EFFECT OF ADMINISTRATION OF *SACCHAROMYCES CEREVISIAE* AND *TRICHODERMA VIRIDE* FERMENTED YAM AND PINEAPPLE PEELS ON ESSENTIAL ORGANS OF WISTAR RATS

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Abstract
Protein-enriched products were manufactured from yam and pineapple peels using solid state fermentation with Saccharomyces cervisiae and Trichoderma viride with a view to converting waste to wealth. The organisms were grown in the two substrates with addition of ammonium sulphate (10gN/kg substrate) as nitrogen source and fermentation was made at tropical room temperature (27±2 °C) for 96 h. The proximate chemical compositions of the products were determined using standard methods. Blood samples from wistar rats fed the products were analysed for biochemical changes. S. cerevisiae fermented yam and pineapple significantly increased the protein content of yam and pineapple peels from 6.60-16.82% and 4.50-21.04% respectively, while T. viride increased protein in the two substrates from 6.60-14.65% and 4.50-15.95% respectively. Rats fed protein-enriched products from yam and pineapple peels fermented with S. cerevisiae and T. viride had similar levels of serum alanine aminotransferase and aspartate aminotransferase activities in their blood as those fed casein diets suggesting no adverse effects. The data on the kidney and liver function tests suggest that none of the formulated experimental feeds is unsafe to both kidney and liver health of the wistar rats and can be recommended as desirable for animal feed.

Keywords: Fermentation, protein-enrichment, biochemical changes, protein-enriched products, casein diet

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1. INTRODUCTION

There is a widening gap in food production and demand in sub-Saharan African countries with annual population growth rates exceeding 2% and high level of poverty. These countries have the greatest food demand but the least supply (Aworh, 2010). For example, it is estimated that about 70% of the population of Nigeria, the most populous country in sub-Saharan Africa with a population of 170 million, live below the poverty line and food insecurity is a major challenge with protein-energy malnutrition and micronutrient deficiencies constituting important public health problems affecting productivity, maternal and infant health (Oshewolo, 2010; Aworh, 2015). Malnutrition, pollution emanating from agricultural and food processing wastes as well as the need for formulation of innovative and alternative protein-rich food sources due to insufficient supply from conventional protein sources like milk, meat and fish have therefore encouraged research into bioconversion of lignocellulosic agro-industrial wastes into commercially valuable products using appropriate technology such as fermentation (Choi and Park, 1999; Gomez *et al*., 2005). Solid state fermentation in which microorganisms are grown on a substrate without requiring aseptic conditions has been used to enrich the protein content of cassava, with the addition of ammonium sulphate and urea as nitrogen sources that provided the necessary nutritional requirements for the growth of the microorganisms in cassava substrate (Noomhorm *et al.*, 1992). This technology is suitable for application at small scale level, and has been used to increase the protein content and add value to cassava peel, a major food processing waste with low protein content, in Nigeria (Iyayi and Losel, 2001; Okpako *et al*., 2008; Ezekiel *et al*., 2010; Ezekiel and Aworh, 2013a). On-farm scale

fermentation of cassava peel using a locally fabricated solid state fermenter, suitable for use in rural areas in Nigeria and other developing countries, increased protein content of cassava peel from 4.21% to 10.93% (Ezekiel and Aworh, 2013b). The protein-enriched cassava peels could be used as livestock feedstock feed, thereby increasing the income of small farmers and cassava processors and reducing poverty.

Nigeria produces 38 million tons of yam annually, accounting for 65% of world production (Verter and Becvarova, 2015), and is the 6th largest producer of pineapples with 800,000 tons annually (Esiobu and Onubuogu, 2014). About 20% of the whole pineapple fruit is juice, the remaining parts in form of crown, peeled skin, base and core are discarded as wastes (Hutagalung and Jalaludin, 1973).Yam and pineapple peels are major wastes from yam and pineapple processing butlittle or no attention is given to their utilization especially in Nigeria. The objectives of this study were to produce protein-enriched products from white yam and pineapple peels by solid state fermentation using *Saccharomyces cerevisiae* and *Trichoderma viride* and evaluate their possible effect on liver and kidney of wistar rats.

