International Journal of Modern Science and Technology, 2019;4(7):186-191.
ISSN: 2456-0235.
www.ijmst.co

Research Article

Use of *Mucor strictus, Rhizomucor Michei* and *Saccaharomyces cerevisiae* for the Detoxification of Cyanide Gotten from Cassava Products through Solid State Fermentation

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Abstract

Microbial fermentation has been reported as an effective means of cyanogens removal of cassava products. The research is aimed at using three species of Fungi (*Mucor strictus, Rhizomucor michei* and *Saccharomyces cerevisae*) for the detoxification of cyanide through solid state fermentation. The results of this study depict the following; the cyanide contents of the cassava leave, tubers and peels are 2.025±0.9, 0.76±0.01 and 2.00±0.08 respectively. After 96 hours of all the three isolated fungi, fermentation time has the best reducing effect on the cyanide content of the cassava samples at varying significance (P>0.05) across the groups. The study depicts that *Rhizomucor michei* had the highest activity after 96 hours in reducing the cyanide content of the cassava tubers and peels where it significantly reduced it (P>0.05) from 0.76±0.01, 2.0±0.07 to 0.17±0.05 and 0.17±0.50 respectively. *M. strictus* had the highest reducing activity on the cassava leaves where it significantly reduced (P>0.05) the cyanide content from 2.00±0.02 to 1.40±0.02. To existing knowledge, this study has contributed that: *S. cerevisiae* and *R. mucor* have the best cyanide reducing activity on the cassava peels, *M. strictus* has the best reducing activity of cyanide on the cassava leaves and *R. mucor* had the best activity in reducing cyanide contents of the tubers.

Keywords: *Mucor strictus; Rhizormucor michei; Saccharomyces cerevisiae*; Detoxification; Cyanide; Cassava; Fermentation.

Introduction

Cassava species are generally classified as sweet or bitter according to low or high cyanide concentration in their root parenchyma tissue. Bitter species contain high concentrations of glycosides distributed throughout the tissue, which forms the poisonous hydrocyanic acid on hydrolysis. This is also present in sweet cassava but in much smaller quantities [2]. A broad range of cyanide concentration has been found among several cassava species and most of them fall within the intermediate range between the sweet and bitter species [2]. Concentration of hydrocyanic acid in cassava tubers ranges from 10 to 490 mg/kg although this depends on variety. Bitter variety has cyanogenic acid levels of about 300 mg/kg in fresh tuber, while the sweet variety has cyanogenic acid level of about 400 mg/kg in fresh tuber. The tuber is processed into garri and cassava flour for human consumption, while the leaves are cooked and eaten especially in Sierra Leone [2]. Sweet cassava tubers can also be eaten boiled. They can be fed raw or billed to pigs, goats, horses and cattle. The main industrial use of cassava is in the production of starch and alcohols [10]. Cassava could be a raw material for the production of fumigants, since HCN it produces on hydrolysis is an active fumigant [15].

Cassava contains the cyanogenic glucosides, linamarine and lotaustralin. These cyanogenic glucosides are hydrolyzed to the corresponding cyanohydrin and glucose by the endogenous enzyme linamarase when cellular damage occurs [5]. Cyanohydrin breakdown non enzymatically at a rate dependent upon pH, temperature and length of time with their stability increasing at acidic pH values [4], [12]. A second enzyme, hydroxynitrilelyase, may also contribute to cyanohydrin breakdown into HCN and acetone [3].

Received: 02.07.2019; Received after Revision: 23.07.2019; Accepted: 24.07.2019; Published: 25.07.2019

The utilization of cassava by products for livestock feeding is highly limited because apart from the low protein and high crude fibre levels of the products, they also contain cyanide [9]. Traditional processing techniques help to reduce the cyanogen levels in these products [13]. Ingestion of cassava product of high cyanogen levels often results in withdrawal of the animal's reserve of sulphur amino acids for detoxification of the cyanide to thiocyanate [16]. The removal of the cyanogen from the product prior to feeding them to livestock is therefore essential.
The utilization of cassava products for livestock feeding is highly limited due to the presence of cyanide ([14]. More or less of these wastes are used as animal feedstuff while others become solid municipal wastes constituting environmental hazards due to their high cyanogenic contents as contamination of our environment arising from indiscriminate disposal of cassava peels has resulted in release of obnoxious smells, surface and ground water pollution and contamination of soils [19].

More of the waste product from cassava processing come from mainly garri and starch processing factories and will only be useful if the peels can be incorporated into the livestock diet formulation and beneficial management practices of these cassava wastes involve analysis and valuation of the nutrients composition and mineral contents of different forms of cassava produced thereby providing documented information that will be useful in cassava waste management, production of improved varieties and raw materials for the production of animal feeds, chemical and pharmaceutical industries [14]. Against this back ground the research is aimed at using three speci es of Fungi (*M. strictus, R. michie* and *S. cerivisi ae*) for the detoxification of cyanide through solid state fermentation.

