UJMR, Conference Special Issue Vol. 10 No. 3. June, 2025, pp. 215 - 220

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.25103.023

Received: 11 April 2025 **Accepted:** 24 June 2025

Valorization of Yam Peel Waste for Single-Cell Protein Production Using *Aspergillus niger*

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Abstract

Agro-industrial waste is a source of nutrients and compounds that can be used to support microbial growth in fermentation processes for the production of bio-products such as enzymes, antibiotics, or single-cell proteins. The microbial biomass is a source of proteins with several advantages over traditional protein sources. This study aimed to produce single-cell protein (SCP) from Aspergillus niger using yam peels as substrate. Aspergillus niger was isolated from garden soil using Sabouraud dextrose agar (SDA) and characterized accordingly. Yam peels a restaurant in processed and analyzed for proximate composition according to standard protocols. The isolate was subjected to submerged fermentation using commercially prepared yeast extract peptone dextrose (YEPD), and the yam peel substrate for a period of 7days on a rotary shaker. Results show that Aspergillus niger isolates had dark to brown colonies with black conidial heads and a pale yellow colour on the reverse of the SDA plate. Microscopically, the conidiophore extended from its hyphae, carrying black globular conidia. Proximate composition of the yam peels substrate was found to contain carbohydrates (81.73%), lipids (4.17%), proteins (3.5%), moisture (5.19%), ash (5.4%), and fiber (1.85%). Support for higher fungal biomass was observed on yam peel substrate, which attained 0.4 OD (optical density), while the maximum growth on the commercially prepared media (YEPD) was 0.23 OD. Thus, the Yam peel substrate supported significantly higher A. niger biomass yield (0.4 OD) compared to commercial YEPD medium (0.23 OD). It is recommended that agro-industrial wastes such as yam peels and related wastes be used to enhance production of SCP, thereby reducing pollution caused by improper disposal of agro-industrial wastes.

Keywords: Aspergillus niger; single cell protein, yam peels; submerged fermentation

INTRODUCTION

Global food demand is estimated to increase by 35–56% from 2010 to 2050. This is attributed to the increase in world population, which will certainly lead to a rise in hunger (Kumar *et al.*, 2024). Several efforts have been made worldwide by various organizations, ranging from governmental to nongovernmental, to increase agricultural output in a sustainable manner (reference). There were many achievements regarding food production processes, especially cereal foods, yet protein foods are still not available at an an affordable cost (Bratosin *et al.*, 2021). The microbial protein known as single-cell protein (SCP), which is made from food, industrial, and agricultural waste, has created new opportunities to satisfy the increasing need for protein (Thiviya *et al.*, 2022). Single-cell protein is a protein substitute made from dried and dead microbial cells from a pure and mixed culture of bacteria, fungi, algae, or yeast (Kumar *et al.*, 2024). These cultures can be used as a protein substitute because they are all cultivated on different carbon residues (Aidoo *et al.*, 2023). In addition to protein, single-cell protein also contains lipids, carbs, minerals, vitamins, and nucleic acids. As a result, it has a far larger nutritional value than typical protein (Bratosin *et al.*, 2021).

The main source of conventional protein is meat, milk, eggs, chicken, beef, cheese, fish, and peanuts. However, the cost of animal protein such as milk, meat, and fish is very high, and poor economic nations are starved of protein (Kumar *et al.*, 2024). By protein composition, the protein-rich food materials such as meat, egg, milk, and fish, have very low protein contents relative to their single cell protein counterpart that is produced using bacteria, yeast, fungi, and algae (Sharif *et al.*, 2021). Numerous microorganisms have been used in

both laboratory and industrial settings for the production of single-cell protein and were grouped into yeast, bacteria, fungi, and algae (Janssen *et al.*, 2022).

