Effects of Mixed Fungal Fermentation in Improving the Nutritional Value of Maize (Zee Mays) Cobs

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Abstract

Background

Fungal fermentation could provide a key opportunity for achieving great benefits of biomass utilization through bioconversion into the simple digestible nutrients and greatly improve nutritive value.

Objective

Mixed fungal fermentation of maize cobs was carried out to determine the effects on nutrients and antinutrients compositions.

Methods

Maize cobs samples were pretreated with sodium hydroxide and solid-state fermentation (SSF) was carried with single and a consortium of four fungi; Lachnocladium flavidum Aspergillus niger, Trichoderma reesei, and Lenzites betulina for 7 days under standard conditions. Proximate composition, amino acid, mineral and antinutrient analysis were conducted on fermented and unfermented cobs. Differences and variabilities in groups were analyzed with the aid of Statistical software Package for the social sciences (SPSS) version 24.

Results

Fermentation of cobs was found to have significantly increased the protein (9.10±0.85g/100g) and ash contents (5.60±0.05g/100g), while decreasing the fiber content (21.00± 0.10g/100g). The mineral levels were significantly ($p<0.05$) increased in potassium, sodium and calcium ions. The levels of some antinutrients were significantly reduced ($p<0.05$); phytate (8.91±0.50mg/100g), saponin (1.84±0.26mg/100g) and flavonoids (1.99±0.12mg/100g). The total amino acid content increased in most fermented cultures, with mixed culture of L. flavidum/ T. reesei having the most significant increase. Amino acid analysis revealed significant increases in essential amino acids Histidine, Leucine and Valine while Alanine, Aspartic and Glutamic acid were among the noticeable increases in non-essential amino acids.

Conclusion

The effects of fermentation on the nutrient and antinutrient composition of maize cobs have been demonstrated. With further studies, maize cobs can serve as a good addition in animal feed production.

Keywords: *Maize cobs, fungi, fermentation, nutrients, antinutrients*

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INTRODUCTION

Significant environmental challenge is posed by wastes generated from the agricultural sector due to economic growth and the rapid increasing population (1). Great attention has been given to the potential for obtaining valuable products from agricultural residues. Maize being one of the very important and common cereal crops in sub-Saharan Africa and result into a large quantum of maize waste estimated at 18-20% per ton (2).

Most biomass have a highly complex recalcitrant structure which is the major barrier to their utilization as nutrient substitutes in animal nutrition and feed

production (3). Digestion by microorganisms and their enzymes in most animals is prevented by the complex and interwoven matrix of cellulose, hemicellulose, and lignin. This matrix contributes to a very high percentage of fibre in animal feeds, thus leading to a poor palatability. The agroresidues are rarely utilization in animal production due to the poor digestive utilization rate of direct feeding (4). Thus, the aims of a successful pretreatment are targeted towards reducing biomass crystallinity, improve porosity, reducing lignin content, avoid developments of inhibitors, and degradation of simple sugars, require lower energy consumption, and more cost effective. In biological pretreatment, the utilization of microorganisms, mostly fungi and bacteria, is employed (5). These organisms produce different types of extracellular degradative lignocellulolytic enzymes which breakdown for the degradation of lignocellulosic biomass in the course of their growth (6). As early as the beginning of the 20th century attempts to improve the nutritional value of crop residues through pretreatment has been undertaken (7). Over the years, continuous discovery and identification of lignocellulolytic microorganisms mainly fungi and bacteria have occurred and the necessity for their extensive study (8).

