

## FTIR analysis for harvested Oyster mushroom

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### Abstract

Oyster mushrooms are non-chlorophyllous saprophytic fungal organisms which have medicinal and nutritional properties along with to producing secondary metabolites. Such mushrooms contain appreciable amount of potassium, phosphorus, copper and iron but low level of calcium. The functional groups of Oyster mushroom was analysed by Fourier Transform Infrared Spectrometry (FTIR). It dealt with identification of compounds and its chemical groups (C-O, C=O, -CH, -OH, -NH and P=O groups of carbohydrates, proteins, nucleic acids and phosphor lipids) showed the intensity of absorption spectra associated with molecular composition or content of the chemical group in the biomass of Oyster mushroom. It provides information about the secondary structure content of protein. Each compound has a characteristic set of absorption bands in its infrared spectrum. Characteristic bands found in the infrared spectra of proteins and polypeptide include the Amide I and Amide II. These arise from the amide bonds that link the amino acids. Based on this analysis we could study about the structural and functional changes in the biomolecules of mushrooms.

**Key words:** Oyster mushroom, protein structure, crude protein and functional groups of biomolecules.

### Introduction

Mushrooms are the fruiting bodies of macroscopic filamentous and epigeal fungi made up of hyphae which interwoven web of tissue known as mycelium in the substrate upon which the fungus feeds; most often their mycelia are buried in the soil around the roots of trees, beneath the leaf litters, in the tissue of a tree trunk and also in other nourishing substrates (Ramsbottom, 1989; Wilkinson and Buezaeki, 1982).

Mushroom cultivation is the classical example of solid state fermentation in biotechnology, and has lots of advantages like low substrate and lignocellulosic waste recycling and high productivity using lignocellulosic substrate approximately reducing environmental pollution. It is established that cultivated mushrooms contain considerable quantities of nutrition and they rank between low grade vegetables and high grade meats. Therefore, they are popularly known as “vegetarian meat”. Moreover, animal products are not always accepted because of many cultural and religious beliefs (Prakash, 2010).

The cultivated mushrooms contain 30-50% protein on dry weight basis, which can play a constructive role in solving one of the main problems in the 20<sup>th</sup> century, which need to feed an increasing population. Edible mushrooms are nutritious and contain proteins, carbohydrates, lipids, vitamins and minerals. Their protein is of a good quality and contains all dietary essential amino acids (Al-Enazi *et al.*, 2012).

Oyster mushroom has both nutritional and therapeutic properties as they produce secondary metabolites, which are exploited in the pharmaceutical industries. These mushrooms also contain appreciable amount of potassium, phosphorus, copper and iron but low levels of calcium. Therefore oyster mushrooms are used in food industries for initiating flavours and taste (Prakash, 2010).

Here an attempt was made with the analysis of functional groups in the harvested oyster mushrooms by FTIR-method.

## **Materials and methods**

### **Mushroom Production Technology**

Oyster mushroom was grown at moderate temperature ranging from 20 to 30° C and humidity 55-70% for a period of 2 to 6 months in a year. It was cultivated in summer months (April to May) by providing the extra humidity required for its growth and also cultivated during the winter season (October to November). The species cultivated here was *Pleurotus ostreatus*. The spawn culture of oyster mushroom (mushroom seed) was collected from the Tamil Nadu Agricultural University, Madurai. It was a source of inoculum for the commercial growing of mushroom under house hold condition.

### **Cultivation Technology**

The procedure for oyster mushroom cultivation can be divided into following four steps:

1. Preparation or procurement of spawn
2. Substrate preparation
3. Spawning of substrate
4. Crop management

### 1) Preparation of spawn:

A pure culture of *Pleurotus sp* was inoculated on the sterilized substrate which was prepared separately from the different sources such as paddy chaffy grains, grains of *Sorghum* and maize. These inoculated spawn bottles were incubated at room temperature for 2-3 weeks.

