

## ***Nutrient Values of Cassava Residual Pulp as Affected by Solid State Fermentation with *Penicillium* spp.***

**R.A. Animashahun<sup>1\*</sup>; S.O. Omoikhoje<sup>2</sup>; M.I. Edokpayi<sup>3</sup> and B.T. Adesina<sup>1</sup>**

<sup>1</sup>Department of Animal Science, Landmark University, P.M.B 1001, Omu-Aran, Kwara State.

<sup>2</sup>Department of Animal Science, Ambrose Alli University, P.M.B. 14, Ekpoma, Edo-Sate.

<sup>3</sup>Department of Agricultural Science, School of Vocational and Technical Education, College of Education, Ekiadolor, Benin City.

\*corresponding author: kunleannies@yahoo.com

### **Abstract**

*This study aimed to determine the influence of a filamentous fungus *Penicillium* sp. using solid state fermentation (SSF) on the nutrient values of cassava residual pulp (CRP). The proximate constituents of the biodegraded CRP were significantly ( $P<0.01$ ) affected. It was also observed that the duration of fermentation significantly ( $P<0.01$ ) influenced the proximate constituents of the CRP. The crude protein has the highest improvement of 78.24% at 28 day fermentation period; the effect on nitrogen free extract (NFE) was opposite to that of CP, with highest reduction (8.67%) at 28 day; ether extract (EE), and ash levels increased with 233.33 and 42.67% respectively at 21 day of fermentation. The detergent fibers were also significantly ( $P<0.01$ ) affected pulp (CRP). The results showed that *Penicillium* sp. under solid state fermentation can enhance the protein value, ether extract and the ash contents of CRP at an optimum duration of 21 days.*

**Keywords:** cassava residual pulp, nutritive values, *Penicillium* sp., solid state fermentation

### **Introduction**

In the last decades, there have been increasing trends towards more efficient utilization of agro-industrial waste products (Akinyele, *et al.* 2011). Uses of these waste products in bio-processes provide alternative substrates, and also help in solving environmental pollutions. Cassava peels, residual pulp, and other by-products from garri processing are normally discarded as wastes, and allowed to rot in the open thereby constituting health hazard (Obob, 2006). About 10 million metric tons of cassava tubers are processed into garri annually in Nigeria, this making the waste products potential important resources for

animal feed, if properly processed using bio-system (Antai and Mbongo, 1994). In an effort to diversify from our mono-culture economy, the Nigerian Government has been intensifying campaigns to encourage the citizenry to produce more cassava for food and cash. An average Nigerian home will likely take cassava products as food every day, while industrially, cassava is used to produce starch, alcohol, fuel, etc. Both the domestic and industrial utilization of cassava lead to generation of large volumes of wastes that can be utilized as animal feed. Africa accounts for more than 90% of the global cassava production in 2004 (FAO, 2006), with Nigeria

contributing 38.4 million metric tonnes, as the largest producer of cassava in the world (Aro, 2008).

The major limitation in the use of cassava residual pulp (CRP) for monogastric animal feeding is its low protein content (Iyayi and Lossei, 2001). With the advent of biotechnological innovations, mainly in the area of enzymes and fermentation technology, many new avenues are opening up for their utilization (Akinyele, *et al.*, 2011). The potential of micro organisms to degrade agro-industrial waste is on the increase (Alva, *et al.*, 2007), and fungal fermentation has been identified as an inexpensive tool for increasing the protein level of substrates in solid state. The use of micro organisms has the advantage of having a fast growth, even in semi-solid state and solid media (Iyayi and Lossei, 2001). These authors reported an enhancement of protein value of cassava flour pulp, peels, and leaves fermented with *Aspergillus niger*, *Sacchamyses cerevisae*, *Rhizopus miebei* and *Mucor strictus*. This study therefore assessed the effect of microbial fermentation with *Penicillium sp.* on cassava residual pulp.

#### Materials and Methods:

Cassava residual pulp (CRP) was obtained from “akpu” processing plants in Ekpoma town. This was sun dried, chopped into pieces, milled and stored.

