Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 10 ~ Issue 2 (2023) pp: 01-05
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Assessment of the proximate and functional constituents of Pawpaw *(Carica papaya)* peels enriched with mycelia of *Cordyceps militaris.*

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ABSTRACT: *Phytochemicals in fungi have played important roles in treatment of diseases of human as well as additives in feeds of animals. Their use especially, the basidiomyceteshas long been ignored until recently, when it became of interest. This study was undertaken to determine and compare the chemical composition and functional constituents of pawpaw peels treated with mycelium of Cordyces militaris by solid state fermentation if it will improve the nutrient contents of the substrate as additive for livestock feeding. Chemical analysis and functional constituents screening were carried out on unfermented and bio fermented pawpaw peel substrates using standard methods of AOAC and HPLC.Proximate analysis revealed that Pawpaw peels fermented by single inoculation of C. militaris recorded an increase in crude protein and ash contents of all substrates. The protein net gains reached 25% when compared with 5%in control. Ash contents also recorded 36 % increase in bio fermented treatments over 13 % in the control. Similarly, C. militaris fermented pawpaw peel mealrecorded the highest significant ($P < 0.05$) decrease in crude fibre content from 75% to18 %.Tannin, phytic acid, total phenol and phenol derivative contents of C. militaris reduced from 55% to 15%,respectively. C. militaris treatment was efficient in enhancing thedelignification of cell walls of pawpaw peels by about34 %compared with the control (5%). Thus, showing its potential of being used in the nutrient improvement of pawpaw peels as feedstuffs.*

KEYWORDS: *Pawpawpeel, Solid State fermentation, Delignification, C.militaris, Nutrient Enhancement.*

Received 25 Jan., 2023; Revised 07 Feb., 2023; Accepted 09 Feb., 2023 © The author(s) 2023.
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I. INTRODUCTION

Livestock production is faced with several challenges one of which is the rising prices of feedstuffs used in animal feed. The surge in prices of these feedstuffs has been attributed tohigh demand and low supply of these ingredients [1]. As such, considering the cost of raising animals, it is expedient to find cheaper alternatives. Several attempts have been made by animal nutritionists to use alternatives and cheaper sources of ingredientsto replace the less available and expensive ones [2].

One of such alternative sources is the use of agro - byproducts which are underutilized wastes from agro allied industries and homes that are abundant in the environment. These wastes poses the problem of improperdisposal thatbecome hazardous and may lead to environmental pollution due to their rapid decay [3].

Pawpaw peels, a waste generated duringits consumption and industrialprocessing had been reported by T.T*Lee et al.,* [4] to be rich in nutrients, high in crude fibre and polyphenols thus, has antioxidant potential, that could serve as energy feed for ruminants. However, the high crude fibre and low crude protein contents ofpawpaw peels constitutes a setback in maximizing its utilization [5, 6].Microbialprocessing of high fibrous feedstuffsthrough solid state fermentation have been documented. Therefore, it could be adopted in reducing waste disposal problems and also add value to products.

II. LITERATURE REVIEW

Some species of fungi (microbes) that is employed in microbial technology, have been found to possess powerful oxidative enzymatic functions by mycelia penetration of plant tissues, structures and chemical bonds under solid state fermentation (SSF) techniques [7].For example,*Ascomycetes* has been widely studied and reported to have potential not only in depolymerizing lignocellulosics but, also reduced

antinutritional factors that are present in agricultural wastes through extracellular enzymes hydrolysis [8, 9]. *Cordyces militaris*, a medicinal fungi, hasalso beenwidely studied as edible fungi with probiotic and nutritional benefits [10].Similarly, some studies have observed that its extracted polysaccharides and bioactives has anti-oxidative and immunomodulatory activities [11]. Little efforts have been made on the use of *Cordyces militaris* as fermentable.

This study therefore, adopted *Cordyces militaris* as bio degrader of pawpaw peels as their mycelium have been reported to be safe for livestock feed. The study aimed to examine the efficacy of the fungi treatment on chemical composition of pawpaw peels for use directly or as additives for animal feeding.

III. MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The research was conducted at the Biochemical Laboratory of the Department of Animal Health and Production Technology, Kogi State Polytechnic, Itakpe Campus. It falls within the Guinea savanna of coordinates of Latitude 7.638^0 N and Longitude 6.335^0 E. Average temperature of 26.64 %, 1280 mm rainfall and 76.4 % relative humidity [12].

3.2 SOURCE AND COLLECTION OF PAWPAW PEELS

Pawpaw peels were collected from fruit vendors within the Polytechnic at season. They were sorted, washed in clean water and air dried for five days.

