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Solid-State Fermentation of Agricultural Wastes into Food Through Pleurotus Cultivation

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ABSTRACT

The technical feasibility of using agricultural wastes (mango and date industry wastes) as a substrate for the cultivation of *Pleurotus ostreatus* NRRL-0366 is evaluated. When comparing the biological efficiency of mushroom production, the highest yield of fruiting bodies was obtained using a mixture of date waste and rice straw at a ratio (1:1) (11.96%), followed by a mixture 3:1 (11.16%). The lowest one was the mixture 2:1 (9.19%). Fungus *Pleurotus ostreatus* NRRL-0366 can also be cultivated on mango waste supplemented with rice straw at a different ratio. The best one was the 1:1 mixture (10.18%), whereas the lowest was a mixture 3:1 (6.4%). Comparing the results obtained favored the use of date waste as a substrate for growing *Pleurotus ostreatus* NRRL-0366. Spawn was cultured on three different substrates as follows: Date waste alone (I); 1:1 (by wt) date waste and rice straw (II); 1:1:1 date waste, rice straw, and corncobs (III). Final dry weight and composition of the fruiting bodies are tabulated for the three sets of conditions. Date waste and rice straw mixture (II) is a good source of nonstarchy carbohydrate (67%) and protein (27.44%) containing amounts of essential amino acids, especially lysine and low RNA (3.81%). Elemental analysis were studied in the fruit bodies of the three media.

Index Entries: Fungus pleurotus ostreatus; agricultural industrial wastes; solid-state fermentation; biological evaluation.

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INTRODUCTION

The diet in developed countries should be changed radically and urgently. This is because of the high consumption of animal products, which are too rich in saturated fats and cholesterol. Dietary changes in developed countries should be initiated by health programs that could promote the partial substitution of animal protein with plant protein, such as mushrooms (*1*). It is well known that mushrooms have a high nutritive value, compared to some other vegetables, for protein (*2*), carbohydrates (*3,4*), fat, and fiber (*5*). Mushrooms, especially *Pleurotus*, have the ability to grow on a wide range of unfermented plant wastes. Low labor cost, low initial investment, and less damage of the fruit bodies by diseases and pests (*6*), encouraged the authors to develop new techniques for growing *Pleurotus* in large quantities, permitting not only internal consumption but also exportation.

The purpose of this work was to study the production of edible mushrooms by solid state fermentation, using Egyptian agricultural wastes (mango juice industry wastes, date wastes, rice straw, and corncobs). The chemical composition of the produced fruiting bodies were investigated.

MATERIAL AND METHODS

Media and Culture

Pleurotus ostreatus NRRL-0366, obtained from Agricultural Research Service (Peoria, IL) was maintained in large tubes containing agar-potato dextrose (*7*). *Pleurotus* species were grown in 250-mL flasks containing 100 mL of medium, autoclaved at 121°C for 1 h, cooled, then inoculated with 1 cm^2 of mycelium (containing 10 mg dry wt). The mycelia were incubated at 28°C for 20–25 d. The medium used for all fermentation experiments, unless otherwise stated, contained the following reagents/100 mL media: 20 g mango or date waste, 20 g small pieces of rice straw, 1.25 g $CaCO_3$, and 100 mL distilled water.

The cultured-spawn mixture was placed in polyethylene bags containing fresh sterilized media. The bags were closed and incubated for 15 d at 28°C. The bags were opened, perforated along the upper sides, and humidified daily by spraying with water. Mushroom pinheads appeared on all sides after 5-7 d. Young mushrooms attained normal size in an additional 3-4 d. The first flush of mushrooms were harvested from each bag. A second crop appeared after an additional interval of 7–10 d. This was followed by a third crop. The three crops were collected, dried in an oven at 60°C, ground, and weighed. The yield based on the total quantity of substrates were compared and the biological efficiency was calculated, as described by Gujral et al. (*8*).

Analytical Methods

The Kjeldahl method was used to determine the crude protein content (total $N \times 6.25$). The true protein was estimated by the Lowry method (*9*) using bovine serum albumin as a standard.

The amino acid profile was determined from the acid hydrolysate of the dried fruit bodies using an amino acid analyzer (Beckman 166 detect). Total nucleic acids were extracted and determined by the methods of Burton (*10*) and Herbert et al. (*11*).

Lipid content was determined by extraction of the dried fruit bodies with cold 1:1 mixture of methanol and chloroform (*12*) followed by purification (*13*). The method of Dubois (*14*) was used to estimate the total carbohydrate of the fruit body hydrolysates (*15*). Minerals (Zn, Fe, Mn, Ca, Cu, Mg, Na, K) in ashed samples were measured with an atomic absorption spectrophotometer (*16*). Phosphorous was measured spectrophotometrically (*17*).

