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Cultivation and Determination of Nutritional Value on Edible Mushroom Pleurotus Ulmarius

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Abstract:

Mushrooms are fleshy, spore-bearing reproductive structures of fungi grown on organic substrates and for a long time, have played an important role as a human food due to its nutritional and medicinal properties. The first commercial cultivation of edible mushrooms was developed in France in the 18th century. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavours. However, several research works on the chemical composition of mushrooms have revealed that mushrooms can be used as a diet to combat diseases making the present use of mushrooms to be totally different from the traditional use . Many research reports described the nutritional compositions of mushrooms as attractive, being good sources of dietary protein, carbohydrate, fats, vitamins, fibre and minerals

Keywords: PDA

I. INTRODUCTION

Hypsizygus ulmarius (elm oyster mushroom) is a high yielding mushroom for which commercial cultivation technology has been released and is gaining popularity, it is widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane *et al.*, 2007).

The origin of *Pleurotus* was first cultivated during the First World War in Germany as a subsistence measure for food storages and the first documentation of cultivation was done by Kaufer (Kaufert F et al.1936). Nowadays, several species of *Pleurotus* are cultivated commercially because of their rich mineral contents and medicinal properties, short life cycle, reproducibility in the recycling of certain agricultural and industrial wastes and low demand on resources and technology (Sibel Yildiz et al. 2002).

Pleurotus species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions (Jandaik and Goyal, 1995). The practice of mushroom cultivation not only produces a nutritious food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemi cellulose, tannin and crude fiber content of straw making it ideal for animal feed (Ortega *et al.*, 1992).

Generally mushroom contains 85-90% water of its dry matter. However amount of water is greatly influenced by relative humidity and temperature during growth and storage. Protein is the most critical component and which contributes to a lot of nutritional value food. The crude fat content ranges from 1-20 % of total dry weight. Besides protein, a large variety of free and combined fatty acids also occur in *A.bispourus* with high concentration of palmic acid, stearic acid and oleic acid. Fresh mushroom contains relatively large amount of carbohydrates (i.e.) 3-28% particularly pentose, hexoses, disaccharides and trehlose (a mushroom sugar). They appear as a good source of several vitamins (thiamine, riboflavin, niacin, biotin, ascorbic acid, vitamin A, B, C, D and minerals sodium, potassium, calcium, iron etc).

Mushrooms are large reproductive structures of edible fungi belonging to either Ascomycotina or Basidiomycotina. They are non-green fungi which occur seasonally all over the world in various habitats. The mushrooms comprise a large heterogeneous group having various shapes, sizes and colours, all quite different in character, appearance and edibility. Of these large groups with more than 2000 edible species, about 300 species belonging to 70 genera are reported from India. Mushrooms have been recognized as the alternate source of good quality protein. They are capable of producing the highest quality of protein per unit area and time from the worthless agro-wastes, which are available to the tune of more than 300 million tonnes per annum in our country.

Mushrooms are the fleshy spore-bearing fruiting bodies of fungi, typically produced above ground on soil or on its food source (substrate). Based on standard morphology, the word "mushroom" was mostly used to describe those fungi that have a stem (stipe), a cap (pileus), and gills (lamellae) or pores on the underside of the cap e.g. (*Basidiomycota* and *Agaricomycetes*). However, it generally refers to a variety of gilled fungi, with or without stems. Mushrooms are also described as macro-fungi with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with the naked eyes and to be picked by hand. Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium.

Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain (Cheung et al. 2008). Nevertheless, until the last decade as compared with vegetables and medicial mushroom species, knowledge of the composition and nutritional value of culinary mushrooms was limited. Because, culinary mushrooms have been perceived only as a delicacy and their consumption in many developed countries has been

marginal and thus of little interest to researches. However, the situation has started to change noticeable; the yearly number of original papers is now several times higher than 10-15 years ago (Kalac et al., 2012). Among the abundant number of edible mushrooms *Pleurotus* genus is a prolific produced of novel "mycochemicals".

