

Full Length Research Paper

Biodegradation of cassava root sieviate with enzymes extracted from isolated fungi

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Received 30 May, 2014; Accepted 1 September, 2014

This study was carried out to investigate changes in proximate and chemical components of cassava root sieviates (CRS) treated with extracted enzymes obtained from (a) *Aspergillus niger* (An); (b) *Rhizopus stolonifer* (Rs); (c) *Trichoderma viridae* (Tv) and (d) *Mucor mucedo* (Mm) applied on autoclaved CRS at 250 ml/kg. Another treatment had a commercial enzyme Roxazyme G2G (RG2G) as the degrading agent. At the end of the seventh day after enzyme application, crude protein, ash and metabolizable energy content increased but crude fibre, pectin, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose and cellulose content decreased. The highest value of metabolizable energy (ME), ash and crude protein were: 2807.81 kcal/kg, 17.32 and 18.32 g/100g dry matter, respectively obtained when enzymes obtained from *A. niger*, *T. viridae* and *A. niger* were added respectively. Results of levels of sugars (mg/mL) in undegraded CRS showed that glucose level increased by 72.4, 67.2, 53.8, 50.1 and 32.5% when enzyme preparations from *A. niger*, *T. viridae*, *R. stolonifer*, *M. mucedo* and Roxazyme G2G were applied on CRS respectively. Results obtained indicate that the use of enzymes from the above named fungi defiberised the CRS and hence promoted better bioavailability of the hidden nutrients in it.

Key words: Cassava root sieviate, enzymes, biodegradation, nutrient enhancement.

INTRODUCTION

Feed insecurity describes a situation whereby there is shortage of feed ingredients in quantity or in quality and this is aggravated by the ever widening demand for conventional feed sources in developing countries. No doubt, there is continuous need for animal protein and low availability of it is the chief factor known for the occurrence of child/infant morbidity, kwashiorkor, poor level of productivity and reduction of life span. Agromisa (2006) revealed that the state of consumption of meat and other animal

protein in Nigeria is estimated at about 8 g per caput per day and this is about 27g less than the 35 g per caput minimum requirements recommended by the Food Agriculture Organization (FAO, 2003). Besides, in Nigeria, Olerede (2005) noted that poultry constitute over 90% of the current national livestock population and are of appreciable economic and social value to the investors and consumers. Large scale egg production is one of the ways to ensure better availability of animal protein. Regmi (2007)

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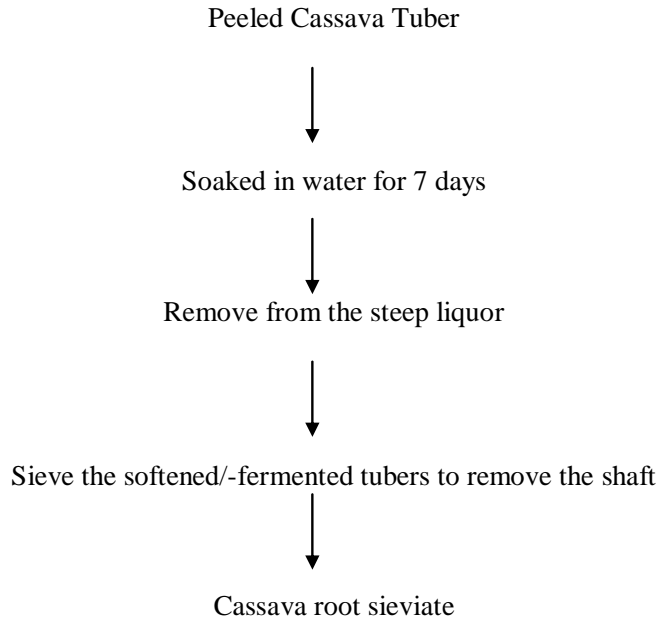


Figure 1. Flow chart for cassava roots sieviate production.

