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Effects of supplementing rice straw growth substrate with cotton seeds on the analytical characteristics of the mushroom, *Pleurotus florida* (Block & Tsao)

M.N. Shashirekha, S. Rajarathnam *, Zakia Bano

Department of Fruit and Vegetable Technology, Central Food Technological Research Institute, Mysore 570 013, India

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Abstract

Incorporation of cotton seed powder (3%) with rice straw substrate, while culturing *Pleurotus florida*, enhanced mushroom yield and net protein yield. The free sugars and polymeric carbohydrates were 13.2% and 39.6% in mushrooms grown on rice straw (RS-M) and 8.3% and 19.8% in the mushrooms grown on cotton seed-supplemented rice straw (CS-M). There was a significant decrease in the total dietary fibre content due to cotton seed supplementation. There was a desired softening (~45% reduction in firmness) observed in the CS-M as measured on a Zenken texturometer. The free amino acids showed about 125% increase in the CS-M compared to the RS-M. There was a significant increase in most of the essential amino acids, such as leucine, isoleucine, valine, cysteine, methionine and phenylalanine. The total protein content (Kjeldahl N × 4.38) showed ~90% increase in the CS-M. The total lipids increased by 35% due to cotton seed supplementation of the rice straw substrate; there was a predominance of unsaturated fatty acids, and linoleic acid in particular.

Keywords: Carbohydrates; Cotton seeds; Fatty acids; Free amino acids; *Pleurotus florida*; Rice straw; Total dietary fibre

1. Introduction

Pleurotus florida (Block & Tsao) is an oyster mushroom, cultured on rice/wheat straw for production of economic yields of white, attractive fruiting bodies of pleasant flavour (Rajarathnam & Zakia Bano, 1987; Rajarathnam, Shashirekha & Zakia Bano, 1988; Rajarathnam, Shashirekha, & Zakia Bano, 2001a; Rajarathnam, Shashirekha, & Rashmi, 2003). In a cycle of 20 days, from spawning the growth substrate, mushroom yields up to 140% (fresh mushrooms yield to dry substrate) are produced (Shashirekha, Rajarathnam, & Zakia Bano, 2001). In earlier findings, with other species of *Pleurotus*, it was found that supplementing the growth substrate with oil seed cakes could greatly influence the production of mushroom yields (Rajarathnam, Zakia Bano, & Patwardhan, 1986; Zakia Bano, Shashirekha, & Rajarathnam, 1993); cotton seed, in particular, was found to double the mushroom yield. This finding has a great impact on the technology of mushroom production since, per unit area, without significant prolongation of the spawn run period, the bioconversion efficiency (BCE) of the species in question, could increase twofold. Cotton seed appears to act as a vital supplementation factor for the growth substrate, which otherwise limits production of enhanced mushroom yields (Shashirekha, Rajarathnam, & Zakia Bano, 2002).

In this context, interest was focussed on the chemical nature of mushrooms produced on rice straw substrate

* Corresponding author. Tel.: +91 0821 2515653; fax: +91 0821 2517233.
E-mail address: rajarathnams@yahoo.com (S. Rajarathnam).

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supplemented with cotton seed powder. Accordingly, analysis was undertaken to establish the quantitative distribution of carbohydrates, proteins and amino acids, lipids and fatty acids. Data on the net output of organic compounds of mushroom fruiting bodies, in comparison to the chemical composition of the rice straw as growth substrate, could be of assistance. Improved compositional characteristics of mushroom fruiting bodies, particularly for protein/amino acids, upgrades the nutritional value. Results obtained along these lines of work are presented in this paper.

2. Materials and methods

2.1. Culture maintenance and growth conditions

Monocultures of *Pleurotus florida* were maintained on malt extract (3%) agar (2%) at 25 °C. The culture was subcultured every 3 months. Using this culture, spawn was prepared on wet, chopped rice straw under aseptic conditions (Rajarathnam, Wankhede, & Patwardhan, 1979). A 3 month-old straw spawn was used for all culturing experiments.

