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Effect of different substrate and supplement on nutritional contents of *Pleorotus Floridanus* Singer

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Abstract

Current manuscript aim to find out effects in nutritional contents of *Pleurotus floridanus* and substrate best favors the growth of *Pleurotus floridanus*. The experiment was designed to cultivate *Pleurotus floridanus* on wheat straw and paddy straw in control and supplemented environment of wheat bran (10%) and lime (4%). Results declare maximum moisture content (92.35%), Protein (25.37%), Carbohydrate (32.57%) was recorded on Paddy straw + wheat bran15%. The highest amount of Ash (8.5%) and Crude fiber (9.32%) was recorded on Wheat straw + Wheat bran15% + lime 4%. The maximum Crude fat (2.86%) was found on wheat straw. In case of mineral the maximum amount of calcium (2.6mg/100g), magnesium (23mg/100g) and potassium (335mg/100g) was recorded on Paddy straw + wheat bran15%. The maximum amount of iron (3.35mg/100g) was obtained from paddy straw and zinc (3.7mg/100g) on wheat straw + wheat bran15% + lime4%. The maximum amount of Sodium (35mg /100g) was recorded on Oyster mushroom grown on wheat straw. Owing to these findings it is concluded that Paddy straw + wheat bran15% is a best substrate for cultivation of *Pleurotus floridanus*.

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Introduction

Oyster Mushrooms are also called as vegetarian meats because they contain high quantity of minerals and proteins (Khan *et al*., 1981). These proteins are intermediate in between that of vegetable and meat regarding their nature (Kurtzman, 1976). *P. florida* can provide more proteins in a very short time than any other crop (Gupta, 1986). Their high yield and biological efficiency are due to their high nitrogen and protein contents (Peksen and Yakupoglu, 2009; Adebayo *et al*., 2009; Fanadzo *et al*., 2010). *P. florida* contains 18 essential amino acids such as methionine, isoleucine, lysine, glutamic acid, cysteine, aspartic acid phenylalanine, tyrosine, tryptophan, valine, arginine, histidine, alanine, glycine serine and proline (Djarijah and Djarijah, 2001). They also contain vitamins like niacin, riboflavin and thiamin. Also, some minerals like ferrous sulfate, phosphorus, sodium and calcium are found in *P. florida* (Pandey and Ghosh, 1996).

Cultivated mushrooms are rich in protein, nutrient, Vit B, Vit D, Vit K, Vit A, and Vit C. (Manzi *et al*., 2001). However, the nutritional value is affected by many factors like, method of cultivation, composition, growth substrate, time of harvesting, specific portions of the fruiting body and time of the interval between harvests (Benjamin, 1995**).** Therefore the present study deals with the cultivation of *Pleurotus floridanus* on different substrates to determine their effect on nutritional composition.

Materials and methods

Proximate analysis of dry fruiting body of *Pleurotus floridanus* grown on different substrates was evaluated. The analysis include Protein, Ash, Crude fat, Crude fiber, Carbohydrate and Minerals (Iron, Zinc, Calcium, Magnesium, Sodium, Potassium) was determined by using AOAC (2000, 2003) method in lab of Agricultural Chemistry, KP, University of Agriculture Peshawar.

Determination of moisture content

The oven drying method was used for determination of moisture content.

A 20g sample of fresh fruiting body was taken in a Petri dish (W_1) and kept it in the oven at 100°C for 6-12 hour until the mushroom becomes fully dried. The Petri dish was then placed in desiccator for 30 minutes to cool and weight again (W_2). Following formula were used for calculation of percent moisture.

$$\%\text{Moisture} = \frac{W1 - W2 \times 100}{\text{Weight of sample}}$$

W_1 = initial weight of petri dish + sample

W_2 = Final weight of petri dish + sample

Determination of ash

First the clean empty crucible were weighted (W_1), then two grams of each sample of powdered dried mushroom were taken in crucible (W_2). The sample was charred by the help of burner. The crucible was then placed in a muffle furnace for 4 hours at 550°C. The white appearance of ash showed the complete oxidation of all organic compounds of the sample. After completion of time the muffle furnace were switched off and the crucible were transferred to desiccator for cooling and then weighted (W_3). Following formula were used for calculation of percent moisture.

