



Conversion of Food waste to Single Cell Protein using *Aspergillus Niger*

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ABSTRACT: The utilization of food waste into products like single cell protein is an alternative solution to global protein shortage and to alleviate pollution problems. This investigation was carried out with food wastes such as orange, pineapple, banana, watermelon and cucumber waste as growth media for *A. niger* using standard techniques. Data obtained showed that Banana waste medium gave a higher yield of *A. niger* biomass and protein content than other waste investigated with values 2.29 ± 0.02 and 0.57 ± 0.01 g/L respectively. Biomass yield from Banana waste medium was statistically significant with the other food waste ($p < 0.05$). Among the various supplemented nitrogen sources in the Banana waste medium, ammonium nitrate (NH_4NO_3) gave the highest biomass and protein yield of 3.20 ± 0.02 and 0.79 ± 0.04 g/L respectively. Thus, the study revealed that *A. niger* biomass can be produced from food waste and optimal yield can be enhanced by supplementing the medium with ammonium nitrate.

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Wastes can be defined as unwanted materials which are discarded from a variety of sources. Waste refers to anything considered useless, but produced by the same action that produces something useful. It could be a by-product of households, industries, agriculture, mining, commercial, and sundry other ventures, activities or sources. When something is unwanted and no longer serves a purpose, it is generally thought of as waste and discarded (Ezejiolora *et al.*, 2014). However, the word waste may have different connotations, since what one considers as waste may not be waste to another person. In other words, waste is not completely useless, since what is considered as waste can be recycled to produce another product (Agunwamba, 1998). In recent years, it has been observed that, the global intensification of food production has resulted in producing large quantities of food and generating lot of agricultural wastes (Elijah and Edema, 2017). Improper management of these wastes can constitute a public health risk and environmental problems, such as diseases and air pollution (Yazid *et al.*, 2017). Nevertheless, this agricultural (food) waste is rich in organic matters that are natural substrate to microorganisms (Azam *et al.*, 2014). Bioconversion of this cheap available substrate to useful products will help in reducing pollutions caused by the waste. The utilization of this cheap available substrate by microorganism into value added products such as fuel and animal feeds

(SCP) thus, enhances food security and sustainable development (Ghosh *et al.*, 2016).

Single cell protein otherwise known as microbial protein is protein obtained from microbial biomass (Hafiza *et al.*, 2011). Using microorganisms for SCP production showed that the protein content is higher compared to plant and animal proteins with good nutritional value (Ezejiolora *et al.*, 2014). Single cell protein technology is designed to solve worldwide protein shortage (Khan *et al.*, 2010). The usefulness of SCP from microorganisms is of great significance such as, food additives, protein supplement, as sources of human and animal feeds, additives in some chemicals and pharmaceutical products (Ukaegbu-Obi, 2016). The advantages of producing SCP through fermentation process as against the conventional methods are as follows; the process is not affected by weather condition, short generation time, it utilize cheap agricultural residues and limited area of land mass (Yunus *et al.*, 2015).

The production of SCP involves a wide variety of microorganisms such as algae, bacteria fungi and yeast (Azam *et al.*, 2014; Yunus *et al.*, 2015; Ukaegbu-Obi, 2016). Algal single cell protein has limitations such as the need for warm temperatures and plenty of sunlight in addition to carbon dioxide, and also that the algal cell wall is indigestible (Azam *et al.*, 2014). Bacteria are capable of growth on a

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wide variety of substrates, they have a short generation time and have high protein content. Their use is somewhat limited by poor public acceptance of bacteria as food, small size and difficulty of harvesting and high content of nucleic acid on dried weight basis. Fungi are becoming widely accepted and used for single cell protein, hence it becomes beneficial to focus on single cell protein from fungi rather than from bacteria and algae. In this investigation, a comparative study was carried out for the utilization of different food waste for the production of *Aspergillus niger* biomass. Also, effect of nitrogen sources on the optimization of biomass yield and protein content was studied.

