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BIOCHEMICAL COMPOSITION OF PLEUROTUS OSTREATUS (JACQ.) P. KUMM. GROWN ON SAWDUST OF LEUCAENA LEUCOCEPHALA (LAM.) DE WIT.

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Abstract

In the present study, the sawdust of *Leucaena leucocephala* was examined to understand its suitability as substratum for the production of oyster mushroom (*Pleurotus ostreatus*). The sawdust contained 86.1, 25.8, 46.9, 27.56, 0.18, 45.36, 20.58, 27.3 and 1.9% of organic carbon, crude protein, neutral detergent fiber, acid detergent fiber, total lipids, cellulose, hemicellulose, lignin, and ash, respectively. Higher growth rate of the fungus was recorded after incubation for eight days on the sawdust of *L. leucocephala* in Petri dishes. However, the weekly production of fruiting bodies (FB) decreased with increase in incubation period, but the accumulated production increased significantly. On the other hand, total production decreased with increase in incubation periods. The contents of ash, fiber, lipid, and nitrogen (crude protein) decreased significantly with increase in growth periods. The alterations in both fatty acid and amino acid contents of FB showed a negative correlation and visible catabolic metabolism with extension of the incubation period.

Introduction

The genus *Pleurotus* (oyster mushroom), an edible mushroom, is widely cultivated due to its favorable organoleptic and medicinal properties, simple and low cost production technology and higher biological efficiency (Chirinang & Intarapichet, 2009). The spent mushroom compost will have value for livestock as well as organic fertilizer (Siddhant & Singh, 2009). *Pleurotus ostreatus* can be cultivated for the production of food, feed, enzymes, as well as medicinal compounds, on a wide variety of agro-forestry by-products, weeds, and wastes (Patil *et al*., 2010). *Leucaena leucocephala* (Lam.) is a rapidly growing leguminous tree, belonging to family Mimosaceae. It is economically an important plant, native to Central America and is cultivated around roadsides and industrial areas for ornamental purposes (Atiq-ur-Rehman & Iqbal, 2007).

The present study was undertaken to test the suitability of sawdust of *L. leucocephala*as substratum for the production of mushroom and to examine if any biochemical changes occurred in *P. ostreatus* due to this substratum.

Material and Methods

The experimental fungus and growth medium: Pure culture of a strain of edible mushroom *Pleurotus ostreatus* was obtained from the Agriculture Research Center, Giza, Egypt. The mold was grown on Malt extract agar medium for 7 days in dark (28±1°C), then 0.5 agar disc was used for inoculation of substratum in both Petri dishes and polyethylene bags.

Nutritional value of the substratum: *L. leucocephala* twigs were ground and then sieved (1.5-2.0mm) to have homogenous substratum.The organic matter, cellulose, hemicellulose, and lignin were estimated according to the methods of AOAC (1995), ASTM (1994), Rozmarin & Simionescu (1973) and Rowell (2005), respectively. Total nitrogen contents were determined using the conventional micro-Kjeldahl method (Allen, 1953). Crude protein calculated by multiplying the total nitrogen content by 6.25. Both acid and neutral detergent fibers were estimated as described by Mertens (1992). Total lipids were extracted with chloroform:methanol (2:1 v/v) according to Fölsh *et al*., (1957). Ash content was determined according to Anon., (2005). The ashing was done at 550°C up to two successive constant weights. Total ash content was expressed as percentage of dry weight.

Determination of moisture content of the FB: The moisture content of each fresh sample was determined by weighing a portion of the sample and dried in an oven for 24h at 105°C, then cooled in desiccators and reweighed, and the process was continued for up to two successive constant weights. The moisture content was expressed as percentage of the wet weight.