Oyster mushrooms are one of the edible mushroom cultivated in the tropics, has gained much importance in the last decade in many countries including India. During 1990 its contribution was 24.1% of the total world production. Oyster mushrooms are cultivated widely as their temperature requirement of 20-30^0 C prevails in most of the areas.

Edible mushroom cultivation is another method of alternative source of increasing the food resources to meet the challenges of increasing population. Mushroom is a simple form of plant life in the group of "Fungi" of the plant kingdom. Those who do not like mushroom miss nothing but as far as the food value is concerned, that cannot readily be obtained from other foods. It is a potential contributor for worlds food supply. Since, they can easily convert the waste into highly acceptable nutritious food in the limited space, labor and environmental conditions.

Edible mushrooms, or wild edible fungi, have been collected and consumed by people for thousands of years. The archaeological record revealed edible species associated with people living 13000 years ago in Chile (Rojas and Mansur, 1995), but it is in China where the eating of wild fungi is first reliably noted, several hundred years before the birth of Christ (Aaronson, 2000). Edible fungi were collected from forests in ancient Greek and Roman times and highly valued, though more by high-ranking people than by peasants (Buller, 1914).

For edibility and safety, proper identification of mushrooms is very important as many mushroom species are poisonous. The colour of their spores (basidiospores) is most helpful in this process. The spore colours of edible mushroom species include white, brown, black, purple-brown, pink, yellow, and cream, with the white colour being the most common, but never blue, green, or red. The presence of juices upon breaking, odours, tastes, bruising reactions, habitat and season are also considered in mushroom identification. However, tasting and smelling mushrooms may be dangerous because of toxins and allergens that may be present. Chemical tests are also used, while molecular identification by professional mycologists is rapidly growing.

A greater percentage of mushroom eaters meet the recommended daily allowance (RDA) and daily recommended intake(DRI) for calcium, copper, iron, magnesium, phosphorus, zinc, folate, niacin, riboflavin, thiamin, vitamin A, B6, B12, C, E, energy, carbohydrate, fiber and protein than non-mushroom eaters. Thus they have a better nutrient profile than do those who do not eat mushrooms.

Without doubt, edible mushroom in fresh, cooked or processed forms are nutritionally sound, tasteful food source for most people and can be a significant dietary component for vegetarians[2]. The nutritional value of edible mushrooms compares favorably to that of most vegetables. Within a single mushroom species, the nutrient content varies widely depending on habitat, the growing medium and handling procedures subsequent to harvest. Regular consumption of whole medicinal and edible mushrooms could introduce a functional or medicinal contribution within the individual's diet. Medicinal mushrooms may prevent or treat "lifestyle-related diseases". The extent of the health beneficial effect will depend on the level and regularity of consumption and the relevance of whole fresh medicinal mushrooms and concentrates to the particular disease.

Edible mushrooms are the fleshy and edible fruit bodies of several species of fungi. Mushrooms belong to the macro fungi, because their fruiting structures are large enough to be seen with the naked eye. They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand. Edibility may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma (Mattila P.2000).

Edible mushrooms are consumed by humans for their nutritional and medicinal values. Mushrooms consumed for health reasons are known as medicinal mushrooms. While hallucinogenic mushrooms (e.g. Psilocybin mushrooms) are occasionally consumed for recreational or religious purposes, they can produce severe nausea and disorientation, and are therefore not commonly considered edible mushrooms (Boa E et al 2004).

Edible mushrooms include many fungal species that are either harvested wild or cultivated. Easily cultivatable and common wild mushrooms are often available in markets, and those that are more difficult to obtain (such as the prized truffle and matsutake) may be collected on a smaller scale by private gatherers. Some preparations may render certain poisonous mushrooms fit for consumption (Jordan P et al 2006).

Before assuming that any wild mushroom is edible, it should be identified and tested. Proper identification of the specie is the only safe way to ensure edibility. Some mushrooms that are edible for most people can cause allergic reactions in some individuals, and old or improperly stored specimens can cause food poisoning. Deadly poisonous mushrooms that are frequently confused with edible mushrooms are responsible for many fatal poisonings. This includes several species of the *Amanita* genus, in particular, *Amanita phalloides*, the *death cap* (Rubel W et al 2008).