3.3 PREPARATION OF SUBSTRATES

Size reduction

The air dried pawpaw peels were then milled to obtain a fine homogenous mixture using a Perten laboratory mill 4100 of 0.8mm sieve size and stored in polythene bag until when needed for analysis.

Fungi selection and Spore Preparation

*Cordyces militaris*was identified, isolated and obtained from the culture bank of the Department of microbiology, Kogi State University Anyigba and maintained onpetri dishes of potato dextrose Agar (PDA), incubated at 25^0 C for 48 hours until mycelia colonized most of the plate's surface as described by P.A Pinto *et al.*[13].

Sterilization and inoculation.

The milled pawpaw peels(substrate)were moistened and about 80g each were weighed separately into 250 ml volumetric flasks in quadruplets and sterilized in autoclave at 121^0C for 15 minutes.After cooling, each bottle is inoculated at the centre with mycelia disc (5mm) of *cordyces militaris* and kept in a cupboard in the laboratory at 30^0C and 100% relative humidity. After 14 days of inoculation, the action of the fungi and the mycelia growth was terminated by oven drying at 50^0C for 36 hours, to a constant weight. The control (T1), has same samples, no inoculum of *c.militaris* but wereoven dried also to constant weight for chemical analysis.

3.4 DETERMINATION OF ANTI-NUTRIENTS CONTENT.

Phytic acid content in each feed sample was determined by the colorimetric method [14]. The absorbance of each sample was measured at 420 nm using spectrophotometer and the quantity of the anti-nutrients estimated from a standard curve obtained by plotting the concentration of the standard anti-nutrients concentration against the absorbance.

The follin-Denis spectrophotometric method as described byG. Lotfi [7] was used to determine the tannin content in the samples. The absorbance of the colour was measured at 760 nm wavelengths with the reagent blank set at zero, using GENWAY model 6500electronic spectrophotometer.

3.5DETERMINATION OF FUNCTIONAL CONSTITUENTS OF SAMPLES

The total phenolic content of samples was estimated using the Folin-Ciocalteu method following the procedure of W. Y Chuang *et al*. [10]. Briefly, the samples were deionized in water at 95^0C for 30 minutes and cooled to room temperature. Afterward, 1 mL of the solution was mixed with 5Ml of folin-ciocalteu reagent and 4 mL 7.4 % Sodium Carbonate. The mixture was incubated under room temperature for 30 mins, total phenol content and its derivatives (gallic acid, epigalocatechin, galocatechin, cafeic acid) were measured at 730 nm absorbance and expressed in quercetin equivalents.

3.6 PROXIMATE ANALYSIS

Unfermented and biofermented samples were subjected to proximate analysis for different chemical characteristics such as moisture, crude fibre, crude protein, fat and percentage ash contents according to standard procedures of AOAC [14].

3.7 DATA ANALYSIS

Data were analyzed with the GLM procedures of SAS as a completely randomized designed experiment using one way analysis of variance (ANOVA), considering fungi treatment performed in quadruplets as main effect. When F test is significant, ($P < 0.05$), tukey test examined the difference [15].

IV. RESULTS AND DISCUSSION

4.1RESULTS

Proximate composition of samples

Table 1 showed the proximate composition of untreated Pawpaw peels and*Cordyceps militaris*treated pawpaw peels. Pawpaw peels fermented by*C. militaris*inoculums recorded an increase in crude protein content and ash content over the control (T_1). There was also a significant decrease ($P < 0.05$) in crude fibre and moisture contentsof *C.militaris* treated samples compared to unfermented samples (T_1). Carbohydrate contents (Calculated by difference) and % fat (ether extract) recorded no concomitant and significant ($P > 0.05$) increase in fermented treatment compared to the control (T_1).

Table 1: Chemical Composition of untreated and *C.militaris* treated pawpaw Peels

(%)

Treatments	Moisture	Protein	Fat	Ash	Crude fibre	Carbohydrate
Pawpaw peel waste only (T_1)	4.38^{c}	4.89^{c}	1.78^{c}	3.31^{c}	13.00^{a}	76.01
Pawpaw peel + *Cordyceps militaris*(T2)	6.41^{a}	7.49^{a}	4.06^{a}	5.08^{a}	7.05^{c}	68.50
SEM	$\pm 0.26^{*}$	0.44^{*}	0.13^{*}	0.15^{*}	0.21^{*}	4.3^{NS}

a, b, c

Values with different letters within a column are significantly $P < 0.05$ different.