Preparation, inoculation and incubation of polyethylene bags: The substratum (500 g/bag) was autoclaved and inoculated with three discs (0.5cm) of *P. ostreatus* (seven day-old culture) obtained from malt extract agar medium. The inoculated bags were incubated at 25°C for three weeks. After that, the temperature was adjusted to 18°C and the humidity of the incubator was adjusted to 90% to facilitate the production of FB. The FB were collected after 30, 40 and 50 days of inoculation and weighed to determine the amount of production per picking and the total production after three pickings. The biological efficiency was expressed as percent (%) according to Mandeel *et al*., (2005) based on the following equation:

$$\text{Biological efficiency (\%)} = \frac{\text{Weight of fresh mushroom fruiting bodies}}{\text{Weight of dry substratum}} \times 100$$

Nutritional value of the FB: The nutritional value of the FB involved the total lipid, total nitrogen, total crude fiber and total ash contents. Total nitrogen (crude protein), total lipid and ash contents were determined as described above. Total crude fiber was estimated according to the method of Triebold & Aurand (1982) and expressed as percentage of dry weight of mushroom. The total metabolizable energy was expressed in kilocalories and calculated considering Atwater's conversion factors: (4 × g crude protein) + (4 × g carbohydrates [total carbohydrates – crude fiber]) + (9 × g total lipids) as described by Sales-Campos *et al*., (2011).

Amino acids analysis: Free amino acids were extracted with absolute ethanol from the FB and the qualitative as well as quantitative determination of amino acids was carried out using LKB 415 alpha plus Amino Acid Analyzer (AAA) according to Christias *et al*., (1975). Standard amino acids (BHD Chemicals, Poole, UK) were used as reference.

Fatty acids analysis: Fatty acid methyl esters were prepared by methanolysis in H_2SO_4-MeOH (Kates, 1972) and methyl esters were analyzed by gas liquid chromatography (GLC) (Perkin-Elmer Model 910, Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector (Johnson & Stocks, 1971). Qualitative and quantitative analysis of peak fatty acid methyl esters was carried out by comparing their retention times with those of an authentic methyl standard (Sigma Co., St. Louis, USA).

Statistical analysis: Treatment means were compared using Fisher's Least Significant Difference (LSD) analysis.

Results and Discussion

Radial growth and growth rate: The results pertaining to the radial growth is depicted in Table 1. The radial growth rate increased with increasing concentration of incubation period. After 14 days of incubation the radial growth observed was 8.56 cm. The growth rate increased from 0.29 after 2nd day of incubation to 1.14 after 8 day of incubation. The growth rate increased with increasing incubation period up to 8 days (Table 1). At incubation period of 10, 12, and 14 days, a decrease of 0.83, 0.73 and 0.24 was observed in growth rate. Our results corroborated with the findings of Alqarawi *et al*., (2013) who reported that optimum mycelial growth and growth rate of *P. ostreatus* were at fourteen and 8 days, respectively. In the same context, Patil *et al*., (2010) reported that the rate of mycelial growth of *P. ostreatus* was different on different substrata.

Harvest yield, moisture percentage and biological efficiency: The results of harvest yield decreased with increasing flushing period (Table 2). The first flush yielded 394.73g/bag, 2nd flush 233.87g/bag and 3rd flush 158.50g/bag. The moisture percentage of fruiting bodies also decreased with increasing flushing periods. The decrease in moisture percentage was 87.36, 84.28 and 78.8 after first, 2nd and 3rd flushing, respectively (Table 2). The biological efficiency decreased from 78.94% after first flushing period to 31.66 after 3rd flushing period (Table 2). The first initiation of FB (pinhead formation) was observed after 23 days of inoculation of polyethylene bags (Table 2). Our results corroborate with the findings of Bhatti *et al*., (2007) and Alqarawi *et al*., (2013) who reported that the optimum incubation period for pinhead formation of FB were 23 and 25 days, respectively.

Table 1. The radial growth (cm) and *growth rate of *P. ostreatus*on ground *L.leucocephala* twigs in Petri dishes (9 cm).

Incubation period (day)	Radial growth (cm)	*Growth rate
2	0.59	0.29
4	1.29	0.35
6	2.68	0.69
8	4.96	1.14
10	6.62	0.83
12	8.08	0.73
14	8.56	0.24
LSD at: 0.05	0.2882	

*: Growth rate was calculated according to Trinci (1969)

Table 2. The harvested yield (g/bag), moisture content (%) and efficiency (%) of fruiting bodies of P.ostreatus collected after different flushing periods.

