



Research Article

Cultivation and Determination of Protein, Elements and Vitamin Content of Oyster Mushroom

Fatimah Buba^{1&2}, Abubakar Sadiq Said², Aisha Hassan², Ali Ali Abdulrahman², Hadiza Musa², Hussaini Bello^{1&3} and Mohammed Adamu Milala²,

¹School of Pharmacy, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, WV1-1LY, U.K.

²Department of Biochemistry, Faculty of Science, University of Maiduguri, Bama Road, Maiduguri, Nigeria

³Department of Science Laboratory Technology, Adamawa state Polytechnic, P.M.B 2146, Yola, Adamawa State Nigeria

*Corresponding author: fatimahbuba@gmail.com, doi.org/10.55639/607hgfed

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ABSTRACT

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Mushrooms are edible substances that are highly appreciated and have nutritional potential, this study focuses on the possibility of using local lignocellulosic wastes as growing medium (sugarcane bagasse) for mushroom cultivation as substrates. The incubation period was 42 days. Chemical compositions of fruit bodies were estimated such as Protein, elemental analysis and vitamin content using the standard method. Protein content was 28.45 ± 1.15 mg/g, the elemental content (1.56 ± 1.14 $\mu\text{m/g}$) for calcium Ca^{2+} (5.04 ± 3.58 $\mu\text{m/g}$) potassium "K" and (0.55 ± 0.53 $\mu\text{m/g}$) phosphorus "P". Mushroom appears to have a high content of potassium than the other element. The cultivated mushroom was found to have vitamin content of (92.60 ± 0.1 $\mu\text{m/g}$ and 52.50 ± 0.06 $\mu\text{m/g}$) for A and E; whereas (11.56 ± 0.8 $\mu\text{m/g}$ and 5.85 ± 0.05 $\mu\text{m/g}$) for B₁ and B₆ respectively. The study provided an insight on chemical composition and its cultivation using sugarcane bagasse as a substrate for the cultivation of oyster mushrooms and further consumption of oyster mushrooms is recommended for optimum protein and vitamin intake.

Corresponding author: Fatimah Buba, Email: fatimahbuba@gmail.com

Department of Biochemistry, Faculty of Science, University of Maiduguri, Bama Road, Maiduguri, Nigeria

INTRODUCTION

Mushroom is a non-traditional horticultural crop with high protein, fibre, vitamins and mineral elements and is mostly grown for food or medicinal purposes (Royse *et al.*, 2017). It contributes greatly to the livelihood of rural and peri-urban dwellers, through food security and income generation with various micronutrient benefits coupled with their medicinal properties (Chang *et al.*, 2018). Mushroom cultivation can represent a small-scale enterprise option (Sanchez, 2004). There have been nearly 14000 species of 1.5 million fungi fruiting large enough to be considered mushrooms and are categorised as edible, medicinal, wild mushrooms with at least 200 species showing various degrees of edibility (Chang, 2006). The mushroom provides a variety of tasty flavours and textures, fresh mushroom contains about 80-95% moisture, 3% protein, 0.3-0.47% fat and 19 minerals and vitamins. The vitamins are not destroyed by cooking, drying, or freezing (Reid *et al.*, 2017), and edible mushrooms are recommended by FAO as food contributing to protein. Oyster mushroom is cultivated worldwide in South East Asia, India, Europe and Africa. On most agricultural waste oyster mushrooms can be grown and can convert the lignocellulose biomass into high-quality food products having high nutritive and flavour content (Chandraprakash *et al.*, 2022). The author reported that oyster mushroom is also rich in carbohydrates, protein fibre, fat and ash. They can be cultivated at both temperate and tropical climatic conditions (Baysal *et al.*, 2013). Varieties of mushrooms were