$$\%\text{Ash} = \frac{\text{Difference in Weight of Ash}}{\text{Weight of sample}} \times 100$$

Difference in weight of Ash= W_3-W_1

Determination of Crude Protein

Procedure

The protein content in the sample of the mushroom was determined by Kjeldhal method. Two grams of dried sample were taken in digestion flask, then 10 ml concentrated H_2SO_4 and 8g of digestion mixture i.e. $K_2SO_4.CUSO_4$ (8:1) were added. For digestion the flasks were kept on a heater until a Blue green color appeared. It took 2 hrs for completion. The digest was allowed to cool and transfer to 100ml volumetric flask and distill water was added to make such volume. Markam Still Distillation apparatus ((Khalil and Manan, 1990) was used for distillation of digest sample. 10ml of the digest sample and 10ml of 0.5 N NaOH was taken in distillation tube.

Distillation was run for 10 min and synthesized NH_3 was collected as NH_4OH in a conical flask having 20ml of 4% boric acid solution with 2-3 drop methyl red indicator. During distillation yellowish color was appear due to NH_4OH. Titration of distillate was occurring against stander 0.1 N HCl until a pink color appeared. Following formula was used for determination of percent crude protein in the sample.

% Crude Protein = 6.25* x %N (*. Correction factor)

$$\%N = \frac{(S - B)xNx0.014xDx100}{\text{Weight of the sample} \times V}$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of sample after digestion

V = Volume taken for distillation

M.W = 0.014 = Milli equivalent weight of Nitrogen

Determination of crude fat

The Soxhlet apparatus was used for determination of crude fat. Two grams of moisture free sample was taken in thimble and kept in extraction tube. Weighted, cleaned receiving beaker was filled with petroleum ether (B.P 40-60°C) and fitted with apparatus. The water and heater were turned on to start the extraction. The ether was evaporated under fume hood and removed the sample and kept in the oven for 30 minutes at 105°C for drying. The sample was then kept in a desiccator for Cooling and then weighted. Following formula were used for determination of percent crude fat.

$$(\%) \text{ Crude fat } = \frac{(W_2 - W_1) \times 100}{\text{Weight of sample}}$$

Where

W_1 =weight of beaker

W_2= weight of beaker + oil

Determination of crude fiber

Two gram fat free sample was taken in a 1000ml capacity beaker and 200ml of 1.25% H_2SO_4 was added and boiled for 30 min under continuous thrilling. The hot water was used for washing of sample, and washed 2-3 times to become acid free. The residue was again took in 1000ml beaker and added 200ml of 1.25% NaOH and again boiled for 30 minutes. The boiled sample was again washed with hot water to become alkaline free. The residues in the crucible were kept in oven for 3-4 hrs at 100°C in order to become completely dried and kept in dessicator to cool and weighed (W_1). The sample was then introduced in the muffle furnace and placed for 3-4 hrs at 550°C until a gray ash was obtained, and then kept in dessicater for cooling and weighted (W_2). Following formula were used for determination of percent Crude fiber.

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2 \times 100}{\text{Wt. of sample}}$$

Total carbohydrate estimation

The carbohydrate was determined after analysis of all other items by the following equation.

Carbohydrate (%) = (100 – % moisture + % crude protein + % crude fat + % ash+ % crude fiber).

Mineral determination

Atomic absorption spectrometry and Flame photometer was used for determination of mineral contents of mushroom.

Wet digestion of sample

For wet digestion of mushroom sample, two grams of the sample were taken in a digestion glass tube and added 12ml of HNO3 and kept for a night at normal temperature. Four milliliters of per Chloric acid ($HClO_4$) were added to the mixture and placed on fume blocks for digestion. Gradually the temperature rises from 50-300°C.

