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Cultivation and determination of nutritional value on edible mushroom *Pleurotus ostreatus*

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Abstract
Mushrooms are a good source of protein, vitamins, and minerals and they also posses anti-cancer, anti-cholesterol and anti-tumour properties. Mushroom cultivation is a profitable agribusiness with minimal capital cost. The research experiment was carried out in order to cultivate oyster mushroom (*Pleurotus ostreatus*) using paddy straw and also analyze the nutrient composition by proximate analysis in both fresh and shade-dried mushrooms. The research further focused on polar and non polar compounds present in oyster mushroom by performing GC-MS analysis. Upon analysing the nutrient content in both fresh and shade-dried mushroom, it was found that shade-dried mushrooms have higher energy, protein and carbohydrate value which is higher than the nutritional values obtained from convective drying techniques such as sun drying, oven drying and low heat air blow drying. Fresh *P. ostreatus* mushroom has energy, protein and carbohydrate. The moisture content in fresh mushroom was 89.69% and 12.27% in shade-dried mushroom. The difference in values of crude fibre, total ash and total fats were significantly negligible. This study concludes that energy, and protein values are comparatively higher and carbohydrate value is lesser in shade dried mushroom.

Keywords: *Pleurotus Ostreatus*, proximate analysis, shade-dried, gas chromatography-mass spectroscopy

Introduction
Mushroom is a fungal fruiting body, technically known as sporophore, which lacks chlorophyll and hence cannot produce its own food. Mushrooms usually depend on dead and decaying plants for its food and typically look like umbrellas with a stem (stipe) and a cap (pileus) that reproduces through spores.

Oyster mushroom (*Pleurotus sp.*) is the second most popular mushroom in the world that can be easily cultivated with minimal investment and requirements. Growing oyster mushrooms is easy as they can grow at temperature ranging from 15-30°C and hence can be well grown in both temperate and tropical climatic conditions (Khan *et al*., 2008) [10]. However nutritional composition is affected by many factors including differences among strains, the composition of growth substrate, the method of cultivation, stage of harvesting, specific portion of the fruiting bodies used for analysis, time interval between harvest and measurement methods (Benjamin, 1995) [4]. Many investigations from different region of the world confirmed that the *Pleurotus* mushroom having highly nutrition and also contains various bioactive compounds including terpenoids, steroids, phenols, alkaloids, lectins and nucleotides, which have been isolated and identified from the fruit body, mycelium and culture broth of mushrooms are shown to have promising biological effects (Lindequist *et al*., 2005) [8].

Materials and Methods
Spawn Collection
Pleurotus ostreatus spawns grown on sorghum grains were collected from Indian Mushroom Farm, Virugambakkam, Chennai, Tamil Nadu.

Substrate Preparation
Paddy straws were collected from local farmers in Thiruvallur district, in Tamil Nadu. At first, these paddy straws were fresh and green and hence needed pre-treatment prior to use. The bundles were spread over a large surface and dried under sun for 3 days and then turned over and was left to dry for another 4 days. The sun dried paddy straws were later chopped into smaller pieces (length varying from 3-5 cm). These paddy straws were then sterilized chemically by adding formaldehyde and carbendazim along with water in an open tank for 18 hours where the ambient temperature ranges from 37-45^{0}C. Later, the sterilized paddy straws were spread to dry, so that the moisture left over is around 65-70% (less to no moisture on hand after touching the dried paddy straws).

Preparation of Mushroom Bag
Paddy straws were made into cylindrical beds and placed into polyethylene bags sealed on the lower side, having 8-12 pinholes. Spawns grown on corn seeds were then spread over the paddy straw along the inner circle. Then, another layer of cylindrical bed was placed above the first layer and spawns were spread on them again. 5 layers were made in each bag. Later, the mushroom bags were sealed on both sides and were incubated in 20-25^{o}C in dark room for spawn and fructification. Mushroom bags were hanged from rope for quick accessing of fruiting bodies. The humidity of bags inside the room was maintained by spraying water twice every day and also by keeping the floor wet throughout the crop cycle.

After 15-18 days, primordia or pinheads developed, they were further allowed to grow into mature fruiting bodies by optimizing the temperature to 17-20^{0}C. The fully grown

mushrooms were removed by twisting along the stipe, weighed and later analyzed for nutritional composition. After weighing, some of the fresh mushrooms were shade-dried at 37-40^{0}C for 2-3 days.

