

Research Article

Evaluation of chemical composition, nutritional value and hydrolytic enzymes of selected cultivated varieties of Mushrooms*Patnala Veeranjanyulu, Boyidi Tirupateswara Rao and Dr. K. S. Chandrashekharaiiah*¹ Department of Biochemistry, Jnana Kaveri PG Centre, Mangalore University,
Chikka Aluvara - 571 232, Kodagu**(Received: 03-02-2025****Revised: 05-05-2025****Accepted: 25-05-2025)**Corresponding Author: *Patnala Veeranjanyulu*. Email: anji8899@yahoo.com**ABSTRACT**

Mushrooms have long been recognized for their dual roles as nutrient-dense foods and sources of bioactive compounds with therapeutic potential. This study investigates the nutritional composition, enzymatic activities, and bioactive properties of four commercially cultivated edible mushrooms *Lentinula edodes* (Shiitake), *Pleurotus eryngii* (King Oyster), *Flammulina filiformis* (Enoki), and *Pleurotus ostreatus* (Oyster mushroom). Proximate analysis revealed that these mushrooms are rich in proteins, carbohydrates, dietary fiber, and essential minerals while being low in fat and calories. The antioxidant potential, assessed via total phenolic and flavonoid content, as well as free radical scavenging assays, confirmed the presence of bioactive compounds contributing to their health benefits. Hydrolytic enzyme activity assays indicated significant levels of amylase, protease, and esterase, highlighting their industrial relevance. Mushrooms cultivated in the Kodagu region exhibited variations in nutrient composition and bioactivity, emphasizing the influence of environmental conditions on their biochemical properties. The findings underscore the potential of these mushrooms as functional foods with applications in nutrition, medicine, and sustainable agriculture.

Keywords: Edible mushrooms, antioxidant activity, hydrolytic enzymes, Proximate analysis.**1. INTRODUCTION**

Edible mushrooms are the delicious fruiting bodies, considered to be healthy food and suitable for all age groups including aged people. Mushrooms have great nutritional significance due to the presence of good sources of carbohydrates, proteins, fats, vitamins, minerals, enzymes and other bioactive compounds. Mushrooms have considerable amounts of water and fiber and low in calorie content. Edible Mushrooms are rich in essential amino acids, nutritionally significant amounts of various vitamins, unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids. Thus, the presence of nutrients and bioactive compounds in the mushrooms promote overall health due to their additive and synergetic effects [1]. More than 3000 species of edible mushrooms are available globally and only 100 are cultivated commercially. A few species of

edible mushrooms which require a simple and inexpensive method, lesser growth period and few environmental controls are grown on an industrial scale.

The most cultivated edible mushrooms are *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus* spp. and *Flammulina velutipes* [2 & 3]. *A. bisporus* (white and brown mushrooms), *Pleurotus ostreatus* (oyster mushroom), *Pleurotus eryngii* (King oyster mushroom), *L. edodes* (Shiitake) and *F. velutipes* (golden needle mushroom) are the most popular cultivated edible mushrooms marketed all over the world [4]. A few studies are available on the nutritional value of species of edible mushrooms from different parts of the world, it is essential to know nutritional value of cultivated species especially of highly appreciated ones. Therefore, the present investigation is carried out to study the chemical composition, nutritional value and

hydrolytic enzymes from *Pleurotus ostreatus* (oyster mushroom), *Pleurotus eryngii* (King oyster mushroom), *L. edodes* (Shiitake) and *F. filiformis* (enoki mushroom).

2. MATERIALS AND METHODS:

2.1 Chemicals:

Acetone, Sodium carbonate, sodium hydroxide, sodium potassium tartrate, Coomassie brilliant blue (G250), Methanol, ortho phosphoric acid, copper sulphate, Bovine serum albumin, disodium hydrogen phosphate, Ammonium sulphate, Acrylamide, sodium dihydrogen phosphate, tris-base, bisacrylamide, tris HCl, glycine, sucrose, TEMED, mercaptoethanol, α -naphthol, 1-naphthyl acetate, diazo- blue B, sodium lauryl sulphate, SDS, 1-naphthyl acetate, bromophenol, ammonium persulphate, coomassie brilliant blue(R-250), acetic acid, sodium acetate, DNS, starch, Maltose, NaCl, Sodium citrate, citric acid, boric acid, borate, Trichloro acetic acid, methanol.

2.2 Mushroom sample collection:

The mushroom samples *Lentinula edodes*, *Pleurotus eryngii*, *Flammulina filiformis*, and *Pleurotus ostreatus* were procured from local markets in Kushalnagar, Madikeri, and Somwarpet, located in the Kodagu district of Karnataka, India. This region is known for its tropical highland climate, characterized by moderate rainfall, high humidity, and temperatures ranging between 15–28°C, creating favorable conditions for mushroom cultivation. The mushrooms were cultivated under controlled or semi-controlled environments such as polyhouses, where optimal humidity (85-95%) and temperature were maintained based on species-specific requirements. *Lentinula edodes* and *Pleurotus eryngii* were typically grown on sterilized sawdust blocks supplemented with rice bran or wheat bran. *Pleurotus ostreatus* was cultivated on pasteurized paddy straw, while *Flammulina filiformis* was grown on sawdust enriched with wheat bran. Mature fruiting bodies at the commercial harvest stage were selected, and only the pileus (cap) portion was used for chemical, nutritional, and enzymatic analyses to

ensure consistency and represent the most bioactive tissue.



