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Nutrient enrichment of pineapple waste using *Aspergillus niger* and *Trichoderma viride* by solid state fermentation

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The enrichment by microbial fermentation of agro industrial waste to alleviate their nutritional problems has been proposed but the nutritional value of the subsequent feed for animal consumption has not been fully elucidated. This study investigates whether solid state fermentation of pineapple waste using the fungi *Aspergillus niger* and *Trichoderma viride* could improve its nutrient content. Results show that fermentation of pineapple waste by solid state fermentation using the fungi *A. niger* and *T. viride* significantly ($P < 0.05$) enriches the nutrient content of the waste, particularly increasing the crude protein and ash content while lowering the crude fiber content. The most significant nutrient enrichment was recorded at 72 h of fermentation using *A. niger* and at 96 h of fermentation using *T. viride*. Indiscernible changes were noted in the mineral content of pineapple waste (PW). Dry matter increased significantly ($P < 0.05$) as fermentation progressed with the highest values recorded at 96 h. This study establishes no significant differences ($P > 0.05$) in the fermentation abilities of the two fungi, *A. niger* and *T. viride*. Fermented pineapple waste may be a potential supplement in compounding animal feed provided that it is acceptable and highly digestible.

Key words: Agro industrial waste, crude fiber, crude protein.

INTRODUCTION

Waste disposal represents a serious problem to many agro industries since it is usually prone to microbial spoilage and causes major environmental problems. The utilization of agro industrial waste by conversion into value added products such as animal feed or manure may be an innovative solution to the environmental waste problem. Agro-industrial wastes in recent times have been the focus of research in animal nutrition especially for monogastric animals (Iyayi and Aderolu, 2004; Iyayi and Fayoyin, 2005). In fact, many feeds that can be fed alternatively at cheaper cost to monogastric livestock are based on the use of agro-industrial waste that are of no food value to humans (Iyayi and Fayoyin, 2005).

The use of microorganisms through fermentation to improve nutritional value of agro-industrial wastes, thereby offering the potential to make dramatic contribu-

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tions to sustainable livestock production has been well documented (Iyayi and Aderolu, 2004; Fasuyi, 2005; Iyayi and Fayoyin, 2005). The conventional commercial feeds are becoming expensive to afford, therefore substitutes for conventional feeds such as crop residues and agro-industrial wastes have been proposed as a sustainable means of livestock production (Fasuyi, 2005; Iyayi and Fayoyin, 2005).

When the pineapple fruits are canned or consumed the crown, the outer peel and the central core are discarded as pineapple waste which accounts for about 50% of the total pineapple fruit weight corresponding to about ten tons of fresh pineapple or one ton of dry pineapple waste per hectare (http://www.fao.org). Pineapple wastes are recommended as tremendous sources of organic raw materials and are potentially available for conversion into useful products such as animal feeds (Hemalatha and Anbuselvi, 2013). Pineapple waste contains high amounts of crude fiber and suitable sugars for growth of microorganisms (Hemalatha and Anbuselvi, 2013).

The utilization of fungi for nutrient enhancement in agro-industrial waste by fermentation has been studied for years and their efficiency shown in substrates such as lignin, cellulose and hemi cellulose polymers found in agro-industrial waste (Howard et al., 2003) A*spergillus niger* and *Trichoderma viride* have been successfully used in a number of fermentation studies towards solid waste management, biomass energy conservation and production of secondary metabolites in various agro-industrial wastes (Omojasola et al., 2008; Femi-Ola et al., 2009; Kareem et al., 2010).

In recognition of this potential, this study was conducted to investigate the use of fungi; *A. niger* and *T. viride* for nutrient enrichment of pineapple waste by solid state fermentation for possible use as animal feed supplement. It is hoped that the results shall provide more knowledge on the application of microorganisms in recovering pineapple waste through fermentation for environmental sustainability and utilization in animal feeds.

MATERIALS AND METHODS

Fungi

The fungi used were isolated pure strains of *A. niger* and *T. viride* grown on SARBORAUD agar medium. Slants of the microbes were obtained from the culture bank of the Department of Veterinary Pathology, Parasitology and Microbiology, University of Nairobi, Kenya. The fungi were then sub cultured in CZAPEK dox broth media, and incubated at 37°C for four days. The grown fungal spores were then maintained in a refrigerator set at 5°C.

Solid state fermentation

Pineapple waste (peelings and core) of 90 g portions were weighed into twenty one 500 ml beakers, covered with aluminum foil and autoclaved at 121°C for 15 min. After sterilization, the samples were then spread on separate foil paper trays in uniform layers of 1 cm thick on open racks allowing sufficient aeration. A set of nine beakers were aseptically inoculated with 15 ml of 2% fungal spore suspension of *A. niger,* another set of nine with *T. viride* and three uninoculated. Sets of three samples were withdrawn after 48, 72 and 96 h, dried at 60°C in an oven for 72 h. The samples were then milled and stored in tightly sealed 100 g plastic bottles. Samples from the three control beakers were treated alike.