One Kilogram of the selected substrate (paddy chaffy grains, grains of *Sorghum* and maize was purchased from grocery shop at Nelpettai, Madurai district) was soaked in water for 3-6 hrs. After this soaking, the substrate was drained off and which was mixed with 3 to 4 grams of Calcium carbonate. It was transferred into the glass container. Only three fourth of the bottle was filled with substrate. Then this bottle was sterilized again in an autoclave. After cooling, they were shaken thoroughly, so that the mycelium spread through the substrate. The bottles were incubated at 24<sup>0</sup> C - 33<sup>0</sup> C for 7-14 days. After incubation, the inoculated bottles were observed for mass colonization of mushroom mycelium. It was used as a source of inoculum for further commercial cultivation.

### 2) Substrate preparation:

Oyster mushroom can be cultivated on a paddy straw based agro-waste having cellulose and lignin which helps in more enzyme production of cellulose that is correlated with more yield.

- Stream pasteurization
- Hot water treatment
- Sterile technique
- Fermentation or composting and

➤ Chemical sterilization.

Paddy straws were collected in a paddy field near Viraganur of Madurai district. It was checked that it was not very leafy, not more than one year old and not unscrambled. Then the paddy straws were cut into small pieces of 2 to 3 cm and it was soaked in fresh water for overnight. Excess water from straw was drained off and removed by spreading it over the filter papers. Meanwhile the water was boiled in a wide mouth container then the paddy straw was mixed with it. After 15 min, the washed water was drained and again spread it on the filter paper and the water was completely removed from the substrate. And five gram of Calcium Carbonate was mixed with the sterilized paddy straw to remove the excess water.

### **3) Spawning of substrate**

Freshly prepared (20-30 days old) grain spawn was best for spawning. Mushroom culturing polythene bags were purchased in which holes were made in the sides of the polythene bags. Then it was packed with paddy straw substrate for one layer which was followed by spawning done in right angles with a crisscross pattern. Again paddy straw substrate was spread over the spawn. This process was repeated for 2 to 3 layers and the final layer was packed with loose straws. It was tied on the top with thick thread and leaving a space above the bag for aeration.

### **4) Crop management**

#### **a) Incubation**

The spawned bags were kept for incubation at 21<sup>0</sup> C to 30<sup>0</sup> C and with a sufficient light and ventilation for 15-17 days for spawn growth and mycelium development. In this place the bags were hanged below the table with thick threads and it was covered with wet jute cloth. Water was sprayed over the bags twice a day to maintain the moisture.

#### **b) Fruiting**

The mycelium was fully colonized on the substrate and the fungus was ready for fruiting. All the bags required high humidity (70-85%) during fruiting. The water was frequently sprayed over the bags in the cropping place depending upon the atmospheric humidity. Sufficient ventilation was provided during fruiting.

#### c) Crop protection

The crop was protected from flies, mites and other diseases by proper monitoring and pruning.

#### d) Harvesting

The right time for crop harvesting was done based on the shape and size of the fruit body by hand picking. The fruiting bodies were harvested before spore release by twisting.

#### Drying and powdering of mushroom

Five gram of harvested mushrooms was dried in Hot air oven at 60<sup>0</sup>C for 3 days. The dried mushrooms ground in pulverizer and made as powder.

#### Crude protein extraction

Five gram of harvested dried mushrooms was ground in a pestle and mortar with 2 different solvents such as water and acetone. The extracted crude protein was centrifuged and collected the supernatant. Then it was treated with Tri chloro acetic acid for proper extraction of such proteins. The extracted crude protein was dried and pulverized.

#### FTIR (Fourier Transform infrared Spectrometry) study

The FTIR technique was applied for the detection of functional groups in the powdered oyster mushrooms.

#### FTIR sample preparation and data analysis

The dried oyster mushroom and protein extract of oyster mushroom (extraction by using two solvents such as acetone and water) was powdered separately by using pestle and mortar. Each sample for FTIR analysis was prepared by mixing the fine ground powder of sample with 2% KBr. The FTIR spectra of each sample were recorded in the range of 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> at a resolution of 400 cm<sup>-1</sup>. The spectral data of each sample was measured.