2. MATERIALS AND METHODS

Yam and pineapple peels are the major substrates used in this study. Thirty male albino rats weighing between 80-120 g and about 6-8 weeks old were used for animal feeding study. The main procedures employed include solid state fermentation and blood analysis for biochemical changes.

2.1. Substrates

Yam peels from white yam tubers (*Abuja* cultivar*)* were collected from a canteen in the University of Ibadan, Oyo State, Nigeria. Pineapple (smooth cayenne cultivar) peels were collected from Fumman fruit-juice manufacturing company, Apata, Ibadan, Oyo state, Nigeria.

2.2. Microorganisms

Saccharomyces cerevisiae (BY4743) was obtained from Department of Microbiology, University of Ibadan, Oyo State, Nigeria and maintained on yeast extract agar slants at 4^{o}C. *Trichoderma viride* (ATCC36316) was obtained from American Type Culture Collection (ATCC) and maintained on potato dextrose agar slants at 4^{o}C.

2.3. Solid-state fermentation of yam and pineapple peels

Yam and pineapple peels were washed, drained, dried at 60^{o}C in a forced-draught oven, dry-milled with a fabricated hammer mill in Mechanical Engineering Department, University of Ibadan and sieved using 3.35 - 4.00 mm sieve mesh sizes. Moisture content of batches of yam and pineapple peels (2 kg each) were adjusted appropriately with distilled water, ammonium sulphate was added as nitrogen source (10 g of N/kg substrate), followed by mixing, sterilization at 121^{o}C for 15 min and cooling to room temperature (27±2^{o}C). Then, 600 ml of fermented yeast broth (2.6 x 10^{8} CFU/ml) was added separately to the cooled, autoclaved yam and pineapple peels (Aruna *et al*., 2017). The *S. cerevisiae* inoculated peels were fermented in a locally fabricated on-farm fermenter, in the shape of a cabinet with fixed racks and capacity for five perforated trays made with 0.2 mm gauge stainless steel pipes, with each tray capable of holding 400 g of substrate per fermentation batch, as previously described (Ezekiel and Aworh, 2013b). Fermentation was carried out at 27±2^{o}C for 96 h. The same procedure was used for the solid-state fermentation of yam and pineapple peels with *T. viride* spore suspension containing approximately 2.7x10^{6} spores per ml. The four fermented peels were spread on metallic trays separately, oven-dried at 60^{o}C and cooled to 27±2^{o}C.

2.4. Compositional analysis

Proximate composition of yam and cassava peels and the fermented products were determined by standard procedures (AOAC, 1984). Moisture content was determined by drying at 105°C to constant weight in a forced-draught oven. Crude protein was determined by the micro-Kjeldahl method (total nitrogen x 6.25) using a Foss Tecator Kjeltec 8200 Analyser and crude fibre by digestion with

H_2SO4 and NaOH using a Foss Fibertec 1020 Analyser. Fat was determined by extraction with petroleum ether in a Soxhlet apparatus and ash by incineration in a muffle furnace at 550°C. Carbohydrate was estimated by difference. True protein content was determined by Folin-Ciocalteau method as described by Lowry *et al*. (1951).

2.5. Basal, experimental and control diets

Basal diet was prepared according to procedure described by Egounlety *et al*. (2002). Experimental and control diets were prepared as described in Table 1. They were incorporated into the basal diet to achieve an isonitrogenous diet at 10% protein level.

