Significance of "GRAS" Fungi in the Fermentation and Enrichment of Cassava Peels for the Production of Low Cost Feeds for Poultry and Domestic Animals

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Abstract: This study used solid-state fermentation methods to nutritionally enhance cassava peel using a strain of *Aspergillus niger*, *Trichoderma viride* and *Saccharomyces cerevisiae.* Cassava peel were collected, dried and made into crunches. This was fermented for 14 days at room temperature and oven dried. Fermented and unfermented crisp of the peels was analysed for crude protein, fat, fibre, dried matter and cyanides. Analysis of the dried fermented peels revealed that there was a significant increase in the protein and decrease in cyanides content of the cassava peel and there were differences in enrichment potency of the test fungi. Protein increased by 50% and cyanide reduced from 46.28mg/kg to 34.85mg/kg when treated with *S. cerevisiae* while treatment with *Aspergillus niger* resulted in more than 90% reduction in cyanides and increased the protein content from 6.25% to 13.39% when compared with the unfermented cassava peels, which had 46.28mg/kg cyanide. Fermentation with *T. viride* resulted in more than 90% reduction in cyanides and increased the protein content from 6.25% to 17.06% when compared with the unfermented cassava peels, which had 46.28mg/kg cyanide. In synergy, fungi fermentation shows slight incremental effect in protein and further reduced cyanide concentration. With the increase achieved in the protein content and the significant decrease in the residual cyanide of cassava peels fermented with these fungi, the end-product could be a good protein supplement in livestock feed production and this method if adequately adopted will help to reduce the cost of livestock feed production, increase the affordability of animal protein and then reduce the menace of agricultural waste pollution in the environment.

Key word: Fungi, Fermentation, Enrichment, Cassava waste.

INTRODUCTION

The use of quality feed for the grazing of domestic animals is important to enhance growth and development of the animal and help to produce quality meat and meat products for human consumption. Adelodun *et al.* (2021) stated that the nutritional quality of feed is paramount to the well-being and quality of meat produced by poultry and domestic animals. Therefore, improving the nutritional quality of animal feed is imperative.

Cassava "*Manihot esculenta"* is a common tuber plant which plays a significant role in food production in Africa and Southern America (Onilude, 1996). For example, cassava supplies about 70% of the daily calorie for over 100 million people in Nigeria, it also serves as a significant source of food for livestock feed globally (Adelodun *et al*., 2021). More significantly is the industrial potential of cassava for the production of starches for textiles, pharmaceuticals, food, alcohol, acetone, and dextrin (Gunorubon, 2012). For these reasons, there is more important use for cassava. Moreover, harvesting and processing of cassava generates large amounts of wastes, leaves, peel and the stem. Peel waste significantly adds to the menace of environmental pollution (FAO, 2001). For example, Aro *et al.* (2010) documented that about 120 tons of cassava tubers are used by starch producing factories in Nigeria and different waste products released comprising about 5 tons of cassava peels and other industrial wastes. Hence, it is imperative to develop biological systems to incorporate and improve waste products from harvesting and processing of cassava into nutritionally enriched livestock feeds.

Utilization of cassava peel as feed is practical among domestic animal farmers (Salami and Odunsi, 2003; Omede *et al.,* 2017; Adeleke *et al.,* 2017; Adelodun *et al*., 2021) of which use was reported ruminant animals (Oloruntola *et al*., 2019) especially goat and pigs (Unigwe *et al.*, 2014). Incidentally, studies have shown that cyanogenic glycosides are more concentrated in cassava parts (Cardoso *et al*., 2005; Burns *et al.*, 2012; Ndam *et al.*, 2019). The low level of protein, high level of toxic cyanogenic compound and fiber (Adeleke *et al*., 2017; Ezekiel *et al.*, 2010) contributed to the factors that limit the use of cassava peel as feed for non ruminant animals. These compositional problems limit the utilization of cassava peel as feed for economic production of poultry animal feeds and animal proteins. It then becomes imperative for the products of cassava to be properly detoxified before they are used as livestock feeds. However, cassava peels when available in excess are often discarded into open space where they cause environmental pollution by releasing foul odor into the immediate environment (Salami and Odunsi, 2003). The advantageous conversion of the excess peels into a nutritious meal for domestic and poultry animals, through fungi fermentation, will help curb pollution problems.