MATERIALS AND METHODS

Sample Collection and Preparation of Fermentation Medium: Banana, cucumber, orange, pineapple and watermelon food wastes were collected from the food section of Uselu market in Benin City, Edo state, Nigeria. These food wastes which include their peels or mesocarp were washed several times with sterile, distilled water and dried before they were weighed and blended with distilled water in the ratio 1:4. The blended fruit wastes were passed through muslin cloth to trap solids residues, leaving behind the fruit waste broth. A 100 mL of each food waste filtrate was transferred in to 250mL Erlenmeyer flasks. The samples thus prepared were autoclaved at 121°C for 15 min. Samples were prepared in duplicates and were designated Food Waste Medium (FWM)

Isolation and Inoculum Preparation of *Aspergillus niger*: The fungus used for this study was isolated from Onion left at room temperature to undergo spoilage. Identification of the fungal isolate was based on cultural and microscopy characterization following procedures from Barnett and Hunter (1972); Larone (1986) and maintained on potato dextrose agar (PDA) slant and stored at 4°C. Inoculum was prepared from a subcultured *A. niger* on potato dextrose agar (PDA) plates and incubated for 5 d. The *A. niger* cultured plates were flooded with 10 mL of sterile 1% v/v tween 80 solution to dislodge the spores from the hyphae. The solution with spores was filtered with a sterile muslin cloth to remove any hyphal fragments present (Ikenebomeh and Chikwendu, 1997). The number of spores was counted using a haemocytometer and inoculum size of 10^6 spore/mL was used to inoculate all the media.

Fermentation process: Submerged fermentation was carried out at room temperature of $28\pm 2^\circ\text{C}$ on an orbital shaker at a speed of 120 rpm using the food waste media. The media were designated as Cucumber waste medium (CWM), Orange waste

medium (OWM), Banana waste medium (BWM), Pineapple waste medium (PWM) and Watermelon waste medium (WWM). The effects of different nitrogen supplements was investigated. The nitrogen sources were NH_4Cl , KNO_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 and NaNO_3 in the concentration of 1.6, 3.0, 2.0, 1.2 and 2.6 g/L respectively for each to supply 0.42 gN/L of medium. The initial pH of all the media was adjusted to 5.0 using 1.0 N H_2SO_4 and/or 1N NaOH. Each medium (100 mL) was transferred into 250 mL Erlenmeyer flask and sterilized at 121 °C for 15 min. The medium in each flask was inoculated with 500 μL of *A. niger* inoculum (10^6 spores/ mL). The media were left to ferment on an orbital shaker at 120 rpm at temperature of $28\pm 2^\circ\text{C}$ followed by determination of fungal biomass and other parameters at every 2 day interval for 8 day.

Analytical methods: Determination of fungi biomass, pH of medium and cell protein content were carried out after 48 h interval for 8 days. The fermented broth was pasteurized at 65 °C for 30 min in a water bath at 2 d interval. The fungal mycelia were collected through filtration using a pre-weighed Whatman No 1 Filter paper and washed twice with 50 mL sterile distilled water. The collected fungal biomass on the filter paper was dried at 90 °C in a Genlab hot air oven (YIA 110 model, England) to a constant weight. Determination of dried biomass protein content was by a modified biuret procedure of Herbert *et al.* (1971) and Tietz (1986). Proximate analysis of the food waste: Moisture, ash, crude fat, carbohydrate and crude protein contents for the fruit waste were determined according to AOAC (2003) method. Data were subjected to statistical analysis for determination of significance using t-test.

RESULT AND DISCUSSION

The isolated fungus culture obtained from Onions was related mainly to the generic nomenclature *Aspergillus* known as *Aspergillus niger*. Proximate analysis of the fruit wastes is summarized in Table 1. Banana waste contained highest content of carbohydrate ($3.25\pm 0.07\%$) and protein ($1.49\pm 0.02\%$) as compared to other waste. Cucumber waste was found to be the least source of chemical composition with values 0.75 ± 0.03 and $0.85\pm 0.02\%$ for carbohydrate and protein contents respectively.

