RESEARCH COMMUNICATIONS IN MICROBIOLOGY
1(1): 32 - 39 (2002)

CHANGES IN THE PROTEIN AND FIBRE COMPONENTS OF CASSAVA AND YAM PEELS AFTER SOLID STATE FERMENTATION BY *ASPERGILLUS NIGER* AND *RHIZOPUS* SP

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ABSTRACT: Dried, milled cassava and yam peels were inoculated separately with *A. niger and Rhizopus* sp in solid state. The changes in crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose were evaluated at 0, 4, 8, and 10 days after inoculation. At the end of the 10th day of fermentation with *A. niger* the crude protein of cassava peel meal (CPM) increased from 3.5% to 7.0% while that inoculated with *Rhizopus* sp increased to 5.25%. Crude protein of yam peel meal increased from 4.38% to 6.38% 10 days after inoculation with *A. niger* and to 7.58% with *Rhizopus* sp. However, the dry matter, crude fibre, NDF, ADF and hemicellulose of both meals decreased.

There was a significant difference ($P < 0.05$) in the degradation of CF, NDF and hemicellulose of both cassava and yam peel meals. The interaction of treatments and days had a significant effect ($P < 0.05$) on the level of crude protein, and degradation of CF, NDF and hemicellulose for cassava peel meal (CPM) and yam peel meal (YPM) on inoculation with the two fungi.

The pH value also decreased with increase in the inoculation period. Simultaneous decrease in the NDF, ADF, hemicellulose and DM with an increase in percentage crude protein content was attributed to the degradation of CF into carbon compounds (possibly simple sugar monomers) by enzymes produced by the fungi, and the utilization of these carbon compounds to build mycelial protein in the substrates. Thus the value of cassava and yam peels which are waste products of yam and cassava processing can be enhanced for livestock feeding through fungal fermentation in solid state.

INTRODUCTION: High feed cost is a major constraint in the Nigerian livestock industry (Iyayi and Yahaya, 1998). This has caused instability in the livestock business in the past few years as many livestock farm businesses are almost paralysed due to low or no profit margin. The possibility of using unconventional feedstuff e.g cassava (Pido *et al.* 1979) and cassava peels (Tewe, 1984; Iyayi and Yahaya, 1998) has been considered. There exists an abundance of cassava and yam wastes from cassava and yam processing units all over Nigeria. These wastes are often allowed to rot away or burnt when dried, sometimes causing environmental pollution.

Despite the potentials these tuber wastes possess in the feeding of livestock in the tropics, their inherently low protein content and crude fibre necessitates supplementation with protein sources (Woolfe, 1992). Some fungi

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have been reported to be beneficial in upgrading the nutritional status of most agro-industrial by-products (Phonboon and Wongwicharin, 1992; Balagopalan, 1996; Abu, 1997).

This study reports an investigation into the changes in the nutritional status of cassava and yam peels for livestock feeding following solid state fermentation with *Aspergillus niger* and *Rhizopus* sp.

2.0 MATERIALS AND METHODS

2.1 Preparation of Samples: Cassava peels were obtained from a local cassava processing plant. The yam peels were collected as wastes of the white yam tuber species from local yam processing centres. The samples were sun-dried separately and milled to 2mm particle sizes.

2.2 Test Organisms and Culture Medium: *Aspergillus niger* and *Rhizopus* sp were obtained from the culture collection of Botany and Microbiology Department, University of Ibadan, Nigeria. The fungi were cultured on petridishes containing potato dextrose agar at 28°C. The organisms were later multiplied by sub-culturing into a liquid medium containing potato, dextrose and distilled water and then incubated at 28°C for 7 days. Patoto dextrose agar was prepared by dissolving 38g of the commercially prepared PDA solid particles in 1 litre of distilled water by melting on a hot plate. The sterilized homogenized mixture obtained was aseptically poured into sterilized petridishes under a microflow.

Potato dextrose liquid medium contained 200g of Irish potato and 20g of dextrose made up to 1 litre with distilled water. The resulting solution was sterilized in an autoclave at 121°C for 15 minutes, and allowed to cool before culturing the fungi on it. This was incubated on a shaker at 28°C for 7 days after which the spores were harvested by filtration through Whatman filter paper.

2.3 Spore Count: One gram of the spores was weighed out and added to a stoppered bottle with sterile distilled water to make up to 10ml and spun thoroughly. About 0.15ml of the mixture was pipetted on to a glass slide and covered with a cover slip. The total number of spores in the 0.15ml mixture was counted including any in the fluid from the slide cover slip. The number of spores counted was adjusted with sterile distilled water to give 10^6 spores per ml.