Proximate analysis

Moisture determination

Moisture content of fresh mushroom was determined by weighing about 20g of both fresh and shade-dried Oyster mushrooms in different dishes and placing them in an electric oven maintained at 105 ± 1°C for five hours. The dishes were later cooled in a desiccator and weighed with the lid on. (AOAC, 1996) [2]

Determination of Total Protein

Five gram of grinded mushroom was taken with 50ml of 1N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 6000 rpm. The supernatant was collected and total protein content was measured according to the method of Lowry *et al.,* (1951) [9].

Determination of Total Lipid

Total lipid content was determined by weighing about 10-30g of mushroom in a thimble and dried for 2 hours at 100±2°C. (Folch *et al.*, 1957) [6].

The total lipid content was determined using the below calculation

Total Lipid =100* (weight of soxhlet with extract- weight of dry soxhlet) / weight of mushroom sample.

Determination of Crude Fibre

Ten grams of moisture and fat-free sample was taken in a beaker and 200 ml of boiling 0.25 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 ml of boiling 0.313 N NaOH added. After boiling for 30minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We). The crucible was heated in a muffle furnace at 600°C for 5-6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fibre (AOAC, 1996) [2].

The Crude fibre content was calculated using the below equation:

Crude fibre (g/100g) = [100-(moisture + fat)] × (W1-W2)/ Weight of mushroom

Determination of Total ASH

Total ash content of mushrooms was determined by accurately weighing one gram of each sample into a crucible and placing the crucible on a clay pipe triangle and at first heating over a low flame till all the material was completely charred, and later by heating in a muffle furnace for about 6 hours at 600°C. It was then cooled in desiccators and weighed.

The total ash was calculated using the following equation (Raghuramulu *et al.,* 2003) [12].

Ash content (g/100g) = Weight of ash × 100/ Weight of mushroom sample taken

Determination of Total Carbohydrate

The content of the available carbohydrate was determined by the following equation (Raghuramulu *et al.,* 2003) [12].

Carbohydrate (g/100g sample) = [100 – weight of (Protein + Fat + Ash + Fibre+Moisture)]

Determination of Metabolizable energy content

Fat, protein or carbohydrates supply metabolizable energy. Metabolizable energy is calculated using the below formula:

ME (Kcal /100g) = [(3.5 X CP) + (8.5 X CF) + (3.5 X NFE)]

Where,

ME = Metabolic Energy

CP = % Crude Protein

CF = % Crude Fat

NFE = % Nitrogen Free Extract (carbohydrate)

Biological efficiency of oyster mushroom

The biological efficiency is the ratio of the weight of the fresh fruiting body (g) per dry weight of substrate (g), expressed as a percentage.

Biological efficiency is calculated using the below formula:

Biological Efficiency (%) = (weight of fresh mushroom/ weight of substrate)*100.

GC-MS Analysis

Qualitative characteristics of the mushroom extract were studied using gas-chromatography/Mass Spectrometry (GC-MS) techniques. The GC-MS analysis was performed with an Agilent Mass Hunter Gas Chromatography equipped with a flame ionization detector (at 230°C). A fused silica capillary column (50 m x 0.22 mm x 0.33 mm CD) coated with methyl silicon (0.3 µm film thickness) was used with nitrogen as the carrier gas (Wekesa *et al*., 2016) [14]. All GC analyses were performed in the splitless mode with the injector temperature at 270°C. The oven temperature was programmed from 60 °C isothermal for 7 min, to 120°C at 5°C per min, then to 180°C at 10°C per min and finally to 220°C at 20°C per min, where it was maintained for l0 min. Peak areas were calculated using an integrator and together with their GC retention times.

Results and Discussion

Morphological Analysis

After 3-5 days the mycelia formation was observed and after 16-17 days pinheads were visible. Full grown oyster mushroom formed after 20-22 days (Figure 1). The fully matured oyster mushroom (*Pleurotus ostreatus*) grown on paddy straw substrate weighed around 410g - 440g during the first flush. Upon two to three consecutive flushes the maximum yield obtained per bag was around 1 ± 0.2kg. The pileus diameter ranged in between 8 - 10cm and stipe length varied from 6 - 12 cm for different oyster mushrooms harvested. The cap (pileus) was yellow to almost white in colour and the stipe was milk white in colour. The values obtained from the morphological analysis in the present study

ranges within the findings of Yang *et al*., (2013) [15]. These results indicate that chemically treated paddy straw used as substrate provides good yield.

Proximate Analysis

The nutritional composition of fresh and shade-dried mushroom is shown in (Table 1).

