Cyanide detoxification in cassava by-products by fungal solid state fermentation.

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Abstract

This study investigated microbial detoxification of cyanide in cassava peels and leaves in solid state fermentation. Three microorganisms, *Mucor Strictus*, *Rhizomucor miehei* and *Saccharomyces cerevisiae* were used to inoculate the cassava by-products. The levels of cyanide in the substrates after 4, 8 and 12 days on inoculation with *R. miehei* were estimated. Cyanide levels in the substrates 7, 14, and 21 days after inoculation with *M. strictus* and *S. cerevisiae* were also estimated.

The three microorganisms caused a significant (P>0.05) reduction in the cyanide of the leaves and peels. *M. strictus* and *R. miehei* caused a 66.67% and 77.13% reduction in cyanide leavel in leaves respectively. Cyanide in the peels was reduced by 49.52%, 80.68% and 76.69% on inoculation with *M. strictus*, *R. miehei* and *S. cerevisiae* respectively. These changes indicated that *R. miehei* had the best potential in reducing cyanide of cassava by-products among the three microorganisms used for the study. Factors such as changes in texture in the plant tissue, increase β-glucosidase activity and utilization of the cyanogenic glucosides and their products of fermentation breakdown by the microorganisms possibly explain the observed reduction in cyanide levels by the microorganisms.

Introduction

The continuous competition between man, the beverage industries and livestock for conventional feed ingredients in developing countries has given rise to a growing need for the utilization of agro-industrial by-products for livestock feeding. In the last 2 decades in Nigeria, there have been concerted efforts at finding ways of complete utilization of these wastes to enhance livestock productivity in the country. These wastes on their own sometimes constitute environmental hazards. Their conversion to useful animal feed will be of advantage to the environment. Cassava peels, leaves and starch residues constitute 25% of the cassava plant. These are usually discarded as wastes after harvesting and procesing.

But their utilization for livestock feeding is highly limited because apart from the low protein and high crude fibre levels of the by-products, they also contain cyanide (Iyayi and Tewe, 1994). Traditional processing techniques help to reduce the cyanogen levels in these by-products (Oke, 1994). Nevertheless residual amount of cyanide are present in many processed products (Cooke and Maduagwu, 1978) and according to Rosling (1988), toxicity from processed cassava depends on the residual content of cyanohydrins and bound glucosides. Ingestion of cassava by-products of high cyanogen levels often results in withdrawal of the animal's reserve of sulphur amino acids for detoxification of the cyanide to thiocyanate (Tewe, 1975). The removal of the cyanogen from the by-products prior to feeding them to livestock is therefore essential.

Microbial fermentation has been reported as an effective means of cyanogen removal from cassava by-products (Bokanga *et al.*, 1990; Padjama *et al.*, 1994 and Essers, 1994). The use of microorganisms which produce linamarase for the detoxification of cassava roots has been reported (Ikediobi and Onyike, 1982; Ikediobi *et al.*, 1985 and Padjama *et al.*, 1993).

The leaves and peels of some Nigerian cassava varieties contain high amounts of cyanide (Oki, 1998). Yet these wastes constitute a potential feed resource for monogastric production. The need for the evolvement of a cheap processing method of these by-products is essential. This study investigated the ability of three microorganisms; *Mucor Strictus, Rhizomucor miehei* and *Saccharomyces cerevisiae* to reduce the total cyanide of cassava leaves and peels.

Materials and Methods

Cassava leaves and peels.

Fresh leaves and peels of a high cyanide cassava variety were obtained from the Cassava Breeding Unit of the International Institute of Tropical Agriculture, IITA, Ibadan Nigeria. They were washed free of sand and screened for removal of all non-organic materials. They were separately shredded into pieces and dried to a constant weight.