2.6. Experimental animals

Thirty male albino rats weighing between 80-120 g and about 6-8 weeks old were obtained from the Animal House of the Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, following clearance from the Animal Care and Use Research Ethics Committee of University of Ibadan, Nigeria. The animals were randomly distributed into six groups (A-F) of five rats, housed individually in metabolic cages, fed with stabilizing diet containing 4% casein for five days and then assigned to the six treatment diets. The animals in Groups A, B, C and D, were fed the basal diet mixed with ground, pelletized *S. cerevisiae* enriched yam peels, *S. cerevisiae* enriched pineapple peels, *T. viride* enriched yam peels and *T. viride* enriched pineapple peels respectively. Those in Groups E and F were fed only basal diet and casein diet respectively. Feed and water were supplied to the rats *ad libitum* for 28 days. The weights of the animals were taken twice a week and their feed intakes were recorded daily. At the end of the test period rats were re-weighed, sacrificed and their blood collected for evaluation of liver and kidney functions.

Preparation of Serum and Tissue Homogenates

The procedure described by Yakubu et al. (2005) was used in the preparation of serum. The rats were thereafter quickly dissected, liver excised and transferred into ice-cold 0.25 M sucrose solution and used as a medium for homogenization. (1:5 wt/vol). The homogenates were kept frozen overnight to ensure maximum release of the enzymes located in the cells of the tissues before being used for the various biochemical assays.

Table 1. Composition of isonitrogenous diets at 10% protein level

Groups	Samples	Protein content of feeds as analyzed (%)	Weight of desired feed (g)	Weight of basal diet (g)	Weight of sample (g)	Final protein content (%)
A	*S. cerevisiae* enriched yam peel	17.83	1000	439.15	560.85	10
B	*S. cerevisiae* enriched pineapple peel	16.64	1000	399.04	600.96	10
C	*T. viride* enriched yam peel	16.95	1000	410.03	589.97	10
D	*T. viride* enriched pineapple peel	15.04	1000	335.11	664.89	10
E	Basal	---	1000	1000		0
F	Casein	87.11	1000	885.20	114.80	10

Determination of Biochemical Parameters

The procedures described in the assay kits from Randox Laboratory for urea, creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in the kidney and liver according to the method described.

2.7. Statistical Analysis

All analyses were performed in triplicates. Data were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) V. 17.0. Means were separated using Duncan Multiple Range Test at 95% confidence level ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

Dried yam and pineapple peels had moisture contents of 10.81% and 14.78% respectively. Their carbohydrate, crude fibre, protein, fat and ash contents on dry weight basis are presented in Table 2. They contained predominantly carbohydrates (74.1-78.8%), are high in crude fibre (9-14%), low in protein (4.5-6.6%) and very low in fat (0.6-1%). Ash content was 4.45% and 6.79% for yam and pineapple peels respectively. Fermentation increased protein content 2-5 fold. Crude protein increased from 6.60% to 14.65% and from 4.52% to 15.95% in yam and pineapple peels respectively when fermented with *T. viride* (Table 3). Greater increases in protein content were observed; from 6.60% to 16.82% and from 4.52% to 21.04% for yam and pineapple peels respectively fermented with *S. cerevisiae* relative to those fermented with *T. viride*.

The level of protein enrichment attained with these products was higher than what was obtained with cassava peel in which crude protein increased from 4.21% to 10.93% in cassava peels fermented with *T. viride* using the same fermenter (Ezekiel and Aworh, 2013b), indicating that the nature of the substrate as well as the type of fermenting microorganism play critical roles in protein-enrichment of agricultural wastes (Noomhorm *et al*., 1992). Solid state fermentation caused substantial reduction in carbohydrate content of yam and pineapple peels (Table 3) as starch and other carbohydrates were used for microbial growth and metabolism (Noomhorm *et al*., 1992; Ezekiel and Aworh, 2013a and Aruna *et al*., 2017).

The measurement of the activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation, diagnosis and tissue cellular damage (Malomo, 2000).

Changes in the levels of normal range of enzymes localized in specific cells indicate functional toxicity of such cells and these alterations occurs prior to obvious cellular architectural degeneration that are observed on histological examination (Wright and Plummer, 1974) and is required in certain amounts for proper functioning of organs.

