

**PROXIMATE COMPOSITION OF AGRO SHELL WASTES AND THEIR  
UTILISATION FOR BIOPROTEIN PRODUCTION WITH *Candida tropicalis*  
MYA- 3404**

**\*OMOGBAI, B. A., OBAZENU, E. I., IDUORIYEKEMWEN, A. S., UBAKA, L. N.  
AND IMONI, A. A.**

Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City,  
Nigeria

\*Corresponding author: barry.omogbai@uniben .edu

---

**ABSTRACT**

An increasing interest for global food shortages for the world's expanding population necessitated the use of agro-waste as food sources or supplement for animal feeds. *Candida tropicalis* MYA- 3404 was isolated from pineapple fruit waste and identified by both phenotypic and molecular methods. Proximate analysis of three agro-wastes: melon, groundnut and walnut shell was carried out by standard chemical analytical methods. The protein content ranged between 3.73 to 12.14 % with groundnut shell having the highest. The shells contained 61.26 to 65.12 % carbohydrate. The moisture, fibre, ash and lipids were in the range 12.5 to 19.07 %, 6.45 to 11.22 %, 1.43 to 6.50 % and 0.43 to 1.14 % respectively. Optimized production of bioprotein by *Candida tropicalis* MYA- 3404 was at pH 7.0, for 72h. Supplementation with nitrogen sources revealed highest biomass production with yeast extract. The optimization of a combination of carbon sources showed highest biomass production of 0.4640±0.053 mg/l for groundnut shell medium. Using submerged fermentation, maximum yield of cell biomass was produced from groundnut shell followed by melon shell as substrate for fermentation. *C. tropicalis* MYA 3404 can be used to produce bioprotein from agro-waste as a cheap source of protein in animal feed.

**KEYWORDS:** Yeast, Shells, Fermentation, Protein

---

**INTRODUCTION**

Dried cell biomass often referred to as bio-protein or Single cell protein (SCP) is a mixture of organic compounds such as proteins, lipids, carbohydrates, nucleic acids, and a variety of vitamins as well as inorganic compounds. The composition of single cell protein depends on the nature of substrate and also the microorganism employed for its production. Cell protein from yeast and fungi comprise 50-55 %

and has high protein: carbohydrates ratio. It contains less methionine and cysteine and more of lysine. It possess a balance of amino acid, high B-complex vitamins hence more suitable as poultry feed (Mchoi and Park, 2003).

Before World War I the interest in SCP had already started (Ugalde and Castrillo, 2002). Using Baker's yeast Germany tried to supplement their protein supply in animal feed during World War

---

1. They were able to substitute as much as half of all the protein sources imported at that time with yeast (Ugalde and Castrillo, 2002). The yeast was cultivated on molasses as carbon source and ammonium salts were used as nitrogen source (Litchfield, 1983). After the end of the World War I the interest in yeast as fodder declined but arose again when World War II started. At this point yeast had been included into the army's diets, and after some time also into the diets of civilians.

However, the determination to produce more than 100 000 tonnes of yeast yearly were never reached (Ugalde and Castrillo, 2002). *Candida utilis* (Torula yeast) was of interest and it was cultivated on sulphite waste liquor from the pulp and paper industries and on wood sugar derived from acid hydrolysis of wood (Litchfield, 1983). Torula yeast production continued even after the World War II in the United States with regards to the utilization of natural sources for fodder (Ugalde and Castrillo, 2002).

The research into SCP production is growing extensively using agro-waste. An increasing interest for global food shortages for the world's expanding population necessitated the use of agro-waste as food sources or supplement for animal feeds. Microbial production is given due attention owing to microorganisms ability to utilize waste materials that cause pollution problems and/ or sanitary hazards.

In the production of microbial protein, several natural products are been tested. The utilization of agro-waste and industrial waste products for microbial cultivation appear to be a general trend in studies of this applied nature. Haider and

EL-Hassy (2000) tested date extract supplemented with nitrogen source as a suitable substrate whereas, cashew and apple juice have also been used. Even though agricultural wastes are useful substrate for production of microbial protein, it is essential that they meet the following criteria; it should be nontoxic, abundant, totally regenerable, non-exotic, cheap and capable to initiate rapid growth of the organisms resulting in high quality cell biomass. Some agricultural and agro industrial waste products used for SCP and other metabolites production include; orange waste, mango waste, cotton salks, kinnow-mandarin waste, barley straw, corn cobs, rice straw, corn straw, onion juice and sugar cane bagasse (Nigam *et al.*, 2000), cassava starch (Tipparat *et al.*, 1995), wheat straw (Abou Hamed, 1993), banana waste (Saquido *et al.*, 1981), capsicum powder (Zhao *et al.*, 2010) and coconut water (Smith and Bull, 1976). The presence of these waste abundantly in nature coupled with their cheap cost could be attributed to their usage as a sole carbon and nitrogen source for the production of SCP. Solid state fermentation (SSF) is currently being used for the production of protein-enriched feed (Rodriguez-Vazquez *et al.*, 1992; Chaudhari *et al.*, 1994). However employing industrial scale application of SSF for production of SCP would help in increased yields and improved conversion efficiency, which would reduce the overall cost of the final product. In the present study an attempt has been made to produce SCP with *Candida tropicalis* MYA- 3404 through submerged fermentation by using shells of walnut, groundnut and melon as energy sources.