observed the unprecedented population growth that has occurred in the last half of the century has created an additional demand for meat and general food in developing countries. The prices of conventional feed ingredients keep rising steadily because of the competition between man and livestock and their increasing populations. The cost of feeds is as high as 70 to 75% of the total cost of production (Oluremi et al., 2007). In an attempt to overcome this challenge, several studies have been carried out on the possibility of using agro industrial by products and crop residues. Therefore, of late, agro industrial by-products are receiving attention. Meanwhile, agro industrial by-products (AIBs) that have their source from plants have cell walls which contain a variety of polysaccharides, the distribution of which varies within primary and secondary cell wall and between mono and dicotyledonous plants. The polymers are interlinked by covalent linkages or via non-carbohydrate compounds. The non-starch polysaccharides (NSPs) comprise 700-900 g/kg of the plant cell wall with the remaining being lignin, protein, fatty acids and waxes. Plant cell wall NSP is a diverse group of molecules with varying degree of water solubility, size and structure which may influence the rheological properties of the gastro intestinal content (Taibipour and Kermanshahi, 2004; Yineth and Mario, 2014). AIBs constitute those parts of crops that are left after removal of value giving components. These residues still contain considerable amount of energy and protein which may be present as intracellular compounds (Huang et al., 2008). AIBs represent potential valuable and renewable resources which find application in various areas that include use as animal feed. AIBs have been successfully incorporated into poultry diets at various levels in developing countries

with resultant effect on reduction of cost of feeding. The use alleviates the existing critical situation of inadequate feed supply. Numerous successful studies on the supplementation of agro-industrial by-products with enzymes have been reviewed by several authors (Kelly-Yong et al., 2005; Khajavi et al., 2005). Lignin has been recognized as the chief barrier to monogastric digestion of cell wall structural polysaccharides (Kumar et al., 2009) and removal of lignin by chemical treatment enhances fibre digestibility (Oms-Oliu et al., 2009). Lignin offers the recalcitrant and adamant posture to fibre by the strength of its chemical nature though lignin may not be solely responsible for the variation in digestibility that is in AIBs (Pandey and Nagveni, 2007; Bachtar, 2005). In Nigeria, large quantities of CRS are produced by the cassava tuber processing industries (Aderemi, 2000). The aim of this study was to investigate possibility of defiberising the polymers that are interlinked by covalent linkages in the CRS and study the changes in proximate and sugars levels components of cassava root sieviates treated with extracted enzymes obtained from *Aspergillus niger* (An), *Rhizopus stolonifer* (Rs), *Trichoderma viridae* (Tv) and *Mucor mucedo* (Mm) and to possibly recommend the most effective biodegrader of CRS among the four fungi and the commercial enzyme.

MATERIALS AND METHODS

The CRS used for this study was obtained from a cassava processing centre in Ibadan, Nigeria. Figure 1 shows the flow chart for cassava roots sieviate production. It was milled with a 0.84 mm sieve and autoclaving was done at 121°C for 15 min.

Sources of fungi used

R. stolonifer and *A. niger* were obtained by exposing a piece of moist bread and a dough made from cassava flour to the air for 24 h and then covered for 3 days. After the third day, growth of the fungi appeared on the bread and cassava dough. Pure *T. viride* was obtained from the Department of Microbiology, University of Ibadan.

Isolation of fungi

A sterile wire loop was used to collect the spores and the mycelia of the actively growing fungi. The spores and mycelia were then inoculated aseptically on the centre of sterile potatoes dextrose agar (PDA) plates. The spores and the mycelia were aseptically spread on the PDA plates using the sterile wire loop in a sterilized chamber. The inoculated plates were then incubated at 34°C in a Gallenkamp incubator. The PDA plates were examined for growth after 48 h. It was observed that there was mix culture of fungi. Spores from each fungus was aseptically isolated and subcultured on fresh sterile PDA and re-incubated for 48 h. Pure culture was then obtained and the mycelium from this was put on slant of sterile PDA. The characterization of the obtained *A. niger* and *R. stolonifer* was known by the use of manual of Barnett and Hunter (1992).

Enzyme production procedure

Enzymes were produced by extraction from cassava root sieviate.

Table 1. Proximate analysis of undegraded and degraded cassava sieviates (g/100g DM).

Parameters	Control (undegraded)	CRS+An	CRS+Tv	CRS+Rs	CRS+Mm	CRS+RG	SEM	P-value
Crude protein	9.63 ^e	16.32 ^a	14.38 ^c	15.32 ^b	14.27 ^c	11.50 ^d	2.23	0.0033
Crude fibre	10.98 ^a	4.82 ^d	5.52 ^d	7.42 ^c	6.28 ^{cd}	8.73 ^b	0.85	0.0024
Ether extract	2.01	2.27	2.09	2.05	2.21	2.24	0.62	0.0830
Ash	9.63 ^d	17.47 ^b	15.32 ^a	14.76 ^b	11.21 ^c	11.52 ^c	2.85	0.0052
Nitrogen free extract	68.75 ^a	60.62 ^d	60.69 ^d	57.45 ^e	64.03 ^c	66.01 ^b	2.62	0.0033
Pectin	11.24 ^a	3.86 ^d	4.21 ^c	3.52 ^d	4.26 ^c	8.21 ^b	0.52	0.0014
Hemicellulose	20.52 ^a	15.70 ^d	14.46 ^d	17.92 ^c	13.71 ^e	18.22 ^b	2.01	0.0041
Cellulose	22.71 ^a	15.70 ^c	14.46 ^c	18.55 ^b	17.26 ^b	19.85 ^a	1.10	0.0032
Metabolizable energy (kcal/kg)	2219.55 ^d	2807.81 ^a	2676.14 ^b	2293.72 ^a	2728.31 ^a	2465.30 ^c	11.85	0.0045

