OPTIMIZATION OF CRUDE PROTEIN PRODUCTION AND REDUCTION IN CYANIDE CONTENT OF CASSAVA PEELS BY PRETREATMENT WITH *Aspergillus niger* ANL301

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Abstract: *Aspergillus niger* ANL301 B-1 β-glucosidase was investigated on its effectiveness in optimizing protein contents and reduction in cyanide content of cassava peels. Freshly harvested cassava tubers peels were dried in oven at 70^{o}C for 36 h and milled into fine particles using micro millers. Two sterilized conical flasks (250 ml), each containing 50 g of the cassava peel flour in triplicates were prepared. Thirty (30) ml of sterile basal medium was added into each flask and thoroughly mixed. The flasks and their contents were sterilized by placing in boiling water at 100^{o}C in a GFL brand water bath for 5 min and in the autoclave at 121^{o}C for 15 min. After cooling, one ml of spore suspension of *A. niger* was aseptically introduced into the first set of the flasks plugged with cotton wool and dried in the oven at 60^{o}C for 24 h. The second set of flasks had no inoculums, were plugged with cotton wool and dried at room temperature. Results showed that fermentation of cassava peels by solid state fermentation using the *A. niger* significantly ($p<0.05$) enriches the protein content of the waste and drastically lowering the cyanide concentration The significant changes in crude protein and cyanide contents after fermentation suggests it could be used as a good source of protein in compounding animal feeds. These findings will facilitate the development of an improved method for enhancing the nutritional value of cassava peels and other waste products.

Keywords: Cassava peel, *Aspergillus niger*, protein, cyanide, fermentation, livestock

Introduction

Livestock and fisheries productivity requires feeds that contain the entire dietary component. However, it is difficult for most farmers to afford the qualities of feeds needed for utmost productivity as a result of high cost. Therefore, there is need to develop non-conventional food sources that will meet the need of all and affordable, hence the needs to exploit agricultural wastes generated by farmers, that is readily available at low or at no cost. Agricultural wastes such as cassava peels, banana peels, plantain peels, and citrus peels can be used for producing the value-added bioprotein. These waste products, however lack nutrients such as protein and vitamins and are rich in fibre with low digestibility (Villa-Boas *et al*., 2005). The need to improve the nutritional content of these by-products using microbes were exploited i.e. utilization of food wastes by biological degradation of the wastes by microorganisms for the production of valuable compounds such as enzymes, citric acid and others as raw materials for medical and industrial uses become vital

Cassava peel is a major by-product of the cassava tuberous root and are normally discarded as wastes and allowed to rot in the open. Bioprocessing of the peels will not only convert the waste to useful products, but it will also minimize environmental problems associated with their disposal and reduces environmental health hazards. Healthy life and cleaner environment is the end result of solving these problems in such a way by processing the waste into value added product (Nigam *et al*., 2009). Thus, efficient bioprocess for underused biomasses is at the forefront of biotechnological research (Rattanachomsri *et al*., 2009; Pan *et al.*, 2011).

Microorganisms produce enzymes that are used in various industrial applications (Mitidieri *et al*., 2006). Isolation of native or production of genetically modified enzyme-producing microorganisms may have substantial impacts on present and future industrial processes (Vermelho *et al*., 2013). Nowadays the most commonly used industrial enzymes belong to the hydrolase group, which exploits several natural substrates (Mitidieri *et al*., 2006). The filamentous fungus *Aspergillus niger* is one of the most common species of mold found inside and outside the home. Although it is one of the most dangerous moulds as it can cause life threatening illnesses and provoke strong allergic reactions, it is a source of one of the enzymes widely used in food products around the world. It produces a variety of enzymes such as cellulase and xylanase (Farinas *et al*., 2010), phytases (Bhavsar *et al*., 2011), amylases (Mitidieri *et al.*, 2006), and peptidases (Morya *et al.*, 2012), and therefore is essential in alcohol and dairy fermentation as well as in the production of citric acid which is a very common food additive.