The act of consuming mushrooms dates to ancient times. Edible mushroom species have been found in association with 13,000 year old ruins in Chile, but the first reliable evidence of mushroom consumption dates to several hundred years BC in China. The Chinese value mushrooms for their medicinal properties as well as for food. Ancient Romans and Greeks, particularly the upper classes, used mushrooms for culinary purposes. Food tasters were employed by Roman Emperors to ensure that mushrooms were safe for consumption (Boa E et al 2004).

Mushrooms are also easily preserved, and historically have provided additional nutrition over winter. Many cultures around the world have either used or continue to use psilocybin mushrooms for spiritual purposes as well as medicinal mushrooms in folk medicine. Some fungi consumed by humans are currently cultivated and sold commercially. Commercial cultivation is important ecologically, as there have been concerns of depletion of larger fungi such as chanterelles in Europe, possibly because the group has grown so popular, yet remains a challenge for cultivation (Jordan P et al 2006).

III. MATERIALS AND METHODS

Collection of Specimens

In the present study *Hypsizygus ulmarius (Pleurotus ulmarius)* were obtained from Tamil Nadu University (T.N.A.U), Coimbatore was used as the parental strain. The mycelia form of colonies of different strains was maintained on PDA medium where inoculated in to 100 ml potato dextrose broth contained in 250 ml conical flask. After four weeks growth to the fungus in the liquid medium were observed. Another type of cultures were maintained from petriplates and test-tubes are used in the culture was maintained potato dextrose agar (PDA) medium.

Preparation of mother spawn

The PDA (Ricker et \al., 1936) medium which was prepared by the following ingredients (Dextrose-20g, infusions from potatoes-200g, agar-18g, distilled water-1000ml) and the spores of *Pleurotus citrinopileatus* was collected directly from the fruit body for inoculation. Then the inoculated petriplates were kept at 15^0 C for three days. The mycelium of Pleurotus *citrinopileatus* was appeared on the petriplates was used as inoculam.

Preparation of spawn bottle:

Spawn was prepared in polythene packets. Sorghum grains were boiled in water bath for 10-15 min in the ratio of 1:1 (Sorghum grains: water) and mixed with 4% (w/w) CaCO3 and 2% (w/w) CaSO4. Sorghum grains were then packed (250g) in polythene bags (of 200x300 mm. size) and sterilized in an autoclave at 121ºC for 30 min. After sterilization, the bags were inoculated with actively growing mycelium of the *P. citrinopileatus* from PDA slants and incubated (at 27±2 ºC) for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains. These spawn bottles/bags were for the seedling of mushroom bed.

Bed preparation (Paddy straw)

The polypropylene bag method was chosen for mushroom cultivation. Fresh paddy straw was chopped into pieces of 2-3 inches length and soaked in water for 10 hours. Water was then drained off from the paddy straw. Afterwards the paddy straw was sterilized using vertical autoclave at 15 Ibs pressure for 20 minutes. The sterilized paddy straw was placed on a wire mesh net for draining off excess water. Polythene bags in the size of 30x60 cm were procured and filled with the treated paddy straw as follows.

Before preparing mushroom beds all the instruments were sterilized with a dilute solution of potassium permanganate and alcohol.

A polypropylene bag was tied at one end and sterilized paddy straw was filled through the open end for about 5cm. A handful of spawn from the bottle was separate towards the periphery of this layer. Again a handful of supplement was applied over the spawn spreader in the bed. Over this some more paddy straw was put and pressed lightly. This process was repeated five times. The mouth of polypropylene bag was rolled and closed with stapler pins. Holes were made over the polypropylene bag for aeration. One bottle of spawn was enough to inoculate two bags and they were kept in the ventilated dark chamber.

