Original Research Article

EFFECT OF DIFFERENT SUBSTRATES ON NUTRITIONAL COMPOSITION OF SHIITAKE MUSHROOM (*LENTINULA EDODES)*

ABSTRACT

Lentinula edodes(Berk.) Peglar, the shiitake mushroom, is worldwide one of the most widely cultivated mushrooms. The cultivation of edible mushrooms is a biotechnological process that uses various residues to produce food of high nutritional value. Two strains of *Lentinula edodes* (DMR-356 and DMR-35) were cultivated on basal substrates wheat straw and poplar sawdust alone and in combination with supplements (Wheat bran, Rice bran and Maize Meal). The fruit body of shiitake mushroom was analyzed for crude protein, crude fiber, Moisture content,crude fat,total ash and total carbohydrates. DMR-356 strain proved best for high nutritional value crude protein (22.44%), crude fat(3.97%), crude fibers(7.69%), total ash(7.75%), and total carbohydrates(63.50%) with substrates wheat straw + wheat bran. The least effective substrate was sawdust alone.

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Comment [a2]: Add some information about conclusion

Key words: *Lentinula edodes*, substrates, supplement, proximate composition

Introduction

Shiitake mushroom is a rich source of protein, containing most of the essential amino acids, minerals and vitamins. *Lentinusedodes* is the first medicinal macro-fungus to enter the realm of modern biotechnology. Its popularity in the global market is attributed not only to its nutritional value but also fortherapeutic applications by Bisen*etal*. (2010). Several by-products of shiitake mushroom have been successfully used for the activation of immunologic system and to improve human health due to its antioxidant and antitumor properties by Wasser and Weis (1999). *L. edodes*is capable of generating stamina, curing colds and may play a role in the cure and prevention of chronic diseases such as heart disease, cancer and AIDS (Dewangan 2005).Cultivation of mushrooms mainly depend on the substrates which act as a source of nutrition. Presently, lignocellulosic substrates along with the various types of farm residues from agriculture, horticulture, forestry, textile and wood industry are explored for cultivation of various types of mushrooms. Sawdust is the most popular basal ingredient used as substrate to produce shiitake by Miller and Jong (1987)along with other such as wheat straw, corn cobs and their mixtures. Regardless of the main ingredient used, starch-based supplements such as wheat bran, rice bran, millet, rye or corn are also added in different proportion to enhance the productivity of shiitake mushroom by Royse (1996), Ivan *et al.*(2003).Addition of organic supplements to the substrate is a promising way of getting higher mushroom yield as supplements enrich the nutrient status of the substrate.

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Despite sporadic research efforts to standardize its cultivation technology, this mushroom has so far not been exploited for its commercial production in India. Keeping in view the agricultural residues available in Jammu, there is an ample scope for its introduction and commercial exploitation. The purpose of the present study was to evaluate thevarious substrates to assess the nutritional value of shiitake mushroom.

Material and Methods

For the study, two strains of *Lentinula edodes* (DMR-356 and DMR-35) were collected from Directorate of Mushroom Research (DMR), Solan. The cultures of these strains were maintained on Potato dextrose agar (PDA) medium and spawn was also prepared on wheat grains.

*Substrate preparation:*Wheat straw, poplar sawdust and sugarcane bagassewere assessed as substrates for the cultivation of *L. edodes*. Wheat straw and sugarcane bagassewere chopped into 3 to 4 cm long pieces. Afterwards, all the substrates were separately soaked in water for

overnight after treating simultaneously with carbendazim (75ppm) and formaldehyde (500ppm). The substrates were taken out of water and dried to maintain 60-70 per cent of moisture. Different supplement *viz*., wheat bran, rice bran, maize meal and chicken manure @ 20 per cent on dry weight basis of each substrates and 2 per cent calcium carbonate were added into each substrate to maintain the pH (5.0) and mixed thoroughly. Wheat straw and saw dust substrate were also taken individually. On dry weight basis, each substrate mixtures (900g) was filled in autoclavable polypropylene bags (9×12inch) and plugged with non-absorbent cotton with glaze paper to protect loss of moisture content from inside the bags (Islam *et al*. 2016). Filled bags were sterilized at 121°C for 3 hours (Christopher and Custodio 2004) and were cooled at room temperature. The substrates were inoculated with 20 g of spawn of *L. edodes*.Each treatment was replicated thrice under CRD factorial design. Standard methodology was followed to raise the shiitake mushroom.