Table 2: Proximate composition of dried yam and pineapple peels

Components (%)	Yam peel	Pineapple peel
Crude protein	6.60 ± 0.04^{a}	4.52 ± 0.27^{b}
True protein	4.38 ± 0.01^{a}	2.84 ± 0.03^{b}
Ash	4.45 ± 0.03^{b}	6.79 ± 0.28^{a}
Crude fibre	9.02 ± 0.09^{b}	13.95 ± 0.14^{a}
Carbohydrate	78.81 ± 0.05^{a}	74.12 ± 0.98^{b}
Fat	1.12 ± 0.01^{a}	0.62 ± 0.03^{b}

Data presented on dry matter basis. Each value is a mean of three independent experiments. Means followed by the same subscript in the same row are not significantly different ($p>0.05$)

Table 3: Proximate composition of fermented yam and pineapple peels

Composition (%)	Samples			
	T. viride Fermented Yam Peel	*T. viride* Fermented Pineapple Peel	*S. cerevisiae* Fermented Yam Peel	*S. cerevisiae* Fermented Pineapple Peel
Crude Protein	14.65±0.02^{d}	15.95±0.01^{c}	16.82±0.12^{b}	21.04±0.01^{a}
Crude Fat	1.81±0.01^{b}	1.29±0.01^{b}	2.16±0.06^{a}	2.08±0.15^{a}
Crude Fibre	11.89±0.30^{c}	18.98±0.75^{a}	9.88±0.15^{d}	15.91±0.20^{b}
Ash	6.91±0.01^{d}	7.60±0.10^{c}	8.11±0.05^{b}	10.54±0.05^{a}
Carbohydrate	64.74±0.3^{a}	56.18±0.06^{c}	63.03±0.1^{b}	50.43±0.04^{d}

Data presented are means and standard deviation of three independent experiments on dry matter basis

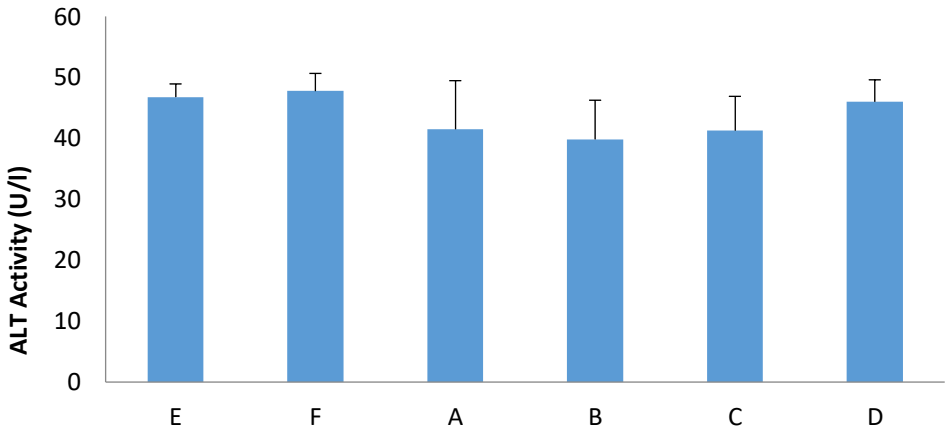

Figure 1 Effect of *Saccharomyces cerevisiae* and *Trichoderma viride* enriched diets on the activity of alanine aminotransferase in male albino rats. Values are mean ± SD n=5

Key
A: *S. cerevisiae* fermented yam peel diet B: *S. cerevisiae* fermented pineapple peel diet
C: *T. viride* fermented yam peel diet D: *T. viride* fermented pineapple peel diet
E: Basal diet F: Casein diet