After 15 days it was observed that the mycelium of Pleurotus has grown all over the paddy straw. Now the polythene cover was peeled off and the compact lump of paddy straw was placed in a cool shady room and sprayed with water 3-4 times per day. The fruit bodies of *Pleurotus* were observed to grow out of the paddy straw were harvested when attained their full growth. The same procedure was followed for the remaining paddy straw substrates in the bag. The harvested mushroom was weighed to estimate the yield.

Sample Preparation:

Cultivated mushrooms were first washed thoroughly to free from mud, ferns and other extraneous material, dried on blotting paper, cut into pieces and dried at 60°C. The mushrooms selected are normally harvested for consumption without division into pileus and stipe. Therefore, the whole mushrooms (Pileus + stipe) were dried, ground to a fine powder and stored under vacuum for further analysis.

Procedure:

Protein analysis:

Protein analysis was estimated as described by Lowry et al., 1951 using Bovine serum albumin as the standard.

Sample was added with 10ml of phosphate buffer and it was allowed to mix in a rotary shaker for 24hrs. Then the filtrate was centrifuged at 5000 rpm for 5 mins. The supernatant was used for further analysis.20ml of the extract was added into the test tubes and it was made up to 100µl with distilled water. Then 5 ml (200ml of 2% sodium carbonate in 0.1 N sodium hydroxide was added with 4ml of the 1% potassium tartrate with 0.5 gm of copper sulphate and was mixed prior to use). To this 0.5ml of 1N Folin- Ciocalteau's reagent was added, was allowed to incubate in dark for 30 mins and the absorbance was determined at 660nm using spectrophotometer.

Carbohydrates analysis:

Sample was sdded with 10ml of 80% alcohol and it was allowed to mix in a rotary shaker for 24 hrs. Then the filtrate was centrifuged at 5000 rpm for 5 mins. The supernatant was used for further analysis. 200µl of the sample was taken in the test tubes. It was made upto 1 ml with distilled water. 4ml of anthrone reagent was added. The tubes were treated over a boiling water bath for 10 min and then cooled down to room temperature. The absorbance of a blue green

solution was measured at 630nm using spectrophotometer and compared from a standard curve preparation with known amounts of glucose.

Lipid Analysis

Total lipid was determined by slight modified method of Folch *et al. (*1957). Five gram of grinded mushroom was suspended in 50 ml of chloroform: methanol (2: 1 v/v) mixture then mixed thoroughly and let stand for 3 days. The solution was filtrated and centrifuged at 1000 g by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. For the determination of total lipid from fresh mushroom, 5 g was taken with 50 ml phosphate buffer and homogenized with a tissue homogenizer. 5ml of homogenized was taken with 50 ml of chloroform: methanol (2: 1 v/v) mixture and lipid content was determined as mentioned above.

IV. RESULTS

Preparation of bed

Sorghum grains are used as spawn for bed preparation. Paddy straw was used as substrate for this cultivation. After 15 days the mycelia of *Pleurotus ulmarius* was observed over the paddy straw. Water was sprayed 3-4 times per day. The fruit bodies of *Pleurotus ulmarius* was observed out of the paddy straw.

Yield of mushroom (*Pleurotus ulmarius)*

Pleurotus ulmarius was harvested when attained their full growth. Harvested mushroom fruit bodies were weighed to estimate the yield. The percentage of harvest was high in *Pleurotus ulmarius* when paddy straw was used as substrates.

Nutritional analysis of mushroom (*Pleurotus ulmarius)*

Pleurotus ulmarius was harvested when attained their full growth. Harvested mushroom fruit bodies were dried for nutritional analysis. In this analysis the presence of carbohydrate, protein and lipids were estimated. In *Pleurotus ulmarius* the presence of protein level was high. It is showed in the table 3 and Fig 2.