Microorganisms and inoculation techniques

Slants of *M. strictus, R. miehei* and *S. cerevisiae* were obtained from culture bank of the Department of Animal and Plant Sciences, The University of Sheffield, UK. The *M. strictus* was sub-cultured on cornmeal agar, the *R. miehei* on malt extract agar and the *S. cerevisiae* on agar medium of yeast extract (1%), peptone (2%) and glucose (2%) in 250ml Erlenmyer flasks after sterilization at 121°C for 15mins. Spore suspensions were then prepared using distilled water. About 30gms of the milled cassava leaves and peels were added to each of 3 sets of flasks and the moisture content adjusted to about 25%. After autoclaving, the set of flasks were aseptically inoculated with each of the organisms and properly labeled. The *M. strictus* flasks were incubated at 15°C, *R. miehei* at 40°C and *S. cerevisiae* at 25°C, corresponding to the optimum temperature growth of the respective organism. Samples were withdrawn at day 4, 8 and 12 from the

substrates inoculated with the fast growing *R. miehei* and at day 7, 14 and 21 from those with the moderately growing *M. strictus* and *S. cerevisiae*. The samples were, freeze-dried overnight and milled.

Determination of Cyanide

Cyanide determination was based on the principle that in the presence of a β-glucosidase and on hydrolysis, cyanide (HCN) is released from cyanoglycosides and trapped by a solution of sodium picrate. The absorbance of the resulting orange-yellow colour was determined spectophotometrically.

About 50mg of the milled samples was weighed and ground to powder with liquid N_2. The powder was dissolved in 2ml 200mM acetate buffer. pH 5.0 (containing 50mM Na acetate and 50µ $MNaNO_3$) and decanted into the outer section of a Warburg flask. 300m sodium picrate solution was pipetted into the inner well of the flask. 1ml β-glucosidase was added to the sample-acetate buffer mixture. The flasks were sealed tightly and incubated at 40°C for 4 hours. Standards were prepared by adding in place of samples in outer section 0-200m KCN. 3ml of acetate buffer were then added to dilute the contents of each of the flasks and sodium picrate was added to the inner well. The flasks were then sealed and incubated as described for the samples. After incubation, the flasks were cooled and to the outer section was added 1ml IM HCl. The flasks were sealed again and incubated at 60°C for 2 hours.

After incubation, 150µl Na picrate from the original 300µl in central well was removed and added to a test tube containing 900µl Na acetate buffer. The contents were thoroughly mixed and read at A540 nm.

Concentration of cyanide in samples was then calculated against the standards taking into account that only 150µl of the 300µl Na picrate in the central well was used in the assay.

Results were subjected to statistical t-test analysis and means separated by least significant difference (lsd).

Results and Discussion

The results of cyanide breakdown in the cassava products by the three microorganisms are shown in figures 1, 2 and 3 and Table 1. Percentage changes in cyanide level brought about by the respective organisms are shown in Table 2.

M. strictus, *R.miehei* and *S. cerevisiae* caused significant reduction ($p<0.05$) in the cyanide of both leaves and peels. *M. strictus* and *R. miehei* significantly reduced the cyanide of the cassava leaves from 450mg to 150mg and 102.9mg representing a 66.67% and 77.13% reduction respectively. Cyanide of cassava peel was reduced from 672.8mg to 340mg, 130mg and 156mg by *M. strictus*, *R. miehei* and *S. cerevisiae* representing 49.52%, 80.68% and 76.69% reduction by the respective microorganisms. These changes indicate that *R. miehei* showed the greatest effect followed by *S. cerevisiae* and *M. strictus* in that order.