## MATERIALS AND METHODS

### *Source of culture and Agro-shell samples*

The microbial culture used for fermentation was *Candida utilis* MYA-3404 isolated from soil sample rich in organic matter. Agro-shell wastes of groundnut and melon were obtained from small holder industries producing fried bottled groundnuts and melon seeds for commercial purpose. Walnut shell was obtained by aseptic mechanical cracking of the nuts in the laboratory. The shells were air-dried for 48 h and converted into powder with a household Lance grinding machine (model: LF80, Germany).

### *Determination of Proximate composition of Agro-shells*

The kjeldahl technique was used for determination of protein content. The quantity of crude protein was calculated by multiplying the % nitrogen obtained by 6.25. The moisture content was determined by drying to a constant weight. Ash content was evaluated by dry ashing using a muffle furnace at 600°C for 6h. The amount of lipid in the shells was determined by using a soxhlet extractor petroleum ether as solvent. The residue obtained from ether extracted was further treated with 1.25 % sulphuric acid and 1.25 % of sodium hydroxide under heating for 30 min. The content was ashed in muffle furnace and reweighed to obtain the fibre content. Carbohydrate was obtained by difference of the other aforementioned components (A.O.A.C., 1995).

### *Isolation and identification of fungal isolate*

One gram (1g) of the soil sample collected was mixed thoroughly with 9ml of sterile distilled water. A ten-fold serial dilution was then carried out. A measured 0.1ml was transferred into Sabouraud

dextrose agar supplemented with 16 µg/ml of chloramphenicol. The plates were incubated at 28±2°C for 72h. The discrete colonies on Sabouraud dextrose agar were selected, counted and examined macroscopically and microscopically using needle mount technique. They were identified based on the procedure outlined by Barnett and Hunter (1998).

### *Identification of Candida Isolates*

Speciation was done by germ tube test, chlamyospore production on corn meal agar and sugar fermentation and assimilation tests using the Microexpress *Candida* identification kit (Tulip, India).

### *Direct Examination of the culture*

**Gram Stain:** Smears were examined for the presence of gram positive budding yeast cells with pseudohyphae.

**Potassium hydroxide (KOH) Mount:** Smears were prepared for each sample by adding a loop full of colony to a drop of 10% KOH on a clean, grease-free slide and placing a cover slip over it. The preparations were slightly warmed to digest the materials and examined under the microscope with X10 and X40 objective for yeast cells, pseudohyphae.

**Germ Tube Test:** All presumptive *Candida* species were subjected to germ tube test by picking colonies from the purity plates. Small test tubes containing about 0.5 ml human serum were inoculated with colonies of test organisms in batches of three replicates per test. Each batch included a known positive and negative control. Inoculated tubes were incubated at 37°C for 2 - 3 h. At the end of incubation, a drop of each serum was transferred to a clean slide, and a cover slip placed over it. These slides were examined microscopically under high power (×40) objective to detect the

presence of germ tubes, which are short hyphal initials.

**Chlamyospore Formation:** All *Candida* isolates were tested for the production of chlamyospores in corn meal agar (CMA) with 1% Tween 80. Subcultures on corn meal agar plates were made from SDA purity plate by furrowing the CMA plates (cut streak method), and incubating at room temperature (28°C) for 2 - 5 days after which they were examined for the production of thick-walled chlamyospores in a lactophenol cotton blue mount of samples taken from the furrows of the corn meal agar plates.

**Sugar Fermentation Test:** All *Candida* isolates were subjected to carbohydrate fermentation and utilization test using *Candida* identification kit (Tulip Diagnostics Limited, India). Each kit contained sterile media for colorimetric identification using biochemical test and carbohydrate utilization tests based on the principle of pH change and substrate utilization designed to identify various metabolic properties of different *Candida* species that can be used to differentiate even closely related *Candida* species. The media were inoculated by adding 100 µl of the *Candida* suspension, incubated at 28°C and read after 24 - 48 h incubation. The *Candidal* suspension was prepared by purifying on a Brain Heart Infusion Agar (BHIA) medium, after which a single discrete colony was picked up and streaked on to BHIA slant for enrichment and incubated at 28°C (room temperature) for 24 - 48 h. The growth on the slant was washed with 2 - 3 ml of sterile saline and the turbidity of the suspension compared with Mcfarland standard number 5. The results were interpreted as per the standards given by the manufacturer in the result interpretation chart.

