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BIOCONVERSION OF CASSAVA WASTES FOR PROTEIN ENRICHMENT USING AMYLOLYTIC FUNGI - *A PRELIMINARY REPORT*

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ABSTRACT

Protein enrichment of cassava wastes using three amylolytic fungi was investigated by solid state cultivation for 6 days. Cassava waste provided the only source of carbon in the medium. Other component mineral nutrients were from $(HN_4)_2$ $S0_4$, Urea and $KHP0_4$. The protein contents of the wastes were improved from 2.03% (w/w) to 6.45, 9.00 and 10.50% (w/w) respectively when treated with *Isaccharomyces cerevisiae, Mucor sp* and *Rhizopus sp.* There were improvements in the protein contents to 9.76, 16.30 and 18.05% (w/w) when extra doses of nitrogenous supplements were added after 48 hours of incubation. Increases in moisture contents were equally observed with slight falls in pH values.

KEY WORDS: Bioconversion, cassava, enrichment, amyloytic fungi

INTRODUCTION

Nigeria is the third largest producer of cassava in the world with an output of over 14 million tons recorded per annum (Komalafe, *et al,* 1980). Cassava has very low protein content of approximately 0.90% and carbohydrate content of 81.10% (Akpan and Ikenebomeh, 1995). This low protein content may lead to protein deficiency diseases such as Kwashiokor if appropriate supplementation is not carried out to increase the protein.

Cassava can be processed into different products which include garri, tapioca, starch and akpu (fufu). During the processing procedure some wastes are produced. These wastes include the peelings (tuber skin), whey and other residues. These by-products expectedly are low in protein. They are used as animals feed mainly for ruminants (Akinrele, 1975). Even at that, large quantities still litter the environment thereby constituting aesthetic problems. These wastes have been converted into useful products such as ethanol, and glutamic acid using microorganisms particularly, fungi (Oyewole and Odunfa, 1988).

In Nigeria, the cost of feeding animals has been on the increase due to the high cost of protein rich feeds. Microbial proteins are far cheaper considering the advantages both in space and time required for their growth. The need for the development of local protein resource for animal feeds from renewable raw materials such as cassava wastes has therefore become urgent. Substrates such as rice, wheat, millet, barley, maize and soybeans have been used for solid state cultivations for the production of protein rich feedstuff in Taiwan (Yang, *et al,* 1993). Amylolytic fungi are able to elaborate the enzyme amylase enabling them to degrade carbohydrate materials thereby yielding protein rich products suitable for feedstuff (Frazier and Westhoff, 1977). This procedure has the potential for reducing the deficit in protein from conventional sources.

Some starchy wastes and residues already investigated include Sago palm in South East Asia (Gumbira, *et al,* 1991), Sweet potato in Taiwan (Yang and Chiu, 1986; Yang, 1988; Yang and Yuan, 1990).

For economic reasons, it is necessary to perform the bioconversion of cassava wastes at the rural level at which cassava production is prominent and the wastes abound. This will bring benefit to the rural dwellers by improving their economic standard and even encourage higher production of cassava.

In this preliminary report, we have shown that cassava wastes can be bioconverted for protein enrichment using amlyolytic yeasts and moulds. The product contains starch (for its calorific value) and protein suitable for livestock.

MATERIALS AND METHODS

Saccharomycess cerevisiae, Mucor sp and *Rhizopus sp* used for the study were locally isolated from moist decaying cassava wastes. They were identified according to the method of Domasch and Gams, (1972). All the organisms have amyloytic activity. The yeast was cultivated at 30°C on yeast/malt extract agar slants while the moulds were cultivated at 30°C on potato dextrose (PD) agar slants.

Before use, the microorganisms were inoculated into potato dextrose broths and incubated at 30°C for 5 days. Portions of each culture broth were adjusted with sterile water to give between $10^3$to 10^5 spores or cells per ml of sterile water. The substrate contained cassava wastes (powder) 80g, $(NH_4)_2$ SO_4, 1.25g, Urea 1.25g and KH_2P04, 1.0g. The cassava waste powder was obtained from oven dried cassava wastes while the other supplements were obtained from Biochemistry and Microbiology Departments of Delta State University, Abraka. The solid substrate was distributed into 250ml conical flasks, autoclaved at 121°C, allowed to cool reasonably before mixing with spores or cells of the

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Table 1: pH, Moisture and Protein content of cassava wastes after the growth of *Saccharomyces cerevisiae, Mucor sp and Rhizopus sp* for 6 days at 30°C

Organism	pH		Moisture% (w/w)		Protein % (w/w)	
	Initial	Final	Initial	Final	Initial	Final
Saccharomyces	4.0	3.00	60	63	2.03	6.45
cerevisiae	+4.0	3.01	60	62	2.03	9.76
Mucor sp	4.0	3.02	60	62	2.03	9.00
	+4.0	3.03	60	622	2.03	16.30
Rhizopus sp	4.0	3.00	60	61	2.03	10.50
	+4.0	3.10	60	63	2.03	18.05

+ = second dose of nitrogenous supplements added after 48 hours incubation.