#### Result and discussion

Oyster mushrooms (*Pleurotus ostreatus*) were cultivated under household condition (Plate:1) and the basidiocarp of mushroom was harvested (Plate:2) and powdered for further

study. And also, the crude protein was extracted by using both water and acetone (Plate:3). The both pulverized mushroom and crude protein applied for functional group analysis study.

Human have been consuming different food groups such as meat and plant products including fungi. Mushrooms are commonly grown in the shady area and propagated through its spores. Even today many people throughout the world consume mushrooms as healthy food, with low calories and fat but high in vegetable proteins, chitin, vitamins and minerals (Aishah and Rosli, 2013).

Mushrooms have been widely known and used as a source of food from ancient time. Many species of mushrooms are used also as medicine (Barros *et al.*, 2007a and Dembitsky *et al.*, 2010). They are very appreciated, not only for their texture, flavor, but also for their nutritional properties and additionally which act as an antioxidant with free radical-scavenging activity (Vidovic *et al.*, 2010). These functional characteristics are mainly due to their chemical composition (Bernas *et al.*, 2006).

Fourier-transform infrared spectroscopy is an analytical technique that enables the rapid, reagents less and high-throughput analysis of a diverse range of samples (Harrigan *et al.*, 2003). Its importance lies in its ability to allow rapid and simultaneous characterization of different functional groups such as lipids, proteins, nucleic acids and polysaccharides (Melin *et al.*, 2004; Bozkurt *et al.*, 2007; Dogan *et al.*, 2007 and Toyran *et al.*, 2007) in biological molecules and complex structures.

The absorption associated with the Amide I band leads to stretching vibrations of the C=O bond of the amide, absorption associated with the Amide II band leads primarily to bending vibrations of the N-H bond. Because both the C=O and the N-H bonds are involved in the hydrogen bonding that takes place between the different elements of secondary structure, the locations of both the Amide I and Amide II bands are sensitive to the secondary structure content of a protein. Studies with proteins of known structure have been used to correlate systematically the shape of the Amide I band to secondary structure content. (Byler & Susi, 1986; and Surewicz & Mantsch, 1988).

FTIR analysis detects the presence of functional groups in binding of particles with biomass. There was a shift in case of treated cells indicating participations of proteins in more quantity. The transport of iron particles may be due to presence of Siderophores. Iron

transport molecules like hydroxamates are mainly present in fungi. They bind the complex molecules and transport them inside of cells. Synthesis of different Nanoparticle by microorganisms such as fungus, yeast, bacteria, algae etc has been reported (Harajyoti and Nabanita, 2011).

In biomass of oyster mushroom, the band region around  $3402.43\text{ cm}^{-1}$  represents  $\text{-OH}$  and  $\text{-NH}$  groups from proteins and carbohydrates (Table:1&Fig:1). The band region from  $2924.09\text{ cm}^{-1}$  to  $2854.65\text{ cm}^{-1}$  represented  $\text{-CH}$  stretching derived from lipids and carbohydrates of the sample. The bands around  $1651.07$  and  $1527.62\text{ cm}^{-1}$  showed  $\text{-C=O}$  and  $\text{NH}$  group of amide-I proteins. The  $1458.18\text{ cm}^{-1}$  region of band contained  $\text{-CH}$  group of proteins. The band range around  $1157.29$  and  $1080.14\text{ cm}^{-1}$  represented,  $\text{-C-O}$  group from pyranose ring of  $\beta(1\text{--}4)$  glucans and  $\beta(1\text{--}3)$  glucans. And also the bands at  $840.96\text{ cm}^{-1}$ ,  $740.67\text{ cm}^{-1}$  and  $678.94\text{ cm}^{-1}$  showed  $\text{-CO}$  group from polysaccharides. This result was similar to Harajyoti and Nabanita (2011), as the FTIR results in change of peaks which may be due to increased in synthesis of proteins in nanoparticles formations. However, participations of proteins/enzymes in biosynthesis of nanoparticles might be determined by absorptions spectra of both U.V and Vis spectrophotometer and FTIR analysis. Relatively more iron was detected in low quantity in untreated biomass and therefore, as minor component of the hyphal cells. It was present in the treated biomass of *Pleurotus* sp.