In this study, food wastes were chosen as potential substrate for SCP production. The bioconversion of these substrates is mostly due to their availability and affordability (Rahman *et al.*, 2016). Food waste is one of the locally available agro-waste rich in organic matters that can be used as carbon and energy sources for fungi growth in SCP production (Adedayo *et al.*,

2011). The study investigated the viable potential of selecting Cucumber, Banana, Orange, Pineapple and Watermelon wastes as substrate for SCP production. The wastes were converted to fermentable sugar through heat treatment. Researchers have used varieties of fruit wastes as a substrate for SCP production. Khan *et al.* (2010); Bacha *et al.* (2011) reported the use of Mango, Apple, Banana, Carrot and Orange wastes as potential substrate for microbial growth and SCP production. The utilization of food waste and other agricultural wastes for fungal biomass production, not only helps to combat pollution but also solves malnutritional problems by

providing protein supplements within the reach of malnourished individuals at an affordable price. The proximate analysis revealed that the food waste contained variable amount of carbohydrate, protein, lipid and moisture content that are useful for fungal growth in the production of single cell protein (Mondal *et al.*, 2012). Banana waste contained higher composition of available carbohydrate and protein content which is likely having a useful effect on the *A. niger* biomass production. This type of substrate composition enhances microbial biomass production (Bacha *et al.*, 2011; Mondal *et al.*, 2012).

Table 1: Proximate composition of various food waste samples

Food waste	Proximate composition (%)					
	Moisture	Ash	Crude fibre	Protein	Lipid	Carbohydrate
Banana	83.11±0.05	2.09±0.01	6.88±0.03	1.49±0.02	1.92±0.02	3.25±0.07
Cucumber	92.94±0.03	0.99±0.03	4.69±0.01	0.85±0.02	0.30±0.02	0.75±0.03
Orange	79.23±0.02	1.42±0.02	11.96±0.05	1.09±0.02	0.48±0.02	2.94±0.03
Pineapple	81.51±0.05	2.49±0.03	13.60±0.04	1.05±0.02	1.04±0.03	1.95±0.03
Watermelon	92.81±0.01	0.20±0.01	5.29±0.02	1.26±0.02	0.10±0.01	0.34±0.01

Five food (Banana, Cucumber, Orange, Pineapple and Watermelon) waste filtrate medium was used as the fermentation medium. The fermentation was carried out in a defined media in order to study the potential of food wastes as an available substrate for fungal biomass and single cell protein (SCP) production. The following defined food wastes media used were: Orange waste medium (OWM), Pineapple waste medium (PWM), Banana waste medium (BWM), Cucumber waste medium (CWM) and Watermelon waste medium (WWM). The *A. niger* biomass cropped in all the fermentation media is illustrated in Fig.1. It was observed that biomass increased gradually as the fermentation progressed. The highest biomass was recorded after day 6 of fermentation in BWM (2.29± 0.02g/L) while the least were from CWM and WWM (1.13±0.02g/L).

The biomass protein content also increased in the course of fermentation with a maximum concentration after 6 d of fermentation in all the media (Fig. 2). The medium that produced highest protein content was BWM followed by PWM and the least was WWM with values of 0.57± 0.01, 0.52 ± 0.01 and 0.31± 0.02 g/L respectively. Comparing the result of BWM and PWM statistically, there was a significant differences (p < 0.05).

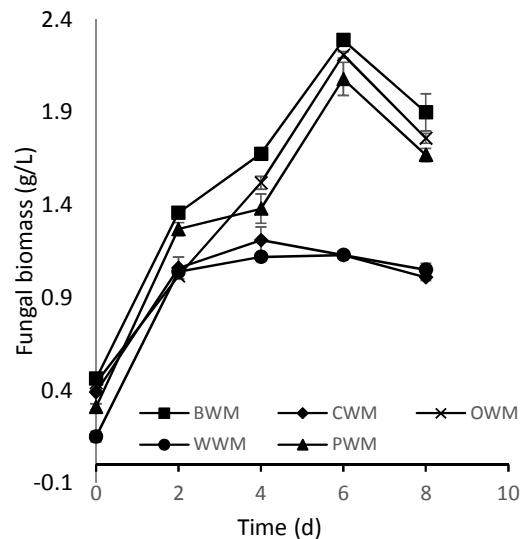


Fig 1: Dried biomass of *Aspergillus niger* yield, produced in the various fruit wastes media in submerged fermentation at 120 rpm on a time course basis. (BWM: Banana waste medium, CWM: Cucumber waste medium, OWM: Orange waste medium, WWM: Watermelon waste medium and PWM: Pineapple waste medium)