Globally, protein consumption has increased by 40% between 2000 and 2018, and is projected to increase at a 9.3% cumulative annual growth rate in the near future (Kumar *et al.*, 2024). Hence, there is an enormous demand and potential market available for human and animal protein (Growth report, 2024). Therefore, newer and more protein sources apart from plants and animals will be required to meet the longer food value chains (Nirmal *et al.*, 2025). Single-cell protein emerged as an additional protein source produced from industrial microbial fermentation techniques (Sharif *et al.*, 2021b). However, the production medium could add cost to the single cell protein product, thereby making it unsustainable (Ye *et al.*, 2024). Hence, a microbial protein produced using such waste biomass as agricultural residues could offer socio-economic benefits and consumer acceptance owing to its vegetarian nature. Agriculture residue is relatively cheap and could be a potential substrate for large-scale protein production when studied. Thus, single-cell protein production will open up new opportunities to meet the growing protein demands for humans and animals.While various agro-wastes have been explored, yam peels remain relatively understudied as a substrate for SCP production using *A. niger*, particularly in direct comparison to standard synthetic media regarding both biomass yield *and* protein content. Therefore, this studied aimed to study the potentials of yam peels in SCP production using *A. niger*.

MATERIALS AND METHODS

Collection of Sample

Garden soil was collected from the Department of Biological Sciences 810106, Zaria Kaduna State (Latitude 11.1511039, Longitude 7.65371178) in sterile polythene bags labeled sterile A-C and transferred to the Laboratory of the Department of Microbiology, Ahmadu Bello University Zaria. Yam peel samples were collected in clean and sterile polythene bags from restaurant catering operations, located at Ahmadu Bello University Social Center, Zaria.

Sample Preparation

Soil samples were collected from three spots in a sterile polytene bag and transported to the laboratory of the Microbiology Department. The samples were homogenized together to achieve a composite soil. One gram (1g) of the soil sample was weighed and poured into a universal bottle, and a ten-fold serial dilution was then carried out. The yampeelswerewashedwithdistilledwatertoremovesandanddirt,airdriedfor12hours,groundintoafinepowderandsievedusing1 mm mesh size (Aruna *et al.*, 2017).

Determination of Proximate Compositions of the Agro-residues

The proximate compositions of the samples would be determined using the methods described by AOAC (2016) for moisture, ash, crude protein, crude fat, crude fibre, and total carbohydrate content.

Isolation of *Aspergillus niger* from Garden Soil

The method of Olawale et al. (2021) was used for the mould isolation. A loop full of 10^{-3} soil dilution was inoculated onto a Sabouraud Dextrose Agar plate and was incubated at room temperature (25±3°C) for 5 days. Subculturing was done on a fresh sterile SDA slant and incubated for another 5 days at room temperature to obtain a pure isolate. Macroscopic and microscopic observations were carried out, where colonial morphology on the plate and reverse side was examined with the aid of "Atlas of clinically important fungi". Lactophenol cotton blue was used to stain the pinch of the mould colony on a microscope slide, and it was examined for conidial, hyphal, and conidiophore morphology using x10 and x40 objectives.

Preparation of Spore Suspension from the Mould Isolate

Spore suspension from the mould isolate was prepared by washing culture of the isolate (*Aspergillus niger*) grown on SDA slants with 5ml of distilled water. The spore washings is standardized to the concentration of 10^6 spore/ml by addition of distilled water using a hemocytometer(Trafimovich, 2023).

Fermentation of Yam Peels using *Aspergillus niger* Suspension

The method of Madika (2016) was adopted, where ten grams (10g) of the prepared yam peel substrate was weighed and poured into a 250 mL Erlenmeyer flask, 100ml of distilled water was added into the Erlenmeyer flask already

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

containing yam peel substrate, it was sterilized by autoclaving at a temperature of 121°C for 15minutes. After autoclaving, the sample was inoculated with 1ml spore suspension (10^6 spores/ml) of Aspergillus niger, and then incubated at room temperature (25±3°C) on a rotary shaker for seven days, while taking optical density readings at 24-hour intervals. Yeast extract peptone dextrose (YEPD) medium, which serves as the control, was prepared by dissolving 5g of yeast extract and 10g of peptone in 500ml of distilled. Dextrose was prepared separately by dissolving 10g of d-glucose, the two solutions were autoclaved at a temperature of 121°C for 15minutes, after autoclaving, both solutions were then mixed together and 100ml of YEPD was aseptically dispensed into 250 ml Erlenmeyer flask, inoculated with 1ml spore suspension of *Aspergillus niger* andincubated at room temperature (25°C) on a rotary shaker for seven days, with result readings taken at 24hours intervals.

RESULTS

Proximate Composition of Yam Peels Substrate

Proximate composition of the yam peels substrate is presented in Figure 1, which revealed a low fibre content of 1.85% and a high carbohydrate content of 81.73%, with the moisture, ash, lipid, and protein contents being 5.19%, 5.4%, 4.17% and 3.5% respectively.