Fungi play an important role in the ecosystem where they are involved in decomposition, nutrient cycling and energy flows (Gulis *et al*., 2017). These fungi could successfully grow on various substrates and break down more complex organic matters (Tunlid *et al*., 2022) to beneficial inorganic nutrients (Nhan *et al.*, 2021). Nhan *et al.* (2021) further report that cellulolytic fungi are important in breaking down cellulose and starchy compounds, converting them into highly absorbable nutrients. *Aspergillus niger* is a mould classified into the genus *Aspergillus* that consist of common moulds which are found throughout the environment and the species of this genus often grow and sporulate quickly (Schuster *et al.*, 2002). *Trichoderma viride* is a fungus that has strong ability to produce extra-cellulolytic enzymes which aid biodegradation of complex organic molecules (Jiang *et al.,* 2011). On the other hand, *Saccharomyces cerevisiae*, often used in brewing and baking industries, is a species of single-celled fungi used since ancient days (Munoz *et al.,* 2005). These classes of fungi are characterized with extensive ability to produce secondary metabolites from complex organic substances and the ability to withstand extreme acidity makes *S. cerevisiae* specifically important in industrial fermentation and production of citric acid (Behera, 2020). *S. cerevisiae, A. niger* and *T, viride* are among moulds classified as "Generally recognized as safe" (GRAS) by the United States Food and Drug Administration (FDA) for use in food production (*Frisvad et al.*, 2018). Using these fungi to enhance protein content and reduce toxigenic cyanides will be significant in improving the nutritional quality of cassava peel. Reports of various studies have shown that fermentation of cassava peels resulted in a product with higher protein content, lower cyanogen and phytate content. It was observed after seven days of fermentation of cassava peels by pure culture of *S. cerevisiae* that protein content increases from 2.4% to 14.1% (Adelodun *et al*., 2021; Antai and Mbongo, 1994). Fermentation using different fungi was studied with *Saccharomyces* spp. and *Lactobacillus* spp. (Oboh, 2006) and *Trichoderma viride* (Ezekiel *et al.*, 2010) reportedly achieved some significant results. Similarly, Iyayi and Losel (2001) obtained, respectively, an increase in crude protein of 7.91% and 9.04% from the fermentation of cassava pulp using *Aspergillus niger* and *Saccharomyces cerevisiae*. Therefore, improving the nutritional quality of this waste and the detoxification, using extra-cellulolytic enzymes producing fungi, could aid the use as animal feed.

This study aims to evaluate the ability of *Aspergillus niger*, *Trichoderma viride* and *Saccharomyces cerevisiae* to ferment, detoxify and enrich cassava peel nutrient. It analysed the nutritional value of cassava peel, fermented peel crunches, evaluated the improvement in the nutritional value, and compared the detoxifying and nutritional enrichment ability of the different fungi. It further provides information in relation to their synergistic effects to ferment and improve the peel nutritionally and evaluate the effect of fungi fermentation on cassava peel nutrient improvement, detoxification and gives reason for us to embrace this technology in the production of animal feeds.

MATERIALS AND METHODS

Sample collection: One kilogram of fresh cassava tuber peels used for this study was obtained from a garri processing yard in Agbale area of Ede, Osun State Nigeria.

Processing of sample: The peels collected were washed and sundried for seven 7 days. The dried peels were then made into crunches and stored dried for further use.

Preparation of inoculum: Strains of *A. niger, S. cerevisiae* and *T. viride* were purchased from Redeemers University Microbiology Laboratory. The inoculums were prepared to 1.0 McFarland standards.