2.4 Inoculation and Incubation Techniques: About 10g of cassava peel and yam peel were oven-dried separately to constant weight, in order to determine their respective moisture contents, and milled. About 50g of the samples were weighed and placed in Erlenmeyer flasks. About 69ml of sterile distilled water containing $MgSO_4.7H_2O$, 0.3g/l; $CaCl_2$, 0.4/g1; and $ZnSO_4.7H_2O$, 1.4g/l as nutrient medium was added to each of the samples to attain a 60% moisture content. The flasks were covered with cotton wool and aluminium foil, and sterilized in an autoclave at 121°C for 15 minutes, and then allowed to cool.

Twelve (12) Erlenmeyer flasks each containing milled cassava peel and yam peel separately were inoculated with 5ml of distilled water containing spores at 10^6 per ml of *Aspergillius niger*. Another 12 Erlenmeyer flasks each containing milled cassava peel and yam peel respectively were also inoculated with 5ml of distilled water containing spores (10^6 per ml) of *Rhizopus* sp. Controls with sterile uninoculated samples for the two by-products were also prepared. Degradation of each sample was carried out in triplicate. The flasks were incubated at 28°C for 0, 4, 8 and 10 days. The growth of the fungi was terminated by oven-drying for 24 hours. The flasks were allowed to cool before further processing for chemical analyses.

2.5 pH Determination: One gram (1g) each of the samples was blended in 10ml distilled water and allowed to stand for 5 minutes before the pH was read using a WPA pH meter.

2.6 Chemical Analysis: Samples were chemically analysed for crude protein, crude fibre, ether extract, ash, acid detergent fibre, neutral detergent fibre, and hemicellulose according to the methods of AOAC (1990). All determinations were carried out in triplicate.

2.7 Statistical Analysis: All data were subjected to analysis of variance, and their significant differences were determined using the Duncan Multiple Range Test.

3. RESULTS: After inoculation of milled cassava and yam peel meals, traces of mycelia of the two fungi became visible after 24 hours. Table 1 shows the proximate composition of the degraded cassava peel with *A. niger* and *Rhizopus* sp. The two fungi caused a significant ($P < 0.05$) increase in the crude protein content while the DM, CF, NDF, ADF, NFE and hemicellulose contents were all significantly ($P < 0.05$) reduced as fermentation period increased. With *A. niger* on cassava peels maximum crude protein level was obtained

Table 1: Proximate analysis of degraded cassava peel meal (%)

Treatment	Day	pH	DM	CF	CP	NDF	ADF	Hemicellulose	NFE
Aspergillus niger	0	5.6	58.40a	5.21a	3.49a	24.79a	15.82a	8.97a	45.72a
	4	5.2	40.16b	3.96b	5.25b	12.44b	11.36b	1.08b	25.69b
	8	4.8	33.52c	3.82b	6.13c	11.20b	10.16b	1.04b	17.65c
	10	4.6	40.73b	2.48c	7.00d	10.55b	9.65c	0.90b	24.89b
Rhizopus sp	0	5.6	58.40a	5.21a	3.50a	24.59a	15.62a	8.97a	45.71a
	4	4.5	40.14b	3.97b	4.92b	14.33b	11.65b	2.68b	26.05b
	8	4.2	36.84c	3.21b	7.88c	10.57b	9.57b	1.00b	19.32c
	10	3.6	32.79d	2.35c	5.25d	6.17c	5.57c	0.60b	19.59c

Note: Means in the same column with different letters are significantly ($p < 0.05$) different.

in 10 days (7.0%) while it was obtained with *Rhizopus* sp in 8days (7.88%) (Fig 1.). With both microorganisms, rapid changes in the composition of the cassava peel meal were obtained within the first 4 days. Thereafter, changes became moderate up to day 10.

Table 2 shows changes in the proximate composition of yam peel following inoculation with *A. niger* and *Rhizopus* sp. Crude protein was significantly ($P < 0.05$) increased after 10 days to 6.38% and 8.05% by *A. niger* and *Rhizopus* sp respectively. DM, CF, NDF, ADF, NFE and hemicellulose all decreased significantly ($P < 0.05$) within the first 4 days. Up to 8 and 10 days, changes became moderate.

Table 2: Proximate analysis of degraded yam peel meal (%)

Treatment	Day	PH	DM	CF	CP	NDF	ADF	Hemicellulose	NFE
Aspergillus niger	0	6.0	60.76a	3.87a	4.38a	32.70a	29.73a	2.97a	45.71a
	4	5.6	36.14b	3.09b	6.13b	8.90b	7.87b	1.03b	21.99b
	8	4.0	23.66d	2.16c	6.25b	7.42b	6.45b	0.97b	10.38c
	10	3.8	30.44c	2.04c	6.38b	5.43c	4.82c	0.61b	17.45d
Rhizopus sp	0	6.0	60.76a	3.87a	4.38a	32.70a	29.73a	2.97a	45.71a
	4	4.8	30.66b	2.46b	8.62b	10.12b	9.06b	1.06b	12.80b
	8	4.0	26.33c	2.05c	8.95b	9.14b	8.14b	1.00b	9.46b
	10	3.8	21.07d	2.00c	7.58c	7.65c	6.66c	0.99b	6.25c

Note: Means in the same column with different letters are significantly ($P < 0.05$) different.

