Protein Enrichment of Cassava By-products Through Solid State Fermentation by Fungi

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Introduction

Nigeria stands as the world's foremost cassava producer with about 26 million tones (FAO, 1993). The leaves and peels, which are by-products of harvesting and processing, constitute 25% of the whole plant. These by-products and the flour constitute a potential source of livestock feed ingredient. The utilization of cassava and it by-products for livestock feeding has long been realized. Various authors have reported their use for feeding poultry (Ravindran, 1991; Sarwat *et al,* 1988, Long and Adetola, 1983), Pigs (Iyayi, 1986; Iyayi and Tewe, 1988) and ruminants (Smith, 1988). But cassava will be most beneficial for feeding monogastric animals.

The major limitation in the use of cassava for monogastric feeding is its low protein content. The flour, for example, contains about 3.6% protein and the peels about 5.5%. Though the leaves are fairly high in protein with an average value of 21%, it is desirable for this level to be improved. Because of the low protein of cassava products, their use in animal feeding usually requires the supplementation of such diets. Protein enrichment of cassava through less expensive means is therefore desirable. Fungal fermentation has been identified as an inexpensive tool for increasing the protein level of substrates in solid state. The attractive characteristics in the use of microorganisms for single cell protein include (1) their fast growth rate even in semi solid and solid media; (2) their high level of protein; (3) their comparable good nutritional values and (4) their easy genetically modification to growth under specific conditions on particular substrates. This study investigated changes in the protein levels of cassava pulp (flour), peels and leaves following solid state fermentation with *Aspergillus niger, Saccharmyces cerevisiae, Rhizomucor miehei and Mucor strictus.*

Materials and Methods

Cassava pulp, peel and leaves were obtained from the Cassava Breeding Unit of the International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria. After being washed, they were separately chopped into pieces and dried to constant weight. The dried samples were then milled and stored.

Inoculation of Samples

A niger, S. cerevisiae, R.. miehei and *M. strictus* were obtained from the culture bank of the Department of Animal and Plant Sciences. The University of Sheffield, Sheffield, UK. The *A .niger* and *R.miehei* were subcultured on 2% cornmeal agar and the *S. cerevisiae* a yeast on a medium of 1% yeast extract, 2% peptone and 2% glucose in 250ml Erlenmeger flasks after autoclaving for 15 minutes at 1210 C. After subculturing, the plates were incubated at 30°C for 3 days. Spore suspensions were prepared in distilled water. About 30gm of the milled samples of leaves and peels were weighed into each of 3 sets of flask. The moisture was adjusted to about 25% and autoclaved. After sterilization, 3 flask containing either peel or leaves were aseptically inoculated with each of the organism and properly labeled. The *A niger* and *S. cerevisiae* flasks were incubated at 35°C. The *R. miehei* flasks at 40°C and the *M. strictus* flask at 15°C. Samples were withdrawn from the *R. miehei* flasks at days 4, 8 and 12 (because of the vigorous growing nature of this fungus); from the *A niger* flasks at days 5, 10, 15 and 20 and from the *S. cerevisiae* and *M. strictus* flasks at days 7, 14 and 21. Withdrawn samples were freeze-dried and milled.

Protein analysis

TCA protein was estimated by the method of Lowry (1962) and percentage crude protein was verified by the method of AOAC (1984). Data were subjected to statistical t-test analysis and means separated by Duncan's multiple range test.

Table 1. Changes in protein of cassava pulp and peels following solid substrate fermentation with *Aspergillus niger* and *Saccharomyces cerevisiae*

	Aspergillus niger					*Saccharomyces cerevisiae*		
Fermentation period (days)	0	5	10	15	20	7	14	21
Cassava pulp	3.60**a** ±0.85	8.10**b** ±1.22	8.15**b** ±1.23	8.40**b** ±1.25	9.04**b** ±1.92	7.58**b** ±1.01	7.79**b** ±1.03	7.91**b** ±1.08
Cassava peels	5.60**a** ±0.95	11.00**b** ±2.66	12.99**b** ±2.80	13.50**b** ±2.95	14.14**b** ±2.99	15.22**b** ±3.00	16.18**b** ±3.05	16.74**b** ±3.08

Means without same letters on the same row are significantly different (P<0.05).