In the powdered crude protein extracts (water soluble) of oyster mushroom, the bands are ranged from  $3425.58\text{ cm}^{-1}$  to  $640.37\text{ cm}^{-1}$ . The bands around  $3425.58 - 2854.65\text{ cm}^{-1}$  represented  $\text{-OH}$ ,  $\text{-CH}$  and  $\text{-NH}$  stretching vibrations that are mainly generated by proteins, lipids and carbohydrates of the sample. The bands derived as  $1658.78\text{ cm}^{-1}$  showed the  $\text{-C=O}$  group of amide  $\text{-I}$  protein. And the band at  $1543.05\text{ cm}^{-1}$  showed  $\text{-NH}$  stretching of amide  $\text{-I}$  protein. The  $1458.18\text{ cm}^{-1}$  absorption regions showed mainly  $\text{-CH}$  group of proteins. The band  $1257.09\text{ cm}^{-1}$  representing the  $\text{-P=O}$  phospho diester groups of nucleic acids and phospholipids (Table:1 & Fig:2). The band range of  $1157.29\text{ cm}^{-1}$  showed the  $\text{-CO}$  group from  $\beta(1\text{--}4)$  glucans and band at  $1072.42\text{ cm}^{-1}$  represented the  $\text{-CO}$  group from  $\beta(1\text{--}3)$  glucans. The absorption band range in this  $833.25\text{ cm}^{-1}$  region was corresponds to the glycosides and  $640.37\text{ cm}^{-1}$  showed  $\text{-CO}$  groups derived from polysaccharides.

The crude protein extract (acetone soluble) of Oyster mushroom showed the bands around  $3402.58 - 725.23\text{ cm}^{-1}$ , of this  $3402.43\text{ cm}^{-1}$  to  $2924.09\text{ cm}^{-1}$  represent the  $\text{-OH}$  and  $\text{-NH}$  group from proteins, carbohydrates and lipids.  $1651.07\text{ cm}^{-1}$  to  $1458.18\text{ cm}^{-1}$  represent the

-C=O, -CH and -NH group of amide- I protein. The band  $1265.30\text{ cm}^{-1}$  representing the -P=O phospho diester groups of nucleic acids and phospholipids. The band range of  $1157.29\text{ cm}^{-1}$  showed the -CO group from pyranose ring of  $\beta(1\rightarrow4)$  glucans and band at  $1080.14\text{ cm}^{-1}$  represented the -CO group from  $\beta(1\rightarrow3)$  glucans. The absorption band range in this  $933.55\text{ cm}^{-1}$  and  $725.23\text{ cm}^{-1}$  region corresponds to the  $\alpha$ -glycosides (Table:1 & Fig:3).

Zhao et al., (2006) studied the identification of *Amanita* mushrooms by Fourier transform infrared spectroscopy. It was used to obtain vibrational spectra of the fruiting bodies of wild growing *Amanita* mushrooms. The result showed that the mushrooms exhibit characteristic spectra, whose strong adsorption bands appear at about 1655, 1076 and  $1040\text{ cm}^{-1}$ . The main compositions of the *Amanita* mushrooms are proteins and polysaccharides by vibrational spectra study.

The present study suggested that the analysis of functional groups in the biomass including crude protein by FTIR method would be helpful for the study of bio-molecules enhancement in the edible mushroom cultivation technology.

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**Plate:1 Oyster mushroom cultivated under household condition(a & b)**

