Biodegradation of cassava root sieviate with enzymes extracted from isolated fungi

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Abstract

This study was carried out to investigate changes in proximate, sugars and cell wall components of cassava root sieviates (CRS) treated with extracted enzymes obtained from Aspergillus niger (An), Trichoderma viridae (Tv), Rhizopus stolonifer (Rs) and Mucor mucedo (Mm) applied on autoclaved CRS at 250ml/kg. Another treatment that had a commercial enzyme Roxazyme G2G (RG2G) as the degrading agent was prepared and the enzyme was applied at 150g/tonne as recommended by the manufacturer. At the end of the 7th day after enzyme application, the chemical analysis showed a higher bioavailability of nutrients in the degraded samples than the undegraded sample. Non starch polysaccharides (NSPs) in the CRS was negatively related to crude protein, ash and metabolisable energy (P<0.05) but positively related to crude fibre, pectin, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose and cellulose content . The highest value of metabolisable energy (ME), Ash, crude protein and phosphorus were : 2807.81kcal/kg, 17.32,18.32 and 0.91 g/100g dry matter respectively; obtained when A.niger, T.viridae, A. niger and R. stolonifer enzymes were added respectively .The lowest values of crude fibre, pectin, NDF,ADF, ADL, hemicellulose and cellulose were: 4.82,3.52,30.74, 17.12, 3.74,13.71 and 14.46g/100g dry matter respectively obtained when enzymes extracted from A.niger , R. stolonifer, T. viride ,A. niger, R. stolonifer, M. mucedo and T. viride were applied . Results of levels of sugars (µg/ml) in undegraded CRS showed that glucose level increased by 72.40% (257.34 to 932.22), 67.18%(257.34 to 784.20), 53.75%(257.34 to 556.40), 50.13%(257.32 to 516.06) and 32.53%(257.34 to 381.43) when enzyme preparations from A. niger, T.viridae , R.stolonifer, M.mucedo, and Roxazyme G2G were applied on CRS for degradation respectively . Other sugars that were significantly (P<0.05) increased after biodegradation were galactose, fructose and sucrose. Significant (P<0.05) differences were also expressed in the mineral analysis. After biodegradation, the degraded CRS had better mineral bioavailability as there were improvements in the minerals quantity. The results revealed that the use of enzymes from the above named fungi and the commercial enzymes defiberised the CRS and hydrolyzed the cell walls and hence promoted better availability of energy, crude proteins and other nutrients which were hitherto unavailable.

Key words: Non-conventional ingredients, biodegradation, nutrient enhancement.

Introduction

Demand is increasing for non-conventional feed sources in developing countries due to an increase in demand for meat. The 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 700-900g/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 et al 2004). 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 (Dusterhoft 1993). 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 (Campbell and Bedford 1992; Marquardt 1997; Bedford 1995; Dierick and Decuypere 1996). Lignin has been recognized as the chief barrier to monogastric digestion of cell wall structural polysaccharides (Kerley et al 1988) and removal of lignin by chemical treatment enhances fibre digestibility (Wang *et al* 1995). 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 (Reeves 1985; Buxton and Russell 1988). Roltz et al; (1986) opined that digestibility changes of fibrous crop residues after fungal incubation were caused by a complex interaction of many factors including cell wall phenolics acids. Phenolics monomers inhibit rumen microorganisms (Hartley and Akin 1989) and are negatively correlated to fibre digestibility (Burritt *et al* 1985; Bohn and Fales 1989). Cell wall phenolics acids, mainly p-coumaric acids and ferulic acids serve to cross link different wall components (Jung *et al,* 1992; Hartley et al 1990), thereby enhancing structural rigidity and also limiting the availability of structural carbohydrates to rumen digestion.CRS is the fibrous leftover when the starchy part of cassava tuber has been removed. In Nigeria, large quantities of CRS are produced by the cassava tuber processing industries (Aderemi, 2000). The objective of this study was to investigate changes in proximate, sugars and cell wall components of Cassava Root Sieviates (CRS) treated with extracted enzymes obtained from *Aspergillus niger (An)*, *Trichoderma viridae (Tv), Rhizopus stolonifer (Rs)* and *Mucor mucedo (Mm)*

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Materials and Methods

The CRS used for this study was obtained from a cassava processing centre in Ibadan. It was milled with a 0.84mm sieve. Autoclaving was done at 121^0C for 15 minutes.