*Estimation of nutritional composition of fruiting bodies of Lentinulaedodes:*Thefruiting bodies of two strains of *L.edodes* harvested from different substrateswere sundried and powdered in grinder andthen analysedfor moisture content, crudeprotein, crude fat, crude fibres, total ash and total carbohydrates.

*Determination of moisture content:*Moisture content in each substrate was determined by hot air oven method (AOAC, 1995). 10g of fresh mushroom sample was dried at 100±2^{0}C for 8-12 hoursin hot air oven and moisture content (%) was calculates as:

$$\text{Moisture content (\%)} = \frac{\text{Weight of original sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of original sample (g)}} \times 100$$

*Estimation of crude protein (CP):*Total nitrogen was measured by Kjeldahl method (AOAC, 1980). Known quantity of sample was taken in Kjeldahl flask and digested with concentrated 25 ml of H_2SO_4. After digestion, the contents were cooled and volume was made to 250 ml. About 100 ml was collected into a conical flask containing 10 ml of 2 per cent boric acid solution having mixed indicator (0.1% methyl red and 0.1% bromocresol green in the ratio of 2:1 in absolute alcohol). The distillate was then titrated against standard sulphuric acid solution

$$\text{N (\%)} = \frac{0.014 \times \text{Volume of N/100 } H_2SO_4 \text{ used} \times \text{Volume made (ml)}}{\text{---------- x 100}}$$

Comment [a5]: Write the equations in proper format

Aliquot taken (ml) X Sample taken (g)

The crude protein (%) sample was calculated by multiplying the N content with factor 6.25.

*Determination of total solids (%):*Total solids (%) in each treatment were determined by hot air oven method in which 10 g of sample in triplicate was weighed and dried at 70±2°C for 12 hours. The left-over residue was determined to calculate the total solids (%).

*Estimation of total ash (%):*Known quantity of sample was taken in pre-weighed silica crucible. After charring the sample (till the smoke disappeared), the crucible was kept in muffle furnace for ignition at 550°C for 2-3h. The crucible was removed on cooling and kept in a desiccators and weighed to find out weight of ash. The ash content was calculated as:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of total carbohydrates:Total carbohydrates were calculated by adding nitrogen free extract and crude fiber contents.

*Statistical Analysis:*The data were statistically analyzed using the R-software at a significance level of 5%.

Results and Discussion

*Nutrient composition of fruiting bodies of Lentinula edodes:*Significant variations were observed in the chemical composition (crude fat, crude fibre total ash and total carbohydrates) of fruiting bodies of *L. edodes*grown on different substrate along with supplements(Table 1). However, there was non -significant difference in the composition of total ash and crude protein content of the fruiting bodies. The highest crude protein content 22.44 and 20.63 per cent was observed in fruiting bodies harvested from wheat straw + wheat bran (20%), however lowest crude protein content of 16.21 and 16.01 per cent was observed on poplar sawdust of DMR-356 and DMR-35 strains of *L. edodes*, respectively.Our results are in concurrence with the finding of Osadchya*et al*. (2002) and Upadhayay and Rai (1999), who reported 23.24 per cent protein in submerged mycelia of *L. edodes*and *L. squarrosulus.* Dewangan (2005) also reported protein

content of 20.65 per cent in Chinese strain of *L. edodes*. Mshandete and Cuff (2007) reported that protein content of edible mushrooms gets influenced by the substrates on which they are grown. It has been reported that not only protein content in fruiting body but also nature of protein is affected by the substrate (Wang *et al.* 2001). The difference in protein content of mushroom grown on different substrates could be due to the varying nitrogen content (Hoa*et al*. 2015). Use of particular strains, time of analysis, choice of substrate used and stage of development of mushrooms are the factors responsible for variation in the protein content of mushroom (Bano and Rajarathnam 1988). Protein content of mushrooms depend on biological, chemical composition and the C:N ratio of the substrates (Ragunathan and Swaminathan 2003).

Comment [a6]: Avoid extra spacing

Moisture per cent in fruiting bodies harvested from different substrates with supplements ranged from 87.00 to 89.19 and 86.90 to 88.44 per cent in DMR-356 and DMR-35, respectively. Maximum moisture content (89.19%) was observed in wheat straw + wheat bran (20%) and the minimum of 87.0 per cent was observed with fruiting bodies grown on poplar sawdustin DMR-356, whereas, maximum moisture content (88.44%) was observed from wheat straw + wheat bran (20%) substrates; however, minimum (86.90%) was from poplar sawdust. Variation in moisture content of fruiting bodies could be attributed to the water holding capacity of substrates. Moisture content also gets influenced by growth stage of the mushroom, growing environmental conditions, mushroom strains and post -harvest environments (Kurtzman 2005).