### **Molecular identification of *Candida* DNA Extraction and PCR Amplification of the Fungal ITS gene**

DNA extraction was carried out on the isolate using the Zymo ZR Fungi DNA miniprep Kit (Zymo Scientific, USA) according to manufacturer's instructions. Polymerase chain reaction was carried out to amplify the Internal Transcribed Spacer ITS gene of the fungi using the primer pair ITS-1 (tccgtaggtgaacctgccc) and ITS-4 (tctccgcttattgatatgcc) (Manter and Vivanco, 2007). The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5X concentration to 1X concentration containing 1X Blend Master mix buffer (Solis Biodyne), 1.5mM MgCl<sub>2</sub>, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), proofreading enzyme, 2µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Eppendorf Vapo protect thermal cycler (Nexus Series) at an initial denaturation temperature of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 55°C and 1 minute 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. During electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight standard.

### **PCR amplification of the 18S rRNA gene and sequencing**

The 18S rRNA amplified was sequenced with a BigDye Terminatory v3.1 cycle sequencing kit using an automated capillary sequencer (model 3100 Avant Genetic Analyzer, Applied Biosystems). Analyses of the gene sequences were carried out as described by Yakimov *et al.* (2006) in conjunction with the similarity rank from Ribosomal Database Project (RDP) Maidak *et al.* (1997). Finally, the FASTA nucleotide database query available through European Bioinformatics Institute were employed to determine the partial 18S rRNA sequences and the degree of similarity to other 18S rRNA gene was estimated.

### **Fermentation**

A batch fermenter was used for the production of Single Cell Protein (SCP) from shell of melon, groundnut and walnut. The experiment set consisted of the fermenter, the air supply and the digital based data acquisition control system. The fermenter and all accessories were chemically treated with 2% potassium metabisulphate solution and then washed with hot water several times. The reactor was filled with one litre (1L) of mineral salt medium (MSM) containing 20g each of melon shell, walnut shell, and groundnut shell as a sole substrate and immediately inoculated with 40 ml of the inoculum. Cell suspensions were prepared from 24h old cultures in sterile media and aseptically introduced to the flask containing MSM. The pH of the medium was adjusted as desired before autoclaving. The pH was maintained with the addition of HCl or NaOH and determined using digital based pH measurement and control system. The

reactor was operated at mixing speed of 300rpm. The samples were checked after 48 h for maximum yield of biomass.

### **Optimization of process parameters for bioprotein production**

**Inoculum Size:** Different concentrations of  $1.5 \times 10^8$  Mcfarland inocula sizes of 2, 3, 4, 5 and 6% v/v were used to analyze for the maximum production of single cell biomass of isolate at pH 6 for a period of 72h at 120 rpm

**Fermentation period:** The effect of fermentation period on bioprotein production was evaluated by harvesting samples at 24, 48, 72, 96 and 120h to check for the maximum yield of biomass at the different incubation period.

**Medium pH:** The initial pH values of 4, 5, 6, 7 and 8 of the growth media was checked before sterilization to enhance the maximum production of biomass of isolate.

**Carbon source addition:** Different carbon source such as glucose, sucrose, maltose, fructose and mixture of the four carbon source (glucose + sucrose + maltose + fructose) each at 0.5 % w/v were used to supplement the shell medium to check for the maximum production of biomass.

**Nitrogen source supplementation:** Organic nitrogen sources (urea, peptone and yeast extract powder) and inorganic (ammonium nitrate, ammonium sulphate,) nitrogen sources were supplemented to the growth media, each at 0.25% w/v to test for the maximum production of yeast biomass.

### **Isolation of yeast bioprotein from fermenter**

A measured 10ml of the fermented media was centrifuged for 20min at 6000rpm. The supernatant obtained was subjected to ammonium sulphate precipitation. Ammonium sulphate [40%

(w/v)] was added to the cell-free supernatant and stirred for 4 h at 4°C. The precipitate obtained was allowed to stand for 2 h and then collected by centrifugation at 15000 rpm for 20 min. The pellet obtained was dissolved in 2ml of 2mM glycine NaOH buffer, pH at 11 and protein was assayed by Lowry's method (Lowry *et al.*, 1951). The blue colour developed by the phosphomolybdic phosphorus in the folincioalteau reagent by the amino acid tyrosine and the tryptophan present in the protein plus the colour developed by the biuretic reaction of the protein with the alkaline cupric tartarate were measured by Lowry's method. Several dilutions of 0.3 mg/ml bovine serum albumin (BSA) was prepared in the same buffer having the unknown of 30 to 150 micrograms/ml (0.03 to 0.15 mg/ml). 1.0 ml was added to each dilution of standard, protein-containing unknown, or buffer (for the reference) to 0.90 ml reagent A (0.1 N of NaOH +0.1N of Na<sub>2</sub>CO<sub>3</sub>) in different test tubes and mixed. The test tubes were incubated for 10 min in a 50 °C bath, and cooled to room temperature. A measured 0.1 ml reagent B (0.02M of CuSO<sub>4</sub>.5H<sub>2</sub>O+0.052M of Na<sub>2</sub>Tartrate) was delivered to each test tube, mixed and incubated for 10min at room temperature. This was followed by rapidly adding 3 ml of reagent C (0.124 M of Na<sub>2</sub>Tartrate.2H<sub>2</sub>O) to each test tube, mixed and incubated for 10 min in the 50