Preparation of fermentation medium: Using a solid state fermentation model, 14 sterile 1L covered plastic rubber containers were purchased commercially. Twenty grams (20 g) of dried cassava peel crunches weighed into each. A 5 cm diameter hole was made in the centre of the container cover, sealed with cotton wool to prevent contamination and to facilitate minimal aeration.

Inoculation and fermentation: Each medium containing 20 g dried cassava peel was aseptically inoculated with 10ml of the 1.0 Mcfarland standardized inoculums and mixed with sterile scalpel;

- the first portion (control) of 20 g dried peel was uninoculated;
- the second portion was inoculated with (10 ml) inoculums of *S. cerevisiae;*
- the third portion was inoculated with (10 ml) inoculums of *A. niger;*
- the fourth portion was inoculated with (10 ml) inoculums of *Trichoderma viride;*
- the fifth portion was inoculated with equal volume (5 ml each) inoculums of *S. cerevisiae and A. niger*;
- the sixth portion was inoculated with equal volume (5 ml each) inoculums of *S. cerevisiae* and *T. viride*;
- the seventh portion was inoculated with equal volume (5 ml each) inoculums of *A. niger* and *T. viride*;

Each chamber was duplicated and incubated at ambient room temperature (27℃) for 14 days using solid state fermentation method.

Proximate analysis: Proximate analysis of the fermented and unfermented cassava peels was carried out to determine its nutritional compositions. The unfermented cassava peel crunches was analyzed for protein, fat, fibre, dry matter and cyanides at the baseline while the fermented cassava peel crunches were analyzed for protein, fat, fibre, dry matter and cyanides after 14 days of fermentation.

The Ash content was determined using the method of Pearson, crude fibre content was determined by the Tricyclic acid (TCA) method, dry matter or moisture content was determined according to the oven method of AOAC, fat content was determined using the Soxhlet extraction, protein content was determined by the Micro-Kjeldahl method as described by Pearson, as reported by Cordenunsi *et al.* (2004), while the cyanide contents of the fermented cassava peels were determined using silver nitrate titration method as described by Oboh *et al.* (2002).

RESULTS

Values estimated from the analysis of nutritional composition of fermented and unfermented cassava peels are presented in the tables and figures below:

Table 1: Nutritional Composition of Cassava Peels Before and After Fermentation

Analysis of cassava peels	average % crude protein	average % crude fat	average % crude fiber	average % dried matter	cyanides (mg/kg)
Unfermented cassava peel	6.25	3.21	13.98	88.94	46.28
Fermented with *S. cerevisiae (SC)*	9.62	2.89	12.48	48.76	34.85
Fermented with *A. niger (AN)*	13.39	1.69	1.34	19.54	1.66
Fermented with *T. viride (TV)*	17.06	1.66	2.14	20.89	0.72
Fermented with *A. niger* and *S. cerevisae*	16.89	1.43	1.56	21.16	0.87
Fermented with *T. viride* and *S. cerevisae*	17.74	1.13	1.41	21.79	0.53
Fermented with *A. niger* and *T. viride*	17.94	1.22	1.36	22.02	0.51