Figure 1. Protein enrichment o cassava products by solid substrate ermentation with *Aspergillus niger*

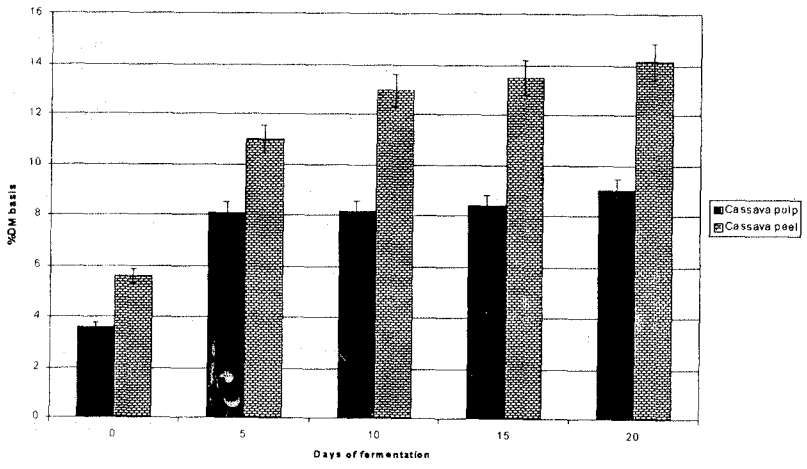


Figure 2. Protein enrichment o cassava products by solid substrate ermentation with the yeast *Saccharomyces cerevisiae*

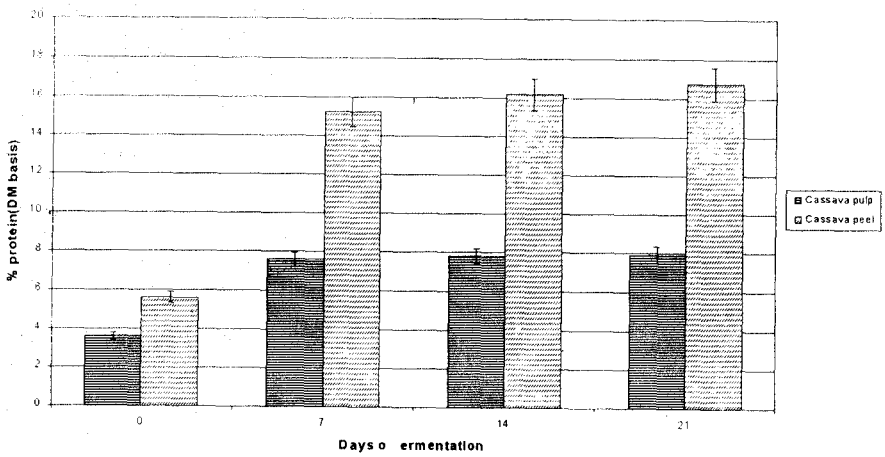


Figure 3. Changes in protein of cassava products on solid substrate fermentation with *Rhizomucor miheI*

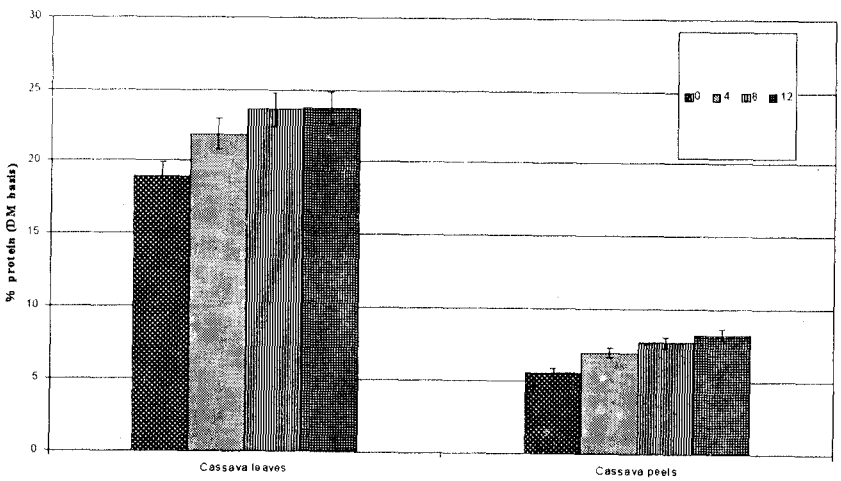


Table 2. Changes in protein of cassava leaves and peels following solid substrate fermentation with *Rhizomucor miehei* and *Mucor strictus*

	Rhizomucor miehei				*Mucor strictus*		
Fermentation period in days	0	4	8	12	7	14	21
Cassava leaves	18.97a ±4.12	21.89b ±5.02	23.63c ±5.36	23.78c ±5.74	20.43b ±5.00	21.95b ±5.01	21.97 ±5.08
Cassava peels	5.60a ±0.95	7.02a ±1.00	7.73a ±1.02	8.26a ±1.24	6.51a ±0.99	8.19a ±1.22	9.54b ±1.96

Means with different letters on same row are significantly (P<0.05) different.