Modification of agricultural wastes produce protein with high nutritional value, that do not compete with food for human consumption, economically feasible and locally available (Uysal *et al.*, 2002). Bioprotein is achievable biotechnologically using microorganisms such as fungi, bacteria and algae. The usage of fungi is most common due to their capability to propagate on agricultural wastes within a short period and ability to produce high protein content in their biomass (Anupama and Ravindr, 2000). Its production can be maximized using potential strain, good substrate and most favourable condition (Jamal *et al*., 2008). Fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein quality and fibre digestibility. It also enhances micronutrient bioavailability and aids in degrading antinutritional factors (Achinewhu *et al*., 1998), thus the objectives of this study is therefore to use *Aspergillus niger* ANL301 to optimizes cassava peels.

Materials and Methods

Sample collection

The freshly harvested cassava (NR 8082) tubers were collected from International Institute of Tropical Agriculture (IITA), Ibadan. The tubers were washed with tap water and peeled with stainless steel knife. The peels were again washed and immediately collected into polythene bags and transported to the laboratory. They were immediately spread in trays and left to dry in the oven at 70^{o}C for 36 h (Ofuya and Nwajiuba, 1990). The dried peels were cooled and then milled into fine particles

using a Willey micromiller with a 2 mm sieve. The fine flour was packed into paper bags, wrapped in a polythene bags and stored at ambient temperature in the laboratory. There were two treatments carried out in three replicates each.

Preparation of inoculums

The *A. niger* was collected from the Microbiology Laboratory, Obafemi Awolowo University, Ile-Ife, and was grown on Potato Dextrose Agar (PDA) slants at 32^{o}C. The PDA slants were kept in the refrigerator at 4^{o}C. Subcultures were made from the slants for use. Sterile distilled water was aseptically poured into 48hour old culture old *A. niger* on PDA plate. Sterile inoculation loop was used to disperse the spores into the water and centrifuged. The supernatant was discarded and suspension containing bacteria spore was obtained.

Vegetative medium for Aspergillus niger

The basal medium used for fermentation contained the following ingredients: KNO_3 (5.0 g), KH_2PO_4 (2.0 g), $MgSO_4.7H_2O$ (0.5 g), $Fe_2So_47H_2O$ (5.0 mg), $ZnSO_4.7H_2O$ (1.0 mg), $Ca_2SO_4.2H_2O$ (5.0 mg), yeast extract (2.0 g), tryptone (5.0 g), thiamine (0.05 mg), Biotin (0.05 mg), nicotinic acid (0.5 mg), riboflavin (0.05 mg 0 in 1,000 ml distilled water. The pH was adjusted to 5.5 before dispensing in 30 ml into Mac Cartney bottles and autoclaving for 15 min at 121^{o}C.

Solid state fermentation (SSF)

Six sterilized 250 ml conical flasks were used with three replicates per treatment. Each containing 50 g of the cassava peel flour. Thirty (30) ml of sterile basal medium was added into each, mixed thoroughly and divided into two groups as stated below

Group A: This group was placed in boiling water at 100^{o}C in a GFL brand water bath for 5 minutes and then sterilized in the autoclave at 121^{o}C for 15 min. After cooling, 1 ml of *A. niger* was aseptically introduced into each flask. After 24 h of fermentation, 10 g of samples were picked from flask under aseptic conditions while subsequently samples (10 g) were picked at 24 h intervals for up to 168 h of fermentation. At the end of each fermentation period, samples were wrapped in appropriately labeled aluminum foil paper and dried in the oven at 60^{o}C for 24 h (Ofuya and Nwajiuba, 1990) for further analysis

Group B: This group was not pre - treated and no inoculation. It serves as the control. This set was allowed to ferment naturally. After 24 h of fermentation, 10 g of samples were picked from flask under aseptic conditions while subsequently samples (10 g) were picked at 24 hourly intervals for up to 168 h of fermentation. The samples were wrapped in appropriately labeled aluminum foil paper and dried at room temperature (Ofuya and Nwajiuba, 1990) for further analysis