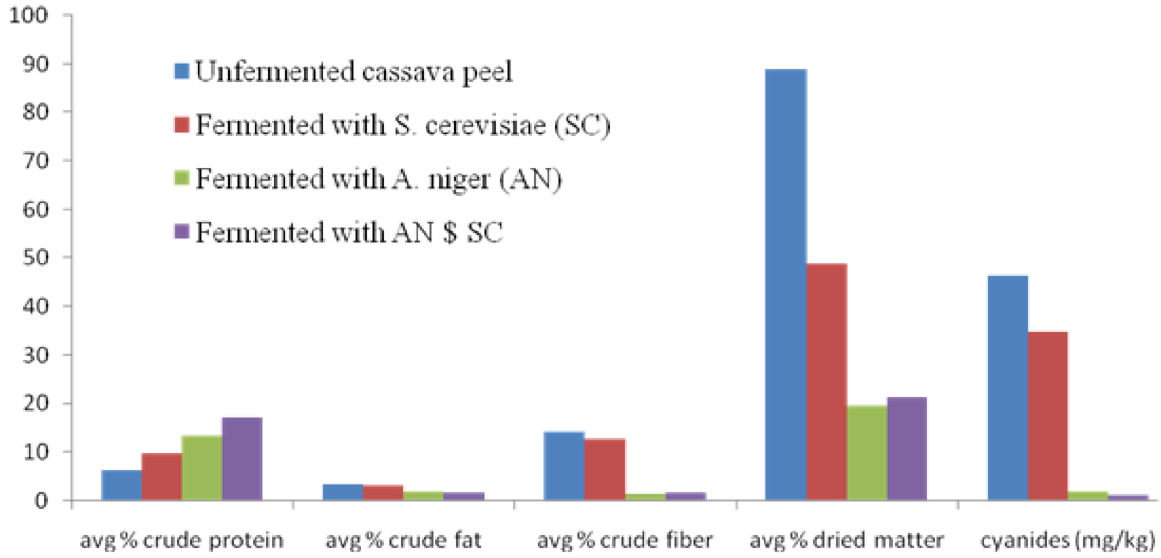

Figure 1a: Synergistic Effect of *S. cerevisiae* and *A. niger* on Cassava Peel Nutrient Composition after Fermentation