In producing fungal enzymes from lignocellulosic residues, Solid state fermentation has been considered to be the best, due to its lower cost of operation and capital investment (9). Solid State Fermentation (SSF) provides an environment with conditions closer to the natural habitat of fungi thus they find it more conducive to grow and produce enzymes and valuable compounds or metabolites which may be produced in lesser yield under submerged fermentation conditions (9). Fungal species produce hydrolytic enzymes, which digest lignocellulosic residues, and also produce variety of metabolites like micronutrients and the microbial population equally serves as whole cell protein. Co-culture with two or more fungi has been documented to enhance multiple enzyme production in SSF, especially when lignocellulosic residues are used as the substrates. In most cases, mixed cultures have proved to be advantageous due to the complexity of these substrates which often requires the action of multiple enzymes and the interaction among different microbes to accomplish the biodegradation (10). This research is thus designed to exploit the advantages of mixed culture fermentation in improving the nutritive value of maize cobs using a consortium of four fungal species (Lachnocladium flavidum Aspergillus niger, Trichoderma reesei, and Lenzites betulina). These are good lignocellulolytic fungi that produce several enzymes to degrade biomass (11).

MATERIALS AND METHODS

Collection and preparation of sample

One and half kilogram of maize cob residues obtained from Samaru farm around Zaria, Kaduna state were air dried to constant weight at 500C and milled to a particle size of 2 -3mm. This was stored in clean polyethene bags for subsequent use. One (1kg) of maize cob was mixed in 4.5L 5% NaOH solution to hydrolyze the sample, at ambient temperature for 1hr. This was neutralized with HCl and subsequently washed with distilled water and the residues oven-dried to constant weight at 500C.

Test organisms and growth of inoculums

Four fungi with lignocellulosic degrading potentials, Aspergillus niger, Lachnocladium flavidum, Trichoderma reesei and Lenzites betulina were stored in PDA slants at 40C. The growth medium was prepared with 0.3% glucose, 2ml distilled water, 0.1% potassium dehydrogenate sulphate, 1% corn steep liquor and finally 0.2% sodium nitrate. After inoculation and substantial growth of medium at room temperature for 5-6 days, the culture was washed with sterile distilled water to serve as the inoculums.

Solid-state fermentation

Biological treatment of maize cob followed solid-state fermentation for a period of 9(nine) days and the fermentation media composition was as described by Ali et al. (12). Organisms were cultivated in mineral salt- agrowastes media containing: CaCl2 0.5 g/L, COC12.6H20 0.0067 g/L, (NH4)2SO4 10.5 g/L, MgSO4.7H2O 0.33 g/L, FeS047H2O 0.013 g/L; MnSG4. H2O 0.004 g/L, ZnS04.7H20 0.004 g/L, yeast- extract, 0.5 g/L and l00g of the maize cobs. The initial pH was adjusted to 5.0 after autoclaving for 15minutes at 121 °C. After sterilizing the medium, it was inoculated with 5 ml of spore suspension, and incubated. Another 50g was weighed and subjected to the same conditions without addition of inoculums to serve as control. Samples were kept in clean polythene bags prior to analysis.

Biochemical analysis

The proximate composition of the fermented samples was carried out, where samples were analyzed for moisture, dry matter, crude protein, ether extract(lipid), Nitrogen free

extract(carbohydrate), fiber, organic matter and mineral matter (ash) using AOAC (13) methods. While the unfermented samples serve as the control. Sodium (Na) and potassium (K) analysis were carried out using flame photometer. Atomic absorption spectroscopy (AAS) was used to determine Calcium (Ca), Iron (Fe), Manganese (Mn), Zinc (Zn), and Magnesium (Mg). The amino acid profile of the samples was determined as described by Speckman et al (14) using Technicon Sequential Multi-sample (TSM) Amino Acid Analyzer. Antinutrients components determined include tannin, using Trease and Evans (16), saponin (14), phytate and oxalate using AOAC methods (13) and cyanide using modified method of Ikediobi (17) carried out at the MultiUser laboratories and the Food Technology Laboratory, institute of Agricultural research Ahmadu Bello University Zaria.