Enzyme production procedure

Enzymes were produced by extraction from the substrates .The fungi used were *A.niger, T.viride, R.stolonifer* and *M.mucedo*. A commercial enzyme (Roxazyme G2G) was also used. Every 50 g of the substrate was moistened with 20 ml of the requisite basal medium (KNO3, 5.0mg; KH2PO4, 2.0g;

Table 1 : Proximate and detergent fibres 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
Dry matter	86.40	89.54	89.24	88.82	88.25	87.33	4.52	0.0711
Crude protein	9.63 ^{e}	15.32 ^{a}	14.38 ^{c}	16.32^{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}	15.47 ^{b}	17.32 ^{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
Neutral Detergent Fibre	38.20^{a}	33.82^{c}	30.74^{d}	35.24^{b}	32.67^{c}	36.4^{b}	2.22	0.0040
Acid Detergent Fibre	19.13^{a}	17.12^{b}	17.28^{b}	17.32^{b}	18.76^{a}	18.99^{a}	1.04	0.0047
Acid Detergent Lignin	10.01^{a}	5.32^{c}	6.52^{c}	3.74^{d}	8.32^{b}	8.88^{b}	0.31	0.0035
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*

$MgSO_4.7H_2O$, 0.5g; Tryptone, 0.5g; FeSO4.4H2O, 3.5mg; Nicotinic acid, 0.5mg; Thiamine, 0.05mg and Biotin, 0.05mg per litre of distilled H_2O) and then 1.0ml 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 33^0C for 7 days. After the growth of the fungi, the content of each flask were mixed with 100ml 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 (4^0C) to prevent denaturation of the enzymes. It was then centrifuged at 3000 rpm for 15 minutes by using the MSE centrifuge. The supernatant was collected and taken as raw enzymes (Onilude and Oso, 1999).The raw enzymes were concentrated 5-fold by means of a vacuum rotatory evaporator. The concentrated enzymes were dialyzed using phosphate buffer of pH 7.4 (0.1M). The *in vitro* dry matter enzymic degradation (IVDMED) was carried out in 3 replicates per treatment. The soluble sugars were determined spectrophotomerically by the methods of AOAC (1995). The ground and sterilized CRS was degraded by various enzymes got from the mentioned fungi. The enzymes were directly applied on CRS at 250 ml/kg and allowed to stay for 7 days. The mouths of the conical flasks that contained the CRS and the enzymes were plugged with sterilized cotton wool to prevent contamination. This was carried out in a sterilized environment. At the end of the 7th day, the samples were oven dried at 70 ^{0}C for 16 hours to stop further action of the enzymes. This was tagged biodegraded CRS.

Chemical and statistical analyses

After drying, all the samples were milled with a 0.84mm sieve and the following analyses were carried out: crude protein, crude fibre and ether extract, using 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 for each sugar.

Dry matter was determined by drying the samples at 105^{0}C for 8 hours. Hemicellulose was estimated as the difference between NDF and ADF. Crude protein was determined as Kjeldhal nitrogen x 6.25. The data collected was subjected to Analysis of Variance (SAS, 1999). Significant differences between means were determined using the Duncan Multiple Range Test (SAS, 1999). The metabolizable energy (ME) of degraded and undegraded GNP samples was determined with the use of Pauzenga (1985) method: ME = 37 x %CP + 81.8 x %Fat + 35.5 x %NFE.

Table 2: Levels of soluble sugars (µg/ml) in undegraded and biodegraded cassava

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$^{a b}$	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

root sieviate

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*

Results and Discussion

The results of the enzymic biodegradation of Cassava Root Sieviate (CRS) as shown in proximate and detergent fibre composition are presented in table 1. The enzymes increased the crude protein content of
the CRS by 37.14% (9.63 to 15.32), 40.99% (9.63 to 16.32) 32.61% (9.63 to 14.38), 32.52% (9.63 to 14.27) and 16.26% (9.63 to 11.50) with *A.niger*, *R. stolonifer, M.mucedo,* T. *viride* and Roxazyme G2G respectively. The values were significantly different (P<0.05) among the fungi. The increase in the crude proteins value of the degraded AIBs was partly due to 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 et al 2005). The crude fibre content in the CRS was also significantly (P<0.05) reduced. *A. niger* caused the highest reduction in the crude fibre from 10.98g/100g to 4.82g/100g which represented a 56.10% reduction and this was followed by the reduction effected by T. viridae 49.73%. The cellulose content in the degraded CRS decreased by 36.33% (22.71 to 14.46), 30.86% (22.77 to 15.70), 23.99% (22.71 to 17.26) and 18.31 %(22.71 to 18.55) and 12.59% (22.71 to 19.85) 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 Losel2001;