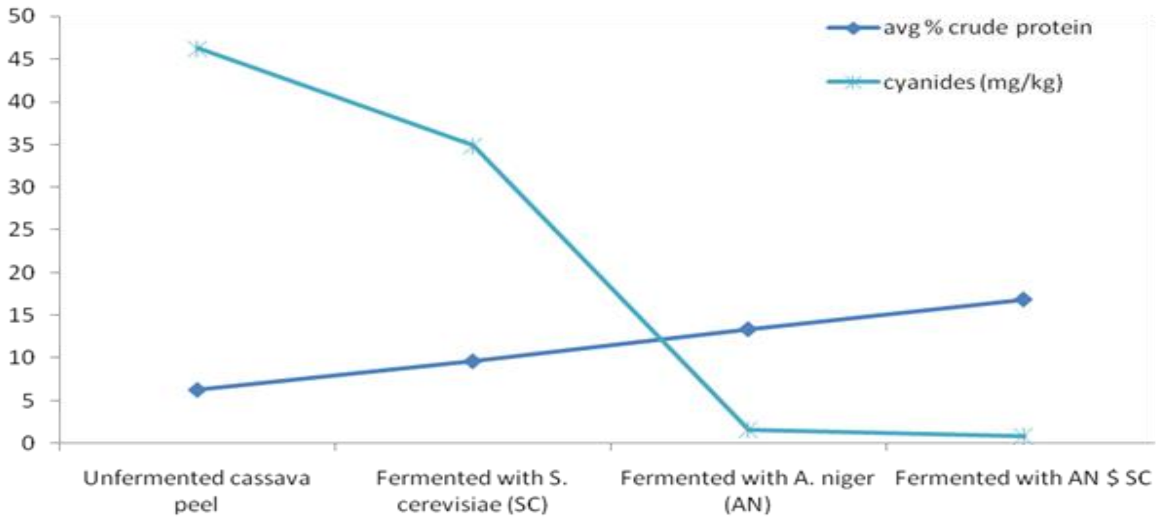

Figure 2a: Comparing Effect of Fermentation on Protein and Cyanide Composition

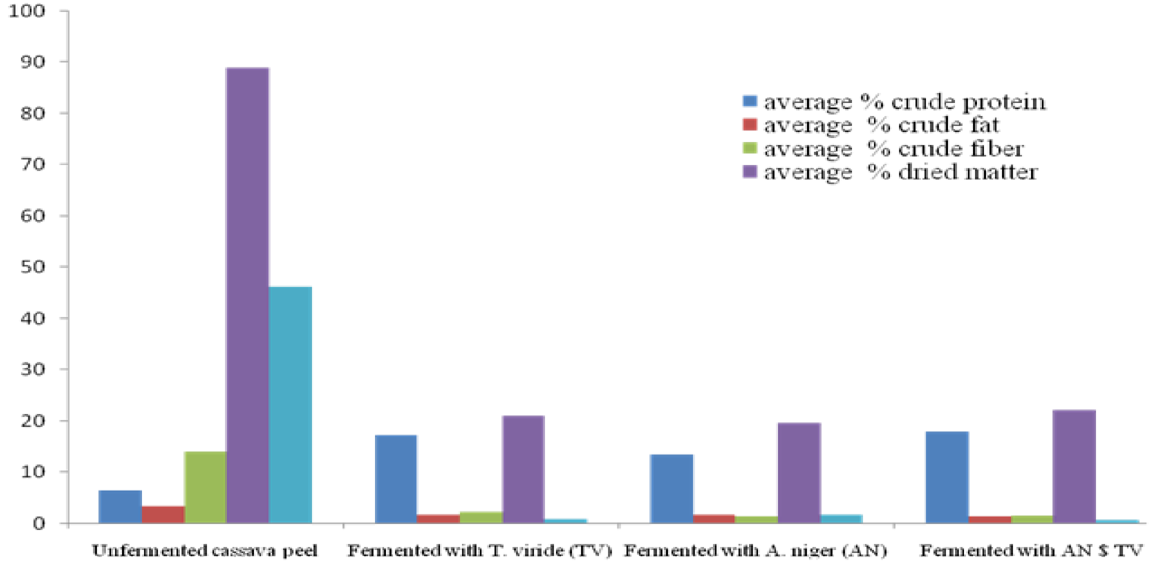

Figure 1b: Synergistic Effect of *T. viride* and *A. niger* on Cassava Peel Nutrient Composition after Fermentation