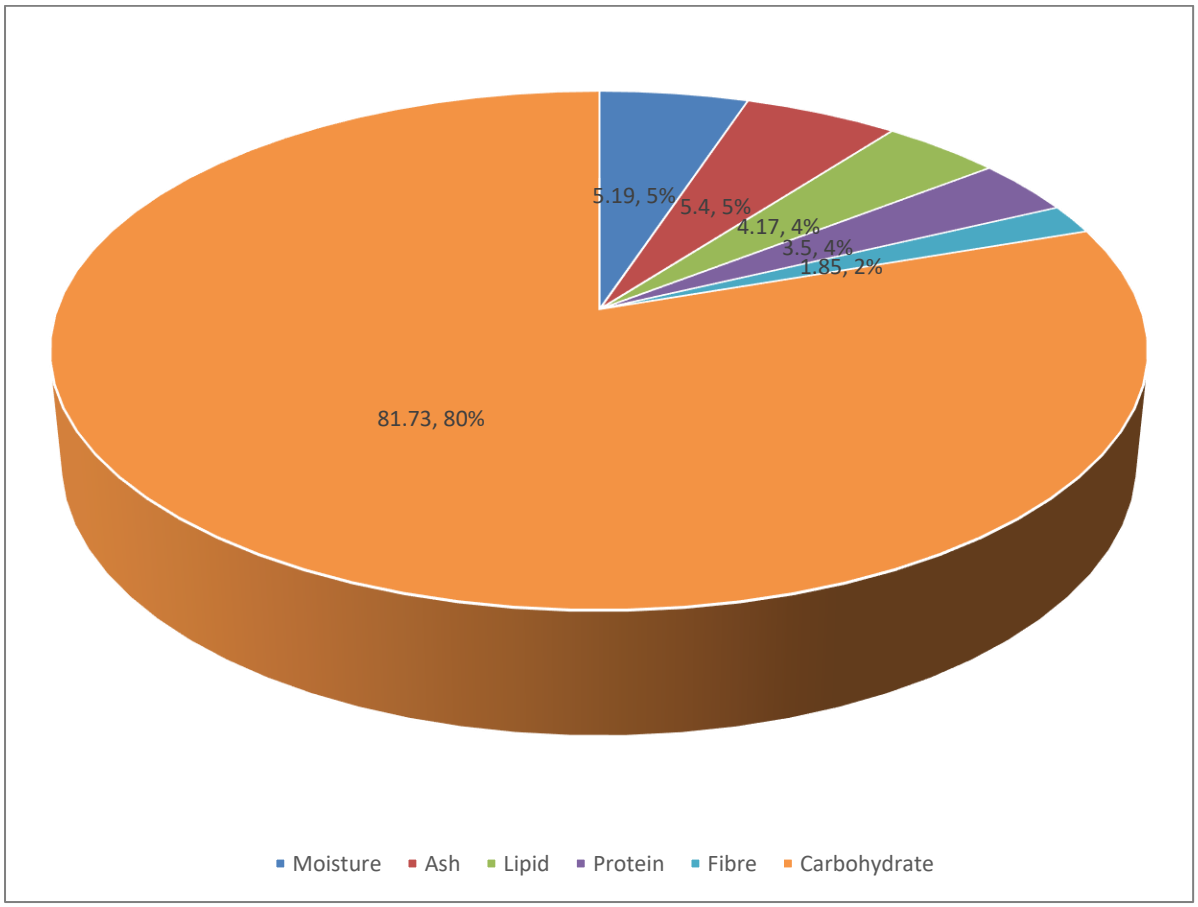

Figure 1: Mean Proximate Composition of the Yam Peels Collected from a Restaurant in ABU Zaria.

Isolation of *Aspergillus niger* from Garden Soil

The macroscopic morphology of *the Aspergillus niger isolate began as a white colony, then rapidly developed into a* dark brown to black colour. Colonies were fluffy and matured on the 5th day. Also, for the microscopic morphology, the hyphae were found to be septate with long conidiophores arising via a foot cell, ending with a swollen vesicle carrying black globular conidia.