cultivated using different agricultural waste. Zhang *et al.* (2002) also reported the cultivation of *Pleurotus Sajor-cajun* on rice and wheat straw without nutrient supplementation where there was a variation in the dry matter loss of the substrate after mushroom growth that varied from 30.1%- 44.3%. However, waste paper supplemented with a corn stalk and wheat bran for oyster mushroom cultivation was reported by Tesfay *et al.* (2020) where higher biological efficiency and total yield of oyster mushroom was observed. In addition, Jarial and Jarial (2022) reported the influence of spawn substrate and doses on the yield and biological efficiency of a variety of blue oyster mushrooms (*H. ulmarius*) on different agricultural waste where bajra grain substrate is the most excellent substrate for spawn production. In Nigeria mushroom is known for their nutritional, medical and mythological purposes (Baros *et al.*, 2017). The fruit bodies, cultured mycelium and culture broth of high basidiomycetes mushroom constitute biologically active polysaccharides that have proceeded through phase I, II, and III clinical trials and are utilised by Asians in treating different cancer and some illness (Wasser *et al.*, 2010). Thus, there is an urgent need to consider our mushroom species in Nigeria to showcase their potential in line with global findings about mushroom cultivations. Therefore, the aim of this study was to cultivate oyster mushroom species using Sugarcane bagasse, the objectives were to determine the vitamin, protein and mineral element contents.

MATERIALS AND METHODS

Sample collection and preparation of substrate

Mushroom seed (spawn) was obtained from the Federal Institute of Industrial Research Oshodi, Lagos Nigeria and was identified by a plant mycologist at the Department of Biological Sciences Faculty of Science University of Maiduguri. Sugarcane bagasse was used as the substrate. The substrate was chopped and pounded to reduce the size to enable spawning, to simulate a tree trunk and for optimum mushrooms fruiting inside the bag, which helps in water uptake and Packaging (Conigilio *et al.*, 2022). The plastic sheet or flexible material sheet is spread out on the ground. Stones or logs are added to each corner or end to prevent the sheet from being lifted by the force of the wind. The cutting instrument is then used to chop the substrate on the log with the chopped pieces falling onto the laid-out plastic sheet (Okere *et al.*, 2022).

Mushroom cultivation

A double door system was adopted with insect-proof nets, Substrate was prepared aseptically bleach solution, and healthy spawn free from contaminants were used. All machinery, work floors and tools must be disinfected before tilling with a 2% formalin solution. Cook out the compost and casing soil at the end of each harvest. The compost temperature was kept at 70° Celsius for 8 hours. Remove all the used compost, casing soil and mushroom stalks were taken off, and the disposing area was designed 2 km away from the farm (Maheswari *et al.*, 2021).

Pre-treatment of the samples

After the substrate was collected and chopped, it was then dried. Drying of the substrate also removes the waterproof layer or "Vaseline" which enables the subsequent process of watering the substrate to occur. The substrate was placed into a drum after being chopped to an ideal size. The substrate was soaked overnight (12-24 hours). This resulted in moisture content of 70-80%. Manually by toughing with hand, the substrate dryness was observed (Chandraprakash *et al.*, 2022).

Pasteurisation

The substrate was heated for an hour at 71-82°C, the water is removed and the substrate is laid out in a shallow layer on clean surfaces to rapidly cool. Pasteurization of the substrate does not kill all the organisms. The utensils for mushroom cultivation were sterilised with bleach. The dilution rate was 20 ml per 20 litres of distilled water, and the temperature of the substrate was around 25-30°C. The *Agaricus bisporus* species (white button mushroom) mycelium would not withstand temperature above 40°C. The mycelium can only survive up to 37°C and is ideal to spawn at a prescribed temperature. However, holes were created by punching the entire body of the bags for an easy supply of oxygen (Moda *et al.*, 2005).

Estimation of protein by micro-Kjeldahl method Jackson, (1967)

About 100mg of fine powdered homogenous sample was immersed into a 30 ml digestion flask and 2 g K₂S₀₄, 100 ml of H₂O, and 20ml of concentrated H₂S₀₄ were added and mixed well. The Flask was left for digestion over the digestion rack;

for approximately 40 min at 37°C till the solution of the flask was white. A Final volume was made to 100ml with distilled water. An aliquot of 10ml of this solution was transferred to the flask of the distillation apparatus. Conical flask of 100ml was placed containing 5 ml of boric acid solution with a few drops of mixed indicator in such a way that the tip of the condenser dipping inside the solution. Further sodium hydroxide solution of 10ml was added to the aliquot in the distillation apparatus through the funnel. It was distilled and collected as ammonia in the boric acid flask. The colour changed from violet to green is an indication of ammonia absorbed, collected at least 20 ml of distillate. The condenser tip was rinsed with water and titrated, the distilled sample against the standard H₂SO₄ 0.01 Nom/100 until the appearance of the original violet colour was the endpoint. Reading at the endpoint was taken.

