Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Technology

Elixir Bio Tech. 89 (2015) 37197-37201

ISSN: 2229-712X

Nutritional enhancement of whole cassava starch residue by biodegradation with fungi SPP

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ARTICLE INFO

Article history:

Received: 26 November 2012;

Received in revised form:

18 December 2015;

Accepted: 23 December 2015;

Keywords

A. niger,
T. viride,
Biomass loss,
Crude protein,
Cellulose,
Hemicellulose.

ABSTRACT

Treatment of crop residues with some species of fungi can enhance their digestibility. This study investigated changes in the nutrient composition of Cassava Starch Residue (CSR) biodegraded with two selected fungi: *Aspergillus niger and Trichoderma viride.* The experimental designed was a 3x4 factorial arrangement. Biodegradation of CSR for 21days at 30^0C recorded a significant biomass loss of 33.11% (*A. niger*), followed by 30.01% (combination of *A. niger x T. viride*) compared to 27.44% (*T. viride*). The crude protein increased significantly (P>0.05) from 4.05% to 7.16%, 6.84% and 6.88% within 14days for *A. niger, T. viride* and combination of *A. niger x T. viride* respectively. Similarly, the fibre in Cassava Starch Residue decreased from 17.07% to 10.31%, 12.83% and 11.89% for the *A. niger, T. viride* and combination *A. niger x T. viride* treatments respectively with a corresponding effect of 2897.1%kcal/kgDM, 2719.2kcal/kgDM and 2739.1kcal/kgDM in the level of metabolisable energy. Cellulose was extensively degraded in all the treatments (P>0.05) than the hemicellulose. Results of this study suggests that fungal biodegradation of whole cassava starch residue is regulated by a complex combination of various factors and consequently enhanced its nutritional profile.

Introduction

The major ingredients in feed for poultry in Nigeria are maize and soybean or groundnut cake to supply energy and protein (Tewe, 2004). Fish meal is a good source of animal protein which is used to complement the amino acids of vegetable protein sources. However, the cost of these main ingredients along with competition for their use as food by man may limit the maximum quantity used, giving way to alternatives. Despite the potential of these low-grade feedstuffs as ingredients in the tropics, their utilization is dependent on their chemical composition. Their low protein and high crude fibre contents limit their use. The crude fibre content is high in non-starch polysaccharide (NSPs) (Longe, 2006) both water-soluble (pectins, gums and mucilages) and water-insoluble (other hemicelluloses, cellulose and lignin). As regards the poor nutritive values and non-suitability of Agro-industrial by products (AIBs) such as cassava starch residue for animals use, research results have shown that pretreatment prior to its use in feed to digest rigid cell walls of plant origin enhances their digestibility by making possible the release of readily digestible monomers (Onilude, 1999; Ologhobo et al., 2007;2008).

Currently, Nigeria is leading the production of cassava in the world with about 44.6million metric tones (FAO 2008) and Cassava starch residue constitutes 10-20% of the processed roots (Adedoyin et al., 2010). Its characterized by low protein content and metabolisable energy and low digestibility. Upgrading these residue by biological means to produce a utilizable product from a substance of little initial feedings value might be needed. Fungi, a saprophyte, capable of extracellular digestion of fibre components (NSPs) through the enzymes secreted by it, has been found to be promising in enhancing the nutritional profile of the choice feedstock. This study assessed the changes being made in CSR after degradation with *Aspergillus niger* and *Trichorderma viride*, in a bid to harness more unconventional feedstuffs to resolve the shortage of livestock feeds and reduce environmental hazards.

Materials And Methods

Experimental site

The degradation process was carried out in the Department of Microbiology laboratory, University of Ibadan, Ibadan.

Experimental Feedstuff

The unconventional feedstuff used for this study was dried Cassava (*Manihot esculenta* Crantz) starch residue.

Source of Fungi

The fungi spp (*Aspergillus niger* and *Trichoderma viride*) used were isolated from cassava by – products contaminated soil, and a decayed wooden tree respectively in the Department of Microbiology, University of Ibadan. Identification was done using the Compendium of soil fungi (Domsch et al., 1980). The fungi were kept on potato dextrose agar (PDA) at 30^OC and sub-cultured every two weeks to ensure viability and active growth.