Comment [a7]: Use percent symbol

Maximum crude fat (3.97%), crude fiber (7.69%) and ash content (7.75%), were observed in fruiting bodies of DMR-356, grown on wheat straw + wheat bran (20%) while minimum crude fat (2.53%), crude fiber (5.34%) and ash content (6.24%) were observed in fruiting bodies harvested from poplar saw dust substrate. Similar trend was observed in DMR-35 strain in which maximum crude fat (3.28%), crude fiber (7.11%) and ash content (7.56%), were observed from wheat straw + wheat bran (20%) substrate while minimum crude fat (2.45%), crude fibre (5.09%) and ash content (6.08%), were observed in fruiting bodies harvested from poplar saw dust substrate. Maximum total carbohydrates (58.95%) of *L. edodes* strain DMR-356 were observed in fruiting bodies grown on poplar saw dust substrate and minimum of 49.25% were observed on wheat straw + wheat bran (20%) substrate. Similar trend was observed in case of DMR-35 strain where maximum total carbohydrates (60.46%) were observed on fruiting bodies grown on poplar saw dust substrates and minimum total carbohydrates (52.62g) were

observed in fruiting bodies grown on wheat straw + wheat bran (20%) substrate. These results are in accordance with the results by Regula and Siwulski (2007), who reported that ash content (7.04%), total carbohydrates (66.0%), soluble fiber content (2.01%) and fat content (2.89%) were observed in *L. edodes.*Dewangan (2005) also reported similar results for crude fat, crude fibre, carbohydrate and ash content of *L. edodes* strain.

Table 1 Nutrient composition (per cent DM) of fruiting bodies of *Lentinula edodes* strains grown on different substrates

Treatment	Moisture (%)		Crude protein (%)		Crude Fat (%)		Crude Fibers (%)		Total Ash (%)		Total Carbohydrates (%)	
	Strains		Strains		Strains		Strains		Strains		Strains	
	DMR-356	DMR 35	DMR 356	DMR-35	DMR 356	DMR 35	DMR 356	DMR 35	DMR 356	DMR 35	DMR 356	DMR 35
Wheat Straw + Wheat Bran	89.19	88.44	22.44	20.63	3.97	3.28	7.69	7.11	7.75	7.56	49.25	52.62
Wheat Straw +Rice Bran	87.76	87.65	21.75	19.79	3.73	3.09	7.28	6.91	7.26	7.21	51.22	54.24
Wheat Straw + Maize Meal	87.43	87.58	21.47	19.71	3.21	2.88	6.71	6.47	7.17	7.09	52.70	55.10
Wheat Straw	88.48	87.31	19.38	18.11	2.86	2.59	6.11	6.11	7.13	7.00	55.68	57.46
Sawdust + Wheat Bran	88.10	87.92	18.67	17.57	2.63	2.47	5.76	5.21	6.29	6.16	57.84	59.80
Sawdust + Rice Bran	87.24	86.99	18.22	17.23	2.53	2.45	5.34	5.09	6.24	6.08	58.95	60.46
Sawdust + Maize Meal	87.13	87.19	17.80	16.87	2.19	2.14	4.88	4.52	6.14	5.95	60.28	61.81
Sawdust	87.00	86.90	16.21	16.01	2.00	2.00	4.24	4.04	5.35	5.12	63.50	64.23
C.D ($p \leq 0.05$)	Strains = NS Substrates = 0.65 Interaction = NS		Strains = NS Substrates = 0.31 Interaction = NS		Strains = 0.70 Substrates = 0.17 Interaction = 0.24		Strains = 0.14 Substrates = 0.14 Interaction =0.21		Strains = 0.68 Substrates = 0.68 Interaction = 0.09		Strains = 1.76 Substrates = 1.76 Interaction = 0.46	

Conclusion

It may be concluded from the study that *L. edodes* strain DMR-356 proved to be the best strain for nutritional composition. Among the different substrates used alone and in combination with different supplements for the cultivation of shiitake mushroom, wheat straw + wheat bran

(20%) proved to be the best substrate followed by wheat straw + rice bran (20%) for commercial cultivation of shiitake mushroom.

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Comment [a8]: References should be as per guidelines, use some latest references.

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