2.2. Culturing conditions

Chopped rice straw (2–3 cm long) was soaked in hot water at 60 °C for 15 min to ensure that it was free of contamination (Rajarathnam, Singh, & Zakia Bano, 1979). Wet straw, containing ~75% water, was spawned (at 10% rate on wet weight basis) along with 1% coarse "horse gram" (*Dolichus biflorus*) powder (mill size ca. 0.5–1.0 mm). The mushroom beds were made in perforated polyethylene (50 μm) bags (25 × 40 cm). For each treatment, starting with a dry straw substrate of 2.5 kg, eight replicate mushroom beds were prepared, allotted in randomized blocks (Rajarathnam et al., 1979; Zakia Bano et al., 1993).

2.3. Oil seed cake supplementation

The rice straw substrate ramified by the mushroom mycelium was supplemented with cotton seed powder at 3% of wet rice straw substrate (≡75% water content) and the mushroom beds were again reformed in polyethylene bags as above. This was found optimum to enhance the biomass yield (details in the patent filed (Rajarathnam, Zakia Bano, & Shashirekha, 2001b)).

2.4. Mushroom yield and protein content

The mushroom yields were recorded on the third day of formation of fruiting primordia, and yields were expressed as grammes (fresh) per kilogramme dry rice straw. Total nitrogen in the fruiting bodies (dried at 60 °C) was estimated by the micro-Kjeldahl method (AOAC, 1975). In view of the fact that mushrooms contain a significant amount of non-protein nitrogen in the form of glucosamine in their chitinous cell walls, besides other ninhydrin-positive compounds (Altamura, Robbins, Andreotti, Long, & Hasselstrom, 1967), and the finding of Fitzpatrick, Esselen, and Wein (1946) that the purified mushroom protein hydrolysate contained 11.8% nitrogen rather than the expected 16%, a conversion factor of 4.38 is suggested to be more appropriate for estimating mushroom protein, if the nitrogen content of the chitin and free amino acids is to be ignored (Zakia Bano & Rajarathnam, 1988).

2.5. Estimation of carbohydrates

Freeze-dried material of the mushroom fruiting bodies, grown on cotton seed supplemented rice straw, was subjected to extraction in 70% alcohol and the free and total sugars determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

The alcohol – insoluble residue was hydrolyzed using 72% sulphuric acid and the total sugars estimated, as above, in the hydrolysate, represented the polysaccharide (alcohol – insoluble carbohydrates) (Rajarathnam et al., 1979).

2.6. Measurement of fresh mushroom texture

The degree of hardness was measured on a Zenken texturometer, using a molar tooth shaped plunger, and readings expressed as kg per volt. Caps and stalks were used separately for measuring texture.

2.7. Estimation of total dietary fibre

The total dietary fibre (TDF) content in the mushroom samples was determined according to AOAC (1970), in three separate experiments.

2.8. Extraction, fractionation and estimation of free amino acids

The 70% alcohol – soluble fraction of the freeze-dried mushroom samples was fractionated on a Dowex – $50H^+$ column into sugars and amino acids, as described by Rajarathnam, Wankhede, and Zakia Bano (1987).

The amino acid fractions were pooled, ammonia expelled under flash evaporation and pH brought down to 4.3 using 2 N HCl, and the whole concentrated under low pressure. Twenty microlitres were injected into a Schimadzu Automat Analyzer. The resolved peaks were identified and computed with the standards run under similar conditions.

2.9. Extraction and estimation of lipids and fatty acid analysis

The total lipids from the freeze-dried mushroom samples were extracted by the method of Folch, Lees, and Solane-Stanely (1957), using chloroform–methanol mixture in a ratio of 2:1 (v/v). Methyl esters were prepared from the total lipids by the method of Craig and Murthy (1959). Fatty acid analysis was performed by gas–liquid chromatography, run on a Hewlett Packard 5750 chromatograph fitted with a flame ionization detector. The glass column (2.15 m × 0.535 cm) was packed with 15% diethylene glycol succinate on 80–100 mesh acid – washed chromosorb – W. The carrier gas was nitrogen at a flow rate of 32 ml min^{-1}. The column temperature gradient was 4 °C min^{-1} from 190 to 240 °C. Heptadecanoic acid (17:0) was used as an internal standard.