Filamentous fungi have been known to be potentially useful for treating industrial effluents. Species of *Giberella* either as crude or immobilized mycelium are used on commercial basis to breakdown

Figure 1. Changes in total cyanide of cassava products following solid substrate fermentation with *Mucor strictus*

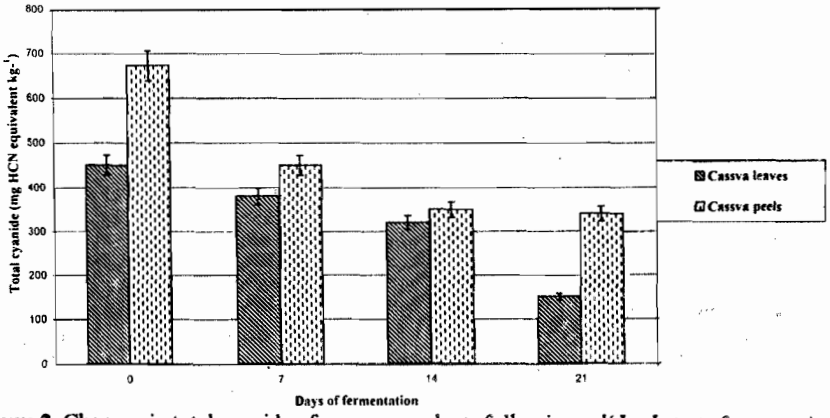

Figure 2. Changes in total cyanide of cassava products following solid substrate fermentation with *Rhizomucor micei*

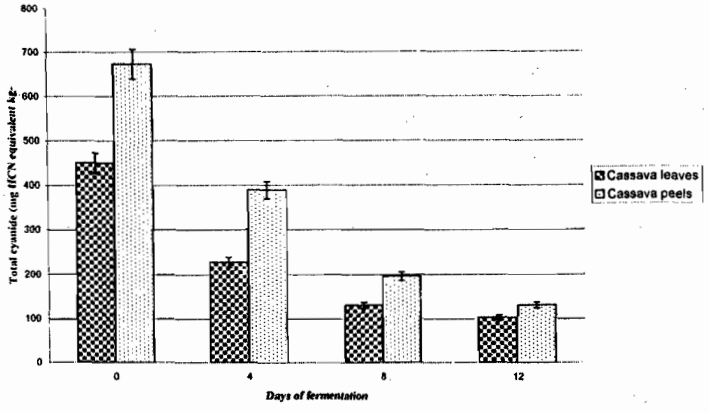

Figure 2. Changes in total cyanide of cassava products following solid substrate fermentation with *Saccharomyces cerevisiae*

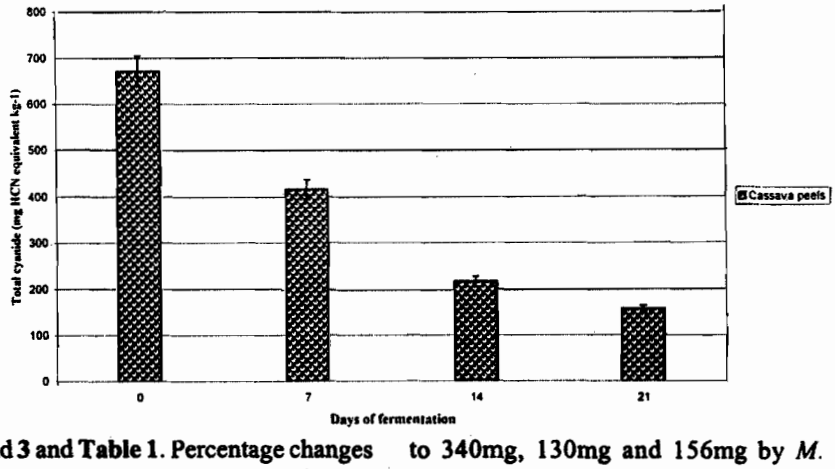

Table 1. Total cyanide (mg HCN equivalent kg -1) of cassava products following solid substrate fermentation with *Mucor strictus, Rhizomucor miehei* and *Saccharomyces cerevisiae.*