Figure 1: *Lentinus edodes*



Figure 2: *Pleurotus eryngii*



Figure 3: *Flammulina filiformis*



Figure 4: *Pleurotus ostreatus*

2.3 Preparation of dried mushroom powder:

The samples were cleaned and chopped into tiny pieces before dried in a hot air oven at 50°C for 6 hours. The dried samples were then ground into a fine powder and analyzed for proximate composition, antioxidant activity, and bioactive protein content.

2.4 Proximate analysis of mushroom samples:

2.4.1 Moisture content

The fresh weight of each mushroom sample was measured using an electronic balance. The samples were then desiccated separately in an oven at 80°C for 48 hours. The weight loss after drying was recorded as the moisture content of the fruiting bodies [5].

2.4.2 Crude protein content

The crude protein content of the mushroom samples was determined using the Kjeldahl method [6]. “The protein content was calculated using a conversion factor of 4.38, specific to mushroom protein”.

2.4.3 Ash content

Approximately 5g of each sample was accurately weighed into a porcelain crucible, which had been preheated to around 100°C and then cooled. The sample was then placed in a muffle furnace at 550°C for 3–4 hours and allowed to cool inside a desiccator. The ash content was then determined.

2.4.4 Fat content

Approximately 10 g of each dried sample was extracted using petroleum ether in a Soxhlet apparatus for about 16 hours”. “The ether was then evaporated, and the remaining residue was

dried in an oven at 80–100°C, cooled in a desiccator, and weighed [7].

2.4.5 Crude fiber content

The crude fiber content was determined in accordance with the protocols outlined by AOAC [8]. Approximately 5 grams of the mushroom sample were initially subjected to extraction using petroleum ether to eliminate fat content. The fat-free material was subsequently transferred to a beaker, where it was combined with 200 milliliters of dilute sulfuric acid and heated to a boil for a duration of 30 minutes. The resulting residue underwent thorough rinsing with boiling water, followed by treatment with boiling sodium hydroxide solution (1.25%) for an additional 30 minutes.

After filtration, the contents were washed once more with boiling water and then rinsed with ethanol. The residue was then carefully placed in a crucible and subjected to drying in a hot air oven at a temperature of 100°C for a period of 3 to 4 hours. Subsequently, it was subjected to heating in a muffle furnace at a temperature of 550°C, following which the crucible, along with the resultant ash, was weighed to determine the fiber percentage.

2.4.6 Carbohydrate content

The total carbohydrate content of mushroom samples was estimated as per AOAC (1990) protocols. AOAC protocol [8] used for estimation of total carbohydrate content of samples by difference method (100-total moisture + total ash + total protein + total fat + total fibers) the percentage of carbohydrate was measured.

2.5 Analysis of minerals:

2.5.1 Preparation of Ash:

About 5g of dried sample placed in a muffle furnace at 1000°C. The residue was cooled in a desiccator and used for further mineral estimation.

2.5.2 Spectrophotometry:

Calcium, magnesium, manganese, phosphorus, iron, copper, and zinc were analyzed using an atomic absorption spectrophotometer (AAS).

The ash from each mushroom sample was digested in a nitric acid (HNO_3) solution and then passed through the AAS system, utilizing specific lamps and calibration with mineral standards at varying concentrations for different micronutrients. Sodium and potassium were determined using the flame photometry method with standard sodium and potassium solutions [9]. All samples were analyzed in triplicate, and the results were recorded as mean \pm S.D.

2.6 Determination of total flavonoid and phenolic content

2.6.1 Preparation of aqueous and solvent extraction

One gram of each dried mushroom sample was separately mixed with 10 mL of boiled water and 10 mL of methanol. The mixtures were stirred for 15 minutes and then centrifuged at 2000g for 15 minutes. The resulting supernatants were designated as the water extract (WE) and methanolic extract, respectively, and were subsequently used for further analysis.

2.6.2 Total flavonoids

Total flavonoid concentration was determined in accordance with the methodology outlined by Barros *et al.* [10]. The fungus extract was blended with distilled water and a solution of sodium nitrite ($NaNO_2$). Subsequently, after a 5-minute interval, a 10% aqueous solution of aluminum chloride ($AlCl_3$) was introduced. Following an additional 5-minute incubation period, a 1M solution of sodium hydroxide ($NaOH$) was incorporated, accompanied by the addition of distilled water. The amalgam was meticulously mixed, and the optical density was gauged at 510 nm against a reagent blank. The total flavonoid content was computed utilizing a quercetin calibration curve, and the outcomes were delineated as milligrams of quercetin equivalents (QEs) per gram of mushroom extract.

