

Original Research Article

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Variability in Protein Content of Different Species of the Genus *Pleurotus* Collected from the North Western Himalayan Regions of India

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ABSTRACT

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In the present study, nine species of *Pleurotus* and their isolates were procured /collected from different sources/locations and cultivated under mushroom house conditions. The crude protein content of various species viz. *P.ostreatus*, *P.sapidus*, *P.eryngii*, *P.florida*, *P.flabellatus*, *P.florida*, *P.cystidiosus*, *P.hypsizygos* and *P.sajor caju* was determined on dry weight basis using micro-kjeldahl method. The protein contents varied from 23.0 per cent to 33.7 per cent in 21 species/isolates of *Pleurotus* analysed. Five isolates belonged to *P.cystidiosus*, four to *P.ostreatus*, three to *P.flabellatus*, two each to *P.eryngii* and *P.fossulatus* and one each to *P.sapidus* and *P.florida*. Lowest protein content was found in *P.eryngii* (23%) and highest in *P.ostreatus* (33.7%) indicating a high variability among various species/isolates.

Introduction

Pleurotus spp. constitute one of the choicest edible mushrooms, it is commonly known as “Oyster Mushroom” and in India it is commonly called as “Dhingri”. The genus *Pleurotus* has important medicinal, biotechnological properties and environmental applications (Cohen *et al.*, 2002. The species of *Pleurotus* grow in the forests, attacking both cellulose and lignin components of wood (Zadrazil and Kurtzman, 1982; Croans, 2004) and are widely cultivated in temperate, subtropical regions of the world. Representatives of genus *Pleurotus* form a heterogeneous group of edible species of high

commercial importance (Zervakis, 2004). The total world production of mushrooms in 2010 is around 3.5 million tons as per FAO statistics (Anonymous, 2007 and Singh *et al.*, 2011). Mushrooms are increasingly being utilized as important food products for their significant role in human health, nutrition, and disease control (Randive *et al.*, 2012.) *Pleurotus* spp. constitute 25 per cent and ranks second among the cultivated mushrooms. In India, mushroom production has crossed over 1,00,000 tons in 2012. *Pleurotus* production is highest in Tamil Nadu and Punjab each with 2,000 tons each. Himachal Pradesh has an annual *Pleurotus* production of 110 tons. (Anonymous, 2015). The species of genus

Pleurotus show great diversity in their adaptation to the varying agro-climatic conditions. This flexible nature of the genus and its ease of cultivation gives it importance than any other cultivated mushroom (Zadrazil and Dube, 1992). Oyster mushroom can grow at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55–70%, on various agricultural waste materials used as substrate (Block *et al.*, 1959). According to Croans, 2004, these mushrooms are a good source of non-starchy carbohydrates, with high content of dietary fiber and moderate quantity of proteins, including most amino acids, minerals, and vitamins. Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. (Antonio *et al.*, 2016). Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties (Cuniff, 1995). Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods. Many food proteins are enzymes which are capable of enhancing the rate of certain biochemical reactions. These reactions can have either a favorable or detrimental effect on the overall properties of foods. Food analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in foods. Thus, the present experiment was done to increase the intake of vital amino acids which cannot be synthesized by our human body but are very vital for the sustenance of different life processes. Such amino acids are found in the protein blocks which are present in

desirable quantities in *Pleurotus* mushroom and the idea is to motivate the common masses about the benefits of mushroom based diet esp those rural farmer or tribal communities which suffer malnutrition.

Materials and Methods

The experimental fruiting trials were conducted in the Mushroom House of the Department of Plant Pathology and protein estimation studies were done in the Soil Science Laboratory of the Department of Soil Science of Chaudhary Sarvan Kumar Himachal Pradesh Krishi Viswavidyalaya, Palampur, India. *Pleurotus* mycelia colonized packets, weighing 200 g each, obtained from the Mushroom spawn unit of the university were used. The cultivation substrate consisted of wheat straw in a polypropylene bag sealed with cotton wool and sterilized at 121°C for 20 min. Mycelial culture of the nine *Pleurotus* spp/ strains collected from Directorate of Mushroom Research, Solan and from wild areas of the Himachal Pradesh were used for the fructification trials. For each *Pleurotus* spp./strain, three replicate bags were prepared and incubated at $25 \pm 2^{\circ}\text{C}$ in the Mushroom House, for full colonization of the substrate. Once fully colonized, induction of fruiting was done by removing the plastic bags with help of sterilized blade while keeping the upper portion of the bag intact. The packets were tied upside down with a rope along the wooden bamboo stands equidistantly (Fig. 1). After, the pinheads starts and fruiting matures, first flush of mushrooms from each bags was harvested. Water was sprayed regularly to maintain humidity (80–85%). Three flushes of each packet were harvested and recorded (Fig 2). Fruiting bodies of all nine spp/ strains of *Pleurotus* were analyzed for nutritional composition according to the Association of Official Analytical Chemists (Chang and Miles, 1989). In the present study, the total crude protein estimation was done using the

micro-Kjeldahl method (Anonymous, 1960), which involved three major steps viz. Digestion, Neutralization and Titration.