RESULTS

The effect of fungal solid-state fermentation using mono and co-cultures on proximate composition of maize cobs is shown in Table 1. The dry matter, ether extract and the nitrogen free extract were not significantly affected (P>0.05) by the fermentation process, as no statistical difference was observed between the unfermented sample (control) and the several mixed and mono-culture fermenting set ups. The crude protein, crude fiber and ash content were all significantly (P<0.05) affected by the fermentation process. The mixed culture of L. flavidum / T. reesei had the highest protein content of 9.10±0.85g/100g, while L. flavidum had the highest effect on the crude fibre reduction (29.00±1.00g/100g) L. flavidum and A. niger mixed culture exerted the highest effect on increasing the ash content, with 5.60±0.50 mg/100g.

Effect of solid-state fermentation using fungal mono and co-cultures on mineral composition of maize cobs is shown in Table 2. Iron, copper, zinc and magnesium were not significantly (P> 0.05) affected or increased by the mono and co-culture fermentation, while the calcium, sodium and potassium were significantly (P< 0.05) increased. Mixed culture of Lach. flavidum /T. reesei had the most significant increase in calcium with 2.51±0.09 mg/100g and potassium (4.40±0.60mg/100g), while the mixed culture of L. flavidum / A. niger had the most significant increase (P< 0.05) in sodium (1.43±0.08mg/100g).

Table 1: **Effect of fungal fermentation on proximate composition of maize cobs**

	DRY MATTER (g/100g)	CRUDE PROTEIN (g/100g)	CRUDE FIBER (g/100g)	ETHER EXTRACT (g/100g)	ASH CONTENT (g/100g)	NITROGEN FREE EXTRACT (g/100)
Control	90.00±2.00^{a}	4.90±0.50^{a}	41.00±1.50^{d}	9.56±0.50^{a}	2.90±0.10^{ab}	48.00±2.00^{a}
Lachnocladium flavidum	92.10±2.30^{a}	9.0±0.70^{b}	29.00±1.00^{a}	9.90±0.40^{a}	3.50±0.30^{c}	47.00±3.00^{a}
Aspergillus niger	90.30±1.95^{a}	8.10±0.90^{b}	31.00±1.50^{ab}	9.10±0.65^{a}	3.40±0.40^{bc}	49.00±1.00^{a}
Trichodermer reseei	91.00±2.50^{a}	8.00±0.60^{b}	35.00±1.30^{c}	10.20±0.80^{a}	3.60±0.20^{c}	51.00±2.00^{a}
L. flavidum & A. niger	90.20±1.60^{a}	8.90±0.75^{b}	30.00±0.50^{ab}	9.0±0.40^{a}	5.60±0.50^{d}	51.00±1.50^{a}
L. flavidun & T. reseei	92.20±2.60^{a}	9.10±0.85^{b}	32.00±1.00^{b}	9.70±0.60^{a}	3.90±0.30^{c}	50.50±1.50^{a}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05.

Table 2: Effect of fungal fermentation on mineral composition of maize cobs

	Iron (mg/100g)	Copper (mg/100g)	Zinc (mg/100g)	Magnesium (mg/100g)	Calcium (mg/100g)	Sodium (mg/100g)	Potassium (mg/100g)
Control	3.50±0.24^{ab}	0.100±0.010^{a}	1.60±0.09^{a}	0.070±0.001^{a}	1.21±0.15^{b}	0.67±0.02^{a}	2.00±0.10^{a}
Lachnocladium flavidum	4.50±0.30^{d}	0.096±0.010^{a}	1.90±0.07^{b}	0.085±0.005^{cd}	1.98±0.05^{c}	1.11±0.05^{d}	3.10±0.20^{b}
Aspergillus niger	4.10±0.25^{cd}	0.260±0.020^{d}	2.50±0.10^{c}	0.088±0.007^{d}	0.94±0.06^{a}	0.81±0.09^{b}	3.50±0.50^{bc}
Trichodermer reesei	4.00±0.20^{c}	0.200±0.015^{c}	2.10±0.20^{b}	0.079±0.005^{bc}	1.88±0.22^{c}	0.99±0.07^{c}	3.90±0.60^{cd}
L. flavidum. & A. niger	4.30±0.30^{cd}	0.160±0.020^{b}	1.90±0.15^{b}	0.080±0.001^{bc}	1.24±0.16^{b}	1.43±0.08^{e}	3.20±0.30^{bc}
L. flavidum. & T. reesei	3.90±0.20^{bc}	0.110±0.010^{a}	2.90±0.30^{d}	0.077±0.002^{b}	2.51±0.09^{d}	1.00±0.05^{c}	4.40±0.60^{d}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05.