°C water bath and cooled to room temperature. Five millilitre (5ml) of samples was added to each test-tube and 5 ml of distilled water was added to the blank test tube. It was thoroughly mixed and allowed to stand for 10 minutes The 5 ml of the alkaline cupric tatrare reagent was added to each tube including the blank. It was well mixed and incubated at room temperature in the dark for 30 min to allow for blue colour development. The reading was taken at 660 nm in the colorimeter. Working standard of tyrosine and tryptophan of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 1mg/l were also prepared into the series of test tubes which were treated in the same way as the samples and run alongside with the samples.

## RESULTS AND DISCUSSION

The phenotypic characteristics of the yeast isolate used in this experimentation is shown in Table 1. The Gram reaction showed it as positive blue cells and growth on corn meal tween 80 agar revealed the formation of pseudohyphae or chlamydospore. The organism fermented glucose and sucrose but was negative for others. In plate 1 is shown the result of the molecular characterisation with band at 600bp positive for *Candida tropicalis*. The base sequence identity of *Candida tropicalis* strain MYA-3404 is shown in Table 2 with Ascension number NW\_0030200391 and 94 % homology.

**Plate 1:** Polymerase chain reaction results for fungal isolate analyzed with 1.5% agarose gel electrophoresis. Lane M is 100bp-1kb DNA ladder (molecular marker). Lane 1 is positive for *Candida tropicalis* with band at 600bp.

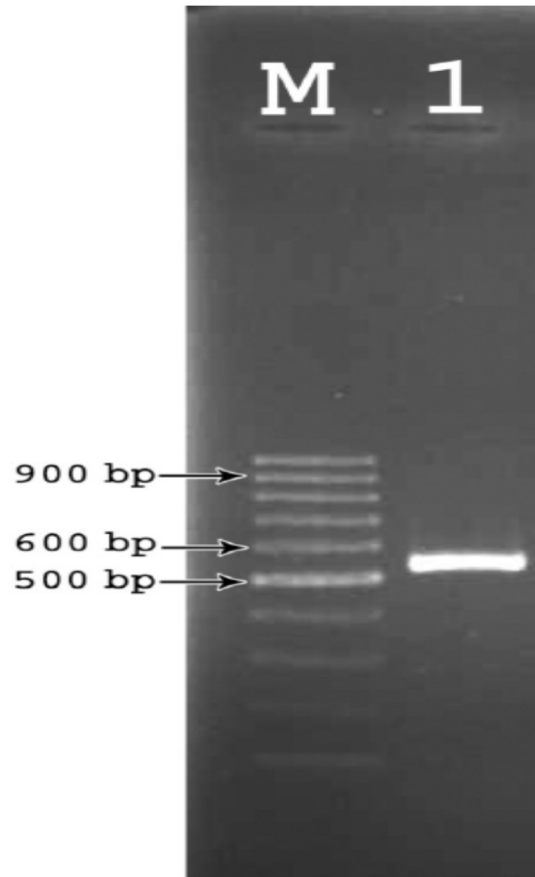


Table 2: Base sequence of *Candida tropicalis* strain MYA-3404

Sequence blast	Ascension no.	Sequence identity	Query coverage length	Score bits/percent homology
<p>GCACCACATGTGTTTTTTATTGAACAAATTTCTTTGGTGGCGGGAGCAATCCTACCGCC            AGAGGTTATAACTAAACCAAACCTTTTTATTTACAGTCAAACCTTGATTTATTATTACAAT            AGTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAA            ATGCGATACGTAATATGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACA            TTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACC            CCCGGGTTTGGTGTTGAGCAATACGCTAGGTTTGTGTTGAAAGAATTTAACGTGGAAAC            TTATTTTAAGCGACTTAGGTTTATCCAAAACGCTTATTTTGCTAGTGGCCACCACAATT            TATTTTCATAACTTTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA            ATAAGCGGAGGAAAAG</p>	<p>NW_003020039.1</p>	<p><i>Candida tropicalis</i> strain MYA-3404</p>	<p>745</p>	<p>460/487(94 %)</p>



The proximate analysis of the three agro-waste with respect to their percentage occurrence are shown in Table 3. Groundnut shell had the highest crude protein content of (12.14±0.92 %) and walnut shell (3.73±0.31 %) the least. Moisture content analysis showed a range between 12.50±0.50 % to 19.07±1.52%. Walnut shell had the highest crude fibre content of 11.22±1.60 % compared to groundnut shell which had the least of 6.45±0.80 %. The result of lipid content indicated that groundnut shell had the highest (1.14±0.01 %), followed by walnut shell (0.49±0.00 %) and melon shell had the least (0.43±0.00 %). Crude ash content of walnut shell was highest (6.50±0.21 %), while melon shell had the least (1.43±0.15 %). The three agro-waste shells had high carbohydrate content which ranged from 61.26±0.32 % to 65.12±1.14 % with melon shell having the least.