2.6.3 Total phenol content

The total phenolic content of the water extracts from all mushroom samples (*Lentinula edodes*, *Pleurotus eryngii*, *Flammulina filiformis*, and *Pleurotus ostreatus*) was determined using a

colorimeter following the Folin-phenol method [11].

The following ingredients were added to the sample: 1 mL of Folin-Ciocalteu reagent, 900 µL of water, 2 mL of a 10% sodium carbonate solution, and 100 mL of the sample. After being well combined, the mixture was left to incubate for one hour at room temperature. Then, a UV-visible spectrophotometer was used to detect the absorbance at 765 nm. A standard curve was established with gallic acid concentrations ranging from 10-100 µg, and the total phenolic content was measured in milligrammes per gramme of sample as gallic acid equivalent (GAE).

2.7 Antioxidant activities Determination:

2.7.1 Free radical scavenging (FRS) DPPH assay:

The antioxidant properties of aqueous extracts from mushroom specimens against DPPH radicals were assessed following the protocol outlined by Lai et al.,[12]. Varying amounts ranging from 10 to 250 µL of the aqueous extracts were utilized, with each volume adjusted to 500 µL using deionized water. This solution was then combined with 100 mM Tris-HCl at pH 7.4. Subsequently, 1 mL of 500 µM DPPH in ethanol was introduced to the mixture. “After vigorous agitation, the concoction was left to incubate at room temperature in darkness for 20 minutes. The absorbance was determined via spectrophotometry at 517 nm against a blank reagent. The radical scavenging efficacy of the extract against DPPH was then computed”.

2.7.2 Reducing power assay (RPA):

The reducing power of the water extracts from all mushroom samples was observed following the “method of Yen and Chen [13]”. Water extract volumes ranging from 10 to 250 µL were adjusted to a final volume of 500 µL using deionized water. “Each sample was then mixed with an equal volume of 100 mM sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes”.

After the incubation period, an equivalent amount of 10% trichloroacetic acid was

introduced, followed by centrifugation at 3000g for a duration of 10 minutes. The supernatant was then amalgamated with distilled water and 0.1% ferric chloride (FeCl₃) in a proportion of 1:1:2 (v/v/v). Subsequently, the absorbance was gauged at 700 nm, with an elevation in absorbance denoting heightened reducing efficacy. A higher absorbance value corresponded to stronger reducing power. Gallic acid (2-10 µg) was used as a control.

2.8 Purification of bioactive proteins/peptides

2.8.1 Preparation of acetone powder from dried, powdered fruiting bodies of mushroom samples:

The acetone powder was prepared following the method of Wetter [14]. A 10% acetone powder was obtained by blending the powdered mushroom sample in a homogenizer with chilled acetone for 5 minutes. The mixture was then filtered using a suction pump. The resulting residue was dried at 37°C, ground into a fine powder, and stored at 4°C for future use.

2.8.2 Preparation of crude protein extract:

A 10% protease extract was prepared from the acetone powder using 0.05 M sodium phosphate buffer (pH 7.0). The mixture was stirred on a magnetic stirrer for 2 hours at 4°C, then centrifuged at 10,000 rpm for 30 minutes. The resulting supernatant was collected and subjected to quantitative analysis of proteins, as well as assessments of amylase, protease, and esterase activity.

2.8.3 Determination of total soluble protein content:

Total protein content was determined using the dye-binding method represented by MM Bradford [15]. Protein concentration was estimated to be using BSA (bovine serum albumin) as the standard. The protein content was measured by recording the optical density at 590 nm.

2.8.4 Determination of the activity of amylase enzyme

Amylase activity was measured based on the amount of reducing sugars released using the

DNSA method, following a modified protocol by Strumeyer [16]. The assay involved incubating a mixture of 0.5 mL of each enzyme source and 0.5 mL of 1% soluble starch (“prepared in 0.1 M phosphate buffer, pH 7.0”) at 55°C for 15 minutes.

The reaction was halted by the addition of 1 mL of 3,5-dinitrosalicylic acid (DNSA), followed by boiling for 10 minutes. The final volume was adjusted to 7 mL with distilled water, and the quantity of reducing sugars released was quantified at 540 nm. One unit of amylase activity was defined as the enzyme quantity necessary to liberate 1 μ mol of glucose equivalent per minute under the assay conditions”. The concentration of reducing sugar (maltose) was determined by utilizing a standard curve under identical conditions, with glucose serving as the reference standard.