Alanine aminotransferase (ALT) activity in the blood serum of rats fed protein-enriched yam and pineapple peels from fermentation with *S. cerevisiae* and *T. viride* were comparable to those of rats fed basal and casein diets (Figure 1) with values ranging from 39.8-47.7 u/l. Similarly, there were no significant differences in the serum aspartate aminotransferace (AST) activity in the blood serum of rats fed protein-enriched yam and pineapple peels from fermentation with *S. cerevisiae* and *T. viride* relative to those fed basal or casein diets (Figure 2). The major differences in enzyme biomarker activities were observed in the serum of rats fed casein diet that had very low alkaline phosphatase (ALP) activity (<200 u/l) when compared to rats fed protein-enriched fermented yam and pineapple peels that had values exceeding 450 u/l or basal diet with over 700 u/l (Figure 3).The reduction in the activity of the serum ALP observed in this study may be due to synthetic ability of fermented yam and pineapple peels and this was reflected with a mild increase in the liver ALP.

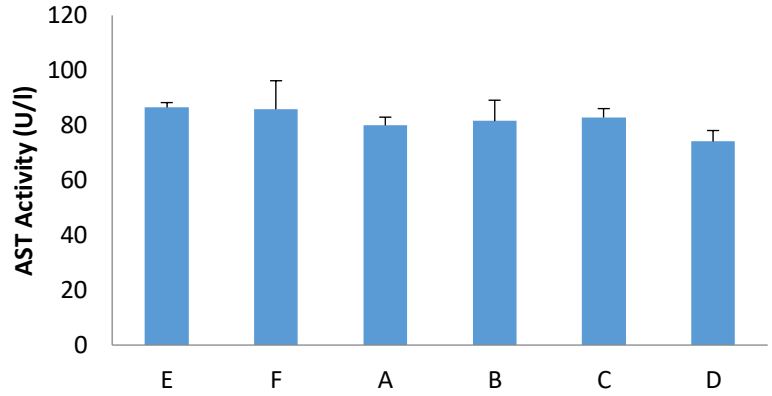

Figure 2 Effect of *Saccharomyces cerevisiae* and *Trichoderma viride* enriched diets on the activity of aspartate aminotransferase in male albino rats. Values are mean ± SD n=5

Key
A: *S. cerevisiae* fermented yam peel diet B: *S. cerevisiae* fermented pineapple peel diet
C: *T. viride* fermented yam peel diet D: *T. viride* fermented pineapple peel diet
E: Basal diet F: Casein diet

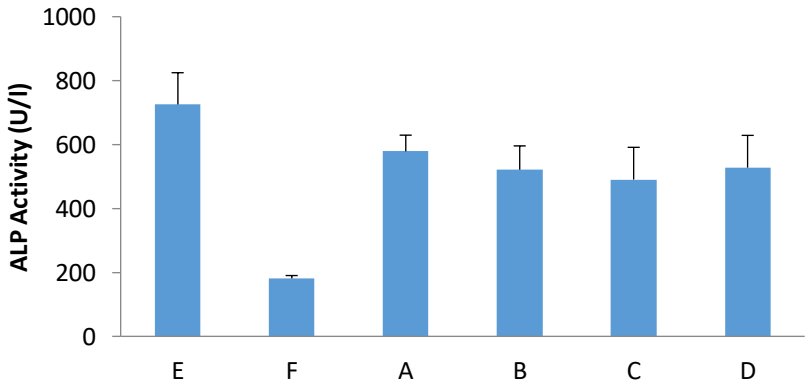

Fig. 3 Effect of *Saccharomyces cerevisiae* and *Trichoderma viride* enriched diets on the activity of alkaline phosphatase in male albino rats. Values are mean ± SD n=5

Key
A: *S. cerevisiae* fermented yam peel diet B: *S. cerevisiae* fermented pineapple peel diet
C: *T. viride* fermented yam peel diet D: *T. viride* fermented pineapple peel diet
E: Basal diet F: Casein diet