The percentage protein was calculated using the formula:

$$\% \text{Crude Protein} = \frac{A \times N \times F \times 14.007}{\text{Weight of sample} \times \text{aliquot taken}}$$

Where:

A=Volume of the acid used, N= Molarity of the acid and F=Factor 6.25

Elemental analysis

Elemental analysis was carried out using (AOAC, 1990), element such as potassium "K", Calcium Ca²⁺, phosphorus "P".

Extraction of samples for vitamin analysis

One gram (1 g) of the raw sample was taken into a centrifuge tube and 10ml of distilled water was added to the sample and the

sample was shaken for 30 minutes using the ultrasonic machine. The sample was then centrifuged at 2500rpm for 30min. The samples were then removed and filtered. And the filtrate was then stored at 40 degrees for subsequent analysis.

Preparation of working standards for vitamin

10mg of each of the vitamin reference standards (vitamins A, E, B1, and B6) were weighed and transferred into a 25ml volumetric flask and dissolved accordingly. Thiamine (B1) was dissolved in 95% alcohol, riboflavin (B) was dissolved in distilled water, and fat-soluble vitamins A and E were dissolved in ethanol. The standard was shaken ultrasonically for 5 min and allowed to cool, it was further made up to the mark with the same solvent used in dissolving them respectively. Aliquots of 1ml of each of the standards were transferred to a 10ml volumetric flask and diluted with their respective diluents to obtain concentrations

Scanning of vitamin sample

UV-visible spectrophotometer Jenway was used to scan the vitamins, the machine was blanked with distilled water for water-soluble vitamins and ethanol for fat soluble vitamins and the sample was scanned wavelength range of 200-400 nm for all the vitamins.

Determination of absorbance of sample and standards using UV-visible spectrophotometer The system (spectrophotometer) was put on and allowed to calibrate for about 10min. The wavelength range of 200-400 nm was selected. The spectrophotometer was blank using the diluents used in preparing the standard (95% alcohol for thiamine and distilled water for B1 and B6). About 2ml of the working standard were transferred into a cuvette and scanned. The absorbance was recorded at 270 nm. The actual concentration of the vitamins in the sample was calculated using the equation below:

The absorbance of Standard / Concentration of Standard = Absorbance of Unknown / Concentration of Unknown.

RESULTS AND DISCUSSION

Table 1: present the morphological characteristics of mushroom cultivated. The table showed the caps and stem colour of the cultivated mushroom were white; having a diameter of 4-8cmcap. It has special pleasant taste with average fruit ranging from 20-70 g in weight. The stem is cylindrical, thicker at the bottom while less at the top. The result is based on cultivation using sugarcane bagasse.

Sugarcane bagasse has been the best substrate for oyster mushroom cultivation. This is in agreement with the reported findings of Mdachi *et al.* (2004) who described the morphological characteristics of different mushroom species for different type’s substrates in which oyster mushroom caps and stems are white and the cap diameter is 4-9 cm and the average fruit ranging from 18-67 g in weight and the stem is semi-circular.

Table 1: Morphological Characteristics of Cultivated Oyster Mushroom

Item	Description
Cap Colour	White
Cap Diameter	4-8 cm
Stem Colour	White
Body Weight Taste	Special pleasant taste
Average Fruit	Within the range of 20-70 g
Fruit Occurrence	individually, sometimes grown in small clusters (2-4 fruit bodies together)
Cap Shape	Small button carried centrally on large, very thick stem at first, and then cap expanded to give funnel thick shape with white short gills.
Stem Shape	Thick cylindrical, thicker at the bottom, while less at the top (tapering downward).
Stem Dimension	3-8 cm in length, while diameters vary from 1.5-3.0 cm at bottom to 1.0 -2.0 cm at top.