Inoculum Preparation

7 day-old slants of each fungal isolate was used for the fermentation process. 10ml of sterile distilled water introduced onto slants of sporulating culture of each isolate.The spores were rinsed into the water using sterile inoculating loop in an aseptic environment. Spore size per-ml was determined using haemocytometer to obtain $1.0x10^8$ spores/ml which was used for the Solid State Fermentation

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Solid state Fermentation

Solid state fermentation was carried out in 250ml Erlenmeyer flasks, in a controlled temperature chamber of 30^{O}C. The solid substrates contained 30g of milled cassava starch residue in separate flasks which was sterilized by autoclaving at 121^{O}C for 20 minutes before the fungi were added. 15 mls of sterile distilled water was added to each flask prior inoculation to enhance fungal growth. Three ml of each of the inoculums was used to inoculate the solid substrate in their respective flasks and the mixture incubated at 30^{0}C. The flask for each treatment was replicated three times for sampling at each period of 0,7, 14 days and 21 days of fermentation. At the end of each fermentation period, sampling was effected from the replicates and obtained samples were steamed at 100^{O}C for 30minutes to terminate fungal activity of the fungi, oven-dried at 60^{O}C for 72 hours and stored at room temperature for chemical analysis.

Biomass Measurement

The biomass loss was monitored according to the method of Kanthasamy et al., (1992) for the number of days the fermentation exercise lasted. The initial weight at day zero and the final weight on the last day of the experiment were noted. The final weight was then subtracted from the initial weight and the difference was then divided by the value of day zero and the percentage obtained using the formular outlined by Kanthasamy et al, (1992).

Chemical Analysis

The samples were analyzed for Dry matter, Crude Protein, Crude fibre, Ether Extract, As, Acid detergent Fibre (ADF), Acid Detergent Lignin (ADL) and Neutral Detergent Fibre (NDF) (A.O.A.C 1990). Hemicellulose of the samples were estimated by difference between NDF and ADF, while Cellulose was estimated by the difference between ADL and hemicellulose. Metabolizable energy was calculated using the equation of Pauzenga, (1985).

Statistical Analysis

The data obtained were subjected to statistical analysis using ANOVA (SAS,1999) while significant means were separated using Duncan Multiple Range test of the same package.

Experimental Design

The experiment was designed as a 3 x 4 factorial arrangement.

Results

Effect of interactions of days of fermentation and organisms on Biomass loss (%)

The biomass loss of fungi-degraded cassava starch residue for different days; 7, 14 and 21 is shown in Table 1. Results showed that for each of the fungi treatment, there were significant differences between biomass loss observed in 7, 14 and 21days. A significant increase in biomass loss from 23.80% (day 7) to 33.11% (day 21) in *A. niger* while losses for *T. viride* and combination of inoculum of *A.niger + T. viride* were 15.86% to 27.44% and 17.65% to 30.01% respectively. Between the three treatments (*A.niger*, *T.viride*, and *A. niger + T.viride* *A. niger* degradation of CSR substrate was significantly (P<0.05) higher than *T. viride* degradation of CSR and combination of inoculum of *A. niger + T.viride* degraded CSR throughout periods.

Effect of days of fermentation and organisms on the Nutrient composition of Biodegraded CSR

The effects of days of fermentation and organisms on the nutrient compositions of cassava starch residue are shown in Table 2 and 3 respectively. Table 2 shows that generally the significant (P<0.05) higher contents of CP, (7.16%), ME (2785.4Kcal/Kg) and least CF (11.67%), cellulose, hemicellulose and ADL were observed in biodegraded CSR substrate on (day 14).

In table 3, effects of *A. niger*, on CP and ME contents of cassava starch residue were higher (P<0.05) than that of *T.viride* and combination of *A.niger + T.viride* inoculum, although, the cellulose (12.01%) and hemicellulose (34.00%) contents of *T. viride* fermented substrate were similar (P>0.05) to that of *A. niger*.

Table 1: Effect of Interactions of Days of Fermentation and Organisms on Biomass Loss (%) Of Biodegraded Csr

DAYS				
Organisms	7	14	21	SEM
A. niger	23.80^{cx}	30.20^{abx}	33.11^{ax}	0.06
T. viride	15.86^{cz}	21.42^{by}	27.44^{ay}	0.08
A.niger +T.viride	17.65^{bcy}	19.89^{by}	30.01^{ax}	0.05

abc. Means on the same row with different superscripts are significantly different (P<0.05)

x,y,z. Means on the same column with different superscripts are significantly different (P<0.05)

Effect of Interactions of days of Fermentation and Organisms on the Nutrients Composition of Biodegraded CSR

The interractions of days of fermentation and organisms on the crude protein, crude fibre and ME contents of cassava starch residue were shown in table 4, 5 and 6. The results in (day 14) showed a significant (P<0.05) increase in CP contents of biodegraded CSR with *A. niger* inoculum with mean value of (7.16%) compared to *T.viride* (6.84%) and combination of *A. niger* and *T.viride* inoculum (6.88%) biodegraded CSR respectively.