The effect of pH in this study revealed that maximum cell biomass yield was produced at pH 6. The cell yield of 0.1086 mg/l was highest with melon shell as the substrate for fermentation. Followed by 0.1054 mg/l cell yield obtained at pH 7 using melon shell containing medium. The least cell biomass (0.0188 mg/l) was produced with walnut shell as substrate for fermentation at pH 5.0. Although bioprotein production was on the increase from pH 5.0 for the agro shell media, a decrease occurred with progression from pH 7.0 to higher values (Table 4).

The higher the inoculum the greater the biomass yield at first. Among various inoculum sizes, 4 ml (v/v) inoculum size gave a maximum yield of cell biomass of 0.0956 mg/l with groundnut shell as

substrate fermentation. This was followed by cell yield of (0.0874 mg/l) from melon shell containing medium. The least biomass obtained (0.0028 mg/l) was from walnut shell substrate inoculated with 2 ml (v/v) inoculum size of *C. tropicalis* MYA-3404 as shown in Table 5.

The result of the effect of incubation time showed that 72 h incubation period using groundnut shell as substrate yielded in maximum cell biomass of 0.1546 mg/l as shown in Table 6. The least cell biomass of 0.0050 mg/l was produced after 24 h with melon shell containing medium.

Supplementation of the three substrates with organic and inorganic nitrogen sources showed that the maximum cell biomass of 0.4840 mg/l was produced with yeast extract addition using walnut shell as substrate for fermentation. This was followed by 0.3410 mg/l cell biomass from groundnut shell as substrate supplemented with ammonium sulphate in Table 7. Peptone addition produced the least cell yield (0.0430 mg/l) from walnut shell containing medium.

The incorporation of all the carbon sources (glucose, maltose, sucrose and fructose) gave the maximum biomass (0.4640 mg/l) after fermentation of the medium containing groundnut shell as substrate for fermentation. Medium supplemented with glucose had the second highest biomass yield (0.3166 mg/l) after inoculating groundnut shell containing medium as shown in Table 8. The least cell yield of (0.0743mg/l) was obtained from walnut shell containing medium that was supplemented with maltose.

Table 3: Proximate analysis of agro-shell wastes

Parameter (%)	Walnut shell	Groundnut shell	Melon shell
Moisture	12.50±0.50 <sup>b</sup>	13.7±0.80 <sup>b</sup>	19.07±1.52 <sup>a</sup>
Protein	3.73±0.31 <sup>c</sup>	12.14±0.92 <sup>a</sup>	9.26±0.71 <sup>b</sup>
Fibre	11.22±1.60 <sup>a</sup>	6.45±0.80 <sup>b</sup>	7.57±0.40 <sup>b</sup>
Lipid	0.49±0.00 <sup>b</sup>	1.14±0.01 <sup>a</sup>	0.43±0.00 <sup>b</sup>
Ash	6.50±0.21 <sup>a</sup>	2.79±0.11 <sup>b</sup>	1.43±0.15 <sup>c</sup>
Carbohydrate	65.12±1.40 <sup>a</sup>	63.52±0.81 <sup>b</sup>	61.26±0.32 <sup>c</sup>

**Legend:** Values are means of triplicates ± standard deviations (SD). Mean differences are presented across column and values with significant difference carry different alphabets a, b and c.

Table 4: Optimization of pH for the biosynthesis of single cell protein from agro-wastes

pH	Walnut shell	Groundnut shell	Melon shell	p-value
5	0.0188±0.007 <sup>A</sup>	0.0287±0.009 <sup>C</sup>	0.0382±0.010 <sup>C</sup>	0.000
6	0.0628±0.023 <sup>B</sup>	0.0888±0.041 <sup>C</sup>	0.1086±0.051 <sup>D</sup>	0.000
7	0.0568±0.014 <sup>B</sup>	0.0804±0.009 <sup>CD</sup>	0.1054±0.017 <sup>E</sup>	0.000
8	0.0496±0.043 <sup>B</sup>	0.0766±0.031 <sup>C</sup>	0.0927±0.019 <sup>D</sup>	0.000
9	0.0386±0.062 <sup>BC</sup>	0.0402±0.046 <sup>C</sup>	0.0468±0.058 <sup>C</sup>	0.008

**Legend:** Values are means of triplicates ± standard deviations (SD). Mean differences are presented as <sup>A</sup>, <sup>B</sup>, <sup>C</sup>, <sup>D</sup>, and <sup>E</sup> across column and values with significant difference carry different alphabets.

