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Comparative Study on the Proximate and Amino Acids Levels in Selected Edible Mushroom Species

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Abstract

Mushrooms represent an ancient group of heterotrophic organisms classified under Thallophytae, and based on their chemical composition and utility, they are broadly categorized as edible or poisonous. Edible mushrooms can further be divided into wild and cultivated types. This study compares the amino acid profiles and proximate compositions of two edible mushrooms, tea tree mushroom (*Agrocybe aegerita*) and oyster mushroom (*Pleurotus ostreatus*), collected from Takum Local Government Area of Taraba State, Nigeria. Freshly harvested samples were air-dried for three days, pulverized, and subjected to amino acid analysis using isocratic high-performance liquid chromatography (HPLC) and proximate analysis following standard protocols from the Association of Official Analytical Chemists (AOAC). Results showed that *Agrocybe aegerita* exhibited higher concentrations of amino acids compared to *Pleurotus ostreatus*, with a non-significant decrease ($p > 0.05$) observed in *Pleurotus ostreatus* for essential and non-essential amino acids including lysine, methionine, threonine, isoleucine, leucine, phenylalanine, valine, tryptophan, histidine, arginine, serine, cysteine, tyrosine, alanine, aspartic acid, glutamic acid, glycine, and proline. Conversely, *Pleurotus ostreatus*

demonstrated slightly higher values in proximate components such as carbohydrate, fiber, ash, and moisture, whereas *Agrocybe aegerita* showed higher but non-significant values in protein, fat, and energy content. The study concludes that *Agrocybe aegerita* is nutritionally superior in terms of amino acid composition, while *Pleurotus ostreatus* is marginally better in proximate nutritional content, supporting their complementary roles in dietary applications.

Keywords: Mushroom; Nutrition; Amino acids; Proximate composition.

INTRODUCTION

Mushrooms have been considered an ingredient of gourmet cuisine across the globe, especially for their unique flavor, and have been valued by humankind as a culinary wonder. More than 2,000 species of mushrooms exist in nature, but around 25 are widely accepted as food, and few are commercially cultivated. Based on their chemical composition and benefits, mushrooms can be classified as poisonous and edible, where edible mushrooms can also be categorized into wild and cultivated edible mushrooms (Singh *et al.*, 2019). Mushrooms are considered a delicacy with high nutritional and functional value, and they are also accepted as nutraceutical foods. They are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance (De Silva *et al.*, 2020). However, there is no easy distinction between edible and medicinal mushrooms because many of the common edible species have therapeutic properties, and several used for medical purposes are also edible (Zhang *et al.*, 2021). The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinula edodes, Pleurotus spp.*, and *Flammulina velutipes*. Mushroom production continuously increases, with China being the biggest producer worldwide (Hyde *et al.*, 2019). However, wild mushrooms are becoming more important for their nutritional, sensory, and especially pharmacological characteristics (Kalac, 2021).

Mushrooms could be an alternative source of new antimicrobial compounds, mainly secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones, but also of some primary metabolites like oxalic acid, peptides, and proteins. *Lentinula edodes* is the most studied species and has antimicrobial action against both gram-positive and gram-negative bacteria (Phan *et al.*, 2020). They have great

nutritional value since they are rich in protein, with a significant content of essential amino acids and fiber, low in fat but with an excellent essential fatty acids profile. Moreover, edible mushrooms provide nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) (Boa, 2020; Cheung, 2021). Thus, they could be an excellent source of many different nutraceuticals and might be used directly in the human diet to promote health due to the synergistic effects of all the bioactive compounds present (Chang & Wasser, 2019).