The CF decreased significantly (P>0.05) with increase in CP contents in all the three treatments (*A. niger, T.viride* and combination of *A. niger + T.viride*), the least CF values was observed in CSR biodegraded with *A. niger* inoculum (10.31%) followed by CSR biodegraded with combination of *A. niger + T.viride* inoculum (11.89%).

The ME for CSR biodegraded with *A. niger* had the highest mean value of 2897.1Kcal/kg DM. compared to 2739.1kcal/kgDm for the CSR inoculated with combination of *A. niger + T.viride*. The same trend was observed for other periods.

Table 2: Effect of days of fermentation on the nutrient composition of biodegraded CSR

DAYS					
Parameter	**0**	**7**	**14**	**21**	**SEM**
Crude Protein (%)	4.05^{y}	6.03^{x}	7.16^{w}	6.93^{w}	0.15
Crude Fibre (%)	17.00^{w}	12.86^{x}	11.67^{y}	11.50^{y}	0.04
Metabolisable energy (Kcal/kg DM	2589.3^{y}	2718.8^{x}	2785.4^{w}	2757.6^{w}	116.5
Neutral Detergent fibre(%)	68.03^{w}	62.20^{x}	54.55^{y}	53.65^{z}	0.06
Acid Detergent fibre	32.00^{w}	27.45^{x}	20.60^{y}	20.38^{y}	0.07
Acid Detergent lignin (%)	14.93^{w}	13.50^{x}	12.44^{y}	12.37^{y}	0.37
Cellulose (%)	17.06^{w}	13.95^{x}	8.06^{y}	8.00^{y}	0.01
Hemicellulose (%)	36.03^{w}	35.73^{w}	33.93^{x}	33.28^{x}	0.24

Wxy: Means on the same row with different superscripts are significant (P<0.05)

Table 3: Effect of organisms on the nutrient composition of biodegraded CSR

Parameter	Aspergilus niger	Trichoderma viride	Aspergillus niger +Trichoderma viride	SEM
Crude Protein (%)	7.16[a]	5.88[b]	5.93[b]	0.10
Crude fibre (%)	12.29[c]	14.14[a]	13.34[b]	0.03
Metabolisable Energy (Kcal/kgDM)	2764.2[a]	2675.6[b]	2698.20[b]	87.4
NDF (%)	58.76[c]	59.58[b]	60.48[a]	0.04
ADF(%)	24.48[b]	25.58[a]	25.26[a]	0.05
ADL(%)	12.98[b]	13.51[a]	13.44[a]	0.28
Cellulsoe (%)	11.49[b]	12.07[a]	11.75[b]	0.01
Hemicelulose (%)	34.28[b]	34.00[b]	35.22[a]	0.18
abc: Means on the same row with different superscripts are significant (P<0.05)				

Table 4: effect of interactions of days of fermentation and organisms on the crude protein (%) composition of biodegraded CSR

DAYS					
ORGANISMS	0	7	14	21	SEM
Aspergillus niger	4.05[z]	6.40[ay]	7.17[aw]	7.07[ax]	1.25
Trichoderma viride	4.05[z]	5.72[cy]	6.84[bx]	6.90[bw]	0.03
Aspergillus niger + Trichoderma viride	4.05	5.97[by]	6.88[bw]	6.81[cx]	0.01
SEM	0.01	0.01	0.03	0.01	
abc: Means on the same column with different superscripts are significant (P<0.05)					
wxyz: Means on the same row with different superscripts are significant (P<0.05)					

Table 5: Effect Of Interactions Of Days Of Fermentation And Organisms On The Crude Fibre (%) Composition Of Biodegraded CSR

DAYS					
ORGANISMS	0	7	14	21	SEM
Aspergillus niger	17.07[w]	11.63[cx]	10.31[cy]	10.24[cy]	1.06
Tirchoderma viride	17.07[w]	13.97[ax]	12.83[ay]	12.78[ay]	0.07
Aspergillus niger + Trichoderma viride	17.07[w]	12.99[bx]	11.89[by]	11.48[by]	0.06
SEM	0.27	0.56	0.02	0.05	
abc: Means on the same column with different superscripts are significant (P<0.05)					
wxyz: Means on the same row with different superscripts are significant (P<0.05)					

Table 6: Effect of interactions of days of fermentation and organisms on the metabolisable energy (kcal/kgdm) content of biodegraded CSR

