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REGULAR ARTICLE

INORGANIC NITROGEN SUPPLEMENTATION AND MICRO-FUNGAL FERMENTATION OF WHITE YAM PEELS (FLOUR) INTO SINGLE CELL PROTEIN

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ABSTRACT

Solid state fermentation (SSF) technique was employed for the production of single cell protein (SCP) using white yam peels as a substrate. Commercial baker's yeast (*S. cerevisiae*) and a local isolate of *A. niger* were used for the fermentation process to generate crude protein. The effect of supplementation by various inorganic sources of nitrogen on the crude protein yields was compared. Ammonium sulphate [$(NH_4)_2SO_4$], one of the inorganic nitrogen source used, supported the highest crude protein yields of 16.78% and 21.30% respectively for *A. niger* and *S. cerevisiae* while the lowest yield of 13.92% and 18.00% were supported by potassium nitrate and sodium nitrate respectively.

Key words: fermentation, microscopic fungi, microbial proteins, nitrogn salts, white yam peels

INTRODUCTION

Fermentation, a relatively efficient, low energy preservation process is one of the oldest forms of food preservation technologies in the world (**Battock and Azam-Ali, 1998**). The basic principle of fermentation involves the utilization of different raw materials by microorganisms to produce varieties of end products. Agricultural crops are processed for many different reasons ranging from the removal of anti-nutritional components and increasing the storage life of the final product to adding value to the processed final product. Value-added products significantly enhance income by creating steady sales throughout the year, even when the crop is no longer in season. They also create employment opportunities for individuals who gather the fresh crops, develop and process the end product themselves and do not work through a distributor or middle person to distribute. Fungi have been exploited to convert carbohydrates, lignocelluloses and other industrial wastes into feedstuffs rich in protein due to the following characteristics: ability for a very fast growth rate which can be easily modified genetically for growth on a particular substrate under particular cultural conditions; high protein content varying from 35 to 60 percent; ability to grow on solids and their nutritional values are as good as other conventional foods rich in protein.

Solid-state fermentation (SSF) involves the growth of microorganisms on predominantly insoluble substrates, with low moisture content (no free liquid) (**Anupuma and Ravindra, 2001**). SSF is a low energy technique that has played vital role in the use of microorganisms to produce beneficial and commercially viable products. It has been successfully used in the transformation of agricultural wastes such as yam peels (**Aderiye and Ogunjobi, 1998**) and has been exploited for the production of feed from carbohydrate substrates (wastes), which has great potential as animal and livestock feed **(Ravinder *et al*., 2003**).

This study therefore, sought to investigate and evaluate the chemical quality of the single cell protein produced from yam peels by *Aspergillus niger* and *Saccharomyces cerevisiae*. The effect of various nitrogen sources on the biomass crop was also studied.

MATERIALS AND METHODS

Substrate collection and preparation

The yam peels used for this study were obtained from kitchens and fast food facilities within the Calabar metropolis. Samples were collected in 4.5l plastic container previously

cleaned and rinsed with 70% ethanol. The samples were washed with sterile distilled water and dried in an oven at $60^{O}C$ for 48 hours until it was crispy. The dried peels was then milled in a hammer mill, (Thomas Wiley Mill, Model ED-5, USA) and sieved to obtain 0.5mm mesh sized flour and kept ready to be used for SSF process.

Microorganisms and Inoculum preparation

Local isolate of *A. niger* were obtained from decaying yam and maintained on potato dextrose agar (PDA) slants after subculturing; while commercial dried baker's yeast *(Saccharomyces cerevisiae)* was purchased at Watt market in Calabar metropolis. The yeast was reactivated with 100 ml of warm ($50^{O}C$) sterile distilled water and maintained on slants of sterile Sabouraud dextrose agar (SDA) medium after subculturing. The inoculated slants were allowed to incubate at $30^{O}C$ for two days after which they were stored at $4^{O}C$ and sub-cultured once every fort-night. Spore suspensions were prepared in five ml sterile distilled water.