A large variety of mushrooms have been utilized traditionally in different cultures for the maintenance of health, as well as in the prevention and treatment of diseases through their immunomodulatory and antineoplastic properties (Patel & Goyal, 2021). In the last decade, interest in the pharmaceutical potential of mushrooms has increased rapidly, and it has been suggested that many mushrooms function as mini-pharmaceutical factories producing compounds with significant biological properties (Rai *et al*., 2020). More than 100 medicinal functions are attributed to mushrooms and fungi, with key medicinal uses including antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protection, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects. They also protect against tumor development and inflammatory processes (Ferreira *et al*., 2022). Numerous molecules synthesized by macrofungi are known to be bioactive, and these bioactive compounds found in fruit bodies, cultured mycelium, and cultured broth include polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, folates, lectins, enzymes, ascorbic, and organic acids. Among these, polysaccharides are the most important for modern medicine and the commercial potential of the most cultivated edible mushrooms worldwide (Xu *et al*., 2023). This research work evaluate the gross energy levels, amino acids and proximate levels of selected edible mushrooms *Pleurotus ostreatus* (Oyster Mushroom) and *Agrocybe aegerita* (Tea-tree mushroom).

MATERIALS AND METHODS

Materials

Kjeldahl catalyst tablet, hot drier, hammer mill, ash, crude fibre, polythene bags, Beaker, Fume cupboard, Heater, Test tube, Cuvet, coulorimeter, Pipette, desiccators, Micro

Kjeldahl digestion flask, Distillation unit, Markham 230 macro Distillation type Foss USA, oven.

Reagents

2,2-Diphenyl-1-picrylhydrazy(DPPH),Folin & Ciocalteu's phenol Amino acid

gallic acid, Petroleum ether (40 – 60 °C), Boric acid solution, methylene blue, methyl Red and 0.825% of methylene value in 1 litre of ethanol 90% v/v, Sodium Hydroxide 50-60%, Hydrochloric acid 0.OIN/M

Sample Collection

The fully matured mushroom species were collected from different parts of Southern Taraba such as Wukari, Takum, Donga and Ibi they were collected at different times of the day: Morning, afternoon and sometime in the evening by uprooting its substratum with the aid of a scalpel.

Preservation of Samples: The samples were sun air dried for some days and stored in transparent polythene bags that are loosely tightened to allow for proper aeration, before it was transported for analysis in science laboratory.

Identification: The samples were identified in the herbarium.

Sample Preparation

About 100gof oyster mushroom was weighed into mortar, and then pounded to fine particle. 240mls of distilled water will be added to sample inside a 250ml beaker make it up to 250mls with distilled water, then allow to settle for 4-6minutes then filter, using filter paper.

Determination of Amino acids by Isocratic HPLC `

Sample preparation

The samples being air-dried and crushed into a powder with a mixer/grinder, 2.0 g of the sample were accurately weighed and quantitatively transferred into a beaker. The beaker was placed in a water bath at 40°C to melt the mass. Then, the digested sample was transferred into a 50 mL calibrated flask; the volume was made up by adding enough water to the mark and the beaker was kept in an ultrasonic bath for 30 min at 40°C. The extract was centrifuged and 30 ml of water was added to the residue, which was then sonicated for 30 min. The solution was then passed through a 0.22 µm Millipore membrane, and the

filtrate was transferred to a 100 mL volumetric flask and diluted with water to make up the volume. This solution was passed through a 0.22 μm Millipore membrane, and the filtrate was used for the following experiments.

Preparation of standard amino acid solutions:

Standard solutions of the amino acids (all essential and the entire non-essential) were prepared. The solutions were prepared by adding 0.1 M HCl. The concentrations of the standard solutions were serially diluted to give 25, 20, 15 and 10nM each. They were stored in a freezer at 4°C till use. The mixed standard solution contained 25 pmol of each amino acid derivative.

Derivatization of the samples:

The mixed amino acid solution or filtered sample (10 μL) was transferred to a full recovery sampler, to which 70 μL sodium hexane sulphonic acid buffers was added; the solution was vortexed briefly and then 20 μL of reconstituted buffer, and the solution was mixed by vortexing for several seconds. It was then heated on a heating block at 55°C for 10 min. Derivatives were stable at room temperature for up to 1 week.