DAYS					
ORGANISMS	0	7	14	21	SEM
Aspergillus niger	2589.3[z]	2772.3[ay]	2897.1[aw]	2798.3[ax]	6.12
Tirchoderma viride	2589.3[y]	2682.8[cx]	2719.2[cw]	2711.3[cw]	15.28
Aspergillus niger + Trichoderma viride	2589.3[z]	2701.4[by]	2739.1[bx]	2763.1[bw]	0.05
SEM	0.02	6.81	14.97		
abc: Means on the same column with different superscripts are significant (P<0.05)					
wxyz: Means on the same row with different superscripts are significant (P<0.05)					

Discussion

The treatments to which cassava starch residue (CSR) were subjected resulted in different rate of biomass loss. The CSR substrate treatment which had the least biomass loss might be due to the resistant starch formed that affects microbial activities. This must have resulted in the low biomass loss as observed in *T. viride*-degraded CSR substrate. Since the degradation of fibre proceeds stepwise with the breakdown of polysaccharide into oligosaccharides, these can be hydrolyzed by glucosidase into their component monomers which are not as bulky and crystalline as the original polysaccharide. Zadrazil and Brunnet, (1980), Sharma (1987), Karunananda et al., (1991) and Onilude, (1999) proposes the possibility of the different strains microbes producing different amount of polysaccharide degrading enzymes which result in variation in biomass loss within the same fibre feedstuff.

The effects of fermentation periods of fungi incubation on the nutritional compositing of cassava starch residue showed that *A. niger* was able to increase crude protein content and decrease cellulose content (Table 2) of CSR significantly better than *T. viride* and the combination of *T. viride* and *A. niger*. This suggested the multi-enzyme production ability of aspergilli organisms as recognized by de Vries and Visser (2001), and Adebiyi et al., (2008). However, fourteen days of fermentation was also observed to produce better nutritional status of the fermented CSR than seven days and 21 days, and this might have been connected with the active growth of microorganisms which was alluded to by Iyayi and Aderolu (2004). Thus, the ME Kcal/kgDM of *A. niger* degraded CSR substrates were significantly improved than other treatments on day (14).

In the interactions of days of fermentation and organisms, *A. niger* inoculated substrates in all the periods produce significantly higher degradation percentage above 20.00% than *T. viride* and combination of *T. viride* + *A. niger* inoculates substrate. Bada (2003) concluded that digestibility changes in fibrous crop residues after fungal incubation were caused by a complex interactions of many factors including cell wall phenolic acid. The crude protein increased by 76.80% (4.05% to 7.16%) on the CSR when fermented with *A. niger* for 14 days. The change in the protein of fermented product could have resulted from slight protein synthesis and substrate utilization by the microorganisms used. Alternatively, it could have been from a synthesis of enzyme protein from a rearrangement of the different proportion following the degradation of other constituents. Iyayi and Losel (2001) obtained 152.00% increase in crude protein of cassava peel after 20 days using *A. niger* and 161.10% increase in pulp. Abu et al., (1997) obtained increase of 134.98% (4.95% to 11.83%) using *A. niger* on sweet potato and 35.15% (4.97% to 6.69%) using *A. oryzae*. The increase in *A. niger* inoculated CSR substrate could be due to the production of α-amylase which was able to break the α-glycosidic bond present in root tuber residue. The optical CP content of the degraded substrates increased with period of fermentation up to day (14) because the set period in this study (0- to 21) days and the active period of the fungi when enzyme production and biodegradation are at 14th day. Furthermore, Aderolu (2000) submitted that at the initial stage day 0 to 14 days, none of the growth limiting factors of microbes, like oxygen, moisture and heat requirement were lacking. The reduction in crude fibre content of all the fungi-degraded CSR substrate in this study could be the disruption of the large molecules of fibre unit, reduction in their viscosity and total encapsulation of fibrous

feed by microbes as recognized by Campbell et al., (1986), Howard et al., (2003). The primary sources of industrial enzymes are about 53% from fungi and yeast, 35.00% for bacteria and 12.00% to 15.00% from plant or animal origin. The most efficient lignin-degrading microorganisms are fungi (Falcon et al., 1995; Badal, 2003) and are known to have a multienzyme activities which brings about synergistic actions in degrading NSPs.

The CSR-degraded with spores of *A. niger* gave the highest crude fibre loss (33.96%) compared to *T. viride* degraded CSR and combination of *A. niger + T.viride* degraded substrate. This is an indication of efficient degradation of CSR by enzymes produced during fermentation by *A. niger*. Afolabi (1999), reported crude fibre reductions of 23.94% (day 4), 26.63% (day 8) and 52.39 (day 10) in cassava peel when inoculated with *A. niger* and with *R. Stolonifer* 23.81% (day 4), 38. 36% (day 8) and 54.47% (day 10) were observed. The organisms used for the degradation of CSR (*A. niger, T. viride* and combination of *A. niger and T.viride*) were able to reduce the CF further even up to 14 days of the fungi incubation because the microbes were still within their active growth stage and growth-limiting factors had not set in. The enzymes produced by these microbes, cellulase by *A. niger* (Badal, 2003), and lignase by *T. viride* (Howard, et al 2003) resulted in varying degree of CF reduction.