Means with different superscripts along the same row are significantly different ($P < 0.05$). An=Aspergillus niger, Tv=Trichoderma viride, Rs=Rhizopus stolonifer, Mm=Mucor mucedo, RG=Roxazyme G2G.

The fungi used were *A. niger*, *R. stolonifer* and *T. viridae*. A commercial exogenous enzymes (Roxazyme G2G) that contained cellulase (endo-1, 4-(β -glucanase), β -glucanase (endo-1, 3 (4)- β -glucanase) and xylanase (endo-1, 4- β xylanase) was also used. Every 50 g of the substrate was moistened with 20 mL of the requisite basal medium (KNO₃, 5.0 mg; KH₂PO₄, 2.0 g; MgSO₄.7H₂O, 0.5 g; Tryptone, 0.5 g; FeSO₄.4H₂O, 3.5mg; C₆H₅NO₂, 0.5 mg; C₁₂H₁₇ClN₄OS, 0.05 mg and C₁₀H₁₆N₂O₃S. Biotin, 0.05 mg per litre of distilled H₂O) and then 1.0 ml of an aqueous spore's suspension of each isolate was added. The conical flasks were covered with sterilized cotton wool and kept in the incubator at 330C for seven days. After the growth of the fungi, the contents of each flask were mixed with 100 mL of the requisite buffer (Phosphate; pH of 7.2) and then filtered through double layered muslin clothe. The filtrate in the flask was in a chilled environment (4°C) to prevent denaturation of the enzymes. It was centrifuged at 3000 rpm for 15 min by using the centrifuge manufactured by Measuring and Scientific Equipment, MSE (UK) Ltd named MSE Cellsep 6/720R centrifuge. The supernatant was collected and taken as raw enzymes (Onilude and Oso, 1999). Raw enzymes were concentrated 5-fold by means of a Vacuum Rotator Evaporator at 3000 rpm. The concentrated enzymes were dialyzed using phosphate buffer of pH 7.2 (0.1M). The *in vitro* dry matter enzymic degradation (IVDMED) was carried out in three replicates per treatment. Ground and sterilized CRS was obtained from the mentioned fungi. The enzymes were directly applied on CRS at 250 ml/kg and allowed to stay for seven days. Mouths of conical flasks that contained the CRS and enzymes were plugged with sterilized cotton wool to prevent contamination. This was carried out in a sterilized environment. At the end of the seventh day, samples were oven dried at 70°C for 16 h to stop further actions of enzymes. This was tagged biodegraded CRS. Soluble sugars were determined spectrophotometrically using the methods of Association of Official Analytical Chemists (1995).

Chemical and statistical analyses

After drying, all the samples were milled with a 0.84 mm sieve and the following analyses were carried out: crude protein, crude fibre and ether extract, using Association of Official Analytical Chemists, AOAC (1995) method while acid detergent fibre, neutral detergent fibre, cellulose and acid detergent lignin were determined using the method of Van Soest and Robertson (1991). The absorbencies were read from the spectrophotometer at the specific wavelength 105°C for 8 h. Hemicellulose was estimated as the difference for

each sugar. Dry matter was determined by drying the samples at between neutral detergent fibre and acid detergent fibre. Crude protein was determined as Kjeldahl nitrogen x 6.25. metabolizable energy (ME) of degraded and undegraded GNP samples was determined with the use of Pausenga (1985) method: ME = 37 x CP% + 81.8 x fat% + 35.5 x NFE%. Data collected were subjected to analysis of variance (SAS, 1999). Significant differences between means were determined using the Duncan multiple range test (SAS, 1999).

