African Journal of Biotechnology Vol. 5 (3), pp. 302-304, 2 February 2006
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB05.360
ISSN 1684–5315 © 2006 Academic Journals

Short Communication

Fungal enrichment of cassava peels proteins

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Accepted 12 December, 2005

About 60% of the cassava produced all over the world is used for human consumption. These peels waste were found to contain 42.6% carbohydrate, 1.6% protein, 12.1% ether extract, 5.0% total ash and 22.5% crude fibre. With the advent of biotechnology approaches, there are opportunities for economic utilization of agro-industrial residues such as cassava peels waste. The Microorganisms isolated from the fermenting cassava waste were *Aspergillus niger*, A. *flavus*, and *A. fumigatus*. These and *Trichoderma* sp. from soil were studied for their ability to increase the protein content of cassava peels waste.

Keywords: Cassava peels, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus.*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most important crop in Nigeria and many tropical countries. Cassava roots play an important role in the African diet and they are processed using simple traditional methods into products such as “gari” and fufu, of lafun flour, some of which are fermented products (Odunfa, 1985). As a rough estimate, about 10 million tones of cassava are processed for gari annually in Nigeria alone (Okafor, 1992). In the processing of cassava fermented products, the roots are normally peeled to rid them of two outer coverings: a thin brown outer covering, and a thicker leathery parenchymatous inner covering. These peels are regarded as wastes and are usually discarded and allowed to rot. With hand peeling the peels can constitute 20-35% of the total weight of the tuber (Ekundayo, 1980). The wastes generated at present pose a disposal problem and would even be more problematic in the future with increased industrial production of cassava products such as cassava flour and dried cassava fufu. Since these peels could make up to 10% of the wet weight of the roots, they constitute an important potential resource if properly harnessed by a biotechnological system. The objective of this work is therefore to utilize the peel as a substrate for microbial protein enrichment.

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MATERIALS AND METHODS

Sample collection

Solid cassava wastes were collected from solid waste dumping sites at the University of Agriculture, Abeokuta (UNAAB) cassava processing site. The *Trichoderma* sp., originally isolated from soil, was obtained from the Department of Microbiology, University of Agriculture, Abeokuta, Nigeria.

Enumeration and isolation of microorganisms

5 g of the collected solid wastes were taken under aseptic conditions. The samples were aseptically homogenized with 50 ml sterile 0.1% peptone under aseptic conditions. Appropriate dilutions were plated on duplicate plates of Malt Extract Agar (MEA) for fungi counts. Plates were incubated at 30°C for 4 – 5 days. After incubation, the colonies on the plates were counted. Colony types and numbers were also noted. Representative colonies were picked at random from the plates and streaked out to obtain pure cultures. The isolates pure cultures were also kept on slants of PDA for fungi before characterization.

The isolated fungi were classified according to their method of (sexual) reproduction. They were identified according to their micro-morphology as well as the colour and morphology of their sporulating structures and conidia. Young, actively growing moulds were picked with a sterile needle unto clean glass slides and prepa-

red for microscopic observation using lactophenol as mountant and cotton blue as stain (Barnett and Hunters, 1992; Harrigan and McCance, 1976). The slides were carefully covered with slips to exclude air bubbles. Microscopic examination of the prepared slides was carried out first using the low power objective followed by the 40 magnification objective lens for a closer examination of a selected field. Microscopic identification was on the basis of the structures bearing the spores and on the spores themselves. Other features observed included presence or absence of septation, rhizoid and other tissues.

Trichoderma sp., originally isolated from soil, was obtained from the Department of Microbiology, University of Agriculture, Abeokuta, Nigeria.

Production of protein

100 g sample of cassava solid wastes were collected from the local processors dump site and mixed thoroughly with 5 ml of isolated or obtained growing culture. The fermentation was carried out in 250 ml conical flasks for 72 h. At intervals of 24 h the waste fermentation medium was centrifuged at 4000 rpm for 30 min. The sediment waste residue was dried in an oven at 105°C for 24 h and ground in a mortar. The crude protein, crude fibre and total carbohydrate were determined on duplicate samples

Chemical analysis of solid cassava wastes inoculated with fungi

These were determined by the methods of Association of Official and Agricultural Chemists (A.O.A.C., 1990) on dry matter basis.