Result are means of triplicate experiments

organisms. Required amount of water was added to give suitable moisture level (60% w/w). They were incubated statically at 30°C for 6 days. Cultures were mixed daily by manual rotation of the flasks. Similar experiments were also set up. In this set a second dose of nitrogenous supplements were added after 48 hours incubation at 30°C. This was done to observe the effect of a boaster dose of the supplements. The pH of each culture medium was determined by mixing aliquots with distilled water and dipping the electrode of pye - model 291 pH metre into it. Moisture contents were determined by drying a 20g sample at 104°C in an oven to constant weight and subtracting this from the original weight (Yang, 1988). Protein contents were determined using the Kjedahl method as described by Meloan and Pomeranz, (1980). All experiments were carried out in triplicates. Results were therefore the average of the three values recorded. Controls were the initial values for all the parameters being investigated viz pH, moisture and protein.

RESULTS AND DISCUSSION

Saccharomyces cerevisiae, Mucor sp and *Rhizopus sp* have shown ability for protein production from cassava wastes as shown in Table 1.

With *Saccharomyces cerevisiae* the protein content increased from the initial of 2.03% (w/w) to 6.45% (w/w), *Mucor sp* to 9.00% (w/w) and *Rhizopus sp* to 10.50% (w/w), when the nitrogenous supplements were added at the beginning of the experiment. When a second dose of the supplements were added after 48 hours incubation the protein content recorded on the 6th day were 9.76,16,30 and 18.05% (w/s) with *S. cerevisiae, Mucor sp* and *Rhizopus sp* respectively. Generally *Rhizopus sp* showed better ability for protein production from the cassava wastes followed by *Mucor sp* and *Saccharomyces cerevisiae*.

There were slight decreases in pH from 4.0 to values ranging from 3.00 to 3.03 as seen in the Table. Moisture contents on the other hand showed a remarkable decrease from the initial 60% (w/w) to values ranging from 61 to 63% (w/w) on the 6th day.

Bioconversion of cassava waste to protein was higher in the moulds than the yeast. All the three organisms are amyloytic. The better performance by *Mucor sp* and *Rhizopus sp* are likely due to their filamentous nature. Because of the growth of their hyphae, the moulds are able to penetrate and spread through the solid substrate and hence grow better. This is in line with the report of Yang, *et al* (1993) which showed that filamentous fungi performed better in protein enrichment of sweet potato. Similar reports had earlier been made by Sukara and Doelle (1988); Ghanem, *et al* (1991); and Gumbira, *et al* (1991) for various other starchy waste products. The amount of protein produced from *Rhizopus sp.* is higher than that produced from *Mucor sp.* This suggest that *Rhizopus sp* grows faster.

Yeast generally have been shown to produce protein from starchy materials such as sweet potato, being amyloytic but not as high as in moulds (Yang and Yuan, 1990). The lower performance by *Saccharomyces sp* here is therefore not unexpected. The sharp increase in protein content when nitrogenous supplements were added after 48 hours incubation could be due to the fact that the supplements acted as booster doses. This effect was observed by Yang, *et al* (1993) in their studies with potato residue. They suggested that it might be due to better maintenance of substrate pH, to decreasing inhibition by nitrogen source and/or to increasing the efficiency of nitrogen utilization.

Changes in pH values recorded can be attributed to metabolic activities of the individual microorganisms. The changes were not too different for the three microorganisms since they are all fungi. They are therefore likely to be elaborating similar enzymes and metabolic wastes.

The initial moisture content of the medium in this study was 60%, hence a suitable condition for aerobic growth existing for the microorganisms. Yang, (1988) reported that when the moisture content is less than 72% which is the water holding capacity of sweet potato, then aerobic growth can occur. The increase in moisture content may however be due to the production of metabolic water or release of water arising from the oxidation of carbohydrates. This is in agreement with the report by Wang (1981).

In conclusion this preliminary report has shown that protein content of cassava wastes can be increased using some microorganisms that are capable of utilizing the wastes as sole carbon source. The product of such bioconversion is suitable as animal feed being rich in protein. In addition, if the procedure is employed on a large scale, it will help in getting the environment rid of the surplus wastes thereby solving the aesthetic problems originally posed by their dumping.

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