RESULTS AND DISCUSSION

The production of edible mushrooms, using a variety of agricultural and forestry residues as substrates (e.g., straw, woodshavings, vegetable fruit wastes, and a host of other cellulosic wastes), has gained prominence in the last few years. Besides being a delicacy, mushrooms are also an important source of food protein for human consumption (*8,18*).

Three crops of *P. ostreatus* NRRL-0366, cultivated on different substrates, were collected, dried, and weighed. The analytical results on the dried fruits were as follows.

Table 1 shows the effect of different types of substrates at different ratios using mango or date wastes supplemented with small pieces of rice straw at ratios 1:1, 2:1, and 3:1, as described in the Methods section. The maximum biological efficiency value (11.96%) was obtained when using date wastes and rice straw at a 1:1 ratio followed by a 3:1 ratio (11.16%). The 2:1 ratio showed the lowest value. *P. ostreatus* also grew well on mango wastes and rice straw (at different ratios), but with a lower biological efficiency value.

The crude protein content in the fruit bodies of *P. ostreatus* cultivated on date waste plus rice straw at different ratios (1:1, 2:1, 3:1) were 27.44, 22.90, and 20.83%, respectively. Gujral et al. (*8*) observed a significant variation in the protein 26.6–35.5% when *P. sajor-caju* was cultivated on different substrates. Also, the crude fat content of fruit bodies grown on the three media varied within reasonable limits, ranging from 2.6–5.76%. The changes in total carbohydrate content of the fruit bodies varied within narrow limits (60.8–64.0%). These results suggest that the substrate does not alter the composition of fruit bodies to any great extent. Further

Table 1
Effect of Substrate Type on the Composition of Fruit Bodies of *P. ostreatus* Calculated at 100 g Dry Wt

Substrates	Total protein	Total carbohydrate	Crude fat	Biol.[a] efficiency, %
Date waste + rice straw				
at a ratio of 1:1	27.44	64.85	5.76	11.96
ditto 2:1	22.90	61.28	2.60	9.19
ditto 3:1	20.83	60.80	5.60	11.16
Mango waste + rice straw				
at a ratio of 1:1	23.90	66.33	2.96	10.18
ditto 2:1	25.99	56.22	9.52	9.80
ditto 3:1	25.44	63.93	11.68	6.40

[a]Biological efficiency % = (weight of dry mushrooms harvested / weight of dry substrate taken) × 100.

Table 2
Chemical Composition of *P. ostreatus'* Fruit Bodies Cultivated on Different Substrates[a]

Substrates	Crude protein, %	True protein, %	Total carbohydrate, %	Pure fat, %	Nucleic acids, % RNA	DNA	T
(I) Date waste alone	27.14	20.00	63.6	2.6	6.03	0.02	6.05
(II) I + rice straw (1:1)	27.44	17.17	67.00	4.1	3.81	0.01	3.82
(III) II + corncobs (1:1:1)	21.88	18.79	68.00	2.2	11.24	0.02	11.26

[a]All data expressed as percentage of dry wt.

studies were carried out by cultivating *P. ostreatus* on date waste alone (I) or supplemented with rice straw at a ratio 1:1 (II) and with addition of ground corncobs at a ratio of 1:1:1 (III) (i.e., II + corncobs).

Table 2 shows that total carbohydrates ranged from 63.6–68.0%. This represents the major constituent of the fruit bodies using different media. These results are in accordance with those mentioned by Bano et al. (*19,20*), who found that, using different species of *Pleurotus*, total carbohydrate content was ranged from 57.4–81.8%.

The pure fat content in fruit bodies of *P. ostreatus* cultivated on the three media were nearly the same in samples I and III (2.6, 2.2%, respectively), whereas in the fruits of medium II, it was about double the others (4.1%). These results were consistent with those mentioned by Bano and Rajaratham (*21*), who stated that the fat content in a different species of *Pleurotus* ranged from 1.08–9.4% on a dry wt basis. On the average, *Pleurotus* species contain 2.85% fat. Since fats and carbohydrates are rarely lacking in a diet, protein constitutes the most critical component contributing to the nutritional value of the food.

It was noticed that the crude protein values in media I and II were 27.14, 27.44% which are higher than that of fruits of medium III (21.88%). These values are higher than that found by Chang (*22*), who reported that on cultivating *Pleurotus* species on rice straw as a substrate, the protein content was 22.5%. Compared to other foods, the protein content of dried *Pleurotus* is about twice as high as that of most vegetables and such standard protein sources as hen (11.9%), pig (12.0%), sheep (14.0%), and cattle dairy (3.5%) (*23*).

Ribonucleic acid was found to be the predominant nucleic acid in all the substrates cultivated on *Pleurotus* as compared to *A. bisporus, L. edodes,* and *V. volvacea.* The DNA and RNA values were reported to be higher in *P. cystictiasus* and *P. sajor-caju* (*24*).