**Inoculation of samples:** This was done as described by Iyayi and Aderolu (2004). *Penicillium sp.* was obtained from the culture bank of department of Microbiology, Ambrose Alli University, Ekpoma. A piece of mycelium of the fungus was then subcultured in potato Dextrose Agar (PDA) in petri dishes and incubated at 30 °C for 3 days. 30g of CRP was weighed

into each of fifteen 250ml. conical flasks. Moisture was adjusted to 25%, the mouth of the flasks clogged with cotton wool and then covered with aluminum foil. The flasks were then autoclaved at 121 °C for 15 minutes for sterilization. A piece of mycelium of the subculture of the fungus (*Penicillium sp.*) was aseptically inoculated into four sets (3 flasks per set) of the conical flasks, while the last set of conical flasks that were not inoculated served as the control. The inoculated flasks were incubated at room temperature. A set of flasks were withdrawn per week (at 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day); the fermentation process terminated by oven drying at 60 °C for 24 hours. The samples were thoroughly mixed and stored in sterilized bottles.

**Analysis of samples:** The nutritional composition (crude protein, crude fibre, nitrogen free extract, ether extract, ash, and detergent fibre constituents were determined according to the standard methods of AOAC (2000).

**Statistical Analysis:** Data generated were subjected to a one-way analysis of variance (ANOVA) of a completely randomized designed model (Steel and Torrie, 1980). Means were separated by Duncan’s new multiple range tests with the aid of SAS (1999) package.

#### Results and Discussion

Table 1 presents the total viable count per gramme (TVC/g) and the hydrogen ion index (pH), while Table 2 shows the proximate components of the samples after the fermentation periods. The pH was significantly affected ( $P < 0.05$ ) by the duration of the mycotic fermentation as the acidity increased progressively from 7.8 in the control to 5.1 obtained at 21<sup>st</sup> day of fermentation. Thereafter, it began to

decrease, with a value of 5.4 obtained on the 28<sup>th</sup> day. Total viable count per gramme (TVC/g) followed the same trend increasing from  $1.91 \times 10^9$ /g in the control to  $9.70 \times 10^{21}$ /g at 21<sup>st</sup> day, then the TVC/g reduced

significantly ( $P < 0.05$ ) to  $9.34 \times 10^{15}$ /g at 28<sup>th</sup> day of fermentation. Similar results have been reported by Biniyam, *et al.* (2010).

**Table 1:** The effect of fermentation period on total viable count (TVC) and hydrogen index pH

Fermentation period in days	Total viable count (TVC)/g	Hydrogen index (pH)
Control (0)	$0.00 \times 10^e$	7.8 <sup>a</sup>
7	$1.91 \times 10^{9c}$	5.9 <sup>b</sup>
14	$7.89 \times 10^{16b}$	5.6 <sup>c</sup>
21	$9.70 \times 10^{21a}$	5.1 <sup>e</sup>
28	$9.34 \times 10^{15b}$	5.40 <sup>d</sup>
SEM	$3.96 \times 10^3$	0.001

<sup>a, b, c, d, e</sup> Treatment means in the same column with different superscripts are significantly ( $P < 0.05$ ) different

**Table 2:** Proximate components of control and fermented CRP over a 28-day fermentation period

Parameters	Fermentation period in days					SEM
	0	7	14	21	28	
Moisture	9.13 <sup>d</sup>	12.62 <sup>a</sup>	10.86 <sup>b</sup>	7.86 <sup>e</sup>	9.62 <sup>c</sup>	5.50
CP	2.39 <sup>e</sup>	3.25 <sup>d</sup>	3.45 <sup>c</sup>	3.93 <sup>b</sup>	4.26 <sup>a</sup>	3.67
CF	11.42 <sup>c</sup>	9.63 <sup>e</sup>	11.55 <sup>d</sup>	14.09 <sup>b</sup>	14.47 <sup>a</sup>	3.67
NFE	72.97 <sup>a</sup>	70.12 <sup>b</sup>	68.93 <sup>c</sup>	67.76 <sup>d</sup>	66.64 <sup>e</sup>	9.00
EE	0.27 <sup>e</sup>	0.34 <sup>d</sup>	0.50 <sup>c</sup>	0.90 <sup>a</sup>	0.53 <sup>b</sup>	3.83
ASH	3.82 <sup>e</sup>	4.05 <sup>d</sup>	4.71 <sup>b</sup>	5.46 <sup>a</sup>	4.48 <sup>c</sup>	4.50
NDF	30.67 <sup>a</sup>	20.06 <sup>b</sup>	16.47 <sup>c</sup>	16.40 <sup>d</sup>	15.83 <sup>e</sup>	4.67
ADF	18.61 <sup>a</sup>	10.21 <sup>e</sup>	12.20 <sup>d</sup>	18.74 <sup>b</sup>	15.83 <sup>c</sup>	2.17
ADL	4.86 <sup>b</sup>	2.83 <sup>e</sup>	3.97 <sup>c</sup>	3.24 <sup>d</sup>	4.98 <sup>a</sup>	3.17