Total protein and cyanide contents determination

Bovine serum albumin (BSA) used for the determination of protein quantity was purchased from Sigma Chemical Company. Protein determination was performed using both the original Lowry method (Lowry *et al*., 1951) and protein determination kit (Bio-Rad, Hercules, CA) following the manufacturer's instruction as described by Bradford (1976). The protein content was also verified using using the micro-Kjeldhal method (N x 6.25). The cyanide was measured spectrophotometrically at 585nm using a cyanide determination kit (Spectroquant Cyanide 14800, Merck, Darmstadt, Germany (Hughes *et al*., 1992), and verified using silver nitrate titration method (Oboh *et al*., 2002)

Data analysis

One-way analysis of variance SPSS (16.0 version), SPSS Inc, Chicago, USA, was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant (Fisher, 1950). Multiple bar graphs were also used in this study for the pictorial representation of assessment endpoints.

Results and Discussion

Cassava peels is readily available at no cost, but markedly low in protein, high in crude fiber, deficient in nutrients other than energy and contain high concentrations of toxic cyanogenic glucosides, caused by the conversion of acetone cyanohydrins in cassava to cyanide, and the presence of linamarin. Ingestion of a lethal dose may leads to death as a result of inhibition of cytochrome oxidase of the respiratory chain by cyanide as reported in goats ingested cassava leaves and in non-ruminants, like pigs, when fed fresh uncooked tubers and cassava peels (Obioha, 1972). Thus the need to detoxify the peels to enhance its economic status in compounding livestock and fish feeds.

In this investigation, crude protein content increased in the inoculated cassava peel flour and ranged between (4.40±0.11 and 29.20 ± 0.24) %, while in naturally fermented peels, it ranged between (4.50 ± 1.20 and 9.80 ± 0.24) %. There was steady increase in the inoculated peels with increasing number of days of fermentation until day 6 and slightly declined afterwards, while in the naturally fermented, there was slow increase but not comparable with the treated peels. At the end of this study, protein production was significant ($p<0.05$) in naturally fermented cassava peels and highly significant ($p<0.01$) in inoculated peels when compared with unfermented peels. Comparing the treated and the untreated peels, there were significant ($p<0.05$) different in protein production on day 5th, 6th, and 7th (Table 1).

The significant increase in the protein contents in inoculated cassava peels could be due to *A niger* ANL301 B-1 β-glucosidase attribute in the production of proteases, an enzyme that breaks down proteins and peptides by catalyzing the hydrolysis of peptide bonds (Oboh *et al*. 2002). Also, the increase observed in the protein contents could be as a result of the bioconversion of sugar into proteins, this is because some fungal are known to convert green plant carbohydrates into fungal protein, which is turn can be converted to animal protein (Balagopalan, 1996, Akinyele and Agbro, 2007). The finding corroborated the observation of Sekeri *et al*. (1973), where carb bean carbohydrates was converted to fungal protein using *A. niger*. Similar findings was reported by Shide *et al.* (2004), in wood sawdust treated with *Lentinus squarrosulus* (Momt) Singer, a basidiomycete also known as a white rot fungi. They observed that the fungus was able to degrade 0.1M HCl-pretreated wood sawdust to protein and ethanol by average value of 72.0% over untreated wood sawdust samples, after hydraulic retention time of 72 h. Also, similar results have been reported by Iyayi (2004) where an increase of 61% in protein content was recorded in Corn bran fermented with *Trichodermaviride*. Similarly, Iyayi (2004) also observed an increase of 41% in the protein level of Wheat offal after 14 days of fermentation using *A. niger*. Ofuya and Nwanjiuba (1990) observed an increase of 185% in the protein content of cassava peels (from 5-6-16%) when *Rhizopus* sp. was cultured on the peels. The fungus can also produce some extracellular enzymes such as, amylases, lipases, cellulase, citric acid , xylanases,

galacatosidase, glucoamylase and linamarase (Oboh and Akindahunsi, 2003, Ganiyu, 2005).These enzymes are considered generally recognized as safe (GRAS) by the United States Food and Drug Administration and is excused from the Federal Food, Drug, and Cosmetic Act food additive tolerance requirements (Schuster *et al.*, 2002). The enzymes are secreted into the cassava mash so as to make use of the starch produced by the cassava peels in the production of carbon and other essential functions (Raimbault, 1998). For instance, the citric acid serves the purpose of improving taste, nutrition and shelf-life of food products and alpha- galactosidase is capable of breaking down certain non-digestible oligosaccharides in the digestive tract.