Table 2 shows the difference in total nucleic acids with the different media. It was observed that on using date waste alone (I) or supplemented with rice straw and corncobs (III), the total nucleic acids are high, 6.5 and 11.26%, respectively, but similar to the nucleic acid content of algae, yeast, and other microbes (*25*). While only using date wastes supplemented with rice straw (II), the total nucleic acid was quite low, 50–70% less than the amount found when using the other two media. These results agree with Bano and Rajarathnam (*6*), who reported that the total nucleic acids of *Pleurotus* species ranged from 2.46–2.93%.

The ease with which this mushroom can be cultivated economically on a large scale, within a period of about 8 wk offers the possibility of its use in food. With this object in view, and the knowledge that the nutritional quality of a protein depends on its amino acid composition, which may be characterized as its "potential nutritional value," and on the availability of these amino acids for utilization, the amino acid composition of the fungal biomass of the three media is presented in Table 3. From these data, it can be seen that the essential amino acids, threonine, methionine, leucine, phenylalanine, tryptophan, and lysine are present in high quantities. Sulfur amino acids, which are lacking in the diet, are present in reasonable amounts. The increase in these amino acids was observed in spite of the fact that hydrolyzing the protein samples by heating to 110°C for 24 h was found to decrease the amounts of sulfur-containing amino acids, as indicated by Broderick (*26*). Glycine, histadine, 1/2 cysteine, alanine, and valine are in greater amounts in sample I than in samples II and III.

Table 3
Amino Acid Composition in Fruit Bodies
of *P. ostreatus* Cultivated on Different Substrates

Amino acids	mg A.A./g dry wt		
	I[a]	II[b]	III[c]
Aspartic acid	12.22	12.85	12.19
Threonine	11.30	11.09	18.00
Serine	8.71	7.22	6.96
Glutamic acid	37.15	21.14	26.50
Glycine	11.52	9.4	8.91
Alanine	5.26	2.7	1.74
Valine	7.24	2.48	0.48
Cysteine	2.56	1.98	7.68
1/2 Cysteine	10.30	—	—
Methionine	0.83	0.74	1.15
Isoleucine	4.68	2.11	—
Leucine	10.15	13.87	5.92
Tyrosine	4.28	4.13	7.65
Phenylalanine	6.16	6.05	6.50
Lysine	17.28	26.89	15.49
Histadine	13.32	7.96	9.75
Arginine	8.71	3.15	6.34
Tryptophan	13.46	9.76	11.5
Proline	trace	trace	trace

[a] I: Date waste alone as a substrate.
[b] II: Date waste + rice straw (1:1 w/w).
[c] III: Date waste + rice straw + corncobs (1:1:1 w/w).

These results agreed with those mentioned by Hadar and Cohen-Arazi (27), who found that *P. ostreatus* "Florida" F_6 fruit bodies contained about 17.2 g% dry wt of amino acids, and the essential amino acids were about 14.54%. It can be concluded that mushroom is a good source of protein containing lysine, arginine, and threonine in high concentration. Since mushrooms are considered delicacies, their supplementation with a cereal diet may help to overcome lysine deficiency. Table 4 shows that the fruit bodies of *P. ostreatus* generally contain about 4.7–7.2% ash, which, in turn, represents the minerals. The effect of substrate types on the mineral contents of *P. ostreatus* fruit bodies' ash is shown in Table 4.

It is evident from Table 4 that magnesium and phosphorus are the main constituents of ash in the three media. These results are similar to those of Bano et al. (*28*) using *Pleurotus* species, *Agricus*, and other mushrooms. Sodium represents the third major mineral of fruit bodies. Compared to other minerals, copper, manganese, and zinc are present in low concentrations in all the media.

Table 4
Effect of Substrate Type on the Mineral Content of *P. ostreatus* Fruit Bodies

Substrates	Ash, %	Minerals mg/100 g dry fruit bodies								
		Ca	Zn	Mn	Cu	Na	Fe	Mg	K	P
I (date wastes)	7.2	31.10	2.80	0.18	4.86	114.00	20.88	255.60	26.46	1560.20
II (I+rice straw)	6.3	28.80	8.60	3.60	5.94	94.32	137.34	167.40	16.56	1251.30
III (II+corncobs)	4.7	32.04	3.60	5.76	6.66	123.12	209.88	399.60	30.96	1462.70

Iron is present in high amounts, especially when using media II and III, but iron is present in a low concentration on using medium I. These results are important, since Bano and Rajarathnam (*6*) indicated that iron, present in the *Pleurotus* species, is available and is helped in its utilization by the presence of other enhancing factors, like protein and ascorbic acid. From Table 4, we can conclude that cultivating *P. ostreatus* on a substrate of date waste supplemented with rice straw and corncobs is a good source of important minerals.

It was concluded that a mixture of date waste and rice straw, which are argicultural pollutants, were suitable for the commerical cultivation of *P. ostreatus* NRRL-0366. The fruit bodies harvested from these substrates have good nutritive value, and contain carbohydrates, fats, and proteins containing a substantial amount of essential amino acids.

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