2.8.5 Determination of activity of protease enzyme

We used 1% casein (pH 7.8) dissolved in 0.1 M phosphate buffer as the substrate to assess proteolytic activity, following the approach of Banik *et al.* [17]. For 10 minutes at 30°C, a mixture of 1 mL of the crude enzyme sample and 5 mL of substrate was incubated. After centrifugation at 1500 rpm for 5 minutes, 5 mL of a 1% trichloroacetic acid (TCA) solution was added to halt the process. After that, the supernatant was left to incubate for 10 minutes with 5 mL of alkaline copper sulphate and then for 30 minutes with 0.5 mL of Folin-Ciocalteu (FC) reagent. We measured the absorbance at 660 nm.

The quantity of enzyme that generates an absorbance value equal to 1 μ g of tyrosine per minute at 30°C was deemed as one unit of protease activity.

2.8.6 Determination of activity of esterase enzyme

Esterase activity was determined using the “method of Gomori [18], as modified by Van Asperen [19]. The assay mixture consisted of 5 mL of 0.3 mM 1-naphthyl acetate, which was prepared by diluting a 30 mM stock solution (dissolved in acetone) with 0.05 M sodium

phosphate buffer (pH 7.0)”. The reaction was initiated by adding 10–100 μ g of enzyme and incubating the mixture at 27°C for 15 minutes.

The reaction was halted by the addition of 1 mL of DBLS reagent, a blend comprising 2 parts of 1% diazo blue B and 5 parts of 5% sodium lauryl sulfate. In the control group, enzymatic activity was quenched with DBLS prior to incubation with the substrate. The optical density of the resultant hue was gauged at 600 nm.

3.RESULTS AND DISCUSSION:

Mushrooms have long been recognized for their dual roles as nutrient-dense foods and sources of bioactive compounds with therapeutic potential. Among them, *Lentinula edodes* (Shiitake), *Pleurotus eryngii* (King Oyster), *Flammulina filiformis* (Enoki), and *Pleurotus ostreatus* (Oyster mushroom) stand out for their exceptional nutritional profiles, rich mineral content, and diverse pharmacological properties. This study focuses on the nutritional composition, enzymatic activities, and therapeutic potential of these mushrooms cultivated in the Kodagu region; a biodiversity hotspot known for its unique agro-climatic conditions. By comparing the Kodagu-grown samples with established global benchmarks, the study aims to highlight the impact of local cultivation practices on the mushrooms' bioactive and nutritional attributes. Furthermore, the findings underscore the significance of these mushrooms as functional foods, providing solutions for both human health and sustainable agricultural practices.

3.1 *Lentinula edodes*:

Lentinula edodes, known as the shiitake mushroom, belongs to the *Marasmiaceae* family and is among the most widely consumed edible mushrooms globally. Renowned for its rich umami flavor and impressive nutritional profile, *L. edodes* has been an integral part of Asian cuisine for centuries. “In addition to its culinary appeal, shiitake mushrooms are valued for their bioactive compounds and health-enhancing properties, making them a significant functional food and therapeutic agent”.

Lentinula edodes is highly nutritious, providing a rich source of proteins, dietary fiber, essential vitamins, and minerals. It is particularly fat and low in calories, making it ideal for weight management and heart-healthy diets. Approximate composition of *Lentinula edodes* (dry weight basis): Protein: 18–25% [20 & 21] Carbohydrates: 50–60% [22 & 23] Dietary Fiber: 8–10% [24 & 20] Fat: 1–3% [25] Ash: 5–7% [21].

In our study, *L. edodes* cultivated in the Kodagu region demonstrated a slightly different composition, with 56.7% carbohydrates, 35% protein, 13.6% fiber, 9.8% ash, and 2.4% fat (Table 1). Mineral analysis of these samples revealed significant concentrations of essential minerals, including calcium (11.2 mg), magnesium (2.1 mg), iron (15.3 mg), phosphorus (4.3 mg), potassium (13.8 mg), zinc (54.96 mg), copper (15.2 mg), sodium (26 mg), and manganese (7.8 mg) (Table 2). These findings align with previous reports, such as Thongbai *et al.* [26], which also highlighted the high mineral content of *L. edodes*.

Phenolic compounds are vital for the antioxidant properties of *Lentinula edodes*. These compounds are quantified using the Folin-Ciocalteu method. Total phenolic content typically ranges from 2.0 to 12.0 mg GAE/g dry weight depending on extraction method, solvent, and sample origin. [27 & 28] Flavonoids contribute to the antioxidant potential of *Lentinula edodes*. They are commonly measured using the aluminum chloride colorimetric method. Total flavonoid content ranges between 1.5 and 6.5 mg QE/g dry weight (QE: quercetin equivalents). [29 & 30] Antioxidant activity in *Lentinula edodes* is attributed to phenolic compounds, flavonoids, and other bioactive molecules like polysaccharides. DPPH: IC₅₀ values range from 0.5 to 3.5 mg/mL depending on extraction methods. FRAP: Values between 10 and 40 mmol Fe²⁺ equivalent/g dry weight [27 & 31].