Table 5: Optimization of inoculum sizes for the biosynthesis of single cell protein from agro-wastes

Inoculum size (1.5x10 <sup>8</sup> ) McFarland (ml)	Walnut shell	Groundnut shell	Melon shell	p-value
2	0.0028±0.005 <sup>A</sup>	0.0108±0.050 <sup>B</sup>	0.0042±0.002 <sup>AB</sup>	0.1884
3	0.0346±0.007 <sup>B</sup>	0.0658±0.005 <sup>E</sup>	0.0611±0.010 <sup>D</sup>	0.000
4	0.0508±0.043 <sup>B</sup>	0.0956±0.031 <sup>D</sup>	0.0874±0.048 <sup>D</sup>	0.000
5	0.0276±0.036 <sup>AB</sup>	0.0494±0.050 <sup>C</sup>	0.0485±0.052 <sup>C</sup>	0.001
6	0.0238±0.007 <sup>A</sup>	0.0432±0.013 <sup>C</sup>	0.0487±0.049 <sup>C</sup>	0.000

**Legend:** Values are means of triplicates ± standard deviations (SD). Mean differences are presented as <sup>A</sup>, <sup>B</sup>, <sup>C</sup>, <sup>D</sup>, and <sup>E</sup> across column and values with significant difference carry different alphabets.

Table 6: Optimization of incubation period for the biosynthesis of single cell protein from agro-wastes

Incubation period (hr)	Walnut shell	Groundnut shell	Melon shell	p-value
24	0.0112±0.039 <sup>A</sup>	0.0096±0.012 <sup>A</sup>	0.0050±0.006 <sup>A</sup>	0.635
48	0.0708±0.054 <sup>A</sup>	0.1320±0.020 <sup>C</sup>	0.1044±0.027 <sup>B</sup>	0.000
72	0.0936±0.036 <sup>A</sup>	0.1546±0.025 <sup>D</sup>	0.1283±0.045 <sup>C</sup>	0.000
96	0.0502±0.057 <sup>A</sup>	0.0877±0.054 <sup>CD</sup>	0.0682±0.009 <sup>B</sup>	0.001
120	0.0487±0.050 <sup>A</sup>	0.0751±0.036 <sup>BC</sup>	0.0682±0.019 <sup>AB</sup>	0.000

**Legend:** Values are means of triplicates ± standard deviations (SD). Mean differences are presented as <sup>A</sup>, <sup>B</sup>, <sup>C</sup>, <sup>D</sup>, and <sup>E</sup> across column and values with significant difference carry different alphabets.

Table 7: Optimization of nitrogen sources for the biosynthesis of single cell protein from agro-wastes

Nitrogen Sources	Walnut shell	Groundnut shell	Melon shell	p-value
Yeast extract	0.1916±0.008 <sup>A</sup>	0.4840±0.022 <sup>D</sup>	0.2950±0.006 <sup>B</sup>	0.000
Peptone	0.0430±0.012 <sup>A</sup>	0.1173±0.010 <sup>A</sup>	0.0643±0.013 <sup>A</sup>	0.025
Urea	0.0866±0.006 <sup>A</sup>	0.2126±0.002 <sup>C</sup>	0.1360±0.004 <sup>B</sup>	0.000
Ammonium nitrate	0.0650±0.005 <sup>A</sup>	0.1433±0.004 <sup>C</sup>	0.0883±0.006 <sup>B</sup>	0.000
Ammonium sulphate	0.1586±0.007 <sup>A</sup>	0.3410±0.039 <sup>C</sup>	0.2666±0.028 <sup>B</sup>	0.000
Control	0.0629±0.023 <sup>B</sup>	0.0888±0.041 <sup>C</sup>	0.1086±0.051 <sup>D</sup>	0.000

**Legend:** Values are means of triplicates ± standard deviations (SD). Mean differences are presented as <sup>A, B, C, D, and E</sup> across column and values with significant difference carry different alphabets.

Table 8: Optimization of carbon sources for the biosynthesis of single cell protein from agro-wastes

Carbon source	Walnut shell(mg/l)	Groundnut shell(mg/l)	Melon shell(mg/l)	p-value
Glucose	0.2153±0.015 <sup>A</sup>	0.3166±0.029 <sup>C</sup>	0.2433±0.006 <sup>AB</sup>	0.000
Maltose	0.0743±0.021 <sup>A</sup>	0.1583±0.040 <sup>B</sup>	0.1673±0.055 <sup>B</sup>	0.006
Sucrose	0.1833±0.017 <sup>AB</sup>	0.2506±0.045 <sup>C</sup>	0.1890±0.005 <sup>AB</sup>	0.002
Fructose	0.1200±0.004 <sup>B</sup>	0.1860±0.005 <sup>C</sup>	0.1376±0.006 <sup>B</sup>	0.000
G+M+S+F	0.2926±0.011 <sup>B</sup>	0.4640±0.053 <sup>D</sup>	0.3803±0.041 <sup>C</sup>	0.000
Control	0.0629±0.023 <sup>B</sup>	0.0888±0.041 <sup>C</sup>	0.1086±0.051 <sup>D</sup>	0.000

**Legend:** Values are means of triplicates ± standard deviations (SD). Mean differences are presented as <sup>A, B, C, D, and E</sup> across column and values with significant difference carry different alphabets.