Table 1: Proximate Analysis of fresh and shade-dried Oyster mushrooms

S.No.	Parameters	Fresh Mushroom	Shade-dried Mushroom	Units
1	Carbohydrate	3.03	49.75	g/100g
2	Crude Fibre	0.12	0.11	g/100g
3	Crude Protein	5.27	30.0	g/100g
4	Energy	37.79	324.67	Kcal/100g
5	Fat	0.51	0.63	g/100g
6	Moisture	89.69	12.27	%
7	Total Ash	1.50	7.35	%

Fig 1: Primordia formation of Oyster mushroom

Fig 2: Shade-dried oyster mushroom

Moisture Content

The moisture content in fresh oyster mushroom was found to be 89.69% which is greater than the value obtained from the study carried out by Alam *et al* (2007). Khan *et al* (2008) [10] also observed similar levels of moisture content (87.2 ± 0.5 %) in *Pleurotus ostreatus*.

The moisture content in shade-dried oyster mushroom (Fifure 2) was found to be as low as 12.27% which is similar to the results obtained by Aishah *et al* (2013) [1]. The moisture content present in shade-dried mushroom is higher than that of the moisture content recorded by Kumela *et al*., (2017) [7] in three different convective methods of drying with 0% pretreatment values.

Protein Content

Crude protein content in fresh mushroom was found to be 5.27g/100g which is greater than 3.8 g/100g obtained from the previous studies made by Sharmila *et al*., (2015) [13].

The maximum protein content in shade-dried mushroom was found to be 30g/100g which is greater than 23.84 g/100g obtained from the previous studies made by Aishah *et al* (2013) [1] and 24.91 g/100g obtained from the previous studies conducted by Alam *et al*., (2007). The maximum crude protein according to Kumela *et al*., (2017) [7] was calculated to be within 25-27 g/100g. This value is lesser than the protein content obtained upon shade-drying. The results indicate that oyster mushrooms when shade dried helps reduce the loss of nutritional values when compared to other convective drying techniques such as sun drying, oven drying and solar drying.

Crude Fat Content

The total fat content found in this study from fresh mushroom was only 0.51 g/100g which is similar to the findings of Zahid *et al*., (2010) [16]. The total fat content determined from this study is lesser than the findings of Khan *et al*., (2008) [10] and Alam *et al*., (2008) [3]. The total fat content in shade-dried mushroom is only 0.63g/100g which is non-significantly different from the fat content obtained from fresh mushroom. This result suggested that the fat content in both fresh and shade-dried mushrooms is present in negligible quantities. The low fat content of oyster mushroom makes it a health benificial food, especially against heart disease and diabetes Khan *et al*., (2008) [10].

Crude Fibre Content

The Crude fibre content present in fresh mushroom was caculated to be 0.12g/100g which is significantly less than the value (0.63 g/100g) obtained from the studies carried out by Zahid *et al*., (2010) [16]. From shade-dried mushroom, the crude fibre content was calculated to be 0.11g/100g which is similar to value obtained from fresh mushroom, but significantly less than the value (2.42 g/100g) obtained by Kumela *et al*., (2017) [7] The results obtained from the present study has resulted in lesser fibre content than other convective

drying techniques.

Total ASH Content

The total ash content obtained from fresh mushroom was found to be 1.5% which is higher than the value (1.41%) obtained from the studies made by Zahid *et al*., (2010) [16]. In case of shade-dried mushroom, the total ash content obtained was 7.35 % which is within the range of the findings of Khan *et al*., (2008) [10]; Alam *et al*., (2008) [3]; and Aishah *et al*., (2013) [1]. The results from the ash content determination obtained from shade-dried mushrooms indicates that oyster mushroom is a good source of minerals that plays an important role in terms of physiochemical and nutritional point of view.

Carbohydrate content (Nitrogen free Extract)

The carbohydrate value was calculated to be 3.03g/100g in fresh oyster mushroom. This value is lesser than the findings (5.17g/100g) of Alam *et al*., (2008) [3]; Adebayo *et al*., (2017) [7] and Zahid *et al*.,(2010) [16], whereas the carbohydrate content of shade-dried mushroom was calculated to be 49.75g/100g which is higher than the value (39.4g/100g) obtained by Kumela *et al*., (2017) [7]. The result obtained from the present findings indicate that shade-dried oyster mushroom contain lesser carbohydrate than the values obtained from other convective drying techniques. The result obtained from the present study, indicates that oyster mushroom, a source of high protein and low carbohydrate content can be consumed even by diabetics regularly.