Chromatographic conditions and procedure

Chromatographic separation of prepared samples was carried on a Buck scientific BLC10/11 – model HPLC equipped with UV 338nm detector. A C18, 2.5 x 200mm, 5um column and a mobile phase of 1:2:2 (100mM sodium phosphate, pH 7.2: Aceton nbitrile: methanol v/v/v) at a flow rate of 0.45 mL/minute and an operating temperature of 40°c. Standard solutions were analysed in a similar manner. In terms of retention time, the composition of each peak was confirmed and using peak area of each amino acid the concentrations were determined in accordance with the external standard method (by extra plotting from the calibration curve, prepared by plotting a graph of peak area versus concentration of each amino acid standard solution).

Method and Proximate composition of Edible Mushroom Species

Proximate composition was done on crude fiber, ash and Nitrogen free Extract used in determining crude protein.

Crude Fiber Determination

2ml of the defatted sample were weighted and dispensed into a quick fit glass. 50ml of glacial acetic acid was added to the sample and it was placed in a heater in the fume

cupboard (digestion flask) at about 200-350 degree Celsius for 45- 60minutes for proper digestion. After digestion, the digested sample was filtered thoroughly with an already weighed filter paper and dried in an oven at 100 degree Celsius for 24hurs after which it was weighed and recorded. The residue was ash in a weighed crucible at 580 – 600 degree Celsius for 3– 4 hours in a furnace and was weighed and recorded.

Calculation of fiber content was as follows;

Weight of residue = weight of filter paper + residue – weight of filter paper Weight of ash = weight of ash + crucible – weight of empty crucible

Weight of crude fiber = weight of ash – weight of residue

Ash Determination

An empty crucible was weighed and recorded; 2ml of the oyster mushroom sample from 240mls beaker prepared were added into the crucible and cooled in a desiccator after which it was weighed and the new weight of the crucible plus ash was recorded. The ash content was calculated as follows;

Weight of ash = (weight of crucible + ash) – weight of crucible % weight of ash = ×100

Determination of Crude Protein

Over 2g of the oyster Mushroom sample from the prepared 240g beaker was weighed into micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixture was digested on an electrothermal heater until clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50ml and 5ml of it was transferred into the distillation apparatus, 5ml of 2% boric acid was pipetted into a 100ml conical flask (the receiver flask) and four drops of screened methyl red indicator were added. About 50% NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Then distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the dark blue colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask was about 50ml after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1M HCl (AOAC, 2005).

The crude protein (N × 6.25) of the mushroom flours was estimated by micro-Kjeldahl method (Humphries, 1956). The total lipid content was determined gravimetrically by extraction in petroleum ether using Soxhlet extractor (AOAC, 1990). The crude fibre and ash contents were also gravimetrically determined (AOAC, 1990). The carbohydrates were calculated according to Müller and Tobin (1980). The gross energy was determined based on Eknayake *et al.* (1999), while the total phenolic was determined using tannic acid standard (Rosset *et al.,* 1982).

RESULTS

Amino Acids Profile of Samples (Mg/100g) Of Dried Oyster Mushroom and Tea Tree Mushroom Species.

The result of the amino acid profile of the studied samples is presented in Table 1.0 The amino acid composition of both mushroom species indicates their richness in essential and non-essential amino acids. The tea tree mushroom exhibited slightly higher concentrations of all analyzed amino acids compared to the oyster mushroom. Notably, leucine (13.54 mg/100g) and glutamic acid (13.29 mg/100g) were the most abundant amino acids in the tea tree mushroom, while oyster mushrooms also contained significant levels of leucine (12.41 mg/100g) and glutamic acid (12.44 mg/100g). These amino acids play crucial roles in protein synthesis and flavor enhancement. The presence of substantial amounts of lysine, valine, and isoleucine suggests the mushrooms can contribute to muscle maintenance and repair. The observed differences, though minor, suggest that the tea tree mushroom may offer a slightly superior amino acid profile compared to the oyster mushroom.

Table 1. Amino Acids Profile of Samples (Mg/100g) Of Dried Oyster Mushroom and Tea Tree Mushroom Species.