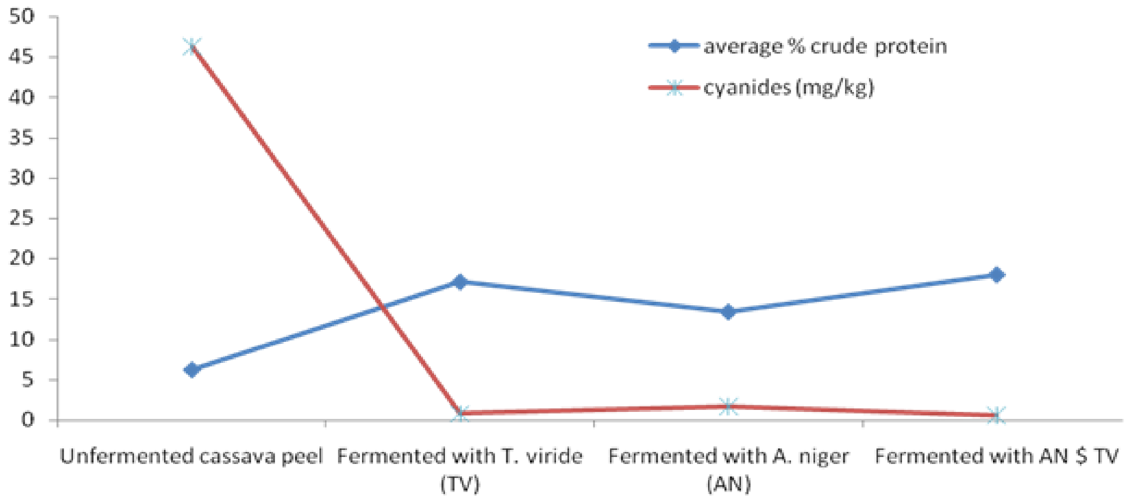

Figure 2b: Comparative Effect of Fermentation on Protein and Cyanide Composition

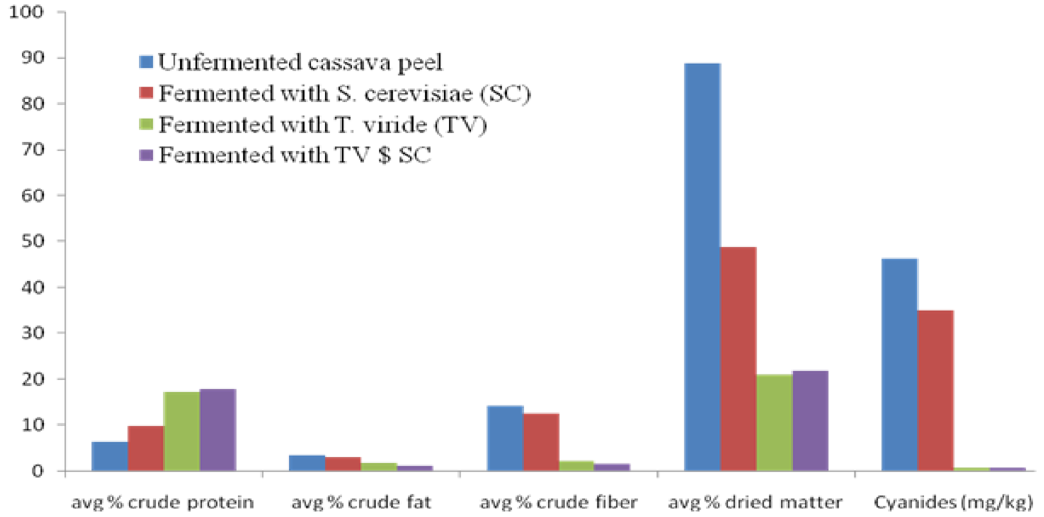

Figure 1c: Synergistic Effect of *T. viride* and *S. cerevisiae* on Cassava Peel Nutrient Composition after Fermentation

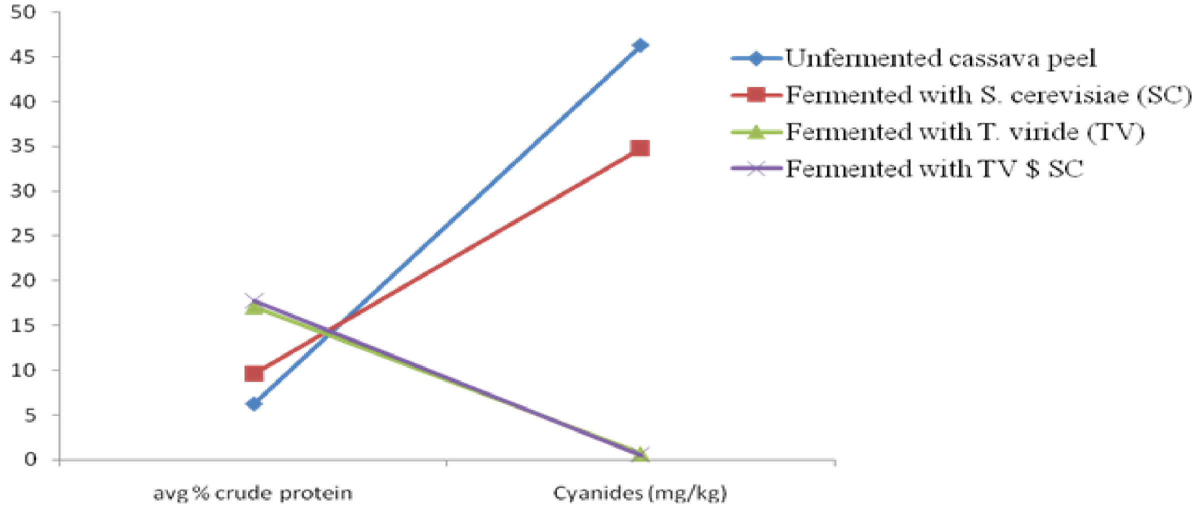

Figure 2c: Comparative Effect of Fermentation on Protein and Cyanide.