Energy Content

In the present study, the energy of oyster mushroom was calculated to be 37.79 Kcal/100g which ranges within the findings of Zahid *et al*., (2010) [16]. The results indicated that fresh oyster mushrooms are a good source of energy. The energy content of shade-dried oyster mushroom was calculated to be 324.67 Kcal/100g which is significantly higher than the findings of Dundar *et al*., (2008) [5]; and Khan *et al*., (2008) [10]. The result indicated that shade-dried oyster mushrooms are an excellent source of energy, which indicates that mushrooms can be used as an energy supplement for vegetarians and as an alternative to fish and meat Khan *et al*., (2008) [10].

GC-MS Result of Oyster Mushroom-Analysis and Identification of Non Polar Compounds

GC-MS analysis of mushroom extract showed the presence of 9 non-volatile compounds in Oyster mushroom. The identified compounds can be mainly divided into three groups according to the diverse functional groups. They are alkanes, alcohols, fatty acids and other organic compounds (Figure 3). Among these volatile compounds, non-polar components dominated over alcohols and acid derivatives. GC-MS results indicated that the presence of some non-polar and volatile compounds such as Ethyl-10H-acridin-9-one(40.881), followed by Furfurylmethyl amphetamine (40.563), 11H-Naphtho[1,2-b]thieno[3,4-d]pyran-11-one, 1-amino-3-methyl (40.461), and Isophthalic acid, 2-formylphenyl propyl ester (40.359). These compounds enhance the flavor and odour of *P. ostreatus*. The finding that, phenyl ethyl alcohol was the major constituent in the fresh P. ostreatus sample was reported by Overton (2010). Phenyl ethyl alcohol is known to provide fresh, sweet aromatic floral, strongly reminiscent of rose with a hyacinth nuance in mushrooms.

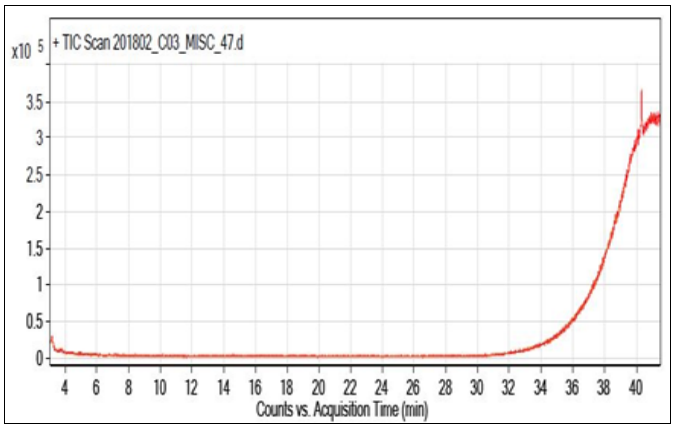

Fig 3: GC-MS analysis of mushroom extract

Conclusion

This project was carried out to analyze and compare the nutritional composition of both fresh and shade-dried oyster mushroom. From the above provided data, it is evident that shade-dried mushrooms have higher energy, protein, carbohydrate values than that of fresh oyster mushrooms. The moisture content in fresh oyster mushroom was recorded to be as high as 89.69%. Fresh oyster mushrooms (*Pleurotus ostreatus*) have high protein content (5.27 g/100g) which indicates that it is a good source of protein. Upon comparison of both fresh and shade-dried mushroom, the difference in crude fibre, total fat and total ash values were found to be less significant. The compounds obtained from GC-MS analysis of cyclohexane extract obtained from *Pleurotus ostreatus* are volatile non- polar compounds. The major volatile non-polar compounds obtained from GC-MS analysis are 10-Ethyl-10H-acridin-9-one with a retention time of 40.881 min, followed by Furfurylmethylamphetamine (40.563 min), 11H-Naphtho[1,2-b]thieno[3,4-d]pyran-11-one, 1-amino-3-methyl (40.461 min), and Isophthalic acid, 2-formylphenyl propyl ester (40.359 min). From the above data recorded and studied it is evident that oyster mushrooms have lower fat, and carbohydrate content which indicates that it can be consumed by diabetics and obese people all around the world with having much of deleterious side effects. The higher protein content indicates that oyster mushroom can be used as an excellent source of protein supplement. Shade-dried oyster mushroom has lesser discoloration and can retain much of its protein content present in fresh oyster mushroom than any other convective drying techniques.

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