Materials and methods

Study area and sample collection

The study samples were taken from a cassava farm by river Benue site at court 5 quarters Makurdi, Benue state, Nigeria; the cassava specie is TMS/O1/1368. The samples were collected in a clean polyethene bag and taken to biological sciences research laboratory of the Federal University of Agriculture Makurdi, Benue state, Nigeria for analysis.

Microbial inoculation and fermentation

This was carried out according to methods described by [20]. Slants of *M. strictus, R. miehei* and *S. cerevisiae* were cultured from local Burukutu joint in Makurdi, Benue State, Nigeria. The *M. strictus* was sub-cultured on corameal agar, the R. miehei on malt extract agar and the *S. cerevisiae* was on agar medium of yeast extract (1%), peptone (2%) and glucose (2%) in 250ml Erlenmyer flasks after sterilization at 121°Cfor 15mins. A spore suspension was prepared using distilled water. The cassava tubers were sorted, peeled, washed with potable water and drained. 10kg of the washed tubers were cut into slices of 3 to 4cm length and soaked in the bowl containing the microbial spore. It was wrapped with polythene bag which was then covered with foil paper leaving space for thermometer. During the fermentation, samples of the fermenting tubers and steep water were aseptically taken out and subjected analysis. After fermentation, the pulp was removed, water squeezed out and spread on a tray and sun-dried

Determination of Cyanide

Cyanide was determined according to a technique used by [6]. The absorbance of the resulting orange-yellow colour was determined spectrophotometrically. About 50mg of the milled samples was weighed and ground to powder with liquid nitrogen. The powder was dissolved in 2 ml of 200 mM acetate buffer pH 5.0 (containing 50 mM Na acetate and 50 M $NaNO_3$) and decanted into the outer section of a Warburg flask. 300 ml sodium picrate solution was pipetted into the inner well of the flask. 1ml (i-gluocosidase) was added to the sample-acetate buffer mixture. The flasks were sealed tightly and incubated at 40°C for 4 h. Standards were prepared by adding in place of samples in outer section 0-200 m KCN. 3 ml of acetate buffer was then added to dilute the contents of each of the flasks and sodium picrate was added to the inner well. The flasks were sealed and incubated as described for the samples. After incubation, the flasks were cooled and to the outer section will be added lml of 1 M HCl. The flasks were sealed again and incubated at 60°C for 2 h. After incubation, about 150µlof Na picrate was taken from the original mix. The contents were thoroughly mixed and read at A540 nm. Concentration of cyanide in samples was calculated against the standards taking into

account that only 150 µl of the 300 µl Na picrate in the central well was used in the assay.

Statistical analysis

Results were subjected to one-way ANOVA and means separated by least significant difference (LSD) using Duncan multiple range tests to determine the differences of the cyanide composition before, during and after fermentation.

Result and discussion

Cyanide content of the cassava leaves, tubers and peels before fermentation

Table 1 shows the cyanide contents of the samples before fermentation (0 h). The cassava leaves had the highest cyanide composition at 2.025±0.9 while the Tubers and Peels had the cyanide composition of 0.76±0.01 and 2.00±0.08 respectively. There is a significant (P>0.05) difference in the cyanide contents of the samples down the group.

Table 1. Cyanide content of the cassava leaves, tubers and peels before fermentation

Sample	Cyanide Composition (0 h)
1. Cassava tubers	0.76 ± 0.01^{a}
2. Cassava peels	2.00 ± 0.08^{b}
3. Cassava leaves	2.025 ± 0.09^{c}

Values indicated by superscripts are statistically significant (P>0.05) down the group.

Effect of fermentation time using S. cerivisiae on cassava leaves, tubers and peels during and after fermentation

Table 2 shows the effect of *S. cerivisiae* on cassava products, 96 h fermentation time had the best reducing effect on the cyanide content of the Tubers, Peels and Leaves at 0.45±0.01, 0.24±0.04 and 1.5±0.05 respectively with the values varying significantly (P>0.05) across the groups.

Table 2. Effects of *S. cerevisiae* on cassava leaves, tubers and peels during and after fermentation

Sample	Control	Cyanide Composition (48 h)	Cyanide Composition (72 h)	Cyanide Composition (96 h)
1. Cassava tubers	0.76 ± 0.01^{a}	0.58 ± 0.20^{b}	0.48 ± 0.10^{b}	0.45 ± 0.01^{b}
2. Cassava peels	2.00 ± 0.08^{b}	0.59 ± 0.03^{a}	0.48 ± 0.02^{a}	0.24 ± 0.04^{a}
3. Cassava leaves	2.025 ± 0.09^{b}	1.4 ± 0.01^{a}	1.9 ± 0.02^{a}	1.5 ± 0.05^{a}

Values indicated by superscripts are statistically significant (P>0.05) across the group

Table 3. Effects of *M. strictus* on the cyanide content of cassava leaves, tubers and peels during and after fermentation

Sample	Control	Cyanide Composition (48 h)	Cyanide Composition (72 h)	Cyanide Composition (96 h)
1. Cassava tubers	0.76 ± 0.01^{b}	0.55 ± 0.01^{a}	0.42 ± 0.04^{a}	0.35 ± 0.02^{a}
2. Cassava peels	2.00 ± 0.08^{b}	0.62 ± 0.02^{a}	0.61 ± 0.05^{a}	0.54 ± 0.01^{a}
3. Cassava leaves	2.025 ± 0.09^{b}	0.14 ± 0.09^{a}	0.11 ± 0.01^{a}	0.4 ± 0.02^{a}