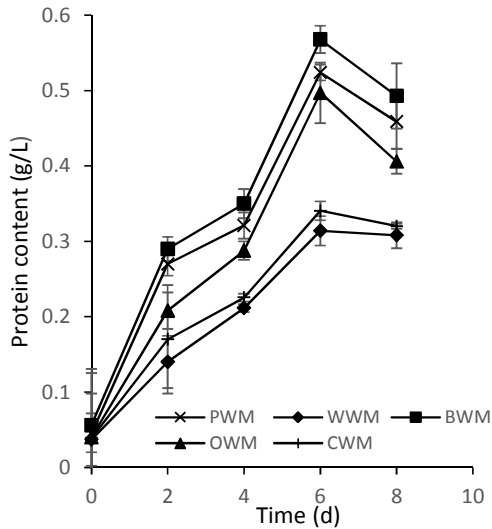


Fig 2: Protein content of *A. niger* biomass using various fruit wastes media in submerged fermentation at 120 rpm on a time course basis. (BWM: Banana waste medium, CWM: Cucumber waste medium, OWM: Orange waste medium, WWM: Watermelon waste medium and PWM: Pineapple waste medium)

The maximum dried biomass cropped was obtained after 6 d of fermentation and it decreased thereafter. Decrease in biomass yield after 6 d of fermentation may be due to nutrient depletion in the growth media. Khan *et al.* (1992); Oshoma and Ikenebomeh (2005) reported that maximum cell biomass was cropped after 6 d of fermentation but, contrary to Aregbesola and Omafuvbe, (2014) who recorded maximum biomass after 4 d.

The investigation revealed that banana waste generated the highest amount of fungal biomass and SCP. Probable reason may be due to the high carbohydrate content in banana waste than other fruit waste. This is in agreement with Khan *et al.* (2010) who reported that banana waste is the best substrate for the production of *Saccharomyces cerevisiae* biomass. Growth of fungi depends mainly on the nutritional composition of the waste that can support the organism biomass (Rahman *et al.*, 2016). Therefore, banana waste contained high amount of chemical compositions than other waste and supported faster growth of the fungus.

The effect of adding nitrogen sources as a nutrient supplements for *A. niger* growth and SCP production was investigated. Dried *A. niger* biomass cropped among the nitrogen sources is shown in Fig.3 after 6 d of fermentation. In all the nitrogen supplementation, NH_4NO_3 medium recorded the highest biomass yield of 3.29 ± 0.02 g/L followed by

$(\text{NH}_4)_2\text{SO}_4$ medium (2.97 ± 0.01) but, statistically not significant ($p > 0.05$). Medium supplemented with KNO_3 gave the least biomass yield of 2.19 ± 0.02 g/L and statistically significant ($p < 0.05$) to that of NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ media.

Analysis of fungal biomass protein content in the nitrogen supplemented media showed that NH_4NO_3 medium had the highest protein content followed by $(\text{NH}_4)_2\text{SO}_4$ and the least was KNO_3 with values of 0.79 ± 0.03 , 0.75 ± 0.01 and 0.45 ± 0.01 g/L respectively. Comparing protein content of NH_4NO_3 and KNO_3 showed a statistically significant difference ($p < 0.05$).

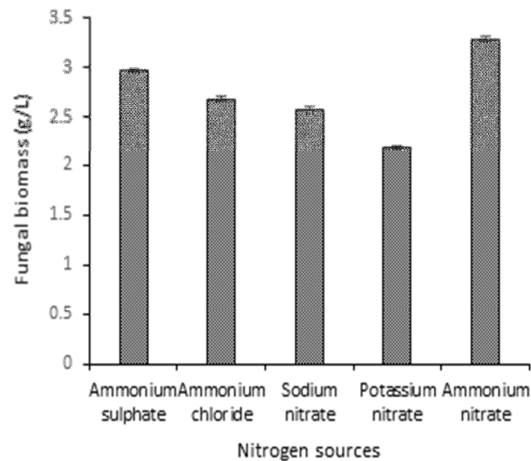


Fig 3: The effects of various nitrogen supplements on *A. niger* biomass yield after 6 d fermentation period on a shaker at 120 rpm and 28 ± 2 °C