Analysis of samples

The nutrient content of the samples were determined quantitatively by proximate analysis and specific mineral analysis. Analysis was done in triplicate for each sample. Proximate analysis was conducted using the methods of AOAC (1998). The specific minerals were determined by the methods outlined by Okalebo, Gathua and Woomer (1993). All data were presented as the mean standard values of three replicates and subjected to a one way analysis of variance (ANOVA) and post ANOVA. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Results in Table 1 show significantly ($P < 0.05$) higher crude protein content in pineapple waste fermented using the fungi *A. niger* and *T. viride* than in the unfermented pineapple waste for the 48, 72 and 96 h fermentation periods. The post fermentation increase in crude protein content could be attributed to the possible secretion of some extra cellular enzymes (protein) such as amylases, xylanases and cellulases into the pineapple waste mash by the fermenting fungi in an attempt to make use of the carbohydrates in the mash as a carbon and energy source (Raimbault, 1998). *A. niger* has been reported to have high specific activity for cellulases and hemi-cellulases (Howard et al., 2003). Additionally, *T. viride* and *A. niger* have found use in the production of extra cellular enzymes including cellulase, amylase and xylanase (Nair et al., 2008).

Fungi colonize substrates for utilization of available nutrients. They synthesize and excrete high quantities of hydrolytic extra cellular enzymes, which catalyze the breakdown of nutrients to products that enter the fungal mycelia across cell membrane to promote biosynthesis and fungal metabolic activities leading to growth (Raimbault, 1998). Therefore, increase in the growth and proliferation of fungal biomass in the form of single cell protein (SCP) or microbial protein accounts for part of the increase in the protein content after fermentation (Raimbault, 1998).

Earlier findings by Guerra et al. (1986) reported similar significant ($P < 0.05$) increase in crude protein content in pineapple waste fermented for 72 and 96 h by liquid state fermentation method using fungi, *A. niger*, *T. viride* and *Myrothecium verrucaria*. Similar findings have been reported with the same methods and fungi using

Table 1. Proximate composition of fermented pineapple waste samples (g / 100 g).

Nutrient content	Unfermented	Fungi	Fermented samples		
			48 h	72 h	96 h
Crude protein	3.69 ± 0.05	*A. niger*	4.53 ± 0.19[a]	10.28 ± 0.14[bd]	8.89 ± 0.28[cef]
		T. viride	4.44 ± 0.14[a]	8.32 ± 0.01[bd]	9.04 ± 0.16[cef]
Ash	2.61 ± 0.23	*A. niger*	5.14 ± 0.03[a]	4.18 ± 0.23[b]	4.79 ± 0.08[c]
		T. viride	4.95 ± 0.06[a]	5.64 ± 0.55[b]	6.48 ± 0.12[c]
Crude fiber	10.80 ± 0.10	*A. niger*	14.09 ± 0.01[a]	5.78 ± 0.01[bd]	2.49 ± 0.01[cef]
		T. viride	9.09 ± 0.01[a]	8.04 ± 0.04[bd]	7.59 ± 0.08[cef]
Dry matter	82.13 ± 0.46	*A. niger*	89.74 ± 0.22[a]	88.81 ± 0.41[b]	90.39 ± 0.27[c]
		T. viride	87.24 ± 0.13[a]	88.16 ± 0.05[b]	90.84 ± 0.41[cef]
Carbohydrate	75.83 ± 1.12	*A. niger*	80.07 ± 0.22[a]	74.36 ± 0.09[d]	76.71 ± 0.05[e]
		T. viride	77.85 ± 0.08	74.20 ± 0.85	75.32 ± 0.33

Values are expressed as mean ± SEM (n = 3) for different determinations. [a]Significant difference of values between 0 and 48 h; [b]significant difference of values between 0 and 72 h; [c]significant difference of values between 0 and 96 h; [d]significant difference of values between 48 and 72 h; [e]significant difference of values between 48 and 96 h; [f]significant difference of values between 72 and 96 h. Significant difference determined at $P < 0.05$.

substrates such as cassava waste (Pothiraj et al., 2006), wheat offal (Iyayi, 2004), maize offal, palm kernel meal (Iyayi and Aderolu, 2004) and rice bran (Oshoma and Ikenebomeh, 2005).

The results in Table 1 indicate significant changes in crude protein content of fermented pineapple wastes at the three fermentation periods in the order 48 < 72 < 96 h using *T. viride* and 48 < 96 < 72 hours using *A. niger*. This is attributed to the increased hydrolytic enzyme activity with prolonged fermentation and increased fungal biosynthesis resulting in increased fungal biomass hence crude protein (Raimbault, 1998). Significantly ($P < 0.05$) lower crude fiber content was recorded in the fermented pineapple waste using both fungi compared to the unfermented pineapple waste for the three fermentation periods (Table 1). The ability of fungi to degrade crude fiber has been reported by several workers (Iyayi and Aderolu, 2004; Iyayi, 2004).