Fermentation of Substrate

Twenty grams of the yam peel flour was weighed into each of three sets of 250 ml Erlenmeyer flasks, the moisture content was adjusted to about 20% and autoclaved for 15 min at $121^{O}C$. After sterilization, the flasks were aseptically inoculated with 2 ml of each of the microorganisms ($3x10^{9}cfu.ml^{-1}$), properly labeled and plugged with sterile cotton wool. The flasks were then left to ferment for 7 days at $28 \pm 2^{O}C$ after which the samples were dried and subjected to chemical analysis.

In studying the effects of various nitrogen supplements and their input on the biomass crop yield, the growth medium was supplemented with each of the following mineral salts: ammonium sulfate [$(NH_4)_2SO_4$], ammonium chloride (NH_4Cl), sodium nitrate ($NaNO_3$) and potassium nitrate (KNO_3) in the concentration of 2.0g, 1.6g, 2.6g and 3.0g/l for each to supply 0.42g of nitrogen per litre of medium. In all the media, initial pH was adjusted to 3.5 for *A. niger* and 5.5 for *S. cerevisiae* using 1N NaOH and 1N HCl. Each medium was transferred to 250 ml Erlenmeyer flasks in triplicates and then autoclaved at $121^{O}C$ for 15 min. Two (2 ml) of inoculum from homogenate of *A. niger* and *S. cerevisiae* were aseptically added into each

medium. Fermentation was at a temperature of 28 ± 2^{O}C followed by determination of biomass and protein yield at 48 hours interval for seven days.

Chemical Analysis

Determination of Crude Protein

The micro-Kjeldahl method for protein determination was employed for protein determination. This is based on three principles:

Digestion: $RNH_2 + 2H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2 + H_2O$

Distillation: $(NH_4)_2\ SO_4 + 2NaOH \longrightarrow 2NH_3 + 2H_2O + Na_2SO_4$

Absorption: $NH_3 + H_3BO_3 \longrightarrow NH_4H_2BO_3$

Titration: $NH_4H_2\ BO_3 + HCl \longrightarrow H_3BO_3 + NH_4Cl$

Procedure

The sample (0.5g) was weighed into the micro-Kjeldahl flask. To this were added 1 Kjeldahl catalyst tablet and 10ml of conc. H_2SO_4. These were set in the appropriate hole of the digestion block heaters in a fume cupboard. The digestion was left on for 4 hours after which a clear colourless solution was left in the tube. The digest was carefully transferred into 100ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the volume of the flask made up to the mark with distilled water. 5ml portion of the digest was then pipetted to Kjeldahl apparatus and 5ml of 40% (w/v) NaOH added.

The mixture was then steam distilled and the liberated ammonia collected into a 50ml conical flask containing 10ml of 2% boric acid plus mixed methyl red-methylene blue indicator solution. The green colour solution was then titrated against 0.01NHCl solution. At the end point, the green colour turns to wine colour, which indicates that, all the nitrogen trapped as ammonium borate have been removed as ammonium chloride. The percentage nitrogen was calculated by using the formula:

% N = Titre value x atomic mass of nitrogen x normality of HCl used x 4

The crude protein is determined by multiplying percentage nitrogen by a constant factor of 6.25 (**AOAC, 1990**).

Crude Fat Determination

The sample (1g) was weighed into fat free extraction thimble and plug lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250ml soxhlet flask which has been previously dried in the oven, cooled in the dessicator and weighed. The soxhlet flask is then filled to ¾ of it volume with petroleum either (b.pt. 40 – 60°C) and the soxhlet flask extractor plus condenser set was placed on the heater. The heater was put on for six hours with constant running water from the tap for condensation of ether vapour. The set is constantly watched for ether leaks and the heat source is adjusted appropriately for the ether to boil gently. The ether is left to siphon over several times at least 10 – 12 times until it is short of siphoning. It is after this is noticed that any ether content of the extractor is carefully drained into the ether stock bottle. The thimble-containing sample is then removed and dried on a clock glass on the bench top. The extractor flask with condenser is replaced and the distillation continues until the flask is practically dried. The flask which now contains the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven (**AOAC, 1990**). If the initial weight of dry soxhlet flask is Wo and the final weight of oven dried flask + oil/fat is W1, percentage fat/oil is obtained by the formula:

$$\text{Oil \%} = \frac{(W1 - Wo)}{\text{Weight of sample taken}} \times 100$$

Crude Fibre Determination

The sample (2g) was accurately weighed into the fibre flask and 100ml of $0.25NH_2SO_4$ added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fibre flask to which 100ml of (0.31NNaOH) was added and heated under reflex for another 1 hour.