Identification of the peaks was based on comparison of retention times of samples and standards. The amount of each fatty acid was calculated by the data – handling and control unit of the instrument. All determinations were done in triplicate and mean values reported.

3. Results and discussion

Extra supplementation of the rice straw growth substrate with cotton seed powder enhanced mushroom yields and protein contents. Besides this, a number of other changes observed in CS-M compared to RS-M are considered here and discussed.

There was a decrease in the carbohydrate content of the CS-M (Table 1). About 38% and 50% reductions in alcohol – solubles and alcohol – insolubles, respectively, were recorded in CS-M compared to RS-M. This is possibly associated with a significant increase in the protein content of the CS-M, an almost twofold increase in the protein content being observed (Table 4).

Supplementation of cotton seed powder to the rice straw substrate caused a decrease in firmness of the mushrooms by 45%, as measured on the Zenken texurometer, compared to RS-M (Table 2). Apparently, the CS-M had a softer texture, while conducting sensory evaluation trials, compared to the RS-M. Supporting evidence was obtained by analysis of the TDF content.

Table 1
Carbohydrates (%)[a] of RS-M and CS-M

Carbohydrates	RS-M	CS-M
Alcohol – solubles (A)	13.2 (1.10)[b]	8.25 (0.75)
Alcohol – insolubles (B)	39.6 (2.15)	19.8 (1.72)
Total (A + B)	52.8	28.1

[a] On dry weight basis.
[b] Each of the values is the mean of three separate experiments. Figures in parentheses represent standard deviations from the mean.

Table 2
Dietary fibre and texture of fresh RS-M and CS-M

Carbohydrates		RS-M	CS-M
Total dietary fibre (g kg^{-1} dry matter)	Cap	382 (36.2)	202 (19.0)
	Stipe	495 (45.6)	269 (23.2)
Texture (kg V^{-1})	Cap	11.20 (10.5)	6.16 (5.9)
	Stipe	24.64 (23.1)	12.12 (11.20)

Each of the values is the mean of three separate experiments. Figures in parentheses represent standard deviations from the mean.

Table 3
Free amino acids[a] in RS-M and CS-M

Amino acid	RS-M	CS-M
Aspartic acid	0.03	–
Threonine	0.77	–
Proline	0.60	–
Glycine	0.29	0.26
Alanine	0.45	0.54
Cysteine	1.16	2.38
Valine	1.30	4.73
Isoleucine	0.28	2.79
Leucine	0.43	1.37
Tyrosine	0.22	2.25
Phenylalanine	0.36	2.89
Histidine	0.02	2.17
Arginine	0.01	0.10
Methionine	–	0.76
	8.92	20.2

Each value is the mean of three separate experiments. The standard errors were estimated to be within ±10% of the mean values.
[a] mg per 100 mg dry sample.

There was a significant decrease in the TDF content of the CS-M, whereas the RS-M had a higher TDF content relative to other mushrooms, such as *Agaricus* and *Volvariella* (Zakia Bano & Rajarathnam, 1988; Rajarathnam & Zakia Bano, 1991).

The free amino acid content in CS-M showed about 125% increase, compared to RS-M (Table 3). Possibly, this is linked to the contribution of several amino acids from the cotton seed powder supplement and any desired protease and *trans*-aminase activities, necessary in this regard to convert substrate proteins, are well known to be operating in *Pleurotus* species (Rajarathnam & Zakia Bano, 1989). A most noteworthy feature and point of practical importance is that the increase in the amino acids is contributed by most of the essential amino acids necessary for the human body. This result underlines the biotransformation implications of employing a mushroom culture for transforming the substrate amino substances.