Table 3, shows the effect of fungal mono and co-culture solid state fermentation on the essential amino acids composition of maize cobs (g/100g). Increases were observed in all amino acids however the most significant increase (P<0.05) were observed in histidine, leucine, phenylalanine, valine and isoleucine. Cultures fermented with *L. flavidum* and its co-cultures with *T. reseei* were found to be most influential in increasing the amino acid composition. Histidine increased from 0.029±0.001g/100g in the control to 0.790±0.035mg/100g in cobs fermented with *L. flavidum / T. reesei*. Alanine (0.39±0.02g/100g), aspartic acid (1.70±0.01g/100g) and glutamic acid (2.21±0.02g/100g) are among the noticeable increases in non-essential amino acids shown in Table 4. Monoculture of *L. flavidum* fermentation increased the non-essential amino acid contents most significantly(P<0.05).

Table 3: Effect of fungal fermentation on Essential Amino Acids composition of maize cobs (g/100g)

Control	0.029±0.001^{a}	0.049±0.002^{a}	1.41±0.02^{b}	0.089±0.004^{b}	0.678±0.080^{a}	0.211±0.020^{a}	0.048±0.003^{a}	1.25±0.05^{ab}	1.11±0.07^{b}
L. flavidum	0.074±0.002^{b}	0.062±0.002^{d}	2.59±0.27^{d}	0.091±0.004^{b}	1.061±0.060^{d}	0.544±0.006^{d}	0.092±0.005^{c}	4.22±0.08^{e}	1.83±0.07^{d} *
A. niger	0.065±0.002^{b}	0.056±0.002^{c}	2.61±0.16^{d}	0.055±0.001^{a}	0.899±0.040^{c}	0.451±0.010^{c}	0.059±0.003^{b}	2.23±0.20^{c}	0.98±0.05^{a}
T. reseei	0.045±0.001^{ab}	0.051±0.002^{ab}	0.99±0.06^{a}	0.110±0.003^{d}	0.71±0.020^{ab}	0.390±0.010^{b}	0.088±0.004^{c}	1.39±0.05^{b}	1.79±0.07^{d}
L. flavidum & A.niger	0.026±0.019^{a}	0.055±0.001^{bc}	1.55±0.01^{b}	0.099±0.002^{c}	0.790±0.030^{b}	0.460±0.020^{c}	0.062±0.002^{b}	1.14±0.02^{a}	1.28±0.05^{c}
L. flavidum &	0.790±0.035^{c}	0.092±0.004*	2.34±0.08^{c}	0.150±0.004*	0.770±0.030^{b}	0.597±0.012*	0.061±0.003^{b}	3.48±0.09^{d}	1.91±0.02*

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05.

Table 4: Effect of fungal fermentation on Non- Essential Amino Acids composition of maize cobs (g/100g)