Samples of 1 g dried powder were digested in 300 ml Kjeldahl flask with 20 ml of concentrated sulphuric acid and 5 g of digestion mixture of following composition in the ratio:

Anhydrous copper sulphate	:	1
Potassium sulphate	:	10
Selenium powder	:	0.1

(1:10:0.1) w/w

One part of the digestion mixture was mixed with 30 parts of sodium sulphate and stored separately in glass stoppered bottle. The digestion was allowed to continue till the mixture became colourless (i.e. free from organic carbon). Digestion mixture was then diluted to 100 ml with distilled water after it had cooled down. Later 5 ml of aliquot was distilled after adding 100 ml of 40 per cent sodium hydroxide. The distillate was then collected in 100 ml flask having 10 ml of 40% boric acid solution. The distillation was

allowed to continue till ammonia ceased to evolve. The excess of acid was neutralized by titrating with N/100 H₂SO₄ (Sulphuric Acid), total crude nitrogen was estimated as per cent dry weight. The amount of total nitrogen in the raw materials were multiplied with the traditional conversion factor of 6.25 (Maehre *et al.*, 2018, Kjeldahl, 1883).

Results and Discussion

Mycelial cultures of six species of *Pleurotus* were procured from DMR, Solan and fifteen were collected from various vegetational zones of Himachal Pradesh during the monsoon months of 2005 and 2006. Thus a total of 21 species/strains were taken for further studies as shown in (Table 1). All the 21 isolates of *Pleurotus* were evaluated for their spawning behaviour following the standard technique (Munjal, 1973). The experimental fruiting trials were conducted under the mushroom house conditions. However, among 21 isolates only twelve showed fructification (Table 2).

Fig.1 Fruiting trials of the collected/ procured *Pleurotus* cultures under Mushroom house conditions



Fig.2 Fruiting Trials of *Pleurotus* spp./ strains



Table.1 Source of collection of various *Pleurotus* species / strains

Source	Name	Species /Strains
Collection from wild	P11	<i>Pleurotus sp.II</i>
	P5	<i>Pleurotus cystidiosus I</i>
	P21	<i>Pleurotus ostreatus IV</i>
	P3	<i>Pleurotus flabellatus II</i>
	P4	<i>Pleurotus cornucopiae</i>
	P12	<i>Pleurotus cystidiosus II</i>
	P6	<i>Pleurotus pulmonarius</i>
	P8	<i>Pleurotus fossulatus I</i>
	P10	<i>Pleurotus fossulatus II</i>
	P18	<i>Pleurotus sp.IV</i>
	P19	<i>Pleurotus sp.V</i>
	P20	<i>Pleurotus ostreatus III</i>
	P7	<i>Pleurotus sp.I</i>
	P15	<i>Pleurotus sp.III</i>
	P17	<i>Pleurotus eryngii II</i>
DMR, Solan	P1	<i>Pleurotus sapidus</i>
	P2	<i>Pleurotus flabellatus I</i>
	P9	<i>Pleurotus florida</i>
	P13	<i>Pleurotus ostreatus I</i>
	P14	<i>Pleurotus eryngii I</i>
	P16	<i>Pleurotus ostreatus II</i>

Table.2 Protein content of various species / strains of *Pleurotus* cultivated under mushroom house conditions

S. No	Species / Strains	Crude Protein Content (N % X 6.25) Dry Weight Basis
1.	<i>Pleurotus eryngii</i> I	27.3
2.	<i>Pleurotus sapidus</i>	31.4
3.	<i>Pleurotus</i> sp.I	29.5
4.	<i>Pleurotus florida</i>	28.4
5.	<i>Pleurotus flabellatus</i> II	30.4
6.	<i>Pleurotus ostreatus</i> IV	31.6
7.	<i>Pleurotus flabellatus</i> I	31.8
8.	<i>Pleurotus</i> sp. II	23.0
9.	<i>Pleurotus ostreatus</i> III	33.3
10.	<i>Pleurotus cornucopiae</i>	23.7
11.	<i>Pleurotus eryngii</i> II	32.1
12.	<i>Pleurotus</i> sp.III	33.7
	C.D (5%)	0.43

* Average of three replications.

The crude protein content on dry weight basis was determined, using micro-Kjeldahl method with a conversion factor equal to 70% of N X 6.25. (Crisan and Sands, 1978) and it was found to be minimum (23.0%) in *Pleurotus* sp.II and maximum (33.7%) in *Pleurotus* sp. III. (Table 4.2). It is usually considered to be the standard method of determining protein concentration. Because the Kjeldahl method does not measure the protein content directly a conversion factor (*F*) is needed to convert the measured nitrogen concentration to a protein concentration.

A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. Similar results were obtained by Crisan and Sands, who concluded that protein content of mushrooms vary from flush to flush (Crisan

and Sands, 1978) and also with the cultivation substrate (Bano and Rajaarthnam, 1982). Khydagi *et al.*, (1998) reported protein content in various *Pleurotus* species between 18.5 to 36.5 which is also in concurrence to our results. Similarly, Gupta *et al.*, (2004) calculated the percentage of nitrogen and crude protein in *P. sajor caju* to be from 4.22-5.89 and 18.46-27.78% respectively. Toro *et al.*, (2006) have reported a protein content of around 27% in strains of *Pleurotus* i.e IE 136, IN 18 and PORO.

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