<sup>a, b, c, d, e</sup> Treatment mean in the same row with different superscripts are significantly different at  $P < 0.05$ . SEM = Standard error of means. CP, CF, NFE, EE, NDF, ADF and ADL are crude protein, crude fiber, nitrogen free extract, ether extract, neutral detergent fiber, acid detergent fiber and acid detergent lignin respectively.

The CP increased significantly ( $P < 0.05$ ) from 2.39 % in the control to 4.26 % at 28<sup>th</sup> day of fermentation representing 78.24 % increment in the CP, while there was

significant ( $P < 0.05$ ) reduction of NFE from 72.97 % in the control to 66.64 % at 28<sup>th</sup> day of fermentation. Changes in CF did not follow any particular pattern. Ether extract

and ash contents increased significantly ( $P<0.05$ ) as the fermentation progressed up to 21 days, while the values dropped on the 28<sup>th</sup> day. The highest EE and ash values were 0.90 % and 5.46 % representing 233% and 42.93% increment in EE and ash of the degraded CRP respectively. Neutral detergent fibre values decreased ( $P<0.05$ ) as the fermentation progressed, from 30.67 in the control to 15.83 on the 28<sup>th</sup> day of fermentation, representing 48.39 % reduction. Acid detergent fibre and ADL however did not follow any pattern. Ofuwa and Nwanjiuba (1990), Iyayi and Lossie (2001), Iyayi (2004) have demonstrated the ability of fungi to degrade cellulose in earlier studies. Hamilyn (1988) also reported that fungi have the ability to provide a variety of enzymes, and that *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium sp.* are the main sources of Cellulase, amylase, hemicellulases, catalases, pectinases, and xylanases. These enzymes help to degrade the non-starch polysaccharides (NSPs) in the substrates, and the fungi in turn can make use of the products as their own source of carbon (Raimbault, 1998). Total viable count (TVC/g) gives a quantitative idea about the presence of micro-organisms in a sample (Dubey and Maheshwani, 2012). The count actually represents the number of colony forming units per gram. (or per ml.). In this experiment, the period between 0 and 21<sup>st</sup> day with values increasing from 0 to  $9.70 \times 10^{21}$ , represented the period of exponential growth of the fungus *Penicillium sp.* Beyond this period, the TVC/g declined representing a period of gradual death of the fungus, as seen on the 28<sup>th</sup> day of fermentation with value of  $9.34 \times 10^{15}$ . This observation agrees with the findings of Jawetz, *et al.* (1984) and Dubey and

Maheshwani (2012). The increase in the CP maybe as a result of the secretion of the enzymes mentioned above (Obboh and Akindahunsi, 2003) into the substrate by the fungus. The increase in the growth and proliferation of the organism in the form of single cell mass may be responsible for the apparent increase in protein (Obboh *et al.* 2002; Obboh, 2006). This fact could also be responsible for the significant reduction in the NFE recorded in this study. Increase in protein could also account for the decrease in the carbohydrate content. The EE represents the fat/lipid content of a sample; it also represents the all important fat soluble vitamins (A, D, E and K). The percentage increase of EE (233%) of the degraded CRP is very significant because one of the main problems in the utilization of cassava products is lack of these fat soluble vitamins, especially vitamin A. Ash content is the rough measure of the inorganic minerals of a sample (FAO, 2006). In this study, the ash increased ( $P<0.05$ ) as the fermentation progressed with the highest value of 42.93 % obtained on the 21<sup>st</sup> day of degradation. This implies that more essential inorganic minerals were made available in the fermented CRP products. The significant reduction in NDF from 30.67 in the control to 15.40 at 21<sup>st</sup> day of fermentation (48.39 %) agrees with the findings of Akinremi *et al.* (2006) who reported protein enrichment and reduction of fibre in cassava peel biodegraded with fungi under solid state fermentation.

## Conclusion

It can be concluded that *Penicillium sp.*, under solid state fermentation, has the potential of enriching the protein content, ether extract, ash as well as reducing the fibre components of cassava residual pulp;

with an optimum fermentation period of 21 days. This could be an important avenue in the use of cassava residual pulp in monogastric nutrition, particularly in poultry.