RESULTS AND DISCUSSION

Results of the enzymic biodegradation of cassava root sieviate (CRS) as shown in proximate and detergent fibre composition are presented in Table 1. Enzymes increased the crude protein content of the CRS by 37.14, 40.99, 32.61, 32.52 and 16.26% with *A. niger*, *R. stolonifer*, *M. mucedo*, *T. viride* and Roxazyme G2G respectively. Values were significantly different ($P < 0.05$) among fungi. Increase in the crude proteins value of the degraded AIBs was partly due to the ability of the enzymes to increase the bioavailability of the protein hitherto encapsulated by the cell walls. Fungal enzymes have the potential of improving not only the NSPs but also the crude proteins as well as other dietary components such as ash and fatty acids (Liu and Baidoo, 2005). Crude fibre content in the CRS was also significantly ($P < 0.05$) reduced. *A. niger* caused the highest reduction in crude fibre level by reducing it from 10.98 g/100 g to 4.82 g/100 g which represented a 56.10% reduction and this was followed by changes orchestrated by *T. viridae* (49.73%). The cellulose content in the degraded CRS decreased by 36.3, 30.9, 24.0, 18.3 and 12.6% with *T. viride*, *A. niger*, *M. mucedo*, *R. stolonifer* and Roxazyme G2G, respectively. This confirms the reports by other authors (Iyayi and Aderolu, 2004; Iyayi and Losel, 2001; Yoshinori et al., 2014). Iyayi and Aderolu (2004) reported reduction in crude fibre content of brewer dried grain, maize ofal and wheat ofal when *A. niger*, *A. flavus* and *Penicillium* sp. were used for their biodegradation.

Table 2. Levels of soluble sugars (mg/mL) in undegraded and biodegraded cassava root sieviate.

Soluble sugars	Undegraded CRS	CRS+Tv	CRS+Mm	CRS+Rs	CRS+An	CRS+RG	SEM	P-value
Glucose	257.34 ^e	784.20 ^b	516.06 ^c	556.40 ^c	932.22 ^a	381.43 ^d	0.41	0.0014
Fructose	89.0 ^d	218.03 ^b	203.20 ^b	137.04 ^c	221.30 ^a	228.44 ^a	0.22	0.0022
Galactose	140.0 ^d	840.0 ^a	230.0 ^c	760.0 ^{ab}	620.0 ^b	200.5 ^c	0.05	0.0016
Sucrose	55.0 ^d	226.20 ^b	209.05 ^b	218.21 ^b	314.54 ^a	178.70 ^c	0.03	0.0020

Means with different superscripts along the same row are significantly different ($P < 0.05$). An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG = Roxazyme G2G.

Table 3. Mineral composition of undegraded and degraded cassava root sieviates mg/kg (PPM).

Minerals	Control undegraded	CRS+An	CRS+Tv	CRS+Rs	CRS+Mn	CRS+RG	SEM
Sodium	0.04 ^c	0.06 ^a	0.06 ^a	0.05 ^b	0.05 ^b	0.05 ^b	0.001
Potassium	0.49 ^d	0.64 ^a	0.51 ^c	0.56 ^b	0.63 ^a	0.55 ^b	0.004
Manganese	6.04 ^c	6.86 ^a	6.84 ^a	6.83 ^a	6.81 ^a	6.64 ^b	0.21
Calcium	0.03 ^c	0.05 ^b	0.05 ^b	0.06 ^a	0.06 ^a	0.04 ^c	0.008
Magnesium	5.60	5.61	5.61	5.62	5.61	5.60	0.02
Phosphorus	31.21 ^c	32.86 ^a	32.84 ^a	31.22 ^b	32.25 ^a	31.41 ^b	1.22
Copper	5.43	5.44	5.44	5.43	5.44	5.43	0.21
Chromium	3.94 ^d	4.50 ^b	4.62 ^a	4.63 ^a	4.52 ^b	4.01 ^c	0.16
Iron	22.83 ^c	22.87 ^{ab}	22.94 ^a	22.94 ^a	22.70 ^b	22.78 ^b	1.95
Zinc	12.86	12.87	12.87	12.87	12.86	12.86	1.14

Means with different superscripts along the same row are significantly different ($P < 0.05$). An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG = Roxazyme G2G.