The mixture was filtered through a fibre sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water twice on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue was oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a dessicator and later weighed to obtain the weight W1. The crucible with weight W1 was transferred to the muffle furnace for ashing at 550°C for 4

hours. The crucible containing white or grey ash (free of carbonaceous material) was cooled in the dessicator and weighed to obtain W2. The difference W1 – W2 gives the weight of fibre (**AOAC, 1990**). The percentage fibre was obtained by the formula:

$$\% \text{ Fibre} = \frac{(W1 - W2)}{\text{Weight of sample}} \times 100$$

Determination of Ash

The sample (2g) was weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550°C and left for about 4 hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100°C in air, then room temperature in a desiccator and weighed (**AOAC, 1990**). The percentage ash was calculated from the formula below:

$$\% \text{ Ash content} = \frac{\text{Weight of ash}}{\text{Original weight of sample}} \times 100$$

Moisture content Determination

The sample (2g) was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100C to dry to a constant weight for 24 hours overnight. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to desiccator, cooled for ten minutes and weighed (**AOAC, 1990**).

If the weight of empty crucible is W_0
Weight of crucible plus sample is W_1
Weight of crucible plus oven dried sample W_3

$$(\% \text{ Moisture}) = \frac{W_1 - W_3}{W_1 - W_0} \times 100$$

Carbohydrate Estimation

Carbohydrate content was estimated by the difference method. This is acheived by deducting the sum of the other individually determined constituents of the food (moisture, ash, protein, fat, dietry fibre) from the total weight.

% Carbohydrates = 100 – (% moisture + % protein + % fat + % ash+ % fibre)

Data analysis

The results are represented as the mean standard values of three replicates each. Students T-test and one-way analysis of variance (ANOVA) were carried out according to the method outlined by **Araoye (2004**). Significance was accepted at $P < 0.05$.

RESULTS

Chemical analysis

Changes in the protein level of the yam peel following the inoculation *with A. niger* and *S. cerevisiae* are shown in Table 1. The chemical analysis revealed a significant increase ($P < 0.05$) in the protein content levels caused by fermentation with the respective fungi. The crude lipid, crude fiber and ash content of the fermented substrate also increased at the end of the seven days fermentation period. However, there was a significant decrease ($P < 0.05$) in the carbohydrate content of the micro-fungi fermented substrate when compared to the unfermented (Tab 1).

Table 1 Chemical compositions of yam peel mash inoculated with *A. niger* and *S. cerevisiae* and fermented for seven days

Sample	Crude Protein (%)	Crude Lipid (ether extract %)	Crude fibre (%)	Carbohydrate (%)	Total Ash (%)
Unfermented	6.56 ± 0.4[c]	1.25 ± 0.2[b]	9.40 ± 0.1[b]	78.81 ± 0.3[a]	3.98 ± 0.1[c]
Fermented with					
A. niger	13.56 ± 0.8[b]	1.45 ± 0.5[a]	12.00 ± 1.1[a]	65.98 ± 3.1[b]	7.01 ± 1.1[a]
S. cerevisiae	14.00 ± 2.7[a]	1.50 ± 0.7[a]	9.95 ± 0.8[b]	66.76 ± 1.2[b]	6.84 ± 0.4[b]

[a] Values with the same alphabet along the same column are not significantly different (P > 0.05)

[b] Values are mean ± standard deviation, based on three replicate values

Fermentation of substrate with micro-fungi.

The biomass in terms of the final crude protein content of the substrate peel mash supplemented with inorganic nitrogen sources is shown in Tab 2, Figure 1 and Figure 2. Yam peel mash when supplemented with inorganic nitrogen sources improved the biomass yield (expressed in terms of crude protein yield). Among all the nitrogen sources investigated, the supplementation of yam peel mash with ammonium sulphate resulted in the highest crude protein yields of 16.78% and 21.30% respectively for *A. niger* and *S. cerevisiae*. This was followed by supplementation with ammonium chloride which resulted in yields of 16.23% and 20.83% protein levels respectively. Supplementation of the yam peel mash with sodium nitrate and potassium nitrate gave lower protein yields. However, the crude protein yields in all the nitrogen supplemented media were significantly (P< 0.05) higher than the control (Tab 2, Figure 1 and Figure 2).