The digestion take 70-80 min for completion and the appearance of white fume occurred. The mixture then allows to cold and introduce to 100ml volumetric flask and such volume were made with distilled water. The wet digested solution was introduced to plastic bottles and labeled then it could be used for mineral determination.

Determination of Calcium (Ca), Magnesium (Mg), Iron (Fe) and Zinc (Zn) by Atomic Absorption Spectrometry

Procedure

The mineral content of digested samples was analyzed by Atomic Absorption Spectrophotometer (Hitachi model 170-10). For each mineral separate electrode lamps were used.

The instrument was run for stander solution of each mineral before determination for accurate working. The dilution factor of all the minerals was 100. For determination of Mg, further dilution occurred by taking 0.5ml of original solution and added enough distilled water to make the volume 100ml.

$$M.W = \frac{\text{Absorbency (ppm)} \times V \times D}{\text{Wt. of sample}}$$

*M. W = Milli equivalent weight

Estimation of potassium (K) and sodium (Na) by flame photometer

The flame photometer was used for analysis of Na and K in the sample.

The Na and K were determined from the same wet digested solution used in AAS. For both Na and K Stander solutions of 20, 40, 60, 80 and 100 milli equivalent/L were taken using following formula for determination of Na and K.

$$M.W = \frac{\text{Absorbency (ppm)} \times V \times D}{\text{Wt. of sample}}$$

*M.W = Milli equivalent weight

Results

Proximate analysis of dry fruiting body of *Pleurotus floridanus* grown on different substrates was evaluated. The results declares that maximum moisture content was found in Paddy straw + wheat bran 15% ranging in value of 92.35% and minimum in Wheat straw + wheat bran15% + lime 4% ranging in value of 88.35%. The highest ash content was found on wheat straw + wheat bran15% + lime 4% having a value of 8.5% and lowest on Paddy straw + wheat bran 15% with a value of 7.2%.

Table 1: Moisture, Ash, Protein, Fat, Fiber and Carbohydrate of *Pleurotus floridanus* on different substrate.

Substrate	Moisture%	Ash%	Protein%	Fat%	Fiber%	Carbohydrates%
Wheat straw	89	7.8	14.87	2.86	7.4	21.93
Wheat straw + wheat bran 15%	91.85	7.4	22.75	1.98	6.23	30.21
Wheat straw + wheat bran 15%+ lime 4 %	88.35	8.5	18.37	1.42	9.32	25.96
Paddy straw	90.11	7.5	15.75	2.48	7.8	23.64
Paddy straw + wheat bran 15%	92.35	7.2	25.37	1.65	6	32.57
Paddy straw + wheat bran 15% + lime 4%	88.61	8	19.25	1.21	9.12	26.19

*mg/100gm dry mushroom.

Analysis of protein contents showed that maximum protein was found in Paddy straw + wheat bran 15% (25.37%) and minimum on wheat straw (14.87%).

The fat content on wheat straw was 2.86% being highest and lowest on Paddy straw + wheat bran 15% + lime 4% with a value of 1.21%.

The highest amount of crude fiber was found on wheat straw + wheat bran15% + lime 4% with a value of 9.32% followed by Paddy straw + wheat bran 15% + lime 4% with a value of 9.12%. The maximum amount of carbohydrate was on Paddy straw + wheat bran 15% having value of 32.57% and minimum on Wheat straw (21.93%). These results are given in Table 1.

Using techniques of atomic absorption spectrometer and flame photometer for determination of mineral contents in mushroom, the values were evaluated such that calcium ranged from 91-2.6 mg/100g). The amount of magnesium ranged from (12-23mg/100g). The maximum Iron (Fe) was observed on paddy straw (3.35mg/100g).

Mineral analysis in mushroom for zinc showed values ranging from 1.1 to 3.7 mg/100 gram. The result showed that maximum amount of sodium was recorded on wheat straw (35 mg/100g). The amount of potassium ranged from 275-335mg/100g. These findings are given in the table 2.