A host of raw materials have been selected as substrate for SCP biosynthesis (Nasseri *et al.*, 2011). The most widely used are carbohydrate sources which are natural microbial substrates which also comprise a renewable feedstock (Ugalde and Castrillo, 2002). Agro-shells of walnut, groundnut and melon are rich in carbohydrate (61.26±0.32 to 65.12±1.40 %) which can provide carbon source for growth and metabolism of *Candida tropicalis* MY-3404. Ezekiel *et al.* (2002) reported that a large scale production of SCP both for human and animal feed depends on cheap and available substrates with carbon source.

The acidity or alkalinity of a fermentation media is important to growth and biosynthesis of materials. A pH

optimum of 6.0 was found for effective biomass formation in this study with the agro-shells. This is in conformity with the results obtained by Rajoka *et al.* (2005). Rosma and Ooi (2006) however reported a pH of 4.5 for *Candida utilis*. Halasz and Radomie, 1991 have noted that increase in bioprotein production increases with pH from 4 to 7. On the whole *Candida* species are capable of good growth in media with pH between 3.0 to 6.2 (Adoki 2007).

Inoculum size is key to breakdown of suitable substrate for biomass production. An inoculum size of 4 ml (v/v) resulted in maximum cell yield (0.0956 mg/ml) in this experiment with groundnut shell. Ravinder *et al.* (2003) obtained the maximum growth yield with 3% (v/v) inoculum size working with deoiled rice

bran. A 10 % (v/v) inoculum size resulted in maximum biomass of *Candida utilis* on rice bran as reported by Rajoka *et al.* (2005). Yunus *et al.* (2015) also obtained a maximum biomass yield for *Candida utilis* and *Rhizopus oligosporus* grown on wheat bran. The differences in maximum yield with different inoculum sizes may be attributed to the diverse organisms, substrates and fermentation techniques employed.

The maximum production of yeast biomass ( $0.0936 \pm 0.036$  to  $0.1546 \pm 0.025$  mg/ml) was found after 72 h of incubation for all agro shells used in this study with groundnut shell taking the lead in production. However no further increase was seen after 72 h. While this corroborates the report of Ravinder *et al.* (2003), it is discordant with Adoki (2007) who reported maximum biomass of *Candida* spp at 96 h as well as Yunus *et al.* (2015) who reported 48h for *Candida utilis* and *Rhizopus oligosporus*. Lubna *et al.* (2004) observed a maximum cell biomass of *Aspergillus niger* after 120 h of incubation.

In other to improve cell mass production of *Candida tropicalis* in this study, different nitrogen sources: organic and inorganic were added to the fermentation media containing agro shells. The results clearly demonstrates that nitrogen compounds influenced the biomass yield. It was observed that the maximum biomass yield of 0.4840 mg/ml was obtained with yeast extract using groundnut shell medium. The least was obtained for peptone. This result is in concordance with the report of Rajoka *et al.* (2004) and Zhao *et al.* (2010) who reported the production of single cell protein using waste capsicum powder produced during capsanthin extraction.

The supplementation with all carbon source resulted in maximum biomass yield of 0.4640 mg/ml during fermentation with groundnut shell medium. The least biomass yield (0.0743 mg/ml) was produced with maltose using walnut shell as substrate. The addition of glucose as a carbon source resulted in an appreciable biomass yield of 0.3166 mg/ml with groundnut shell. This is concordance with the report of Kurbanoulu who applied it on bioprotein production using horn hydrolysate. The use of groundnut shell supplemented with organic nitrogen source such as yeast extract and subsequent fermentation with *Candida tropicalis* MY-3404 can lead to effective bioprotein production using this organism while maintaining other parameters of pH, temperature and inoculum size. Thus instead of burning groundnut shell which can lead to environmental hazard, they can be effectively harnessed into single cell protein as animal feed.