	Mucor strictus				*Rhizomucor miehei*			*Saccharomyces cerevisiae*		
Fermentation period (days)	0	7	14	21	4	8	12	7	14	21
Cassava leaves	450.00a ±25.00	380.00b ±20.00	320.00b ±16.00	150.00d ±9.00	227.2c ±12.77	130.89d ±4.11	102.09e ±2.00	-	-	-
Cassava peels	672.80a ±37.20	450.00b ±25.00	350.00b ±20.00	340.00bc ±18.50	389.00b ±21.00	196.34c ±8.66	130.89d ±4.11	416.00b ±22.0	216.00c ±11.14	156.00d ±6.88

Means with different letter on the same row are significantly different (P<0.05)

Table 2. Percentage changes in cyanide (mg HCN equivalent kg-1) and peels follwing solid state fermentation with *M. strictus, R. miehei* and *S. cerevisiae*

	M. strictus	*R. miehei*	*S. cerevisiae*
Length of fermantation			
Days	0-21	0 -12	0 - 21
Cassava leaves	450-150 (66.7%)	450 - 102.9 (77.13%)	-
Cassava peel	672.8 - 340 (49.52%)	672.8 -130 (80.68%)	672.8-156 (76.69%)

cyanide (Wainwright, 1992). The results of the present study are also in agreement with the findings of Essers (1994) who monitored linamarin breakdown in cassava following heap fermentation with various fungi. The author also reported that 9 days after fermentation, *Mucor racemosus* had broken down 90% of the linamarin and *Rhizopus oryzae*, 45-84%. The ability of most yeasts to release HCN from cassava substrates was also reported by the author.

Padmaja *et al* (1994) reported total cyanide reduction in cassava 72 hours after incubation with mixed culture of lactobacilli, streptococci, cornybacteria and yeast cells. The percentage reduction of the cyanide by the microorganisms in this study was similar to the reported trend obtained by the above author and Essers (1994). Fungi are able to reduce the level of cyanide in two possible ways. In the first stage, they cause release of linamarase enzyme into the medium. The enzyme which is suboptimally active at low pH during fermentation (Padmaja *et al.*, 1994) cause the breakdown of the glucosides linamarin and lotaustralin present in the cassava peel and leaves into a cyanohydrin. This compound then breaks down to the corresponding ketone and HCN (Wainwright, 1992). Because of high linamarase activity in cassava (Padmaja *et al.*, 1993), considerable decrease in the total and glucosidic cyanide occurred in the fermented peel and leaves in comparison with the unfermented samples within the period of fermentation in this study. Tewe (1991) also reported losses of up to 98% in free cyanide during fermentation of cassava roots with poultry litter for 8 weeks. The removal of cyanogens from cassava during fermentation from the report of Bokanga (1995) can be the result of several factors including:

(1) Changes in the texture in the plant tissues which make it possible for vacuole-bound cyanogenic glucosides to diffuse and come in contact with membrane-bound linamarase.
(2) Increase in β-glycosidase activity in cassava tissue, and
(3) Utilization of cyanogenic glucosides and the breakdown products by fermentation microorganisms. The differences obtained in the percentage degradation of cyanide by the microorganisms can be attributed to specie differences as earlier reported by Essers (1994). The highest percentage degradation by *R. miehei* compared to the other two organisms is due to the more vigorous growth of the *R. miehei* and significant biomass production of this organism as observed during the study within a relatively short time. Thus with more of the organisms being produced, faster degradation of the cyanide was obtained with a possible combination of the factors earlier mentioned.

This study has shown that fungal fermentation is an inexpensive and simple technique suitable for cyanide elimination from cassava by-products to make them suitable for use as livestock feed ingredients. *R. miehei*, a fast growing fungus requiring high temperature has been shown as a suitable microorganism for effective cyanide elimination in cassava peels and leaves. This will be useful in most tropical countries where large quantities of cassava peels and leaves abound as farm and processing wastes and where the prevailing ambient temperature is suitable for the growth of microorganisms like *R. miehei* that have ability to degrade cyanide.

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