Table 2 Crude protein yields of *A. niger* and *S. cerevisiae* biomass cropped in the different nitrogen supplemented peel mash over seven days of fermentation

Organism		**Nitrogen source added/ percentage crude protein produced** Crude Protein produced			
	Control	Ammonium sulfate	Sodium nitrate	Potassium nitrate	Ammonium chloride
A. niger	15.68 ± 0.2[b]	16.78 ± 2.8[b]	15.14 ± 3.0[b]	13.92 ± 2.4[b]	16.23 ± 3.0[b]
S. cerevisiae	14.00 ± 2.7[a]	21.30 ± 4.5[a]	18.00 ± 2.7[a]	18.22 ± 3.6[a]	20.83 ± 3.5[a]

[a] Means in the same row with varying superscripts differ significantly (P< 0.05)

[b] Values are mean ± standard deviation, based on three replicate values.

[c] Control: no nitrogen source added.

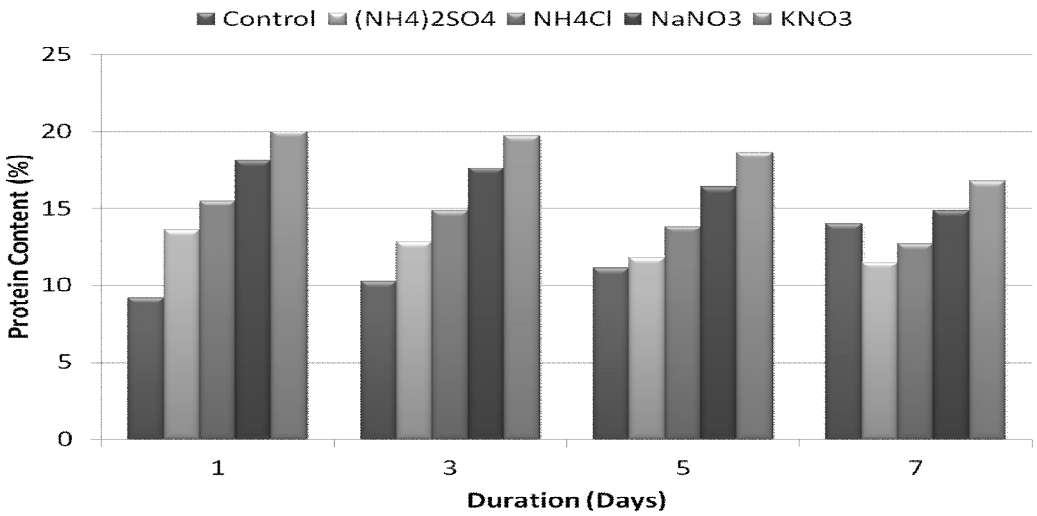

Figure 1 Protein content of *A. niger* biomass crop formed when growing on yam peels supplemented with different nitrogen sources

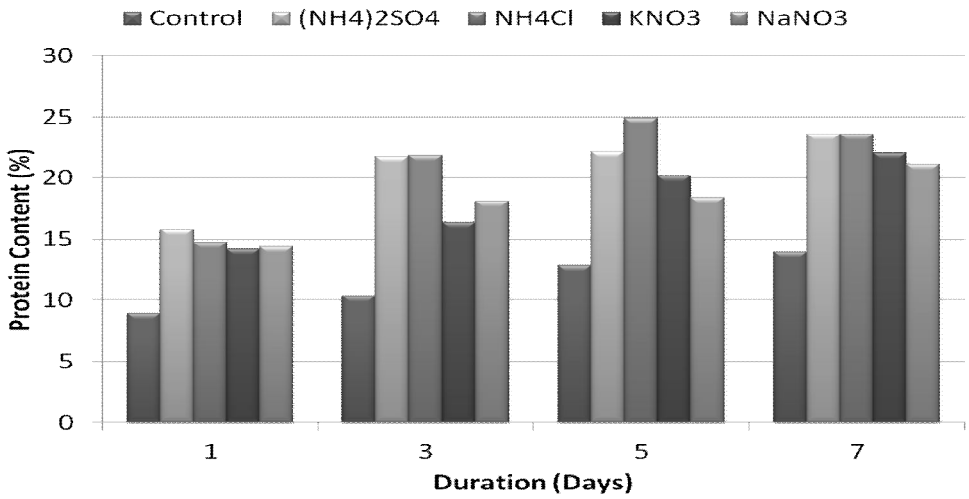

Figure 2 Protein content of *S. cerevisiae* biomass crop formed when growing on yam peels supplemented with different nitrogen sources