Beyond its nutritional benefits, *Lentinula edodes* contains a variety of bioactive compounds with valuable applications in nutrition, industry, and pharmacology. These include polysaccharides, glycoproteins, hydrolytic enzymes, and phenolic

components like *p*-coumaric acid, *p*-hydroxybenzoic acid, and cinnamic acid. Our study revealed that extracts of *L. edodes* cultivated in Kodagu exhibited significant hydrolytic enzyme activity, including amylases (477.9 U), proteases (732.22 U), and esterases (576.66 U).

The therapeutic potential of *Lentinula edodes* is attributed to its bioactive compounds, including lentinan (a β -glucan), eritadenine, phenolic compounds, sterols, and terpenoids. These compounds are known for their wide-ranging pharmacological properties.

Documented Health Benefits: Antioxidant Activity [23 & 27]. Immunomodulatory Effects [32]. Anti-Cancer Potential [33]. Cholesterol-Lowering Properties [22]. Anti-Microbial Activity [20].

Anti-Diabetic Effects [23].

The nutritional and bioactive properties of *L. edodes* underscore its value not only as a dietary component but also as a potential resource for nutraceutical and pharmaceutical applications.

3.2 *Pleurotus eryngii*:

Pleurotus eryngii, normally known as the King Oyster Mushroom, belongs to the *Pleurotaceae* family. Distinguished by its thick, meaty stem and small, smooth cap, this mushroom has gained worldwide popularity for its unique texture, rich nutritional profile, and numerous health benefits. It is a versatile culinary ingredient and is widely recognized as a functional food due to its biologically active compounds and therapeutic potential.

It is rich in essential amino acids, proteins, polysaccharides, and other bioactive compounds, making it a versatile mushroom with culinary and therapeutic applications. Another species, *P. citrinopileatus*, is equally notable for its medicinal attributes, exhibiting anti-cancer, antioxidant, immunomodulatory, anti-inflammatory, and anti-hypertensive activities. Furthermore, *P. citrinopileatus* has shown potential in reducing lipid levels and combating obesity, making it a valuable functional food.

Pleurotus eryngii is a nutrient-dense mushroom, very good in essential proteins, vitamins, dietary fiber, vitamins, and minerals.

Its low-fat content and calorie density make it an excellent choice for weight management and cardiovascular health. Approximate composition of *Pleurotus eryngii* (dry weight basis): Protein: 20–28% [34 & 35] Carbohydrates: 55–60% [24 & 36] Dietary Fiber: 20–24% [37] Fat: 1.5–3% [25] Ash: 5.5–7.5% [38].

In our study, *Pleurotus eryngii* cultivated in the Kodagu region demonstrated a slightly varied composition: Carbohydrates: 60.2% Protein: 23% Fiber: 12% Ash: 7.6% Fat: 1.74% (Table 1). The mineral composition analysis revealed significant concentrations of essential minerals: calcium (3.8 mg), magnesium (1.8 mg), iron (12.4 mg), phosphorus (2.1 mg), potassium (8.1 mg), zinc (37.4 mg), copper (10.4 mg), sodium (21.34 mg), and manganese (4.6 mg) (Table 2). The above findings align with data from Kalac [24] Patel *et al.*, [39], further solidifying *Pleurotus eryngii*'s role as a nutritionally superior food.

Phenolic components significantly impart to the antioxidant properties of *Pleurotus eryngii*. These compounds are commonly quantified using the Folin-Ciocalteu method, and their content varies depending on the extraction method, solvent used, and cultivation conditions. Reported Range: 1.5–10.0 mg GAE/g dry weight (“GAE: gallic acid equivalents”) [39 & 40]. Flavonoids contribute to the antioxidant potential of *Pleurotus eryngii*. These are typically measured using the aluminum chloride colorimetric method, and their levels are influenced by extraction parameters. Reported Range: 1.2–4.8 mg QE/g dry weight (QE: quercetin equivalents) [41]. Antioxidant activity in *Pleurotus eryngii* is attributed to phenolic acids, flavonoids, polysaccharides, and other bioactive compounds. Various assays are used to measure its antioxidant potential: DPPH Radical Scavenging Assay: IC₅₀ values range from 0.8 to 4.0 mg/mL, depending on extraction conditions. FRAP (Ferric Reducing Antioxidant Power): Values range from 5 to 30 mmol Fe²⁺ equivalent/g dry weight [37 & 42].

The therapeutic potential of *Pleurotus eryngii* stems from its bioactive compounds, including polysaccharides, phenolic compounds, ergothioneine, and sterols. These compounds

provide a range of pharmacological activities that contribute to its reputation as a functional food.

Documented Health Benefits: Antioxidant Activity [43 & 44]. Immunomodulatory Effects [45], Anti-Cancer Potential [46], Anti-Inflammatory Properties [47], Anti-Microbial Activity [35], Cholesterol-Lowering Effects [24 & 44].