Values indicated by superscripts are statistically significant (P>0.05) across the group

Effects of fermentation using R. mucor on cyanide content of cassava leaves, tubers and peels during and after fermentation

Table 4 shows the effect of the fungi *R. mucor* on cassava products, 96 h fermentation time had the best reducing effect on the cyanide content of the Tubers, Peels and Leaves at 0.17±0.03, 0.17±0.02 and 2.0±0.01 respectively with the values varying significantly (P>0.05) across the groups compared to the control

Effects of fermentation using R. mucor, M. strictus and S. cerevisiae on cyanide content of cassava leaves, tubers and peels after 96 hours of fermentation

Table 5 shows the effect of the fungi *R. mucor, M. strictus and S. cerevisiae* on cassava products. *Rhizor M*. had the highest activity after 96 h in reducing the cyanide content of the cassava tubers and peels where it significantly reduced it (P>0.05) from 0.76±0.01, 2.0±0.07 to 0.17±0.05 and 0.17±0.50 respectively. *M. strictus* had the highest reducing activity on the Cassava leaves where it significantly reduced (P>0.05) the cyanide content from 2.00±0.02 to 1.40±0.02.

The effects of fermentation by all the organisms in Tables 2,3,4 and 5 showed that *S. cerevisiae* and *R. mucor* have the best cyanide reducing activity on the cassava peels while *M. strictus* have the best reducing activity on the cassava leaves and *R. mucor* had the best activity in reducing HCN contents of the Tubers. This shows that there is a varying HCN reducing activity of the products by the organisms used in fermentation. Nature and duration of processing like chopping, threshing and boiling reduces as much as 95% HCN [7, 8, 11, 17]. The reduction in cyanide content is an indication that the fermenting substrate could be a medium supporting growth for microorganisms involving in detoxification process. Apart from being a low cost inexpensive substrate, easy accessible, the use of cassava peels in food industry can be considered as a significant way to reduce cost. Also, it is noteworthy that the process will no doubt have a major environmental impact as it helps to resolve the problem associated with the disposal of cassava waste. This will create a safe and eco-friendly environment, especially in major cassava processing region of the world [1].

Table 4. Effects of fermentation using R. mucor on cyanide content of cassava leaves, tubers and peels during and after fermentation

Sample	Control	Cyanide Composition (48 h)	Cyanide Composition (72 h)	Cyanide Composition (96 h)
1. Cassava tubers	0.76 ± 0.01^{b}	0.36 ± 0.01^{a}	0.33 ± 0.00^{a}	0.17 ± 0.03^{a}
2. Cassava peels	2.00 ± 0.09^{b}	1.0 ± 0.02^{a}	0.34 ± 0.02^{a}	0.17 ± 0.02^{a}
3. Cassava leaves	2.00 ± 0.02^{b}	1.3 ± 0.05^{b}	0.20 ± 0.03^{a}	2.0 ± 0.01^{a}

Values indicated by superscripts are statistically significant (P>0.05) across the group

Table 5. Cyanide composition of cassava leaves, tubers and peels before and after 96 h of fermentation

Sample	Crude sample	*M. strictus*	*R. mucor*	*S. cerevasae*
1. Cassava tubers	0.76 ± 0.01^{b}	0.51 ± 0.08^{a}	0.17 ± 0.05^{a}	0.5 ± 0.06^{a}
2. Cassava peels	2.00 ± 0.07^{b}	0.54 ± 0.23^{a}	0.17 ± 0.50^{a}	0.24 ± 0.13^{a}
3. Cassava leaves	2.00 ± 0.02^{b}	1.40 ± 0.02^{a}	2.00 ± 0.30^{b}	1.5 ± 0.30^{a}

Values indicated by superscripts are statistically significant (P>0.05) across the group

Conclusions

The fungi used in the study could efficiently reduce the level of cyanide by fermentation. Therefore, fungi fermentation, especially in cassava tuber, peel and cassava leaves could potentially be used to reduce the cyanogenic content of cassava product. *S. cerevisiae* and *R. mucor* have the best cyanide reducing activity on

the cassava peels while *M. strictus* have the best reducing activity on the cassava leaves and *R. mucor* had the best activity in reducing HCN contents of the tubers. Therefore, fermentation of cassava could facilitate the decontamination of waste disposed into the environment for a desirable products formation in food processing industry under normal conditions. Further studies should be carried out on other nutritive components of the products and other anti-nutrients present in the cassava leaves, peels and tubers.

Conflict of interest

The authors declare that there is no competing interest.

Acknowledgements

The authors wish to acknowledge the Management of Biochemistry Department, Federal University of Agriculture Makurdi, Benue State, Nigeria, for granting SA permission to embark on the project. The authors also appreciate Mr. Yakubu Danjuma for his contribution towards the research.

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