The pH of inoculated cassava peel changed from 5.6 to 4.6 with *A. niger*, and to 3.6 with *Rhizopus* sp. The pH of inoculated yam peel changed from 6.0 to 3.8 with both *A niger and Rhizopus* sp. Table 3 shows a summary of the percentage changes in pH and nutrients of cassava and yam peel meals on inoculation with the two fungi. Results indicate that percentage changes in pH, DM, NDF, CP, ADF, hemicellulose (Hem) and NFE in cassava peels were significantly higher ($P < 0.05$) on inoculation with *Rhizopus* sp than with *A. niger*. For CPM, there was no significant ($P > 0.05$) change in the DM, CP, and NFE. The NDF, ADF and Hem were significantly ($P < 0.05$) reduced by *A. niger*. There was no significant difference ($P > 0.05$) in the changes in pH and CF between the two microorganisms, for YPM.

4. DISCUSSION: The appearance of mycelia on the peels after 24 hours was an indication that degradation had commenced, and more conspicuously with *Rhizopus* due to the pattern of its rapidly growing and branched hyphae or stolons. This is in conformity with the observation of Alexopoulus (1962) and Ofuya and Nwajiuba (1990). The pH values were within the range suitable for fungal growth as reported by Sasson (1988) and Ofuya and Nwajiuba (1990).

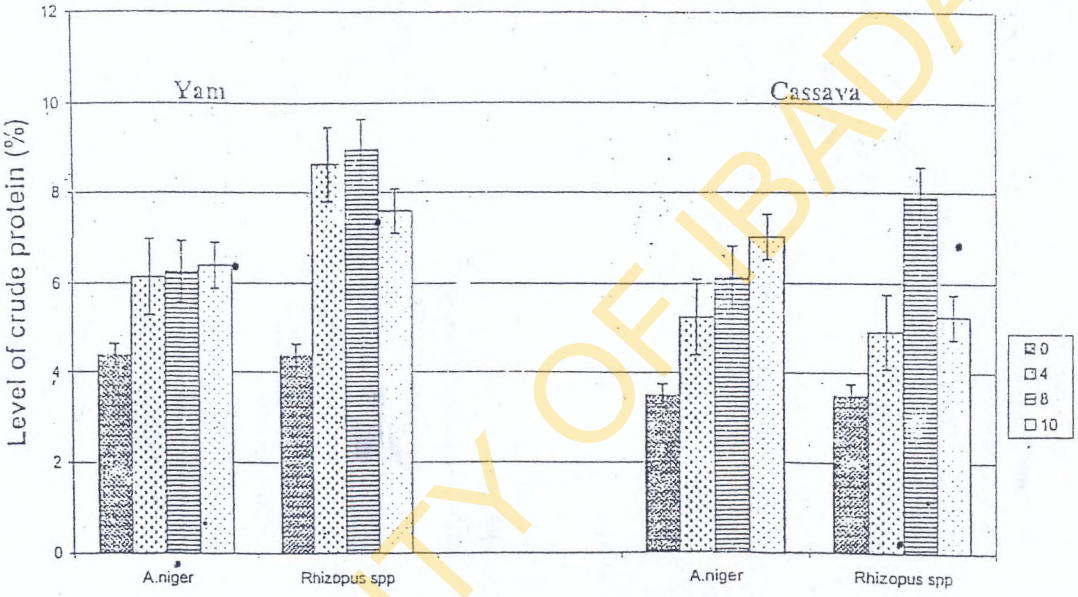

Fig 1. Changes in the crude protein content of yam and cassava in a solidstate fermentation

Table 3: Percentage changes in pH and nutrients of CPM and YPM on inoculation with *A. niger* and *Rhizopus* sp

Treatment	Cassava peel meal								Yam peel meal							
	pH	DM	CF	CP	NDF	ADF	HEM	NFE	pH	DM	CF	CP	NDF	ADF	HEM	NFE
A. niger	18	30.26	52.40	100.57	57.44	39.00	89.97	45.56	36.67	49.90	47.29	45.66	89.99	89.79	79.46	67.87
Rhizopus sp	36	43.85	54.89	125	74.49	64.34	93.33	57.15	36.67	65.92	48.92	93.06	76.60	77.60	66.67	86.92

The DM content of the degraded samples decreased as the fermentation period increased. This can be attributed to the effect of the fungi in degrading the crude fibre and its components, into simple sugars by enzymes secreted by the fungi. This action reduced the dry matter content and at the same time increased the NFE content (Ofuya and Nwajiuba, 1990).