## REFERENCES

- Abou-Hamed, S. A. A. (1993). Bioconversion of wheat straw by yeast into single cell protein. *Egyptian J. Microbiol.* 28(1): 1-9.
- Adoki, A. (2007). Factors affecting the yeast growth and protein yield production from orange, plantain and banana wastes processing residues using *Candida* sp. *Afri. J. Biotech.* 7: 290-295.
- AOAC (Association of Official Analytical Chemist) (1995). *Official Methods of Analysis*, 16th Ed. Association of Official Analytical Chemists, Washington, DC.
- Barnett, H. L. and Hunter, B. B. (1998). *Illustrated Genera of Imperfect*

- Fungi*, 4th ed. Burgess publishing company, Minneapolis, 218p.
- Chaudhari, L. C., Singh, R. K. and Kamra, D. N. (1994). Biodelignification of sugarcane baggase by *Pleurotus florida* and *Pleurotus cornucopiae*. *Indian J. Microbiol.*, 34: 55-57.
- Haidar, M. and EL-Hassy, M. (2000). Biodegradation of orange and pineapple peels for production of single cell protein. *Garyounis J. Sci.*, 1: 7-23.
- Halaszi, A. and Radomie, L. (1991). Use of yeast. *Bioresource Technol.*, 39: 17-22.
- Kurbanoulu, E. B. (2001). Production of single cell protein from ram horn hydrolysate. *Turkish J. Biol.* 25: 371-377.
- Litchfield, J. H. (1983). "Single-Cell Proteins." *Science*, 219: 740-746.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* 193:265-275.
- Lubna, I., Nadeem, M., Baig, S. J., Syed, Q. A. and Rana, F. R. (2004). Bioconversion of citrus waste into protein rich biomass by *Aspergillus niger*. *Pakistan J. Biochem. Molecular Biol.*, 37: 172-176.
- Maidak, B. L., Olsen, G. H., Larsen, N., Overbeek, R., McCaughey, M. J. and Woese, C. R. (1997). The RPD (Ribosomal database project). *Nucleic Acid Res.*, 25: 109-111.
- Manter, D. K. and Vivanco, J. M. (2007). Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *J. Microbiol. Methods*, 71: 7-14.
- Mchoi, M. H. and Park, Y.H. (2003). Production of yeast biomass using waste Chinese biomass bio-energy. *J. Microbiol.* 25: 221-226.
- Nasseri, A. T., Rasoul-Amini, S., Moromvat, M.H. and Ghasemi, Y. (2011). Production of single cell protein from fruits waste. *Am. J. food Technol.*, 6(2): 103-116.
- Nigam, N. M. (2000). Cultivation of *Candidal angeronii* in sugarcane bagasse hemi cellulose hydrolysate for the production of single cell protein. *World J. Microbiol. Biotechnol.*, 16: 367-372.
- Rajoka, M. I., Khan, S. H., Jabba, M. A. and Awan, A. S. (2005). Hashmi Kinetics of batch single cell protein production from rice polishing with *Candida utilis* in continuously aerated tank reactors. *Bioresource Technol.*, 97: 1934-1941.
- Ravinder, R. L., Raol, V. and Ravindra, P. (2003). Production of SCP from deoiled rice bran, *Food Technol. Biotechnol.*, 41: 243-246.
- Rodriguez-Vazquez, R., Villanuevaventure, G. and Riosleal, E. (1992). Sugar cane bagasse pith dry pretreatment for Single Cell Protein production. *Bioresource Technol.* 39: 17-22.
- Rosma, A. and Ooi, K. I. (2006). Production of *Candida utilis* biomass and intracellular protein content: Effect of agitation speed and aeration rate. *Malaysian J. Microbiol.*, 2: 15-18.
- Saquido, P. M. A., Cayabyab, V. A. and Vyenco, F. R. (1981). Bioconversion of banana waste into single cell protein. *J. Appl. Microbiol. Biotechnol.*, 5(3): 321-326.

- Smith, M. E. and Bull, A. T. (1976). Protein and other compositional analysis of *Saccharomyces fragilis* grown on coconut water waste. *J. Appl. Bacteriol.*, 41: 97-107.
- Tipparat, H. and Kittikun, A.H. (1995). Optimization of single cell protein production from cassava starch using *Schwanniomyces castellii*. *World J. Microbiol. Biotechnol.*, 11: 607-609.
- Ugalde, U. O. and Castrillo, J. I. (2002). "Single Cell Proteins from Fungi and Yeasts". *Appl. J. Mycotechnol. Biotechnol.*, 2: 123-149.
- Yakimov, M. M., Cappello, S., Crisafi, E., Tursi, A., Savini, A. and Corselli, C. (2006). Phylogenetic survey of metabolically active microbial communities associated with the deep-sea coral *Lophella pertusa* from the Apulian Plateau, Central Mediterranean sea. *Deep Sea Res.* 53: 62-75.
- Yunus, F., Nadeem, M and Rashid, F. (2010) .Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran) for animal feed. *J. Inst. Brew.*, 121: 553-557.
- Zhao, G., Zhang, W. and Zhang, G. (2010). Production of single cell protein using waste capsicum powder produced during capsanthin extraction. *Letts. Appl. Microbiol.* 50: 187-91.