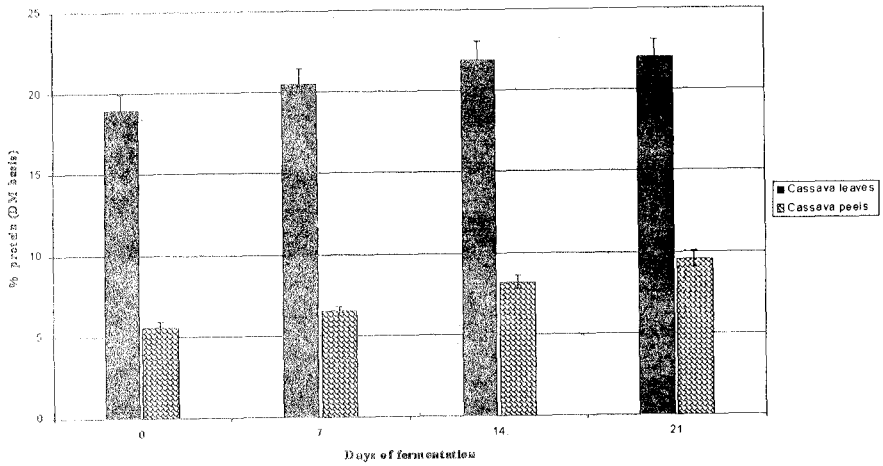
Results and Discussion

Changes in the protein level of cassava pulp and peels following inoculation with *A.niger* and *S.cerevisiae* are shown in **Figures 1 and 2** and **Table 1**. Changes in the leaves and peels on fermentation with *R. miehie* and *M. strictus* are shown in **Figures 3 and 4 and Table 2. Tables 1** and **2** show significant increase (P<0.05) in the level of protein caused by fermentation with the respective fungi.

A. nige. caused a significant increase(P<0.05) in the protein of peels after 20 days from 5.6% to 14. 14% and of the pulp from 3.6 % to 9.40. *S. cerevisiae* caused a significant increase (P<0.05) in the protein of peels after 7 days of fermentation to 15.22%. Beyond this period and up to day 21, the protein level rose non-significantly (P>0.05) to 16.14%. The yeast significantly increased (P<0.05) the protein of the pulp from 3.6% to 7.58% after 7 days and beyond this day non-significantly (P>0.05) to 7.91%. *R. miehei* caused a significant increase (P<0.05) in the protein level of the leaves within the first 7 days but there was no further significant increase (P>0.05) in the protein of the peels on inoculation with the organism. *M. strictus* significantly (P<0.05) increased the protein of the leaves up to day 7 but beyond there was no further significant (P>0.05) increase. The organism brought about significant increases (P<0.05) in the protein of the peels only by day 21.

All the fungi showed potential to increase the protein of the cassava products. The yeast *S. cerevisiae* demonstrated the best ability to enrich the peels, with a change of 171.78% in 7 days and 192.85% after 21 days. This was followed by *A. niger, M. stricus* and *R miehei* in that order. But *A. niger* resulted in a higher percentage change in the protein level of the leaves. Results obtained for *A. niger* are comparable to those of Abu (1997) who reported similar findings using sweet potato in solid state fermentation. According to Wainright (1992), fermentation of cereals leads to improvement in protein content. The author reported that fermenting corn meal with the yeast *S. cerevisiae* and *Candida trpoicalis* increased the protein content from 7.7% to 8.9% and that the protein content can be further increased by adding malt extract to the meals. Balagopalan (1996) reported the potential

Figur 4. Effect of solid substrate fermentation of cassava products with *Mucor strictus* on their protein contents



of most fungi to enrich the protein of cassava product. Similar studies by Essers (1994) showed the ability of fungi to enrich the protein of cassava products.

In the present study, the optimum period for a good yield of protein in the substrate lies between 12 to 15 days. Balagopolan (1996) reported 12 days as the optimum for some cassava products. The increase in protein recorded in the present study is at par with those obtained by other workers reporting on solid sate fermentation; Brook *et al* (1969), Manilal *et al* (1985) Daubresse *et al* (1987). The period between 0 and 15 days represents the period when the growth of the microorganisms is most vigorous. Beyond this period, the microorganisms very quickly use up the materials in the medium and growth is slowed down. This explains why the increase in protein beyond a certain period for the respective organism is only slight. Adding boosters like malt extract as suggested by Wainright (1992) or molasses at the initial stage can ensure a further increase in protein content of the material being enriched.

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