Table 2. Mineral analysis for content like Ca, Mg, Fe, Zn, Na and K of *Pleurotus floridanus* on different substrate.

Substrate	Ca*	Mg*	Fe*	Zn*	Na*	K*
Wheat straw	1	15	3	1.1	35	290
Wheat straw + wheat bran 15%	2.05	19.5	2.25	2.15	15	320
Wheat straw + wheat bran 15%+ lime 4 %	1.7	12	1.85	3.7	20	300
Paddy straw	1.25	17	3.35	1.35	25	275
Paddy straw + wheat bran 15%	2.6	23	2.6	2.55	10	335
Paddy straw + wheat bran 15% + lime 4%	1.5	13	1.45	2.95	10	315

Discussion

The maximum moisture content was found in Paddy straw + wheat bran 15% and minimum in Wheat straw + wheat bran15% + lime 4% similar findings found by Manzi *et al.* (1999) and Patil *et al.* (2010). The highest ash content was found on wheat straw + wheat bran15% + lime 4% and lowest on Paddy straw + wheat bran 15%. Same was reported by El –Kattan *et al.* (1991). However Jonathan *et al.* (2006) found 5.3% ash in wild *P. florida*. The maximum protein was found in Paddy straw + wheat bran 15% and minimum on wheat straw. Our result shows consistency with those of Mandhare (2000), Patil *et al.* (2010) and Mane *et al.* (2007). Fat contents on wheat straw being highest and lowest on Paddy straw + wheat bran 15% + lime 4%. Same was reported by Patil *et al.* (2010) and Jonathan *et al.* (2006). The highest amount of crud fiber was found on wheat straw + wheat bran15% + lime 4% followed by Paddy straw + wheat bran 15% + lime 4%. Such result were observed by Ahmed *et al.* (2009) who reported crude fiber of *Pleurotus florida* on soybean and rice straw. Validity of current findings confirms with findings of Bonatti *et al.* (2004), Khydagi *et al.* (1998), Sharma & Madan (1993) and Singh *et al.* (2003).

The maximum amount of carbohydrate was on Paddy straw + wheat bran 15% and minimum on Wheat straw, similarly Rashad *et al.* (2009) reported 20.9-33.0% carbohydrates in *P. ostreatus* grown on *Citrus limonium* and *Carica papaya* wastes. Patil *et al.* (2008) found carbohydrate on soybean straw and 53.87% carbohydrate on wheat straw + paddy straw. These variations may be due to the reasons of different types of substrate and species.

Calcium Content ranges from 91-2.6 mg/100g. Similar results observed by Akindahunsi & Oyetayo (2006) and Ahmed *et al.* (2009). The amount of magnesium results show consistency with Alam *et al.* (2007) and Bhattacharjya *et al.* (2015). Maximum Iron (Fe) was observed on paddy straw. According to Patil *et al.* (2010) cultivation of *P. ostreatus* on different agro-waste the amount of iron ranged from 13.13 to 15.62 mg/100g. Similar fluctuation of iron content in *Pleurotus sp* was also reported by Ahmed *et al.* (2009). The variation may be due to variety of substrate and mushroom species. Nuruddin *et al.* (2010) found 13.57-16.53 mg/100g zinc in *Pleurotus ostreatus* on paddy straw with supplement of cow dung.

The variation with our find values may be due to differences of substrates and species for conduction of studies. Patail *et al.* (2010) grow *Pleurotus ostreatus* on different agro-waste in pure and mix form. Similar results have been reported for by Ali *et al.* (2010), Nuruddin *et al.* (2010) and Manzi *et al.* (1999) for potassium contents.

Conclusion

This manuscript is helpful to determine a correlation study of substrate and supplement changing parameter and their consequent effects on nutritional contents of *Pleorotus Floridanus* Singer. These parameters were observed to be significant in and play key role in nutritional contents of *Pleorotus Floridanus* Singer.

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