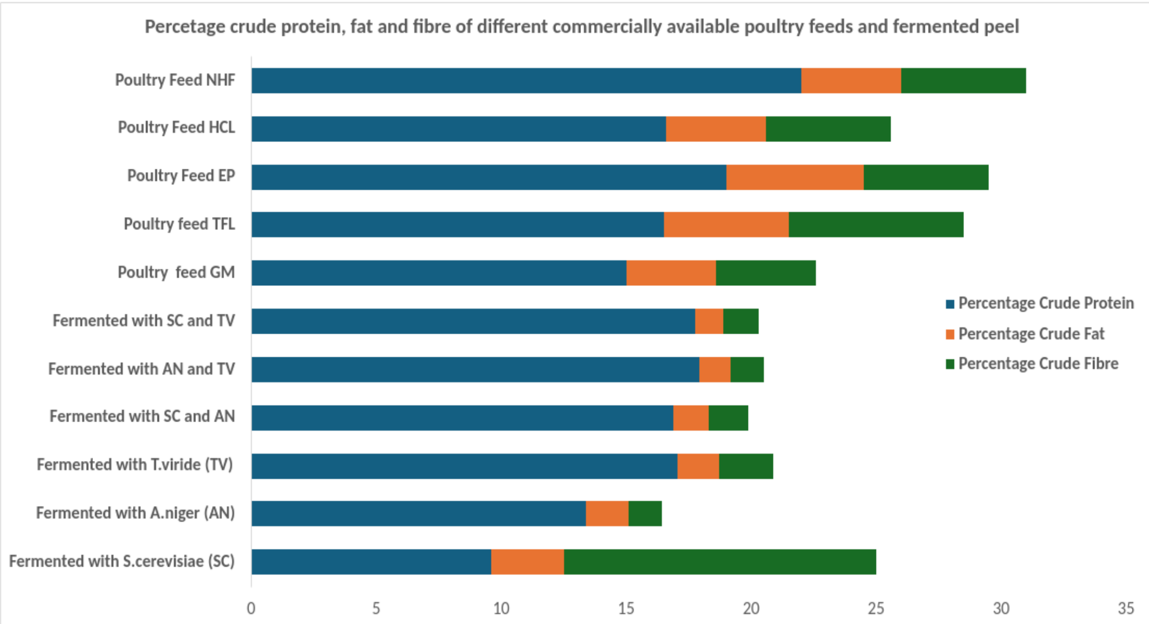

Figure 3: Comparing Fermented Cassava Peel and Commercially Available Poultry Feeds

DISCUSSION

It is observed from the results that fermentation using fungi has effects on the nutritional components of cassava peel. This is supported by studies by Lateef *et al.* (2008), Adeleke *et al*. (2017) and Akinyele (2015). Table. Fermentation using solid state fermentation and fungi technology was able to improve the protein component and reduced the cyanides present in cassava peel which subsequently leads to reduction in crude fat, dried matter and crude fiber percentage of the cassava peel. It was observe that the different fungi species, generally recognized as safe, could have diverse fermentation effect on the nutritional enrichment of carbohydrate rich foods as established by Fajimi *et al.* (2016), Onilude (2017), Olufunke (2013), Ezekiel (2013) and Akinyele (2015).