Quantitative and functional constituents of samples

The results of the contents of anti-nutritional factors and functional constituents in Pawpaw peel meal is presented in Table 2. It indicated that the tannin andPhytic acid contents in bio fermented treatment reduces significantly ($P < 0.05$) when compared to untreated samples. *C. militaris* treatment reduces tannin level by 48 % when compared to the control. Phytic acid level also reduces by 57%. Possibly, bio processing significantly ($P < 0.05$) reduces both tannin and Phytic acid concentrations of the samples. Tannin values ranges between 2.6 - 3.30.

The total phenol content ranged from 1.92 - 3.1 and significantly ($P < 0.05$) decrease in *C .militaris* treated pawpaw peel samples when compared to the untreated (control). Total flavonoids also ranged from 1.2 - 2.34 andshowed no significantly ($P>0.05$) difference between treatments.A significant ($P<0.05$) decrease in concentrations of phenol-like compounds (Gallic acid, Gallocatechin, epigallocatechin, catechin and caffeic acid)was observed in *C.militaris*treatedpawpaw peel samples compared to untreated sample. Similarly, values for cordyceptin, ergosterol and cell wall polysaccharides (mannan oligosaccharides) significantly ($P < 0.05$) increases in bio fermented samples.

Table 2: Content of functional constituents of unfermented and *C.militaris* fermented pawpaw peel meal.

Parameters	T_1	T_2	SEM
Tannin(%)	3.30^{a}	2.60^{b}	0.15*
Phytic acid(%)	1.40^{a}	0.60^{b}	0.05*
Functional components			
Cell wall polysaccharides			
β-glucan			
Mannan oligosaccharides	3.6	1.30	0.30 *
Ergosterol	3.0	2.20	0.20 *
Cordycepin (3'deoxyadenosine)	50.0	30.50	2.13 *
Crude triterpenes (mg/g DM)	6.25	5.05	0.31^{NS}
Total flavonoids (QE mg/g)	2.34	1.20	0.10^{NS}
Phenol -like compounds (µg/g DM)			
Gallic acid	116.2	104.0	2.50 *
Gallocatechin	1132.2	1034.0	0.01 *
Epigallocatechin	1491.3	1276.0	8.00 *
Caffeic acid	110.4	96.80	2.80 *
Total phenol(GAE mg/g)	3.11.	92	0.11*

a, b, c

Mean values on the same row with different superscripts differs significantly P < 0.05DM: dry matter; QE: Quercetin ; GAE: Gallic acid equivalent; * = significant; ^{NS}= not significant; (n = 4)

4.2 DISCUSSION

The general marginal increase in crude protein content of Pawpaw peels fermented by *C. militaris* may be attributed to the secretion of some extracellular enzymes (laccase, xylanase) into the Pawpaw peel substrates/mash by fermentating organisms whose proliferation, according to E.A Iyayi and Z. A Adeolu [16] form a complex in form of single protein that contribute to the nutrient content of the mash. Similarly, A. Rypacek and R.H Kartzman [17] raised the possibility of an increase in nitrogen content of incubated substrate which might be due to the presence of nitrogen fixing bacteria in the mash, a suggestion also supported by A. Pandey *et al.* [6]. On the contrary, G.M Walker and N. A White [18] considered fungi not to have ability to fix nitrogen and need to be supplied with nitrogen containing compound. Increased protein content during fungal treatment therefore, may be justified from literatures that most fungi species possess the ability to use inorganic nitrogen sources (Nitrate, Nitrite), degrade them to ammonium ions that can be assimilated into glutamine and glutamate thus, increasing the net content of nitrogen. The crude protein reported in this study may however, not be adequate enough to support the production of Monogastric animals but can serve as supplementary protein source in Monogastric diets. The level is similar to the 7% CP requirements for ruminants which will provide the required ammonia needed by rumen microorganism to support optimum microbial activity [7].

The reduction in moisture content of bio fermented sample could be attributed to the formation of fruiting bodies of microorganisms which utilizes nutrients as well as water for growth [17]. This is supported by the assertion made by J.A*Bentil et al.* [19] that water forms about 60% of the composition of microbial mash.

The concomitant increase in ash content in biodegraded treatment compared with the control suggested the enrichment of the mash by minerals present in the mycelium of fungi grown on the substrate. The result was similar to that reported by J.A*Bentil et al*. [19] who observed that fungi spraw have richer supply of minerals that is twice higher than in vegetable. The fruiting bodies of most fungi also contain about 10 % ash on dry matter basis which may be incorporated to the diets on decomposing. The ash values obtained for both raw and processed samples were higher than those reported by earlier researchers who documented 2.3 ± 0.11%. Geographical location, stage of maturity and soil type may be the cause of the variation in ash content. The ash content values obtained in this study is however, adequate to enhance or meet the mineral requirements of most livestock diets.