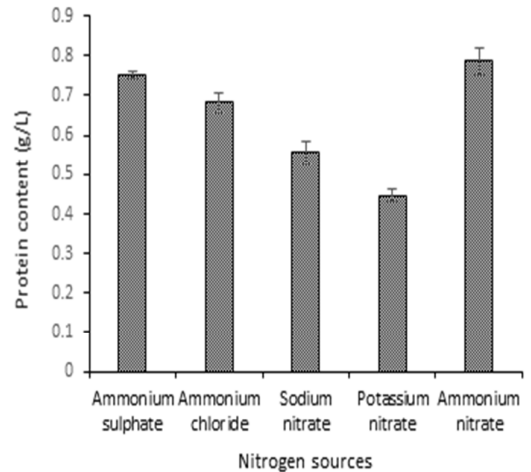


Fig. 4: Protein content of various nitrogen supplements on *A. niger* biomass yield after 6 d fermentation period on a shaker at 120 rpm and 28 ± 2 °C

The agricultural wastes are usually low in nutrients such as nitrogen (Linde *et al.*, 2006). Media supplementation with nitrogen sources could improve fungal growth, hence increase in protein content. Optimization of biomass cropped and protein content of *A. niger* with different nitrogen sources was studied using banana waste that gave higher biomass yield and protein content. Nitrogen source is one of the operational parameters for a successful fermentation processes (Yusun *et al.*, 2015). The effects of various nitrogen sources were investigated to determine the ideal nitrogen source for the maximum yield of fungus biomass and protein content. The result proved that all the nitrogen sources, increased cell biomass yield than the control (without nitrogen). The study of Nadeem *et al.* (2010) observed that medium supplemented with nitrogen enhanced the growth of the organism hence, increase in cell biomass.

In this study NH_4NO_3 was found to crop the highest dried cell biomass followed by $(\text{NH}_4)_2\text{SO}_4$ while KNO_3 gave the least yield. This is in agreement with previous reports when other agricultural and industrial wastes were utilized as substrates and supplemented with nitrogen sources (Anupam and Ravindra, 2001; Oshoma and Ikenebomeh, 2005; Aregbesola and Omafuvbe, 2014). Low yield of KNO_3 was due to the principle of displacement reaction (Anupam and Ravindra, 2001). The principle suggested that K^+ ion is high in the electrochemical series and displaced other ions to form their salts. As such, availability of free nitrogen and other mineral solutions controlling the pH was low for the growth of *A. niger*. This study is contrary to the work of Nadeem *et al.* (2010) who reported that among the various nitrogen sources investigated, NH_4Cl supported maximum yield of 5.78 g/L.

The dried cell biomass protein contents of *A. niger* were estimated and found that the highest content was from medium supplemented with NH_4NO_3 . Similar results had been reported by using $(\text{NH}_4)_2\text{SO}_4$ with *A. niger* (Oshoma and Ikenebomeh, 2005; Nadeem *et al.*, 2010; Aregbesola and Omafuvbe, 2014). Alemu (2013) recorded a maximum protein yield of 24.04 % in *A. niger* cell biomass grown in orange waste medium and supplemented with ammonium sulphate. The low protein content obtained from the control (without nitrogen) could be as a result of the deficiency of nutrient supplementation, mostly nitrogen sources, required for fungal growth. This highlights the importance of nitrogen supplementation to increase protein yield. On the contrary, Mondal *et al.* (2012) reported that nitrogen

supplementation has a suppressive effect on *Saccharomyces cerevisiae* protein yield.

Conclusion: This study proved that Banana waste is the ideal substrate for *A. niger* growth. Thus, the fruit waste should be exploited as a substrate for the production of cell biomass rather than being dumped on the roads and water bodies. The fungus biomass can be enhanced with ammonium nitrate as a supplement in the fruit waste medium for optimal yield. The use of ammonium nitrate will reduce media cost as organic nitrogen sources are costlier than the inorganic nitrogen sources.

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