## References

- Aderemi, F. A., Lawal, T. E., and Iyayi, E. A. (2006) Nutritional value of biodegraded cassava root sieveate CRS) and its utilization by layers. *Journal of Food technology* 4(3): 216- 220.
- Akinyele, B. J., Olaniyi, O. O. and Arotupin, D. J. (2011). Bioconversion of selected agricultural wastes and associated enzymes by *Volvariella volvocea* : An Edible Mushroom. *Research Journal of Microbiology* 6(1): 63-70.
- Alva, S. J., Anupama, J. S., Chiu, Y. Y and Vysholi. (2007). Production and characterization of fungi amylase isolated from *Aspergillus sp.* JGI 12 in solid state culture. *African Journal of Biotechnology*. 6:576-581.
- Antai, S. P. and Mbongo, P. M. (1994). Utilization of Cassava peels as substrate for crude protein formation. *Plant Foods for Human Nutrition*, Vol.48;.345-351.
- AOAC (2000). Association of official Analytical chemists. Official methods of analysis. 12<sup>th</sup> edition Washington D.C.
- Aro, S. O. (2008). Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. *African Journal of Biotechnology*. Vol.7 (25), PP.4789-4797.
- Biniyam, Y., Tesfaye, A. and Amuttha, S. (2010). Solid substrate fermentation and conversion of orange waste into fungal biomass using *Aspergillus niger* KA-06 and *Chaetomium spp.* KC-06. *African Journal of Microbiology Research* Vol.4(12) pp. 1275-1281
- Dubey, R. C., and Maheshwari, D.K. (2012). Methods in Microbiology. A textbook of Microbiology. S. Chand and company Ltd. Ram Nagar, New Delhi 110 085. ISBN: 81- 219-2559-2. Pg. 72-73
- FAO (2006). Food and Agricultural Organization. Production Yearbook, 2006 FAO Rome, Italy
- Hamilyn, P.F. (1988). Fungal Biotechnology. *British Mycological Society Newsletter*. 130 - 134.
- Iyayi, E. A. (2004). Changes in the cellulose, sugar and crude protein contents of agro-industrial by-products fermented with *A. Niger*, *A. flavus* and *Penicillium sp.* *African Journal of Biotechnology*. Vol. (3): 816-188.
- Iyayi, E. A. and Aderolu, A. Z. (2004). Enhancement of the feeding values of some agro- industrial by-products for laying hens after their solid state fermentation with *Trichoderma viride*. *Afr. J. Biotech.* 3: 182-185
- Iyayi, E. A. and Lossei, D. M. (2001) Protein enhancement of cassava by products through solid state fermentation by fungi. *The Journal of Food Technology in Africa*. Vol. 6. (4). 116- 118.

- Jawetz, E., Melnick, J. L. and Adelberg, E. A. (1984). The growth and death of micro-organisms. *A Review of Medical Microbiology* 16<sup>th</sup> edition. Lange Medical Publications. Dravie L. Los Altos, California 94022. Pg. 92-94
- Oboh, G. (2006). Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus* sp. solid media fermentation techniques. *Electronic J.Biotechnology*. Vol. 9 (1): 46-49.
- Oboh, G., Akindahunsi, A. A., and Oshodi, A. A. (2002). Nutrient and anti-nutrient content of *Aspergillus niger* fermented cassava products (flour and garri). *Journal of Food Composition and Analysis* .Vol. 15, (5): 617-622.
- Oboh, G. and Akindahunsi, A. A. (2003). Biochemical changes in cassava products (flour and garri) subjected to *Saccharomyces cerevisiae* solid state fermentation. *Food Chemistry*. Vol.82 (4): 599- 602.
- Ofuya, .C. O. and Nwajiuba, .C. J. (1990). Micro-biodegradation and utilization of cassava peel. *World J Microbiol. Biotechno.* 6: 144-148.
- Rambault, T. M. (1998). General and microbiological aspects of solid substrate fermentation. *Electronic Journal of Biotechnology (online)*.Vol.1, (3)
- SAS (1999). Statistical Analysis System user's Guide. *Statistis SAS Institute*. Cary, NC 27513, USA.
- Steel, R. D. G. and Torrie, J. H. (1980). Principles and procedures of statistics. *A Biometrical Approach, International Students' edn.* McGraw Hill, London.