Cell biomass of *Aspergillus niger* on Yam Peels

The cell biomass of *Aspergillus niger* on yam peel substrate and the commercially prepared yeast extract, peptone dextrose is presented in

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Figure 2. A gradual increase in fungal biomass on yam peel substrate was observed from day 1 to 5, attaining an exponential increase to 0.4 optical density on the 6th day with a decline to 0.32 optical density on the 7th day. Similarly, a gradual increase in fungal biomass on YEPD was observed from the 1st to 7th day with a peak optical density of 0.23.

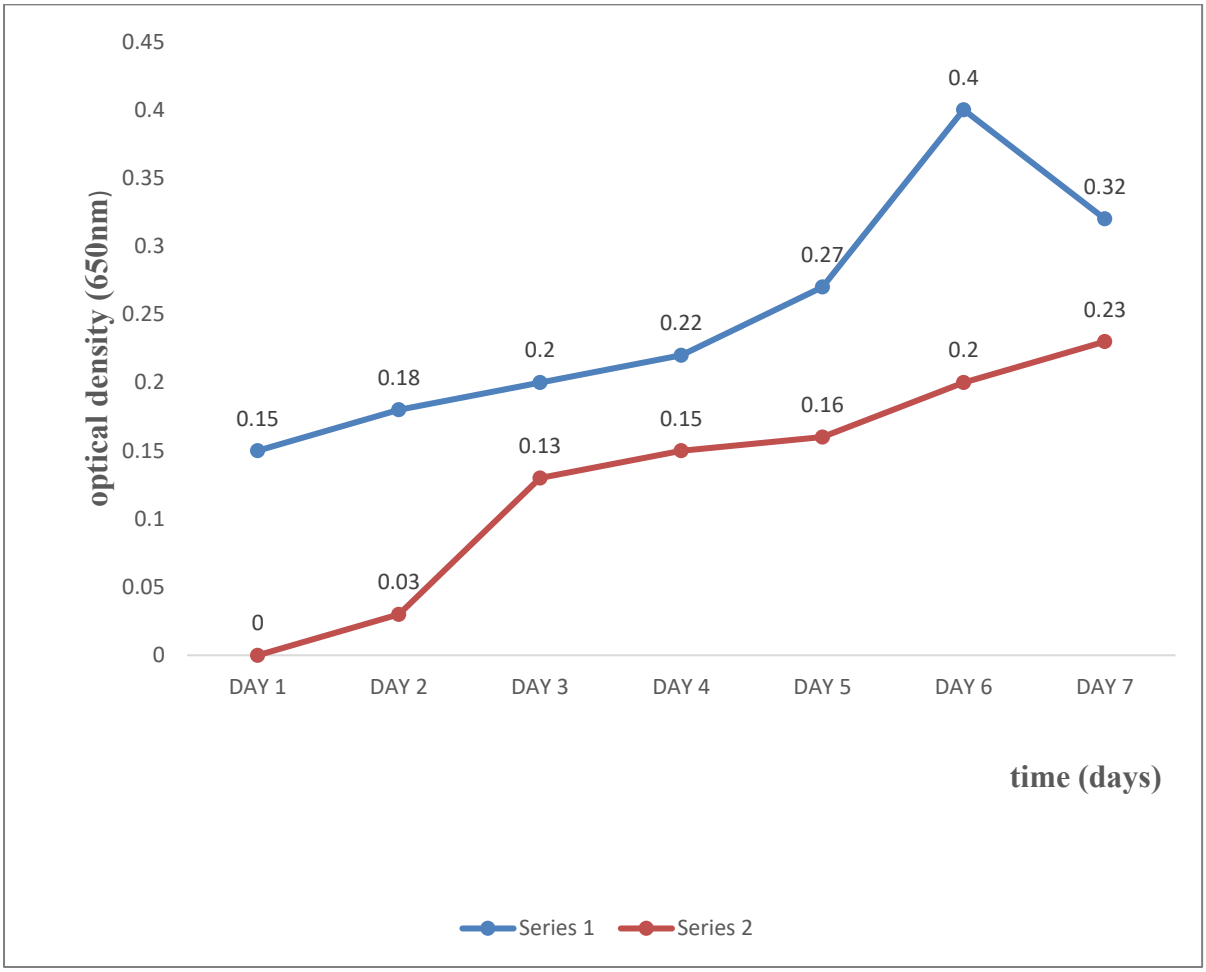

Figure 2: Cell biomass of *Aspergillus niger* on Yam Peels and Yeast Extract Peptone Dextrose (YEPD) Medium
Key: Series 1: yam peels substrate
Series 2: control medium (YEPD)