**a)**



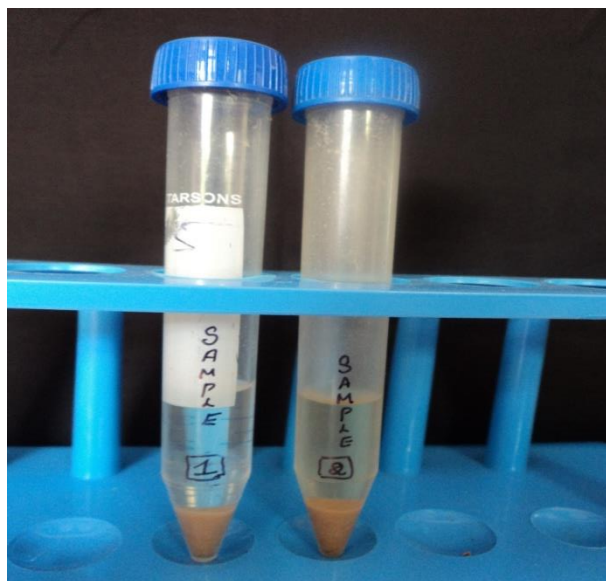
**b)**



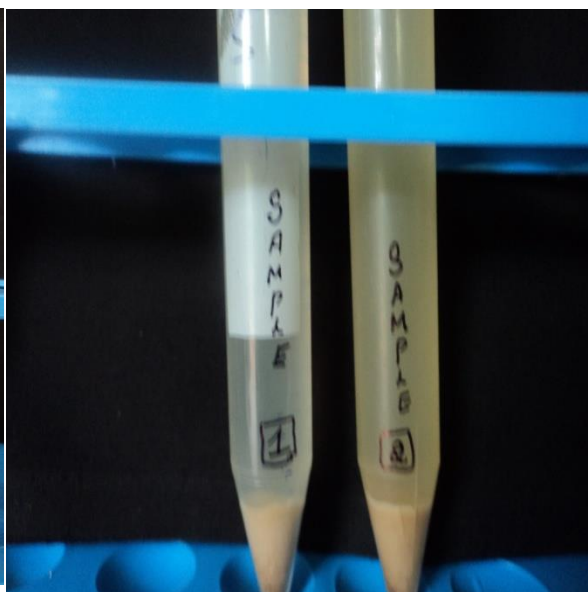
**Plate:2 Harvested Mushroom**



**Plate:3 Extraction of Oyster mushrooms proteins by using various solvents (Acetone and water)**



Sample1:



Sample:2

Table: 1

**FTIR-Analysis of powdered biomass and crude protein extract of Oyster Mushroom showing the functional groups of biomolecules**

Sl.No	Samples	Absorption band region (cm <sup>-1</sup> )	Functional groups of biomolecules
1	Biomass of oyster mushrooms	3402.43	-OH and -NH groups from proteins and carbohydrates
		2924.09	-CH groups from lipids and carbohydrates
		2854.65	- CH groups from lipids
		1651.07	-C= O group of amide- I protein
		1527.62	-NH group of amide -I proteins
		1458.18	- CH group of proteins
		1157.29	-C-O group from Pyranose ring of β (1→4) glucans
		1080.14	-C-O group from β (1→3) glucans
		840.96	- C-O group from polysaccharides
740.67	- C-O group from polysaccharides		

		678.94	- C-O group from polysaccharides
2	Crude protein extract (water soluble) of Oyster mushroom	3425.58	-OH and -NH groups from proteins and carbohydrates
		2924.09	-CH groups from lipids and carbohydrates
		2854.65	- CH groups from carbohydrates
		1658.78	-C=O group of amide- I protein
		1543.05	- CH and NH group of amide II protein
		1458.18	- CH group of proteins
		1257.59	-P=O Phospho diester groups of nucleic acids and phospholipids
		1157.29	-C-O group from carbohydrates
		1072.42	- C-O group from $\beta$ (1 $\rightarrow$ 3) glucans
		833.25	- Group Correspond to $\alpha$ -Glycosides
		640.37	- C-O group from polysaccharides
3	Crude protein extract (Acetone soluble) of Oyster mushroom	3402.43	-OH and -NH groups from proteins and carbohydrates
		2924.09	-CH groups from lipids and carbohydrates
		1651.07	- C= O group of amide- I protein
		1527.62	- CH and NH group of amide- II protein
		1458.18	- CH group of proteins
		1265.30	- P=O Phospho diester groups of nucleic acids and phospholipids
		1157.29	- C-O group from Pyranose ring of $\beta$ (1 $\rightarrow$ 4) glucans
		1080.14	- C-O group from $\beta$ (1 $\rightarrow$ 3) glucans
		933.55	- Group Correspond to $\alpha$ -Glycoside