Determination of crude protein content

An amount of 10 g finely ground dried samples were each transferred into a 50 ml Kjedhal flasks. 2 ml distilled water were added and the flasks were allowed to stand for 30 min. 0.02 g powdered pumice, 1.33 g K_2SO_4 catalyst mixture and 1.5 ml concentrated H_2SO_4 were then added. Heating was done on the digestion rack until frothing stopped. Heating was again increased to gentle boiling so that the H_2SO_4 condensed to about one third way up the neck of the flask. The isolated particles were washed with 30% H_20_2 solution before boiling again for 1 h. On cooling, 10 ml deionized water was added slowly with swirling. 2 ml aliquots of each diluted solution were measured and total nitrogen determined spectrophotometrically using Technicon Auto – analyzer. The Crude protein contents were determined from total nitrogen values using the conversion method of A.O.A.C. (1990).

Determination of crude fibre

Two grams each of dried samples were transferred into clean filter crucibles. 150 ml of 0.128 M H_2SO_4 previously preheated in the reagent system were added to prevent foaming. The contents of the beakers were boiled for 30 min and filtered through a Buchner funnel with the aid of a suction pump. The residues were washed with hot deionized water until acid free. The residues left after acid digestion were carefully transferred into a 400 ml beaker. 150 ml of 0.22 M KOH solution and a few drops of octanol were added to each sample. The mixtures were again heated for 30 min with constant stirring. The contents of the beaker were filtered through a Buchner funnel and 15 cm No 4 Whatman filter paper on cooling. The residues were washed several times with hot water and once with methylated spirit until free from KOH. Finally, the residues were transferred into porcelain crucibles and dried at 130°C for 2 h. They were allowed to cool in desiccators before weighing. The weights represented the crude fibre concepts which were expressed as percentages of the original samples.

RESULTS

Table 1 shows the microbiological assessment of the cassava solid wastes, with a total aerobic count of 2.17 x 10^2, lactic acid bacteria of 1.6 x 10^2 and total fungal count of 3.1 x 10^5. During the isolation the three moulds frequently encountered were *Aspergillus niger*, *A. flavus*, and *A. fumigatus*. The morphological and physiological properties of the major lactic acid bacteria isolated from the cassava solid waste were used in identifying the strains. The isolates were identified and the organisms are *Lactobacillus brevis*, *L. delbrueckii*, *La. sake*, *L. casei* and *L. plantarum*.

Table 1. Microbiological assessment of solid cassava wastes.

Amount of microbes	Cfu
Total aerobic count	2.17 x 10^2
Lactic acid bacteria	1.6 x 10^2
Total fungal count	3.1 x 10^5

Chemical changes in cassava peels inoculated with different moulds

Table 2 shows the changes in the protein, crude fibre carbohydrate yield of the fermented peeled cassava wastes inoculated with different moulds. The fermentation of the wastes with moulds caused an increased in the protein content. The highest protein yield was in the medium fermented by *Trichoderma sp*. followed by *A. fumigatus*, *A. flavus* and *A. niger*.

DISCUSSION

All the moulds isolated from the cassava solid wastes (*A. niger, A. flavus* and *A. fumigatus*) are effective in producing feed of high protein, but their used for this purpose is limited due to the toxic materials produced by these organisms. These fungi have been implicated in animal diseases (Richard et al., 1985) and mycotoxin production (Mossel, 1982). *A. fumigatus* has been previously found to produce high amount of microbial proteins (Odunfa, 1987). The results of microbial protein yield in this work can be compared to that of other studies. Odunfa (1987) obtained overall yields of 7.2 – 19% using different moulds to ferment cassava peels while overall yields of 12.6 – 25.5% were obtained in this work. The moulds make used of the fibre to produce the

Table 2. Protein, crude fibre and carbohydrate yield of cassava solid wastes inoculated with different moulds.

Moulds	Crude protein (%)	Total carbohydrate (%)	Crude fibre (%)
Aspergillus niger	12.6	33.4	30.6
Aspergillus flavus	14.0	31.0	28.5
Aspergillus fumigatus	18.9	30.4	25.8
Trichoderma sp.	25.5	28.6	23.2
Control	1.45	43.5	50.55

microbial protein and this is responsible for the reduction in crude fibre content. It has also been reported in other works of Abiola (1997, 1999) that it is possible to increase the protein content in pigs diet by adding 40% of sun-dried cassava peels into the feed.

From this study it can de deduced that a protein enriched cassava feed for cattle's or pigs can be obtained by using *Trichoderma* sp. for fermenting cassava peels. Efforts should be made for improving peels wastes hydrolysis conditions; its effective conversion into fermentable sugars is an area which needs further inputs in terms of research and development. Cassava peels hydrolysate could serve as a good substrate for production of value-added products.

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