Low metabolisable energy (ME), according to Onifade, (1992) and Iyayi and Losel, (2001) is due to reduce starch digestibility . The low M.E observed in day(O) for the entire treatments may be due to the increased level of soluble NSP as suggested by Choct et.al., (1999) and Iyayi and Aderolu, (2004). Fermentation of this substrate by fungi specie produced enzymes which led to the increase observed in M.E. Iyayi and Aderolu (2004) working with *T. viride* on agro-industrial by-products brewer dried grain, rice bran, palm kernel meal and corn bran obtained 5.0%, 6.3%, 9.0% and 18.5% increases in energy respectively. The increment in M.E of fungi degraded CSR were below the values recorded by Iyayi and Aderolu (2004) on agro-industrial by products. The difference might tell the variation in nutrient composition of choice substrate, and also physical treatment CSR was subjected to. The increased available energy content of fibre feedstuff subjected to microbial treatment may result into their better utilization by monogastric.

Cellulose is an homogenous polymer formed from linear chain of β(1-4) linked D-glucopyranosyl unit. The highest cellulose degradation observed when *A. niger* was inoculated on CSR substrate, for 14 days (32.68%) (17.07 to 11.49%) could be attributed to the enzymes produced by Aspergillus. Combination of *A. niger* and *T.viride* were able to degrade (31.16%) (17.07% to 11.75%) of cellulose in CSR. This could be because of synergistic actions in both fungi (Balagopalan 1996). Cellulose degradation in CSR degraded with *A. niger* is better than in combination *of A. niger + T.viride* and *T. viride* degraded CSR substrates, this could be a function of variability in the quantity and quality of complete cellulase enzyme complex being produce by various organisms. Hemicelluloses on the other hand is an heterogeneous polymers having β (1-4) linked backbone of xylose, mannose or glucose residue that can form extensive H^+ bonding with cellulose. It requires several different enzymes with different specificities for complete hydrolysis, the polysaccharides does not form tightly, packed crystalline structures like cellulose and is, thus, more accessible to enzymatic hydrolysis (Gilbert and Hazlewood, 2003) . Hemicellulose consists of about 20 to 35% DM of root tuber. Badal, (2003), and Howard et al., (2003) recognized endoxylanases, and β xylosidase, and several accessory enzymes, such as α -ι -arabionofuranosi-dase, α -glucuronidase, ferulic acid esterase, and P-coumaric acid esterase, as the enzyme necessary for hydrolyzing various substituted xylans. *A. niger* fermentation brought about (4.88%) loss in hemicellulose content CSR followed by *T. viride* fermentation (5.55%) at 14 days. The fungal preference for cellulose over hemicelluloses and lignin may be indicative of the need for a more readily digestible energy source prior to hemicelluloses and lignin degradation. Also, fungi may need glucose or xylose to form H202 for activating lignin peroxidases.

Generally, the fibre components of the fermented CSR decreased over the periods of fermentation. During biodegradation exercise the hydrolytic enzymes of the Fungus broke-down polysaccharide content of the CSR into various sugars. *A. niger* had the highest (68.03% to 58.76%) NDF and ADF (32.00% to 24.48%) degradation percentages. This is contrary to the assertion by Onilude (1994) that *Trichodema spp* had the highest cellulolytic enzymes secretion in solid state fermentation. The enzyme-substrate specificity could have resulted in better performance of *A. niger* on the CSR. In-terms of ADL degradation, for all the treatments *A. niger* however, gave a significant loss (14.93% to 12.98%) in lignin content in CSR; whereas *T. viride* fermentation resulted in total loss of (14.93% to 13.51%) likewise combination of *T. viride + A. niger* (14.93% to 13.44%) yielded loss of (9.97 %). This suggested the inability of *T. viride* produce lignase which ought to have degrade part of lignin in CSR. *A. niger* , however, was the most efficient and extensive lignin degraders in this study.

Conclusion

Results of this study showed that fungal biodegradation of cassava starch residue is regulated by a complex multi-factors and consequently enhanced its nutritional indices

Acknowledgement

The authors thank the University of Ibadan, Ibadan for financing this study viz Senate Research Grant SRG/FAF/2006/3^B.

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