The trend in the changes in CF after inoculating cassava peel meal with *A. niger* and *Rhizopus* sp showed 52.39% and 54.89% decreases in CF content respectively relative to the control. This is attributed to the effect of degradation by the fungi. Concomitant with this was the corresponding degradation of NDF and ADF, components of which are hemicellulose, cellulose and lignin. The trend is in conformity with the results obtained by Varghese *et al* (1977), Brook *et al* (1969) and Ofuya and Nwajiuba (1990) who reported similar findings using *Rhizopus* sp and *Aspergillus niger*. There was a decrease in the percentage hemicellulose of CPM treated separately with *A. niger* and *Rhizopus* sp from 0 to 10 days, the values of percentage degradation being 89.96% with *A. niger* and 96.33% with *Rhizopus* sp. The corresponding value for YPM for the same period was 79.46% when inoculated with *A. niger*, while 66.67% degradaton was observed when *Rhizopus* sp was used as the degrading organism. These results can be explained by the fact that *A. niger* and *Rhizopus* sp are saprophytic in nature and produce enzymes for extracellular digestion (Alexopoulus, 1962; Cooke, 1979) and these enzymes are capable of degrading structural carbohydrates such as cellulose, hemicellulose and lignin (Rai *et al*, 1988).

In lignocellulosic wastes, polysaccharides (principally cellulose and hemicellulose) provide the carbon and energy required for growth. This is why NDF and ADF levels were observed to fall with fermentation. It is also important to note the reduction in the NFE values. The NFE represents the soluble carbohydrates. The results obtained differ from those of Iyayi and Lösel (1999) who reported an increase within the first 5 days in sugar level when various products of cassava were degraded only for the level to begin to fall after 10 days in a 21- day fermentation period. The rapid decrease in the NFE observed in this study can be attributed to the vigorous growth of the microorganisms within the first 4 days with a consequent utilization of the available soluble carbohydrates as energy source for active multiplication and further degradation of the polysaccharides.

Four days after inoculating both cassava peel meal and yam peel meal with the fungi there was a dramatic increase in crude protein content. In CPM the crude protein had increased from 2.50% at day 0 to 7.0% after 16 days on inoculation *with A. niger* and to 7.88% after 8 days on inoculation with *Rhizopus* sp. The same trend was observed for yam peel. After 10 days the CP of CPM increased by 100.57% and 125% when inoculated with *A. niger* and *Rhizopus* sp. respectively. For the YPM, the CP increased by 45.66% and 73.06% when inoculated with *A. niger* and *Rhizopus* sp, respectively. The increase in crude protein observed was due to the additional crude protein produced in fungal mycelia (Graham, 1973) because mycelial protein is influenced by carbon to nitrogen ratio (Graham, 1973; Ofuya and Nwajiuba, 1990). Similar increase in crude protein has been reported by Gray and Abou-el-Seoud (1996), Brook *et al* (1969), Varghese *et al* (1977), Sasson (1988), Abu (1997) and Iyayi and Lösel (1991), using *A. niger*, *Rhizopus* sp and even other fungi like *Trichoderma harzianum* and *Aspergillus oryzae*.

There was a significant difference ($P \leq 0.05$) in the effect of the days on all the nutrient constituents of both yam peel and cassava peel. As the period of fermentation increases, more mycelial growth, spore formation and spore germination take place. As a result, this increases the enzyme secreted by the fungi for extracellular digestion under this condition; hence the percentage degradation will increase and at the same time mycelial protein will accumulate to increase the final crude protein of the substrates (Graham, 1973). Significant changes in the components of the meals occurred after 4 days after inoculation. Similar findings with sweet potato and cassava peels with *A. niger* have been reported by Abu (1997) and Iyayi and Lösel (1999), respectively.

Rhizopus sp had a significant advantage over *A. niger* in bringing about changes in the nutrient composition of substrates perhaps due to its more vigorous growth as observed in this study. The apparent increase in crude protein content could be due to overall loss in crude fibre from degraded sample, thus changing the relative proportion of the remaining constituents.

5. CONCLUSIONS: The decrease in crude fibre with an increase in crude protein content suggests that *Aspergillus niger* and *Rhizopus* sp can be used to improve the nutrient status of cassava and yam peels as a suitable feedstock for monogastrics and other animals. *Rhizopus* sp had an advantage over *A. niger* in bringing about the changes observed in the substrates.

The method of solid state fermentation is simple and inexpensive and, by using cassava peel and yam peel as industrial raw materials, it will help to convert by-products of low quality to feeds suitable for livestock production. This will at the same time minimize disposal problems often associated with these agro-industrial by-products at the sites of processing.

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