In addition, there were no significant differences ($p<0.05$) in the levels of urea in rats treated with the experimental diets and those in either of the control groups (E or F). Urea levels in the serum of rats fed *Saccharomyces cerevisiae* enriched yam and pineapple peel diets were 36.1 and 36.8 mg/dl respectively while its levels in those subsisted on *Trichoderma viride* enriched yam and pineapple peel diets were 36.0 and 41.2 mg/dl respectively (Figure 4). With respect to creatinine levels, there were no significant differences ($p<0.05$) among the values obtained from rats fed the experimental diets and those

fed the positive control diet (F). However, the creatinine levels of groups A and C rats were significantly lower than that of group E (Figure 5). Low creatinine levels do not have strong clinical significance whereas high levels are diagnostic for severe kidney damage (MedlinePlus, n.d). Taken together therefore, the data on the kidney and liver function tests suggest that none of the formulated experimental feeds is unsafe to both kidney and liver health of the wistar rats.

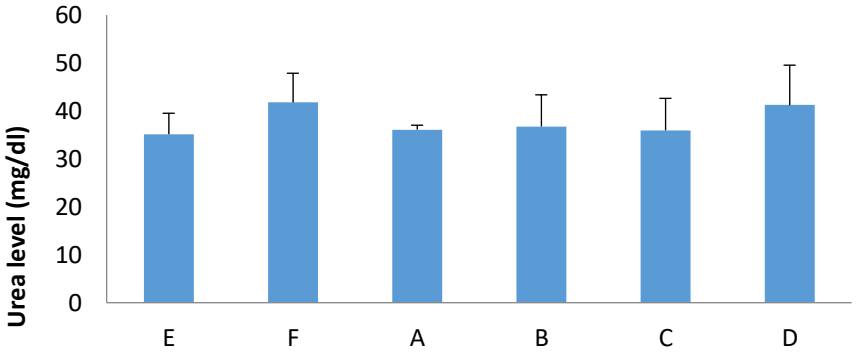

Fig. 4 Urea levels in serum of *Trichoderma viride* and *Saccharomyces cerevisiae* enriched yam and pineapple and control diets fed rats. Values are mean ± SD n=5

Key

A: *S. cerevisiae* fermented yam peel diet B: *S. cerevisiae* fermented pineapple peel diet
C: *T. viride* fermented yam peel diet D: *T. viride* fermented pineapple peel diet
E: Basal diet F: Casein diet

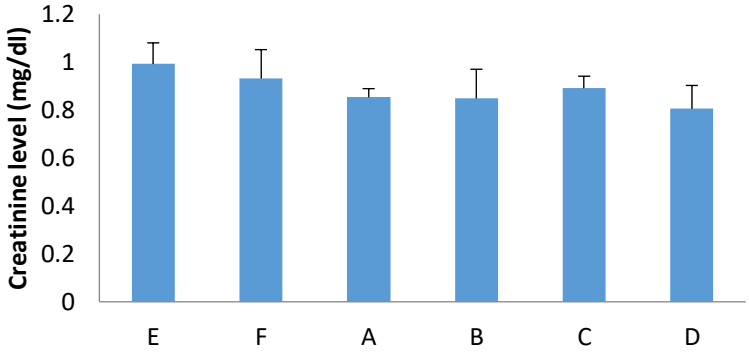

Fig. 5 Creatinine levels in serum of *Trichoderma viride* and *Saccharomyces cerevisiae* enriched yam and pineapple and control diets fed rats. Values are mean ± SD n=5

Key

A: *S. cerevisiae* fermented yam peel diet B: *S. cerevisiae* fermented pineapple peel diet
C: *T. viride* fermented yam peel diet D: *T. viride* fermented pineapple peel diet
E: Basal diet F: Casein diet

4. CONCLUSION

Solid state fermentation with *S. cerevisiae* and *T. viride* significantly increased the protein contents of yam and pineapple peels. Biochemical examination of blood of wistar rats fed the protein-enriched fermented products revealed that the products had no

adverse effects. It would appear that protein-enriched yam and pineapple peels from fermentation with *S. cerevisiae* and *T. viride* are safe for consumption and can be recommended as desirable for animal feed.

5. ACKNOWLEDGEMENT

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