The fermentation process in addition to enriching the substrate with protein also releases oligosaccharides and simple sugars into the medium as a result of microbial degradation of otherwise unavailable polysaccharides (Duru and Uma, 2003). This suggests the production of hydrolytic enzymes in the fermentation culture by fungi that enable them to metabolize complex carbohydrate polymers (Duru and Uma, 2003; Oboh, 2006). This could explain the decrease in crude fiber content. The significant decrease in crude fiber was noted to coincide with significant increase in crude protein. Other researchers have reported similar findings (Duru and Uma, 2003; Oboh, 2006).

The carbohydrate content of which crude fiber is a constituent, acts as the carbon source for the growing microbes hence its depletion results from its utilization to produce fungal biomass, which is microbial protein or SCP (Raimbault, 1998). Despite the degradation of crude fiber by the fermenting fungi, no significant change in carbohydrate content in pineapple waste (Table 1) was recorded at 72 and 96 h of fermentation using A. *niger* and 48, 72 and 96 h using *T. viride,* as expected since fungi metabolize complex sugars to simple sugars. This is attributed to the ability of fungi to further hydrolyze the simple sugars for use as a carbon source to synthesize fungal biomass rich in protein (Oboh, 2006).

Dry matter content (Table 1) was significantly ($P < 0.05$) high in all the fermented samples compared to the unfermented samples. This is in line with the significant ($P < 0.05$) increase in protein content in all the fermented samples compared to the unfermented. This is attributed to the increased fungal biomass in the fermented samples because of inoculation and growth of the fungi in the fermented compared to the unfermented samples. The insignificant changes in dry matter levels at the 48, 72 and 96 h fermentation periods may be explained by the equal utilization and biosynthesis of nutrient during fermentation hence the lack of significant changes on summation.

There were no discernible trends (Table 2) reported in the specific mineral content (calcium, phosphorous and magnesium) in the fermented samples compared with the unfermented. Despite this, a significant increase in ash content was recorded in all fermented samples using both fungi when compared to the unfermented. This may suggest the introduction of specific mineral(s) by the inoculums that were not investigated. There were no significant differences ($P > 0.05$) in the fermentation abilities of the fungi, *A. niger* and *T. viride* with regard to pineapple waste fermentation and fermentation of pine-

Table 2. Minerals composition of fermented pineapple waste (mg/g).

Nutrient	Unfermented sample	Fungi	Fermented samples		
			48 h	72 h	96 h
Calcium	12.83 ± 0.03	*A. niger*	21.54 ± 0.01[a]	10.32 ± 0.04[d]	11.99 ± 0.22[e]
		T. viride	12.50 ± 0.14	9.82 ± 0.03	2.63 ± 0.01[cef]
Phosphorous	1.2 2 ± 0.02	*A. niger*	0.70 ± 0.02	1.92 ± 0.12	2.55 ± 0.02
		T. viride	1.69 ± 0.03	1.99 ± 0.05	1.87 ± 0.05
Magnesium	1.15 ± 0.00	*A. niger*	1.20 ± 0.00[a]	1.05 ± 0.00[bd]	1.47 ± 0.00[cef]
		T. viride	1.17 ± 0.00[a]	1.13 ± 0.00[bd]	1.51 ± 0.00[cef]

Values are expressed as mean ± SEM (n = 3) for different determinations. [a]Significant difference of values between 0 and 48 h; [b]significant difference of values between 0 and 72 h; [c]significant difference of values between 0 and 96 h; [d]significant difference of values between 48 and 72 h; [e]significant difference of values between 48 and 96 h; [f]significant difference of values between 72 and 96 h. Significant difference determined at $P < 0.05$.

apple waste for periods of 72 and 96 h are equally viable as no significant differences ($P > 0.05$) were established when assessing the amount of yield obtained at these two times. The significant ($P < 0.05$) increase in protein content of the pineapple waste after fermentation with *A. niger* and *T. viride* and the significant decrease ($P < 0.05$) in crude fiber concludes that fermentation of pineapple waste by solid state fermentation using the fungi *A. niger* and *T. viride* enriches the nutrient content of the waste and this by product could be good supplement in compounding animal feed provided that it is acceptable and highly digestible. Future fermentation studies using pineapple waste as substrate should be planned with improvements in the fermentation technique by including substrate pretreatment to convert the raw substrate into a more suitable form to increase its utilization by the fermenting microorganism and control of parameters such as pH which have been suggested to slow down the fermentation process (Iyayi, 2006). Further research is also proposed to determine appropriate incubation periods for optimal fermentation results.

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