Amino Acids mg/100g	Oyster Mushroom	Tea tree mushroom
Lysine	5.83±0.01[a]	6.00±0.00[a]
Methionine	2.65±0.01[a]	2.78±0.01[a]
Threonine	3.83±0.01[a]	3.95±0.00[a]
Isoleusine	4.78±0.01[a]	5.16±0.01[a]
Leusine	12.41±0.00[a]	13.54±0.02[a]
Phenylalanine	5.01±0.01[a]	5.15±0.00[a]

Valine	7.82±0.01[a]	8.15±0.00[a]
Tryptophan	5.63±0.01[a]	6.36±0.01[a]
Histidine	4.04±0.01[a]	5.12±0.00[a]
Arginine	7.13±0.01[a]	8.12±0.01[a]
Serine	5.15±0.00[a]	5.77±0.00[a]
Cysteine	3.56±0.01[a]	4.65±0.00[a]
Tyrosine	7.13±0.01[a]	7.15±0.00[a]
Alanine	7.56±0.01[a]	8.25±0.00[a]
Aspartic acid	7.65±0.00[a]	8.01±0.01[a]
Glutamic acid	12.44±0.03[a]	13.29±0.01[a]
Glycine	9.26±0.01[a]	11.43±0.01[a]
Proline	5.55±0.01[a]	6.25±0.01[a]

Each value represents the mean of 2 determinations ± standard deviation values (n=2).

Mean in same row having different letters of alphabets are statistically significant ($p<0.05$).

Proximate levels Of Dried Oyster Mushroom and Tea Tree Mushroom Species.

Proximate and Energy content of samples (%)

The proximate composition results highlight differences in macronutrient distribution between the two mushroom species. The tea tree mushroom had a higher protein content (32.73%) than the oyster mushroom (29.83%), indicating its superior potential as a protein source. Conversely, the oyster mushroom had a higher fiber content (7.16%) than the tea tree mushroom (5.35%), which may suggest better digestive health benefits. The fat content was relatively low in both mushrooms, but the tea tree mushroom had a slightly higher fat concentration (2.56%) than the oyster mushroom (1.72%). The carbohydrate content was marginally higher in the oyster mushroom (45.78%) than in the tea tree mushroom (43.43%), which could influence their energy contribution. The calculated energy values further confirm this, as the tea tree mushroom provided more energy (3115.16 mg/100g) than the oyster mushroom (2771.26 mg/100g). These results suggest that while both mushrooms are nutritious, the tea tree mushroom offers a slightly higher protein and energy yield, whereas the oyster mushroom may be more beneficial for dietary fiber intake.

Table 2 **Proximate levels Of Dried Oyster Mushroom and Tea Tree Mushroom Species.**

Proximate and Energy content of samples (%)

PROXIMATE LEVELS %	OYSTER MUSHROOM	TEA TREE MUSHROOM
Protein	29.83±0.01[a]	32.73±0.01[a]
Fat	1.72±0.00[a]	2.56±0.01[a]
Fibre	7.16±0.01[a]	5.35±0.00[a]
Ash	8.66±0.01[a]	6.64±3.51[a]
Moisture	6.85±0.00[a]	6.80±0.00[a]
Carbohydrate	45.78±0.05[a]	43.43±0.05[a]
Energy (mg/100g)	2771.26±0.01[a]	3115.16±0.01[a]

Each value represents the mean of 2 determinations ± standard deviation values (n=2).

Mean in same row having different letters of alphabets are statistically significant ($p<0.05$).

DISCUSSION

The amino acid composition of dried oyster mushroom (*Pleurotus ostreatus*) and tea tree mushroom (*Agrocybe aegerita*) revealed high levels of essential amino acids, with leucine, lysine, and valine being predominant. Tea tree mushroom exhibited slightly higher concentrations of these amino acids compared to oyster mushroom. This observation aligns with the findings of Mattila *et al.* (2002), who reported that mushrooms are rich sources of essential amino acids, particularly leucine and lysine. Similarly, Kalač (2016) noted that *Pleurotus* species are excellent sources of amino acids, making them suitable protein alternatives in human diets. More recent studies, such as those by Zhang *et al.* (2021), confirm the nutritional superiority of Agrocybe species in terms of amino acid profile, making them a promising dietary supplement for protein enhancement.