Ofuya and Nwanjimba 1990). Iyayi and Aderolu(2004) reported reduction in crude fibre content of brewer dried grain, maize offal and wheat offal when *A.niger, A. flavus* and *Penicillium* sp were used for their biodegradation. According to these workers, crude fibres in the above mentioned AIBs were significantly ($P<0.05$) reduced by all the fungi until after the 14th day. They further reported that *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. Glucan 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 (Ezieshi and Olomu 2004). Apart from 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 (Hughes et al 2000). In the mineral analysis, there was improvement in the Potassium, phosphorus, Calcium, Iron, Chromium and Manganese content after enzymic degradation of CRS. The highest value (32.86mg/kg) for phosphorus was found in the *A. niger* degraded sample. Apparently, it is likely that 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. Phytate is a potent chelator of minerals and, thus, its presence in a feed will strongly dictate the outcome of minerals associated with this molecule (Okon and Ogunmodede, 1996; Eaton and Ramsdell 1995). The results of sugar fractions (glucose, fructose, galactose and sucrose) in the undegraded and degraded CRS are shown in Table 2.0. Glucose was the highest produced sugar. The highest glucose yield was in the CRS degraded with *A. niger* with a value of 932.22µg/ml compared to 381.43µg/ml in the RG2G degraded CRS. The enzymes increased the metabolic energy content of the CRS by 20.95% (2219.55 to 2807.81), 18.65 %(2219.55 to 2728.31), 17.06% (2219.55 to 2676.14), 9.97% (2219.55 to 2465.30) and 3.23% (2219.55 to 2465.30) 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 (Pandey et al 2000; 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 fibrillian complex which renders resistance to the endogenous enzymes. Furthermore, Martins et al (2000) observed a continuous increase in sugar production in the substrates until after the 14th 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 .It was suggested that with fungal biomass increase, the nutrient in the substrate medium are quickly used up. Beyond 14 days, the fungi started using up the products of breakdown of the NSPs, hence, the observed reduction in the sugar level.

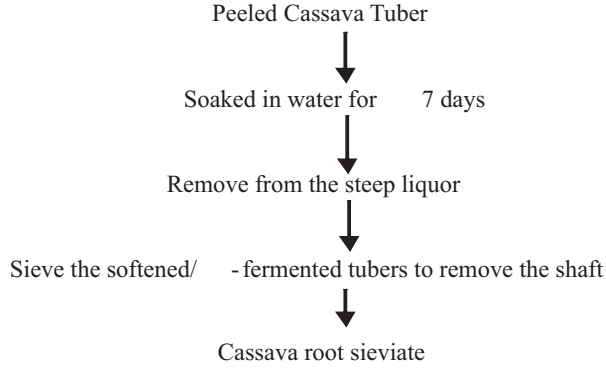
There was increase in the ME because there was increase in the soluble sugars availability. Soluble sugars are the assimilable forms of carbohydrates needed for energy production. However, fungal enzymes added to the AIB were able to break the cell walls. Hence, the degraded CRS had higher values of sugars than the undegraded AlB and this shows the effects of the enzymes on the CRS.

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*

FIGURE 1: FLOW CHART FOR CASSAVA ROOTS SIEVIATE PRODUCTION



Conclusion and application

The study revealed that the treatment of cassava root sieviate with extracted enzymes from *A.niger, T.viride, M.mucedo, R.stolonifer* and Roxazyme G2G improved the crude protein, metabolizable energy, sugars and phosphorus content while the fibre fractions were reduced.

The increase in crude protein and ME of the degraded CRS due to enzymic degradation made the CRS beneficial thereby increasing its nutritive value, minimizing the amount of waste produced and thereby reducing the problem of environmental pollution.

The use of this technique can enhances the use of CRS as animal feed ingredient.

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