Additionally, extracts of *P. eryngii* have been found to contain a variety of hydrolytic enzymes, including amylases (23.15 U), proteases (421.11 U), and esterases (310.56 U). These findings are consistent with studies by Adebayo and colleagues (2012) and Gregori and colleagues (2007), highlighting the ecological and industrial importance of these enzymes.

Pleurotus eryngii exemplifies the synergy between nutrition, health, and sustainability, solidifying its place as an indispensable resource for promoting wellness and environmental stewardship.

3.3 *Flammulina filiformis*:

Flammulina filiformis, popularly known as enoki or winter mushroom, belongs to the family Physalacriaceae. Known for its slender stems and small, white caps, this mushroom is highly regarded for its exceptional nutritional profile and numerous therapeutic properties. It is widely consumed in Asian cuisines and has gained recognition as a functional food due to its bioactive components and health-promoting benefits.

Flammulina filiformis is an excellent source of essential nutrients, offering a rich profile of proteins, dietary fiber, vitamins, and minerals. It is particularly noted for its low calorie and fat content, making it an ideal component of low-fat and weight-management diets.

Studies reveal the following approximate composition (on a dry weight basis): Protein: 19–26% [48 & 49] Carbohydrates: 50–60% [50] Dietary Fiber: 20–25% [43] Fat: 1.0–2.5% [51] Ash: 6.5–7.8% [46].

In our study, *F. filiformis* cultivated in the Kodagu region demonstrated a slightly different composition, with 61% carbohydrates, 25% protein, 8% fiber, 2.8% ash, and 1.25% fat (Table 1). Mineral analysis of these samples revealed

significant concentrations of essential minerals, including calcium (5.2 mg), magnesium (2.1 mg), iron (11.38 mg), phosphorus (2.5 mg), potassium (12.8 mg), zinc (34.6 mg), copper (11.28 mg), sodium (27.40 mg), and manganese (4.3 mg) (Table 2).

The therapeutic potential of *Flammulina filiformis* is attributed to its diverse range of bioactive compounds, including polysaccharides, proteins, sterols, phenolics, and terpenoids. These compounds exhibit a wide array of pharmacological activities, like Antioxidant Activity [33], Immunomodulatory Effects [49], Anti-Cancer Potential [43], Anti-Inflammatory Properties [50], Anti-Microbial Activity [51], Anti-Diabetic and Lipid-Lowering Effects [48].

Phenolic components play a key role in the antioxidant properties of *Flammulina filiformis*. These compounds are often quantified using the Folin-Ciocalteu method. Reported Range: 1.0–8.5 mg GAE/g dry weight (GAE: gallic acid equivalents), depending on the extraction method, solvent, and mushroom cultivation conditions [52 & 53]. Flavonoids in *Flammulina filiformis* are important antioxidants that can be measured using the aluminum chloride colorimetric method. Reported Range: 0.5–3.5 mg QE/g dry weight (QE: quercetin equivalents) [54]. The antioxidant property of *Flammulina filiformis* is attributed to its phenolic components, flavonoids, and polysaccharides. Various assays are used to evaluate its “antioxidant potential: DPPH Radical Scavenging Assay”: IC₅₀ values range from 0.5 to 3.0 mg/mL, depending on the extraction and assay conditions. FRAP (Ferric Reducing Antioxidant Power): Values range from 8 to 25 mmol Fe²⁺ equivalent/g dry weight [55].

Flammulina filiformis produces several hydrolytic enzymes, such as cellulases, lipases, and proteases, which have applications in the food and biotechnology industries. These enzymes play a role in the degradation of agro-waste, contributing to sustainable agricultural practices [46].

Our study found that extracts of *Flammulina filiformis* cultivated in Kodagu exhibited the presence of hydrolytic enzymes such as amylases

(8.39 U), proteases (191 U), and esterases (53.67 U).

The versatile nutritional and medicinal attributes of *Flammulina filiformis* highlight its significance as both a functional food and a therapeutic agent. From its role in cancer prevention to its applications in combating oxidative stress and inflammation, enoki mushrooms continue to attract interest in nutraceutical and pharmaceutical research. Their adaptability to sustainable cultivation practices further enhances their value, making them an indispensable resource for health and wellness.

3.4 *Pleurotus ostreatus*:

Belonging to the Pleurotaceae family, the oyster mushroom is scientifically named *Pleurotus ostreatus*. Recognized for its fan-shaped caps and white to gray gills, this versatile mushroom is widely cultivated and consumed across the globe. Known for its impressive nutritional composition and abundance of bioactive compounds, it is highly regarded as both a functional food and a natural therapeutic agent, attracting significant interest in the fields of nutraceuticals and pharmaceuticals.

Pleurotus ostreatus provides a robust nutritional profile, making it a highly beneficial dietary component. It is particularly valued for its high protein and fiber content, as well as its low fat and calorie levels, which make it suitable for weight management and heart-healthy diets.