DISCUSSION

The successful isolation of *Aspergillus niger* from the garden soil shows the natural diversity of the organisms, i.e., the organisms can be found anywhere with favorable environmental conditions, including soil and plant residues (Habib *et al.*, 2015). Anupama & Ravindra, (2001) isolated *Aspergillus niger* from decaying wood in the soil, this fungal isolate was shown to yield SCP, by utilizing rice bran as its substrate.

The proximate composition of the yam peels was shown to contain high amount of carbohydrate (81.73%) and some amount of protein (3.50%). These two constituents are the major sources of nutrients that support microbial growth, thus indicating the potential of the yam peels for use as a substrate. In a study where "potato waste" was used as a substrate in the production of SCP, it was reported to have a proximate composition of 63.06% carbohydrate, 4.61% protein, 52.35% moisture, 4.98% ash and 6.23% fiber (Helal *et al.*, 2022). Another study by Madika *et al.*, (2016) reported that the proximate composition of the substrates "pineapple peels" used for SCP productionis: carbohydrate (59.65%), protein (8.73%), moisture (10.98%), ash (4.91%) and fiber (13.4%), these are in close relationships with the proximate composition of the substrate used in the present study. Carbohydrate is known to be a great source of energy for fungi/moulds, while protein serves as the source of growth-limiting nutrients such as amino acids, nitrogen, and sulphur (Onu *et al.*, 2022). The yam peels substrate has provided good support for the

growth of *Aspergillus niger*. An increase in cell biomass occurred gradually from day 1 to day 5, thus indicating the organism's adaptation to the substrate utilization. The attainment of peak growth on day 6 may be attributed to the full adaptation of the organism to its fermentation medium, and also to achieving maximum access to available carbohydrates. Moreover, the mould's ability to grow exponentially could be explained as the ability of the Aspergillus niger to secrete enzymes that break down complex starch and non-starch polysaccharides into simple sugars for uptake and utilization (Ezekiel, 2013).

The control medium, yeast extract peptone dextrose (YEPD) showed a constant increase in the cellular growth, with a maximum fungal biomass at 0.23 optical density (O.D). The yeast extract peptone dextrose medium supported fair fungal growth compared to the yam peels substrate, thus signifying the potential of the cheap and readily available yam peels substrate to replace the commercially expensive synthetic medium. This finding is in line with the work of Madika (2016), who reported that pineapple waste has the ability to support the growth of *Saccharomyces cerevisiae* more than the reference synthetic medium. The decrease in overall growth observed in the yeast peels fermentation medium on the 7th day could be attributed to the depletion of nutrients within the medium.

Another study by Roy and Bhowal (2021) on the production of SCP using *Aspergillus niger* from mandarin orange peels revealed maximum biomass increase and peak optical density of 0.92 on day 11 of a 12-day fermentation process. The finding of this study, in addition to the results of the current study, shows that agro-residues can be use as substrate for fungi in industrial production processes involving them. Thus, it serves as a tool for reducing the production cost of related industrial products.

CONCLUSION

Apergillus niger was successfully isolated from garden soil, using Sabouraud dextrose agar (SDA) as the medium of choice. It has a dark brown to black conidial surface, a pale yellow colony on the reverse, with an umbonate elevation and rapid growth between day 3 to day 5. Microscopically, it is made up of branched-septate hyphae, with the conidiophore carrying black globular conidia. Proximate composition of the yam peels substrates was percentage (%) *viz* carbohydrate (81.73), protein (3.50), moisture (5.19), ash (5.40), lipid (4.17), and fibre (1.85). Maximum fungal cell biomass of 0.4 optical density was observed on day 6 of fermentation with a decline to 0.32 optical density on day 7, using yam peels as substrate. The commercially synthetic yeast extract peptone dextrose used as a control medium supported a fair increase in fungal cell biomass compared to the main substrate (yam peel), attaining a maximum optical density of 0.23 on day 7.

RECOMMENDATIONS

It is recommended that further studies should aim at utilizing Yam peels and other agricultural waste for sustainable industrial production of single-cell protein.

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