DISCUSSION

The appearance of fungal mycelia on the substrate mash after 48 hours was an indication of the breakdown of polysaccharides into fermentable sugars. This is in line with the report of **Ofuya and Nwajiuba (1990)**. Fermentation of the yam peel mash with pure strains of either *A. niger* or *S. cerevisiae* for seven days caused a significant increase ($P< 0.05$) in the crude protein, fat and ash content. The increase in the fat content could be attributed to the fact that, the fungi could secrete microbial oil (**Araoye, 2004**). Earlier findings of **Akindumila and Glatz** (**1998**), also agrees with this assertion and concludes that microorganisms could produce microbial oil during fermentation. Increase in the ash could be attributed to the inorganic content of the nutrient (basal) medium, which contains both the salt of magnesium and potassium. Micro-fungi solid substrate fermentation of the yam peel mash caused a significant decrease ($P < 0.05$) in the carbohydrate content. This decrease could be attributed to the ability of the fungi to hydrolyze starch into glucose and ultimately the glucose will be used by the same organisms as a carbon source to synthesize biomass rich in protein. The decrease in the carbohydrate content could also be attributed to the significant increase in the protein content of the peels fermented with microorganisms.

From this study, both fungi showed potential to increase the protein content of the yam peel mash with *Saccharomyces cerevisiae* demonstrating the best ability to enrich the peel mash in seven days. There was an increase in protein content compared to the unfermented peel from 6.56% to 13.56% when fermented with *A. niger*. Also the peels when fermented with *S. cerevisiae* had an improvement to 14.0%. This implied that the fungi and yeast had significant ($P< 0.05$) effect on the protein content. The increase in the crude protein observed could be attributed to the additional crude protein (extracellular enzymes) such as amylases produced by the fungal mycelia (**Nelson *et.al*., 2000; Okafor and Ejiofor, 1990; Onilude, 1994; Akindahunsi *et al*., 1999; Oboh and Akindahunsi, 2003**) and thus secreted into the fermenting mash in an attempt to make use of the starches as a carbon source (**Raimbault, 1998**). Furthermore, the increase in the growth and proliferation of the microorganisms in the fermenting substrates may possibly account for the apparent increase in the protein content of the fermented peels (**Antai and Mbongo, 1994; Akingbala *et al*., 1995**).

Although good growth was obtained without added nitrogen, crude protein yield increased appreciatively with addition of nitrogen sources. From this observation, it is evident that the availability of nitrogen is a major controlling factor in the final biomass and consequently crude protein yields. The lower yields obtained from non-supplemented yam peel mash could be attributed to limited concentration of assimilable nutrients, particularly nitrogen sources required for microbial growth and biomass formation. Ammonium sulphate when added as supplement, to the medium gave the highest crude protein yield for both microorganisms. This could be attributed to the fact that apart from providing nitrogen, it also provides sulphur, an element required by both fungi and yeast for growth in the medium. The work of **Ikenebomeh and Chikwendu (1997)** observed that ammonium sulphate was the choice supplement to improve biomass yield in cassava whey. **Oshoma and Ikenebomeh (2005)** also observed ammonium sulphate as being a better nitrogen supplement for the growth of *A. niger*.

CONCLUSION

A higher yield of single cell protein production from *A. niger* and *S. cerevisiae* was feasible by solid state fermentation of yam peel mash. Supplementing yam peel mash with mineral sources before fermenting enhanced the growth of the microorganisms and led to larger cell mass production. Among the four nitrogen sources utilized, ammonium sulphate supplement gave the best growth and biomass yield of both microorganisms, but more

intensive for *S. Cerevisiae*. In essence, yam peel was utilized as a potential substrate for microbial enrichment of protein in product; hence, it is possible to transform agricultural wastes to protein rich feed and food.

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