Approximate composition of *Pleurotus ostreatus* (dry weight basis): Protein: 19–30% [56, 57 & 24] Carbohydrates: 50–60% [58, 59 & 60] Dietary Fiber: 16–25% [5 & 24] Fat: 1.5–3% [5] Ash: 6–8% [24].

In our study, *Pleurotus ostreatus* cultivated in the Kodagu region exhibited a slightly altered composition: Carbohydrates: 63.7% Protein: 26% Fiber: 7.8% Ash: 8.6% Fat: 1.64%

Mineral analysis of Kodagu-cultivated samples revealed significant concentrations of essential minerals, including Calcium: 1.28 mg, Magnesium: 1.84 mg, Iron: 12.8 mg, Phosphorus: 4.2 mg, Potassium: 11.40 mg, Zinc: 51.26 mg, Copper: 12.3 mg, Sodium: 37.4 mg, Manganese: 5.4 mg

These findings align with studies by Kalac [24] and Barros [10], which highlighted the mineral richness of *Pleurotus species*, underscoring their role in combating micronutrient deficiencies.

The therapeutic benefits of *Pleurotus ostreatus* are attributed to its diverse range of bioactive compounds, including sterols, polysaccharides, flavonoids, and phenolic acids. These compounds exhibit a wide spectrum of pharmacological activities, supporting its role as a functional food.

These compounds exhibit a wide array of pharmacologic activities, like Antioxidant Activity [56 & 60] Anti-Cancer Potential [61] Anti-Inflammatory Properties [59] Anti-Microbial Activity [57] Cholesterol-Lowering and Anti-Diabetic Effects [24 & 58].

The antioxidant properties of *Pleurotus ostreatus* is mainly due to its phenolic components. The Folin-Ciocalteu method is commonly used to quantify these compounds. Reported Range: 1.5–10.0 mg GAE/g dry weight (GAE: gallic acid equivalents), influenced by solvent type and extraction method [62]. Flavonoids are important antioxidants in *Pleurotus ostreatus*. The aluminum chloride colorimetric method is used to measure their concentration. Reported Range: 1.0–5.0 mg QE/g dry weight (QE: quercetin equivalents) [28].

Antioxidant activity in *Pleurotus ostreatus* is attributed to its phenolic compounds, flavonoids, and bioactive polysaccharides. Several assays are commonly used: DPPH Radical Scavenging Assay:

IC₅₀ values range from 0.6 to 3.5 mg/mL, depending on extraction conditions. FRAP (Ferric Reducing Antioxidant Power): Values range from 5 to 30 mmol Fe²⁺ equivalent/g dry weight [63].

Pleurotus ostreatus is a prolific producer of hydrolytic and oxidative enzymes, In our study, extracts of *Pleurotus ostreatus* cultivated in Kodagu exhibited significant enzymatic activity such as amylases (44.79 U), proteases (221.11 U), and esterases (665.55 U).

Pleurotus ostreatus exemplifies the dual benefits of nutritional richness and therapeutic potential. Its role in reducing oxidative stress, combating inflammation, and supporting immune health

positions it as a valuable functional food. Furthermore, its cholesterol-lowering and anti-cancer properties make it a promising agent for addressing chronic diseases.

Table 1: Proximate analysis of mushroom samples

Mushrooms	Crude protein (%)	Crude fat (%)	Ash (%)	Carbohydrates (%)	Crude fiber (%)
<i>L. edodes</i>	35	2.4	9.8	56.7	53.6
<i>P. eryngii</i>	23	1.74	7.6	60.2	12
<i>F. filiformis</i>	25	1.25	2.8	61	8
<i>P. ostreatus</i>	26	1.64	8.6	63.7	7.8

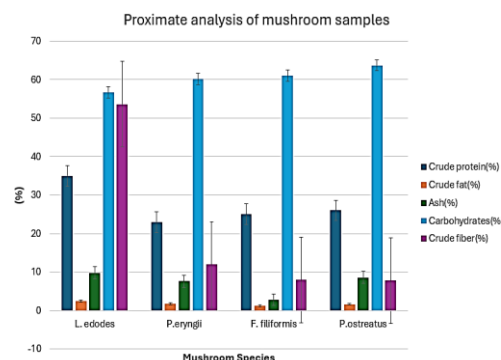


Figure 5: Proximate analysis of mushroom sample

Table 2: Mineral composition of mushroom sample

Mushrooms	Micronutrients (mg/100g)		Macronutrients (mg/100g)			Trace elements (mg/100g)				
	Na	K	Ca	Mg	P	Pb	Cu	Mn	Zn	Fe
<i>L. edodes</i>	26	13.28	11.2	2.1	4.3	0.0	15.2	7.8	54.96	15.3
<i>P. eryngii</i>	21.34	8.1	3.8	1.8	2.1	0.0	10.4	4.6	37.4	12.4
<i>F. filiformis</i>	27.40	12.8	5.2	2.1	2.5	0.0	11.28	4.3	34.6	11.38
<i>P. ostreatus</i>	37.4	11.40	1.28	1.84	4.2	0.0	12.3	5.4	51.26	12.8