Protein

Table 2 shows the protein content of the cultivated oyster mushroom. The content was estimated to be 28.45 ± 1.15. The estimated values correspond to the work of (Khan *et al.*, 2008) with a protein concentration of 33.56±0.84. This makes oyster mushroom a mushroom protein. Mushroom protein is of high quality and it contains an abundance of an amino acids

(Baysal *et al.*, 2003). However, a lesser concentration (6.87mg/100g) of protein was reported by Akter *et al.*, 2022 with sugarcane bagasse and sawdust and rice husk at a 3:1 ratio as substrate. Also, Hassan *et al.*, 2015 reported a closer figure of 30% to our current finding on protein. The variation could be due to the substrate used and also the percentage of spawn (Zhou *et al.*, 2022).

Table 2: Concentration of Protein

Sample	Scientific Name	Mode of Cultivation	Protein Content (mg/mg)
Oyster mushroom	<i>Pleurotusastreatus</i>	Lab cultivation	28.45±1.15

Content of mineral elements obtained from white mushroom *Agaricusbisporus*.

The result in Table 3 has shown the mineral element in oyster mushroom (*Agaricusbisporus*). These mushrooms were found to contain an average mineral content of 1.56±1.14 µm/g calcium Ca²⁺, 5.04±3.58 µm/g potassium “K” and 0.55±0.53 µm/g phosphorus "P". The highest mineral element content among the three elements in the mushrooms was potassium followed by calcium and the least value was phosphorus. These results show that the oyster mushroom *Agaricus bisporus* is high in minerals which correspond to the result of (Quimio *et al*, 1990) that calcium has a value of 2.11±1.10 µm/g followed by potassium

with a value of 6.02±2.18 µm/g and phosphorus having 0.80±0.23 with significant difference observed. However, Karatas (2022) Ca, Mg, Na, Zn, Cd and Cr were recorded in the lime-sawdust substrate. Although, the substrates used differ from the current study but report similar elements. The lignocellulose material utilized could be the reason for the variation in the mineral content. Zhou *et al.*, (2022) justified the aforementioned claim that scallop shell powder (SSP) was adopted to improve the growth of king oyster mushrooms where subsequent addition of 2% SSP increased the calcium content by 64%.

Table 3: Mineral elements content of the oyster mushroom

Element	Concentration (µm/g)
Ca ²⁺	1.56±1.14
K	5.04±3.58
P	0.55±0.53

Data are expressed as mean±SD of three determinations (n=3).

Vitamin content

The vitamin content is shown in Table 4. The result shows the presence of fat-soluble vitamins A and E with a concentration of 92.60 ± 0.1µm/g and 52.50± 0.06µm/g. While water-soluble vitamins determine were B1 and B6 with a corresponding concentration of (11.56 ± 0.8µm/g and 5.85 ± 0.05µm/g) respectively. The current findings related to the work of Jaworska *et*

al., (2015) in which vitamins A and E have a concentration of 84.75±2.91 and 54.50±0.08 with vitamins B1 and B6 to be 11.68±0.05 and 4.81±0.04. The high concentrations indicate a reliable source of vitamins. In another finding reported by Dunkwal and Jood (2009) B vitamins were detected when wheat straw and brassica straw was used to evaluate the nutritional composition of oyster mushroom. The

content obtained was (3.89 and 3.52mg/100g). The variation observed in the number of B vitamins could be due to the difference in the substrate used. The

brassica straw-grown mushrooms exhibited higher vitamin content when compared to wheat straw.

Table 4: Vitamin composition of mushroom

Vitamin	Concentration ($\mu\text{m/g}$)
A	92.60 \pm 0.1
E	52.50 \pm 0.06
B ₁	11.56 \pm 0.8
B ₆	5.85 \pm 0.05

Data are expressed as mean \pm SD of three determinations (n=3).

CONCLUSION

Sugarcane bagasse was used for the cultivation of the oyster mushroom, standard method was used to determine the various composition (vitamin, mineral element and protein). Vitamin A and E were observed to be higher (92.60 \pm 0.1 $\mu\text{m/g}$ and 52.50 \pm 0.06 $\mu\text{m/g}$). whereas water-soluble vitamins B1 and B6

(11.56 \pm 0.8 and 5.85 \pm 0.05) and the protein content were found to be (28.45 \pm 1.15) based on the protein and vitamins profile obtained; readily available sugarcane bagasse was shown to be the best substrate for the cultivation of oyster mushroom. Therefore, the oyster mushroom is recommended for consumption for protein and vitamin intake.

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