Substrate (Paddy straw)

Young sporophore of *Pleurotus ulmarius*

Pleurotus ulmarius

Table 1: Days for completion of spawn running, pinhead formation and fruiting body formation of Pleurotus ulmarius

Substrate	Spawn running	Pinhead formation	Fruiting body formation
Paddy straw	17-20 days	21-23 days	25-27days

Table 2: Yield performance of Pleurotus ulmarius On Paddy straw

Substrate	Species Name	Yield (g)			
		First flush	Second flush	Third flush	Total
Paddy straw	*Pleurotus ulmarius*	310.8	260.5	140	711.3

Table 3: Nutritional analysis of Pleurotus ulmarius

S.no	Parameters	Triplicate value			SD ± SE Value
1	Carbohydrates	0.06	0.05	0.06	0.06 ± 0.004
2	Protein	0.66	0.67	0.64	0.65 ± 0.010
3	Lipids	0.1	-	0.1	0.1 ± 0.01

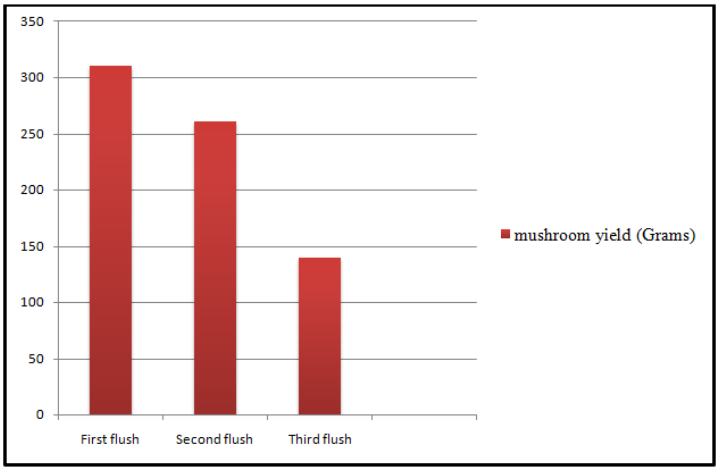

Fig 1: Yield performance of Pleurotus ulmarius On Paddy straw

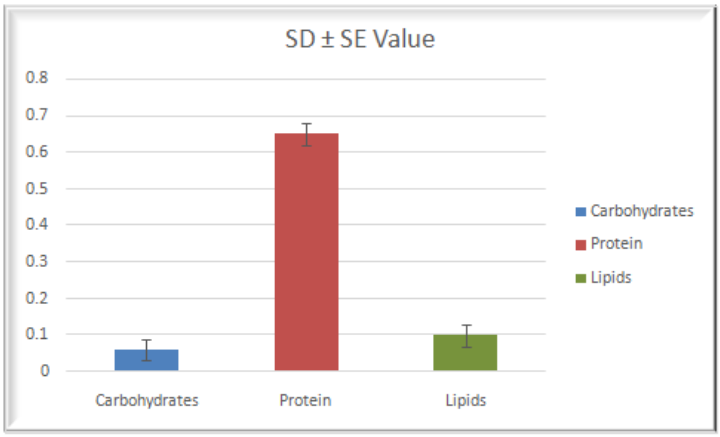

Fig 2: Nutritional analysis of Pleurotus ulmarius

V. SUMMARY

The present study mushrooms are considered to be healthy and highly nutritive food can be compared with vegetables and meat which need less space for cultivation. *Pleurotus ulmarius* potential activity was grown on paddy straw substrates.

The polypropylene bag method was chosen for mushroom cultivation. Mushroom beds were prepared using paddy straw substrate. Before preparing mushroom beds all the instruments were sterilized with a dilute solution of potassium permanganate and alcohol.

A polypropylene bag was tied at one end and sterilized paddy straw was filled through the open end for about 5cm. A handful of spawn from the bottle was separate towards the periphery of this layer. . Holes were made over the polypropylene bag for aeration .After 15 days it was observed that the mycelium of *Pleurotus ulmarius* has grown all over the paddy straw. Paddy straw was placed in a cool shady room and sprayed with water 3-4 times per day. The fruit bodies of *Pleurotus ulmarius* were observed to grow out of the paddy straw were harvested when attained their full growth. The harvested mushroom was weighed to estimate the yield.

After harvesting the harvested mushroom fruit bodies were dried for nutritional analysis. Here Protein analysis was estimated as described by Lowry et al.,method and Total lipid was determined by slight modified method of Folch*et al.*

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