Flushing period	Yield (g/bag)	Moisture content (%)	Biological efficiency (%)
First picking	394.73	87.36	78.94
Second picking	233.87	84.28	46.77
Third picking	158.50	78.8	31.66
LSD at: 0.05	41.51	2.58	8.30

Nutritional value (%): Total metabolizable energy decreased from 298.85 to 252% after 3rd flushing periods (Table 3). The nutritional value of FB collected after different flushing periods showed a wide range of variations (Table 3). With the increased of growth periods, the contents of lipids, crude protein, ash content and total metabolizable energy decreased significantly, however, fiber content increased. A decrease in total lipid content from 0.36% after first flushing to 0.07% after 3rd flushing was observed in the present study. Decrease in ash content was observed as 8.59, 6.93 and 5.64 after first, 2nd and 3rd flushing, respectively. Crude fiber content (CF) increased from 12.55% at first flushing to 16.34% at 3rd flushing. Crude protein content (CP) decreased and the maximum decrease was 11.11% after 3rd flushing period. Total carbohydrate content (TC) increased by increasing flushing periods. The maximum increase in TC was 68.02 after 3rd flushing period. Our results are in agreement with the findings of Patil *et al*., (2010) and Michael *et al*., (2011). A great magnitude of variability in the nutritional composition of mushrooms among the same species has been reported due to mold strain chosen, surrounding environmental conditions and nutritional requirements (Das & Mukherjee, 2007). Toro *et al*., (2006) and Sales-Campos *et al*., (2011) reported large variations in fiber content while working on several *Pleurotus* species. Variable low lipid contents in the mushrooms grown in different residues have been reported by Sales-Campos *et al*., (2011). The higher crude protein content of FB of *P. ostreatus* reported in the present study is in parallel with many previous reports (Shin *et al*., 2007; Alam *et al*., 2008; Sales-Campos *et al*., 2011). The significant decrease in total metabolizable energy has been reported in our results with the increased of incubation periods most probably due to consumption of nutrients during the first initiation of FB (pinhead formation), consequently, the continuous injection of freshly soluble nutrients may extend the production curve with nearly stable total metabolizable energy (Albert *et al*., 2002; Walker & White, 2011).

Table 3. Nutritional value (%) of fruiting bodies of P.ostreatus collected after different flushing periods.

Flushing period	Nutritional value (%)					
	TL	AC	CF	CP	TC	TME
First picking	0.368	8.59	12.55	33.72	52.72	298.85
Second picking	0.240	6.93	14.60	21.19	60.73	271.44
Third picking	0.070	5.64	16.34	11.11	68.02	252.00
LSD at 0.05	0.0784	0.932	0.46	4.391	5.534	33.25

TL= Total lipids content; AC= Ash content; CF= Crude fiber content; CP= Crude protien content; TC= totl carbohydrate content; TME= Total metabolizable energy

Fatty acid profile: Gas chromatographic analysis of fatty acids (methyl ester) revealed the presence of three saturated fatty acids (myristic [C_{14}], palmitic [C_{16}], stearic [C_{18}]) and three unsaturated fatty acids (myristic [$C_{14:1}$], oleic [$C_{18:1}$], linoleic [$C_{18:2}$]) with total unsaturation percent 80.94 in the FB of *P. ostreatus* at first picking (Table 4). Our results are in accordance with Pedneault *et al*., (2007) who reported that oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids were the most common unsaturated fatty acids in lipids of *P. ostreatus*. They are also common precursors as well as intermediates during biosynthesis and metabolism of other fatty acids (Rangan & Smith, 2002). A significant increase in saturated fatty acids such as myristic [C_{14}], palmitic [C_{16}] and stearic [C_{18}] was observed after every flushing period. An increase of 6.40, 18.43 and 5.73% in myristic [C_{14}], palmitic [C_{16}] and stearic [C_{18}] respectively was observed after 2nd flushing. After 3rd flushing the increase was 6.90% in myristic [C_{14}], 23.75 in palmitic [C_{16}] and 10.26 in stearic [C_{18}] (Table 4). Extension of incubation period more than first flushing period caused appearance of saturated fatty acids, lauric (C_{12}), margaric (C_{17}) and arachidic (C_{20}). On the other hand, the unsaturated fatty acids ($C_{14:1}$, $C_{18:1}$, $C_{18:2}$) decreased gradually with the extension of incubation period. It has been reported that the increase in saturated fatty acids can be used as selection criterion for unfavorable conditions (Laoteng *et al*., 2011). Lipid accumulation and synthesis of saturated fatty acids in fungi are complex and closely associated with cell physiology and environmental adaptation of cell (Laoteng *et al*., 2011).