The total lipid showed about a 35% increase in CS-M (Table 4). Linoleic acid was the predominant unsaturated fatty acid that increased considerably in CS-M (Table 5). One of the main reasons why mushroom is a favoured item for human nutrition is its abundance of unsaturated fatty acids and its hypocholesterolemic

Table 4
Calculated energy values (EV[a]) of RS-M and CS-M

Constituents	RS-M (%)	EV	CS-M (%)	EV
Carbohydrates	52.8	184	28.1	97.7
Protein	19.6	51.3	37.2	97.4
Fat	4.0	33.4	5.4	45.1
Total EV		269		240

The values were obtained by multiplying the percentage of carbohydrates, crude protein, and fat by Atwater conversion factors 3.48, 2.62 and 8.37, to correct for the reduced digestibility of carbohydrate (85%), proteins (70%) and fat (90%). (Zakia Bano & Rajarathnam, 1988).
[a] kcal/100 g.

Table 5
Fatty acid composition of RS-M and CS-M

Fatty acids (%)	RS-M	CS-M
Myristic acid (C 14 : 0)	2.87	–
Palmitic acid (C 16 : 0)	12.3	3.12
Palmitoleic acid (C 16 : 1)	0.50	0.93
Oleic acid (C 18 : 1)	13.1	12.9
Linoleic acid (C 18 : 2)	71.2	83.0
Saturated	15.2	3.12
Unsaturated	84.8	96.9

Each value is the mean of three separate experiments. The standard errors were estimated to be within ±10% of the mean values.

property (Rajarathnam, Shashirekha, & Zakia Bano, 1992). Linoleic acid, in turn, is well known to be an essential fatty acid, not synthesized by the human body (Zakia Bano & Rajarathnam, 1988). It is also known to serve as the basis for production and transformation of mushroom flavour constituents (Rajarathnam, Zakia Bano, Berger, & Drawert, 1990).

From a nutritional point of view, the energy value of the carbohydrate content of CS-M is low compared to RS-M; however, the energy values of protein and lipids of CS-M are comparatively high (Table 4). Excluding carbohydrates, because of their non-starchy and poor digestibility nature (because of predominance of β-glucosidic linkages), proteins and lipids are of a quality desirable for human nutrition. Therefore, the nutritive value of CS-M is enhanced due to supplementation with cotton seed powder; however, the net energy value is relatively less. This is in favour of the assertion that "mushrooms are low calorie foods" (Zakia Bano & Rajarathnam, 1988). This is an illustrative example of a fungal culture used for enhanced efficiency of bioconversion and biotransformation of a supplemented growth substrate into a nutritionally upgraded edible biomass.

4. Conclusion

The present investigation clearly demonstrates the impact and influence of the composition of a growth substrate on the chemistry of mushroom crop produced. Rice straw, being dominated by carbohydrates, has a limited content of nitrogen = 0.8% (≡5% protein, based on 6.25 conversion factor) (Rajarathnam et al., 1979) whereas the mushroom fruiting bodies, on a dry basis, contain ~22% protein, and 63% carbohydrates (Rajarathnam, Shashirekha, & Zakia Bano, 1998). As eukaryotes, mushrooms totally depend on growth substrate for nitrogen content (Rajarathnam & Zakia Bano, 1989). Cotton seed powder supplementation, in trace amounts, doubles the mushroom yield through an activated secretion of cellulolytic and ligninolytic enzymes, favouring active biodegradation of rice straw substrate (Zakia Bano et al., 1993). The biomass, produced as mushroom fruiting bodies, shows clear chemical differences from the unsupplemented rice straw substrate. Small degrees of cotton seed powder supplementation modulate the mushroom chemistry in such a way that a doubling of the amino compounds has relatively reduced the carbohydrate content. The 125% increase in FAA is a very vital observation of the present study. This, possibly reflects the adsorption and absorption of the amino acids from the cotton seed powder supplemented rice straw substrate. This implies that chemistry of growth substrate has a direct influence on the chemical composition of the mushroom fruiting bodies. Increase in unsaturated fatty acid, particularly linolenic acids, is of considerable importance in upgrading the nutritive value of the mushroom crop produced.

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