Table 1: Crude protein (% dry weight) production of *A. niger*ANL301fermented and naturally fermented pretreated cassava peels at different time interval

Periods of fermentation (hours)	Protein contents (%) (Naturally fermented peels)	Protein contents (%) (Inoculated cassava peels)
0	4.50±1.20 [a]	4.40±0.11 [a]
24	4.72 ±0.20 [a]	5.02±0.14 [a]
48	6.06±0.26 [a]	12.96±0.12 [a]
72	6.25±0.12 [a]	17.40±0.10 [a]
96	7.28±0.24 [a]	21.80±0.14[b]
120	7.65±0.65 [a]	28.40±0.20[b]
144	8.10±0.03 [a]	32.90±0.13[b]
168	9.80±0.11 [a]	29.20±0.24[b]

Mean with different superscript in the row are significantly different ($p<0.01$)

Table 2: Cyanide contents (mg/kg) in *A. niger* ANL301 fermented and naturally fermented pretreated cassava peels at different time interval

Periods of fermentation (hours)	Cyanide concentration (mg/kg) (Naturally fermented peels)	Cyanide concentration (mg/kg) (Inoculated cassava peels)
0	148 ± 4.23 [a]	152 ± 2.02 [a]
24	139 ± 5.47 [a]	101 ± 3.03 [a]
48	126 ± 5.41 [a]	45 ± 2.45 [b]
72	116± 3.22 [a]	38 ± 3.23 [b]
96	100 ± 4.35 [a]	26 ± 1.03 [b]
120	92 ± 5.12 [a]	19 ± 2.30 [b]
144	65 ± 5.11 [a]	13 ± 1.02 [b]
168	39 ± 3.01 [a]	05 ± 2.03 [b]

Mean with different superscript in the row are significantly different ($p<0.01$)

Reduction in cyanide concentrations varies significantly ($p<0.01$) between the *A. niger* inoculated peels and naturally fermented peels. The cyanide concentrations in naturally fermented and inoculated fermented treatments decreased with increase in the duration of the treatments. Fermentation of the cassava peels with *A. niger* reduced the cyanide content from (152 ± 2.02) mg/kg to (05 ± 2.03) mg/kg while the naturally fermented cassava peel reduced the cyanide concentrations from (148 ± 4.23) mg/kg to (39 ± 3.01 (Table 2). The cyanide concentrations reported in this study was within the WHO cyanide range of 40 to 400 mg/kg fresh weight (WHO, 1996), but in the naturally fermented peels, it was higher than recommended limit of some cassava products in Nigeria; gari (19.0 mg/kg), fufu (25 mg/kg) (Oboh and Akindahunsi, 2003). The reported cyanide concentrations reported in the freshly harvested peels could be detrimental to livestock health as this could cause goitrogenic as demonstrated by Tewe *et al.* (1984), who reported a significant reduction in serum thyroxine levels in growing pigs fed cassava peel diets containing 96 ppm total cyanide.

The findings had revealed *A. niger* possesses the capacity to degrade cassava peels and significantly enhanced protein content in the fermented cassava peels and very efficient in cyanide detoxification. Similar observation was reported by Tweyongyere and Katongole (2002), when the waste water from cassava pulp was used to ferment cassava peels. The cassava peels regarded as having no economic value, could be integrated into animal nutrition. Apart from reducing contamination of the environment, this technique will be essentially useful in converting wastes into useful products, an observation that call for holistic research in fungi and other microbial biotechnology.

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