The proximate analysis showed that the protein content of tea tree mushroom (32.73%) was higher than that of oyster mushroom (29.83%), corroborating the results of Adebayo *et al.* (2018), who reported that Agrocybe species generally have higher protein content compared to Pleurotus species. This makes tea tree mushroom a better candidate for addressing protein malnutrition. The study by Li *et al.* (2022) further supports this claim, stating that Agrocybe aegerita contains essential proteins that enhance muscle development and metabolic activities. The carbohydrate content was slightly higher in oyster mushroom (45.78%) compared to tea tree mushroom (43.43%), consistent with the findings of Manzi *et al.* (2001), who observed that Pleurotus species contain higher carbohydrate levels due to their rich polysaccharide composition. These polysaccharides have been linked to immune-modulating properties, as noted in the research by Sun *et al.* (2023).

The fat content of both mushrooms was low, with tea tree mushroom having slightly higher fat levels (2.56%) than oyster mushroom (1.72%). This is in agreement with the findings of Cheung (2010), who stated that edible mushrooms generally contain minimal lipids, making them suitable for low-fat diets. Additionally, Guo *et al.* (2021) highlighted that the low lipid content in mushrooms contributes to their cholesterol-lowering effects. The fiber content was notably higher in oyster mushroom (7.16%) than in tea tree mushroom (5.35%), supporting the results of Hoa *et al.* (2015), who reported that Pleurotus species tend to have higher fiber content, which aids digestion and gut health. Similarly, a study by Wang *et al.* (2020) showed that dietary fiber from Pleurotus species improves gut microbiota composition, promoting better digestion and overall health.

The energy value of tea tree mushroom (3115.16 mg/100g) was higher than that of oyster mushroom (2771.26 mg/100g), indicating its superior caloric contribution. This aligns with previous research by Nowak *et al.* (2006), who observed that Agrocybe species exhibit higher energy values due to their protein and lipid content. Furthermore, a recent investigation by Xie *et al.* (2022) highlighted the potential of Agrocybe mushrooms in sports nutrition, where high-calorie foods are needed for sustained energy release. The ash content was higher in oyster mushroom (8.66%) than in tea tree mushroom (6.64%), suggesting a higher mineral content, which is consistent with findings by Chang and Miles (2004) on the mineral composition of edible mushrooms. The research by Oliveira *et al.* (2023) further confirms that Pleurotus mushrooms contain substantial levels of essential minerals like calcium, potassium, and magnesium, which are crucial for bone health and metabolic regulation.

CONCLUSION

The results of this study demonstrate that both oyster and tea tree mushrooms are nutritionally rich, with significant amounts of essential amino acids, proteins, and carbohydrates. Tea tree mushroom showed higher protein and energy content, making it a superior choice for protein supplementation. Oyster mushroom, however, had higher fiber and ash content, suggesting better contributions to digestion and mineral intake. Additionally, the low-fat content of both mushrooms makes them excellent dietary choices for individuals seeking heart-healthy foods. Overall, both mushrooms are valuable dietary components with potential health benefits, and their consumption should be encouraged for their nutritional and functional properties. Therefore, nutritional values of tea tree mushroom is characterized by higher levels of amino acid and in connection with the consumption of mushrooms, it is recommended in diet due to high essential amino acid.

Recommendation

Having conducted this research, the following recommendations can be made:

i. Consumption of Tea tree mushroom should be encouraged because of high level of amino acids.
ii. Cultivation of mushroom should be encouraged for economic benefits.
iii. Government should organize seminars to enlighten people on the nutritional values of edible mushroom (Oyster and tea tree mushrooms).

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