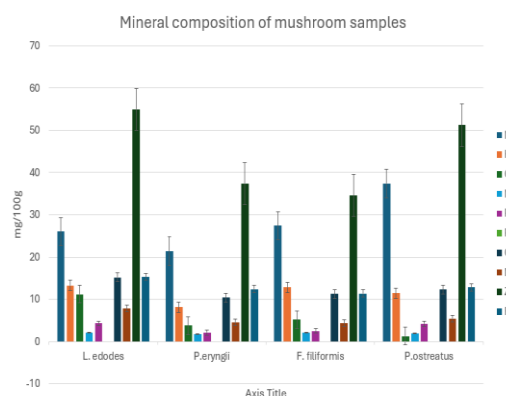


Figure 6: Mineral composition of mushroom sample

Table 3: Total Phenols and Total Flavonoids of mushroom samples

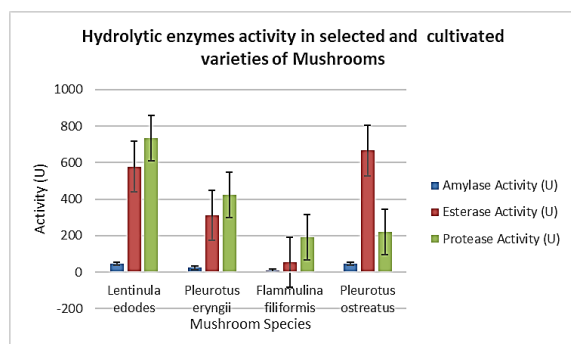
Parameters	<i>L. edodes</i>	<i>P. eryngii</i>	<i>F. filiformis</i>	<i>P. ostreatus</i>
Total phenols (mg/g)	26.79	23.14	24.67	23.47
Total flavonoids (mg/g)	4.9	2.8	3.92	2.94

Table 4: DPPH radical scavenging activity of mushroom samples

Concentration(μ g/ mL)	<i>L. edodes</i>	<i>P. eryngii</i>	<i>F. filiformis</i>	<i>P. ostreatus</i>
	% Inhibition			
10	17	12	20	14
20	36	22	46	32
30	48	34	62	58
40	64	54	78	64
50	82	67	84	77

Table 5: Reducing power activity of mushroom samples

Concentration (μ g/mL)	<i>L. edodes</i>	<i>P. eryngii</i>	<i>F. filiformis</i>	<i>P. ostreatus</i>
	OD at 705nm			
50	0.57	0.28	0.46	0.32
100	0.62	0.52	0.67	0.56
150	0.81	0.60	0.84	0.62
200	0.86	0.64	0.88	0.66
250	0.92	0.69	0.91	0.72

**Figure 7: Hydrolytic enzymes activity in selected and cultivated varieties of Mushrooms****Table 6: Hydrolytic enzymes activity in selected and cultivated varieties of Mushrooms**

Mushroom Species	Amylase Activity (U)	Esterase Activity (U)	Protease Activity (U)
<i>Lentinula edodes</i>	44.79	576.66	732.22
<i>Pleurotus eryngii</i>	23.15	310.56	421.11
<i>Flammulina filiformis</i>	8.39	53.67	191
<i>Pleurotus ostreatus</i>	44.79	665.55	221.11

4. Conclusion:

Mushrooms, including *Lentinula edodes* (Shiitake), *Pleurotus eryngii* (King Oyster), *Flammulina filiformis* (Enoki), and *Pleurotus ostreatus* (Oyster mushroom), represent a remarkable intersection of nutrition, health, and sustainability. Each species boasts a distinct yet complementary nutritional profile, rich in proteins, dietary fiber, essential vitamins, and minerals, while being low in fat and calories. These properties make them ideal dietary components for weight management and cardiovascular health.

Beyond their nutritional value, these mushrooms are powerhouses of bioactive compounds such as polysaccharides, phenolic acids, sterols, and enzymes, which exhibit potent antioxidant, immunomodulatory, anti-inflammatory, anti-microbial, and cholesterol-lowering properties. These bioactivities underscore their potential as functional foods with wide-ranging applications in nutraceuticals and pharmaceuticals.

The comparative analysis of mushrooms cultivated in the Kodagu region revealed slight variations in nutritional composition and enzymatic activities, emphasizing the influence of cultivation conditions on their bioactive potential. Moreover, the significant enzymatic activities of amylases, proteases, and esterases in these mushrooms highlight their industrial and ecological applications, particularly in sustainable agricultural practices.

In conclusion, the nutritional, therapeutic, and industrial relevance of these mushrooms positions them as indispensable resources for promoting health and wellness while supporting environmental sustainability. Continued research and cultivation efforts can further unlock their potential, offering innovative solutions to address global health challenges and support sustainable development.

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Conflict of interest:

The authors declare that there are no conflicts of interest related to this research.

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