Crude fibre in the above mentioned AIBs were significantly ($P < 0.05$) reduced by all the fungi until after the fourteenth day. *A. niger* consistently caused the highest reduction in crude fibre in all the AIBs followed by *A. flavus* and *Penicillium* sp. The use of enzyme has predominantly been related to the hydrolysis of fibre or non-starch polysaccharides fractions in the AIBs. Glucans chains of cellulose are held together in an organized manner by inter and intra molecular hydrogen which renders the carbohydrates and other nutrients insoluble and resistant to enzymic hydrolysis. The structure and properties of β -glucans are described as polymer of glucose with a β -1, 4 linked backbones and β -1, 3 side linkages (Atik et al., 2006; Ezieshi and Olomu, 2004). Apart from the fact that β -glucans and the insoluble NSPs arabinoxylans are also found in crude fibre and they are in endosperm cell walls. Crude fibres were hydrolyzed by the synergetic action of xylanase and glucanases (Dare et al., 2010; Hughes et al., 2000). Table 3 shows mineral composition of undegraded and degraded cassava root sieviates. It reveals improvement in the potassium, phosphorus, calcium, iron, chromium and manganese content after enzymic degradation of CRS. The highest value (32.86 mg/kg) for phosphorus was found in the *A.*

niger degraded sample. Possibly, enzyme phytase was part of the cocktail of enzymes produced by the used fungi and this must have assisted in the liberation of the phytate bound phosphorus. Phytate, like oxalates and tannins, is an organic compound (myo-inositol hexaphosphate) which occurs in all plants and these mineral binding factors possess anti-nutritional properties because they are potential chelators of minerals and, thus, their presence in any AIBs will strongly have negative effect on the digestibility of such materials (Sofia et al., 2014; Hannah et al., 2014). From the results, biodegradation of CRS by the fungi did enhance the bioavailability of the mineral elements (Na, K, Mg Ca, P and Fe). The results of sugar fractions (glucose, fructose, galactose and sucrose) in the undegraded and degraded CRS are shown in Table 2. Glucose was the highest produced sugar (glucose > fructose > sucrose > galactose). The highest glucose yield was in the CRS degraded with *A. niger* with a value of 932.22 g/mL as compared to 381.43 g/mL in the RG2G degraded CRS. The enzymes increased the metabolic energy content of the CRS by 21.0, 18.7, 17.1, 10.0 and 3.2% with *A. niger*, *M. mucedo*, *T. viride*, Roxazyme G2G and *R. stolonifer*, respectively. The values were significantly different ($P < 0.05$) among the fungi. The increase in the sugar content

and the metabolizable energy value of the degraded CRS was partly due to the ability of the enzymes to disrupt the cell wall structure (Ates et al., 2008; Gunal and Yasar, 2004). AIBs are known to have low ME values and they often have a high content of growth inhibiting, viscous, water-soluble, non starch polysaccharides (WNSPs). According to Oluremi et al. (2007), the monogastrics are unable to release the intrinsic energy portion of the AIBs as the energy remains latent in the fibrillan complex which renders resistance to the endogenous enzymes. In addition, Martins et al. (2000) observed a continuous increase in sugar production in the substrates until after the fourteenth day of fungi on the substrates when there was gradual reduction in sugar availability in the substrates. There were significant ($P < 0.05$) increase in sugar production when *A. niger*, *A. flavus* and *Penicillium* species were inoculated on brewer dried grain, maize offal and wheat offal. This study shows that with fungal biomass increase, the nutrients in the substrate medium were quickly used up. Beyond fourteenth days, the fungi started using up the products of breakdown of the NSPs, hence, the observed reduction in the sugar level. There was increase in the ME because there was increase in the soluble sugars availability. Soluble sugars are the assimilable forms of carbohydrates needed for energy production. However, fungal enzymes added to the AIB were able to break the cell walls. Hence, the degraded CRS had higher values of sugars than the undegraded one and this shows the effects of the enzymes on the CRS.

Conclusion

This study leads makes us believe that the treatment of cassava root sieviate with extracted enzymes from *A. niger*, *M. mucedo*, *R. stolonifer*, *T. viride* and Roxazyme G2G may improve the crude protein, metabolizable energy, sugars and mineral and reduce the fibre fractions. Furthermore, the increase in crude protein and meta-bolizable energy of the degraded CRS due to enzymic degradation made the CRS beneficial thereby increasing its nutritive value and solving the problem of environmental pollution. The application of this technique helps in the conversion of AIBs into animal feeds thereby improving their preservation and utilization by livestock animals. Ultimately, this will have direct positive effect on availability of animal protein. Of the four fungi and the commercial Roxazyme G2G, enzymes from *A. niger* appeared to be the most suitable for the biodegradation of cassava root sieviate as it succeeded in effecting the highest improvement in terms of crude protein, crude fibre, ash, sugar and metabolizing energy. Therefore, the use of *A. niger* in enhancement of CRS as animal feed ingredient is preferred to *T. viride*, *M. mucedo*, *R. stolonifer* and Roxazyme G2G.

Conflict of Interests

The authors have not declared any conflict of interests.

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