Table 4. Fatty acid profile of fruit bodies (%) of P.ostreatus after different flushing periods.

Flushing period	Fatty acid profile of fruit bodies (%)									
	C_{12}	C_{14}	$C_{14:1}$	C_{16}	C_{17}	C_{18}	$C_{18:1}$	$C_{18:2}$	C_{20}	TS
First picking	0.00	2.95	1.83	12.79	0.00	3.29	16.90	62.24	0.00	19.03
Second picking	2.62	6.90	1.37	18.43	1.62	5.73	12.77	48.83	1.74	37.04
Third picking	0.00	6.40	0.70	23.75	3.72	10.26	10.95	41.39	2.84	46.97
LSD at 0.05	0.5508	1.1048	0.329	2.6722	1.7306	1.2575	1.2338	3.417	1.213	2.8486

Lauric (C12), Myristic (C_{14}), Myristic ($C_{14:1}$), Palmitic (C_{16}), Margaric (C_{17}), Stearic (C_{18}), Oleic ($C_{18:1}$), Linoleic ($C_{18:2}$), Arachidic (C_{20}) and total saturated fatty acids (TS).

Table 5. Amino acid profile of fruit bodies (mg/g) of *P.ostreatus* after different flushing periods.

Flushing period	Amino acid profile of fruit bodies (mg/g)																			
	Alanine	Arginine	Asparagine	Aspartic acid	Cysteine	Glutamic acid	Glutamine	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine	Total
First picking	0.23	0.64	1.37	1.37	0.06	1.53	0.97	0.09	0.56	0.29	0.98	0.64	0.08	0.75	0.10	0.12	1.16	0.11	0.18	0.24	11.49
Second picking	0.17	0.76	1.81	1.64	0.03	2.20	1.13	0.27	0.81	0.4	ND	0.42	ND	1.22	0.55	0.26	1.47	0.37	0.31	0.37	14.2
Third picking	0.09	1.04	2.15	2.02	ND	3.06	1.42	0.38	1.05	0.62	ND	0.24	ND	1.85	1.09	0.50	1.93	0.47	0.45	0.51	18.88
LSD at 0.05	0.07	0.19	0.16	0.18	0.03	0.23	0.11	0.06	0.20	0.14	0.25	0.23	0.03	0.41	0.20	0.15	0.19	0.08	0.12	0.09	1.25

Amino acid profile: The results related to the amino acid profile of fruiting bodies are depicted in Table 5. The analysis of amino acids revealed the presence of 20 amino acids namely alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine in the FB of *P. ostreatus* at first picking (Table 5). The most abundant amino acids were asparagine, aspartic acid, and glutamic acid followed by leucine and threonine. The least abundant amino acids were proline, glycine, methionine, and cysteine. The amino acid profile shows fluctuations in their appearance after every flushing period. The amino acids arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine showed increase in the amino acids from first flushing to 3rd flushing, however, alanine, cysteine, leucine, lysine, Methionine showed decrease in its content from first flushing to 3rd flushing (Table 5). The results of our research are in accordance with Chirinang & Intarapichet (2009) who reported that the FB of oyster mushroom (*P. ostreatus*) rich in asparagine, aspartic acid as well as glutamic acid and poor in proline, glycine, as well as methionine. The slight qualitative and quantitative alterations in amino acid profile in our study compared with previous reports may be due to the novel substrate material used for the cultivation in this study and genetic properties of our Egyptian oyster mushroom (*P. ostreatus*) as reported by Das & Mukherjee (2007). Glutamate and glutamine are of the primary amino acids involved in the assimilation of ammonia and they play a central role in amino acid biosynthesis by the ready transfer of amino or amide groups, respectively, in the synthesis of other amino acids by transamination or transamidation reactions (Moat *et al*., 2002). It is necessary to mention here that, such a significant increase in free amino acids reported with increase of incubation period of *P. ostreatus* could have been due the hydrolysis (decrease) of protein content (Table 3). Such a profile of proteins and amino acids reported in fungi with consumption of nutrients from the substratum and driving the mold towards decline phase (Gottlieb *et al*., 1968).

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