

Mushroom species		DNA	RNA	Total
V. bombycina	Fruiting bodies	0.26±0.03	3.51±0.10	3.77±0.11
	Mycelia	0.24±0.02	3.76±0.08	4.00±0.09
L. ulmarius	Fruiting bodies	0.21±0.01	3.30±0.08	3.51±0.10
	Mycelia	0.22±0.01	3.42±0.06	3.64±0.08
P. citrinopileatus	Fruiting bodies	0.20±0.01	3.81±0.18	4.01±0.20
	Mycelia	0.19±0.02	3.96±0.11	4.15±0.15

All values are means ± SEM of triplicate determinations.

Table 4. Amino acid composition (mg g^{-1} protein) of the fruiting bodies and mycelia of edible mushrooms

Amino acid	*V. bombycina*		*L. ulmarius*		*P. citrinopileatu*		FAO/WHO (1991) requirement pattern
	Fruiting bodies	Mycelia	Fruiting bodies	Mycelia	Fruiting bodies	Mycelia	
Aspartic acid	150	135	101	112	104	155	
Threonine	46.5 (1.37)	55.2 (1.62)	41.2 (1.21)	38.5 (1.13)	49.2 (1.45)	44.2 (1.30)	34
Serine	0.32	1.01	45.3	49.9	56.3	47.4	
Glutamic acid	272	259	182	114	120	101	
Proline	58.9	50.1	23.1	20.2	34.1	35.6	
Glycine	0.57	1.21	40.1	35.7	51.9	43.2	
Alanine	75.7	69.3	80.2	56.2	73.0	63.1	
Valine	35.8 (1.02)	43.2 (1.23)	56.1 (1.60)	51.3 (1.47)	60.7 (1.73)	15.7 (0.45)	35
Cystine	19.1	22.1	18.2	21.9	15.8	75.3	
Methionine	1.22 (0.81)[a]	2.12 (0.97)	19.1 (1.49)	23.4 (1.81)	25.4 (1.65)	33.2 (4.34)	25[a]
Isoleucine	54.1 (1.93)	48.1 (1.72)	58.9 (2.10)	115 (4.11)	35.1 (1.25)	56.6 (2.02)	28
Leucine	50.1 (0.76)	57.2 (0.87)	101 (1.53)	97.3 (1.47)	71.2 (1.08)	16.8 (0.25)	66
Tyrosine	45.8	50.3	72.1	110	32.2	33.2	
Phenylalanine	60.2 (1.68)[b]	49.2 (1.58)	51.1 (1.96)	45.1 (2.46)	39.7 (1.14)	54.4 (1.39)	63[b]
Histidine	3.67	15.1	23.3	18.5	84.8	105	
Lysine	54.1 (0.93)	60.1 (1.04)	46.1 (0.79)	57.2 (0.99)	56.3 (0.97)	51.3 (0.88)	58
Arginine	26.3	21.3	5.34	6.21	68.1	54.4	
Total essential amino acids	367	388	464	560	386	381	

Values are the average of two determinations.
Figures in parentheses are the essential amino acid score.
[a]Cystine + Methionine.
[b]Tyrosine + Phenylalanine.

different from the mushrooms grown in other media and conditions since it has been reported that the proportions of the amino acids depend on the culture medium (Reusser *et al.*, 1958; Litchfield *et al.*, 1963). In terms of protein nutritional quality, the mycelium of *L. ulmarius* has the highest EAA score among all the mushroom samples.

CONCLUSIONS

Comparison of the chemical composition of mycelial biomass produced in submerged culture and fruiting bodies grown on solid composts revealed a great similarity between the two fungal structures. Being rich in protein, dietary fibre and low in fat with a high P:S ratio, the mushroom mycelia have great potential as possible human food, animal feed and food supplements in the same way as other microbial protein sources, such as single-cell proteins (Litchfield, 1983). However, the nutritional values of the mushroom presented were based on chemical analyses only. Biological evaluation using human or animal feeding studies would be required to establish the actual nutritional value, particularly the *in vivo* protein digestibility and availability of the essential amino acids. Animal feeding studies using the mushroom mycelia are now being carried out to determine their nutritional values. The feasibility of a large-scale bioconversion of soy milk waste into nutritive mycelial biomass would be useful in solving both environmental and food problems.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the financial support of a Direct Grant from the Research Committee of the Chinese University of Hong Kong.

REFERENCES

AOAC (1990). *Official Methods of Analysis*, 14th edn. Association of Official Analytical Chemists, Washington, DC.