Trichoderma viride, Aspergillus niger and *Saccharomyces cerevisiae* were effective in enriching the nutritional composition of cassava. Raw protein percentage was increased to 9.62% from 6.25 using *Saccharomyces* alone, 13.39% using only *Aspergillus niger* and 17.06% using *T. viride* only. In synergy, *S. cerevisiae* and *A. niger* could further increase protein to 16.89% using both fungi in equal inoculation volume. T. viride and A. niger increased protein to 17.94% while *S. cerevisiae* and *T. viride* could increase protein to 17.74% using both fungi in equal proportion. Conversely, *Saccharomyces* was able to reduce the cyanide composition of the cassava peel to 34.85 mg/kg from 46.28 mg/kg, *Aspergillus niger* reduced cyanides from 46.28mg/kg to 1.66mg/kg while *T.viride* reduced cyanide to 0.72mg/kg. Study by Adeleke *et al.* (2017) and Oboh *et al*. (2002) established that *Aspergillus niger* could reduce cyanide and increase protein content of cassava residue significantly. In synergy, cyanide is further reduced to 0.87mg/kg by *S. cerevisiae* and *A. niger, T. viride* and *A. niger* reduced cyanides to 0.51mg/kg while *T. viride* and *S. cerevisiae* was able to reduce cyanides to 0.53mg/kg. The enrichment effect of *S. cerevisiae* is significantly low compared to *A. niger and T. viride* in the nutrient enrichment of cassava peel, this may be due to the ability of *A. niger* and T. viride to digest polysaccharides and lignocelluloses. More so, *Aspergillus* has been widely described as fungi with high potential to hydrolyse cellulotic compounds and breakdown complex carbohydrates compared to Saccharomyses (Lateef *et al.*, 2008) while T. viride are known to hydrolyse high starch compounds. It is evident that fungi fermentation could play a vital role in nutrient enrichment therefore, fermentation of cassava peels in solid state using fungi for 14 days will significantly enrich the protein content and reduce the cyanides. In addition, *S. cerevisiae* is less potent in the enrichment of cassava peel compared to *T. viride* and *A. niger*. increase protein and reduction of cyanides will be better achieved with T. *viride* and *Aspergillus niger* compared to *S. cerevisiae*. Moreover, *S. cerevisiae* in synergy with *T. viride* and *Aspergillus niger* can also boost nutrient enrichment of cassava peel. Furthermore, *T. viride* can be more potent polysaccharide degrading than *A. niger*. Adeleke *et al.* (2017) and Oboh (2006) also describe the enrichment potential of *A. niger* and found it to be very significant in reducing cyanide and enriching protein in cassava residue enrichment while Oboh *et al.* (2002) also established the potency of *Saccharomyces cerevisiae* in the fermentation of cassava pulp and subsequently increase protein and reduce cyanide.

Solid state fermentation with two fungi in synergy (*A. niger* with *S. cerevisiae, A. niger* with *T. viride,* and *S. cerevisae* with *T. viride*) performs better in the process of fermentation and the effect on protein enrichment and cyanide reduction was significantly different to what was observed with individual fungi fermentation chamber. The synergy of two of these fungi could be beneficial to commercialization of this method in the production of animal feed for sustainable animal protein production

It is observed that fermented cassava peel compared to common commercially available feeds has considerable or equivalent protein, fat and fiber content. This is a testament to the potency of fungi in reducing complex polysaccharides to usable nutrients and it shows that fermented cassava peel could rival commercially available animal feeds if this technology can be fortified.

The outcome of this study gives a satisfying result that will make cassava peel and some other agricultural processing wastes, function as alternative raw materials in livestock feed production for poultry and domestic animals. This will inadvertently reduce the cost of animal feeds and the outrageous cost of animal proteins and reduce the menace arising from deposition of agricultural waste in our environment.

CONCLUSION

Fermentation of cassava peel, which is a common agricultural waste, could facilitate the formation of desirable and sustainable products for feeding livestock and decontamination of the environment. Using fungi that are generally recognized as safe with the ability to degrade oligosaccharides compounds to ferment peels of cassava may significantly enhance its nutritional quality, with increase protein content and decrease in residual cyanide and the end-product could be highly digestible and a good supplement in compounding animal feed. If this technology is applied in livestock feed production, it could be a means to a sustainable livestock feed production which could aid feed composition improvement, feed cost reduction, increase animal food availability at reduced cost and a significant means to environmental waste management.

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