The decrease in crude fibre content in fermented samples recorded across *C. militaris* treatment could be as a result of extracellular enzymes secreted by fungus that biodegrades the cell wall components of plants into soluble units such as glucosidases, xylanases & peroxidases which reduces oligosaccharides to their monometric units [1]. This finding is similar to that reported by A .O Ojokoh [20] who also reported a reduction in crude fibre content of ground nut peels meal fermented by A*scomycetes* .However, the values documented by him were higher than the ones obtained in this study. The variations in values may be as a result of differences in strain of fungi used for fermentation process. Literatures reported better delignification of fibres in *Basidiomycetes* fungi than ascomycetes. The values obtained for bio fermented pawpaw peel samples were higher than the reported value for jack beans (3.4%), maize (2.0%) and guinea corn (3.5%) as documented by T. Annon [5] but, similar to conventional feedstuff like soya bean meal (6.5%). Thus, may tend to be advantageous to some Monogastric animals especially rabbits, since they have innate ability to tolerate and digest fibrous materials.

The reduction in values of anti-nutritional factors (Phytics& tannin) observed in this study may be as a result of heat treatment during sterilization. Studies have established that most anti-nutrients can be denatured by heat. Similarly, fungi from *Basiodiomycetes* and *Ascomycetes* families are capable of not only degrading lignocellulosics but, also denaturinganti-nutritional factors such as tannins, alkaloids, lectins and others that may be present in the agricultural wastes through extracellular enzymatic hydrolysis [9]. It is likely that the increase in anti-nutritional factors in the control when compared to bio fermented feed samples, shows that drying feedstuffs alone cannot decrease extractable tannin content of tree legumes [13]. The range of phytic acid from 0.60 to 1.40 for bio fermented groundnut peel samples recorded in this study is lower than the values reported for jack beans(4.2%) and most conventional feedstuffs.Therefore, the values for anti-nutrients recorded in this study was found to be within permissible range tolerated by livestock.It is obvious that bio treated pawpaw peel meal had significantly (P< 0.05) lower tannin and phytic acid contents and the products were better detoxified.

The significant decline in values for total phenol content and it's like compounds could suggested detoxification and break down of substrate by fermentating fungi through the enzymatic action of *C. militaris*. However, the high content of total phenol in untreated substrate is understandable as fungi by nature is rich in polyphenols as functional substance which have been harnessed in the production of enzymes, antioxidant and anti-inflammatory agents [21]. Furthermore, the increase observed for values of ergosterol, mannan oligosaccharides and cordyceptin in bio treated substrate informed of the enrichmentof the mash by fruiting

bodies and mycelia of *C.militaris.*This agrees with the findings of W.Y Chuang *et al*. [10] who reported 3'-deoxyadenosine to be the end product from break down of ATP in fungi cells. As such, the end product of fungi degradation is often energy and protein that binds to G-proteins (A1, A2A, A2B, A3)in tissues to up regulates pro-apoptosis genes and down regulates antioxidant gene expression. The surge in ergosterol in bio treated substrate might have been as a result of reduction in fat content of the substrate, as ergosterol has capacity to scavenge for fats and enhances Vitamin D secretion [21,11,4] .

The low values for contents and non-significant difference in treatments for triterpenes and total flavonoids is understandable as both are functional sterol metabolites commonly found in mushrooms at lower quantities depending on species and stages of development. V. K Pandey *et al*. [6] found that the flavonoid content of extracts from different stages of development in mushroom was low and no flavonoids detected in the fresh fruiting body and Primodium water extracts. However, flavonoids were found to be numerically significant in concentration in this study. The difference in observed reports could be due to absorbed nutrients and compounds from the medium in which they grow.

V.CONCLUSION

Data presented in this study show that there are differences within the fungi degradation mechanism and chemical composition of the substrates. *C.militaris* treatment was effective in enhancing efficient delignification process, which resulted in low crude fibre content, decline in anti-nutritional factors and secondary metabolites in pawpaw peels. Solid state fermentation therefore, increase the protein content and ash content of substrate. Thus, improving its nutritional qualities.Biodegraded pawpaw peels can serve as good source of animal feeds supplement. However, the effectiveness of fungi treatment depends on factors such as choice of fungi strain, chemical and structural features of the substrates and the culture condition which needs to be evaluated.

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