Bartnicki-Garcia, S. (1968). Cell wall chemistry, morphogenesis and taxonomy of fungi. *Annu. Rev. Microbiol.*, **22**, 87–108.

Chang, S. T. & Mao, X. *Hong Kong Mushrooms*. The Chinese University Press, Hong Kong.

Cheung, P. C. K. (1995). Biological conversion of soybean waste to edible mushroom mycelium by submerged fermentation. In *Proceedings of the Fourth Pacific Rim Biotechnology Conference*, Sydney, Australia, 6–9 February 1995, pp. 96–97.

Cheung, P. C. K. (1996). Dietary fibre content and composition of some cultivated edible mushroom fruiting bodies and mycelia. *J. Agric. Food Chem.*, **44**, 468–471.

Cirillo, V. P., Hardwick, W. A. & Seeley, R. D. (1960). Fermentation process for producing edible mushroom mycelium. US Patent 2,928,210.

Crisan, E. V. & Sands, A. (1978). Nutritional value. In *The Biology and Cultivation of Edible Mushrooms*, eds S. T. Chang & W. A. Hayes. Academic Press, New York, pp. 137–168.

Danell, E. & Eaker, D. (1992). Amino acid and total protein content of the edible mushroom *Cantharellus cibarius* (Fries). *J. Sci. Food Agric.*, **60**, 333–337.

El-Kattan, M. H., Afify, A. S. & Aly, A. Z. M. (1991). Evaluation of *Pleurotus sajor-caju* fungal pellets as food. *Mushroom J. Trop.*, **11**, 13–22.

FAO/WHO (1991). Protein quality evaluation. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 51. FAO/WHO, Rome, Italy, 23 pp.

Gray, W. D. (1973). *The Use of Fungi as Food and in Food Processing*, Parts I & II. Butterworths, London.

Hadar, Y. & Cohen-Arazi, E. (1986). Chemical composition of edible mushroom *Pleurotus ostreatus* produced by fermentation. *Appl. Environ. Microbiol.*, **51**, 1352–1354.

Haytowitz, D. B. & Matthews, R. H. (1984). *Composition of Foods: Vegetables and Vegetable Products. Raw, Processed, Prepared.* USDA Agriculture Handbook Nos. 8–11. USDA, Washington, DC.

Kihlberg, R. (1972). The microbe as a source of food. In *Annual Review of Microbiology*, eds C. E. Clifton, S. Raffel & M. P. Starr. Interscience Palo Alto, CA, pp. 427–466.

Kurkela, R., Koivurinta, J. & Kuusinen, R. (1980). Non-protein nitrogen compounds in the higher fungi—a review. *Food Chem.*, **5**, 109–130.

Li, G. S. F. & Chang, S. T. (1982). The nucleic acid content of some edible mushrooms. *Eur. J. Appl. Microbiol. Biotechnol.*, **15**, 237–240.

Litchfield, J. H. (1967). Submerged culture of mushroom mycelium. In *Microbial Technology*, ed. H. J. Peppler. Reinhold, New York, pp. 107–144.

Litchfield, J. H. (1983). Single-cell proteins. *Science*, **219**, 740–746.

Litchfield, J. H., Overbeck, R. C. & Davison, R. S. (1963). Factors affecting the growth of morel mushroom mycelium in submerged culture. *J. Agric. Food Chem.*, **11**, 158–162.

Moore, S. (1963). On the determination of cystine as cysteic acid. *J. Biol. Chem.*, **238**, 235–237.

PAG (1970). Single-cell protein guideline. Report of FAO/WHO/UNICEF Protein Advisory Group No. 4. United Nations, New York.

Reusser, F., Spencer, J. F. T. & Sallans, H. R. (1958). Protein and fat content of mushrooms grown in submerged culture. *Tricholoma nudum* as a source of microbiological protein. *Appl. Microbiol.*, **6**, 1–8.

Senatore, F., Dini, A. & Marino, A. (1988). Chemical constituents of some Basidiomycetes. *J. Sci. Food Agric.*, **45**, 337–345.

Vedder, P. J. C. (1978). Cultivation. In *The Biology and Cultivation of Edible Mushrooms*, eds S. T. Chang & W. A. Hayes. Academic Press, New York, pp. 371–392.

Zakhary, J. W., Rafik El-Mahdy, A., Abo-Bakr, T. M. & El Tabey-Shehata, A. M. (1984). Cultivation and chemical composition of the paddy-straw mushroom (*Volvariella volvaceae*). *Food Chem.*, **13**, 265–276.