	Glycine	Serine	Proline	Tyrosine	Alanine	Cysteine	Aspartic acid	Glutamic acid
Control	0.20±0.02^{a}	0.039±0.003^{a}	0.021±0.001^{a}	0.097±0.003^{a}	0.17±0.02^{a}	0.039±0.002^{b}	0.91±0.02^{a}	1.66±0.01^{a}
Lach. flavidum	0.43±0.01^{d}	0.076±0.001^{e}	0.042±0.002^{b}	0.199±0.004^{c}	0.25±0.01^{b}	0.058±0.002^{c}	1.70±0.01^{d}	2.55±0.02^{c}
A. niger	0.22±0.01^{a}	0.049±0.002^{b}	0.039±0.001^{b}	0.100±0.030^{a}	0.25±0.01^{b}	0.045±0.001^{b}	1.35±0.01^{b}	2.40±0.01^{b}
T. reseei	0.42±0.02^{d}	0.077±0.003^{e}	0.360±0.020^{c}	0.990±0.020^{d}	0.31±0.02^{c}	0.028±0.001^{a}	1.29±0.02^{c}	2.02±0.01^{b}
L. flavidum & A. niger	0.32±0.01^{c}	0.058±0.002^{c}	0.044±0.002^{b}	0.110±0.010^{a}	0.29±0.02^{c}	0.040±0.010^{b}	1.02±0.01^{b}	1.99±0.01^{b}
L. flavidum & T. reseei	0.29±0.02^{b}	0.065±0.003^{d}	0.045±0.003^{b}	0.150±0.010^{b}	0.39±0.02^{d}	0.055±0.002^{c}	1.32±0.02^{d}	2.21±0.02^{d}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05.

The effect of solid-state fermentation using fungal mono and co-cultures on some antinutrient composition of maize cobs is shown in Table 5. Tannin was not significantly (P>0.05) affected at P>0.05 while significant (P<0.05) reduction was observed in cyanide, phytate, saponin and oxalate contents. The co-culture of *L. flavidum* and *T. reesei* had the most significant effect on cyanide (0.39±0.01ppm) and saponin (1.84±0.26mg/100g) while single culture of *Lach. flavidum* had the most significant (P<0.05) effect on oxalate (0.09±0.01) and phytate (8.91±0.50) contents.

DISCUSSION

Fungal fermentation was found to have affected the crude protein, crude fiber and ash significantly (P>0.05). Increases in growth of fermenting organisms, the proliferation of the fungi in the form of single cell proteins and their constitution into

Table 5: Effect of fungal fermentation on some antinutrient composition of maize cobs

	Cyanide (ppm)	Phytate (mg/100g)	Tannin (mg/g)	Saponin (mg/100g)	Oxalate (mg/kg)	Flavonoids (mg/100g)
Control	2.31±0.08^{f}	20.98±2.10^{b}	0.022±0.001^{b}	10.50±0.96^{d}	0.56±0.03^{f}	8.53±0.40^{c}
Lachn. flavidum	0.66±0.04^{b}	8.91±0.50^{a}	0.028±0.001^{c}	3.13±0.45^{c}	0.09±0.01^{a}	3.34±0.20^{b}
A. niger	1.21±0.04^{c}	11.34±0.92^{a}	0.013±0.001^{a}	2.67±0.30^{b}	0.38±0.01^{e}	2.93±0.18^{b}
T. reseei	1.58±0.09^{e}	9.21±1.00^{a}	0.026±0.001^{c}	2.01±0.25^{ab}	0.25±0.01^{c}	3.12±0.60^{b}
L. flavidum & A. niger	1.39±0.08^{d}	9.11±0.79^{a}	0.011±0.001^{a}	2.98±0.16^{bc}	0.18±0.01^{b}	2.88±0.70^{b}
L. flavidum & T. reseei	0.39±0.01^{a}	11.25±0.90^{a}	0.023±0.001^{b}	1.84±0.26^{a}	0.30±0.01^{d}	1.99±0.12^{a}

Values are Mean ± SD, Values with different superscript letter down the column are significantly different at p <0.05.

structural proteins could all have resulted in the observed increase in protein contents. This is in addition to the possible synthesis of enzymes arising from rearrangements of components after most of the structural components are degraded thus a decrease in carbon ratio in the total mass (18). The utilization of carbohydrates as energy sources by the fermenting organisms leads to a concentration of nitrogen in the fermented sample thus another contributing factor to the increase in the proportion of protein (19). A significant decrease was observed in the crude fiber in the current research, from cultures fermented with L. flavidum and its mixture with A. niger. Microorganisms in the course of fermentation secrete hydrolytic enzymes which proportionally lead to a decrease in crude fiber. Crude fibre constitutes on the average 30 to 36% of plant biomass. (19). This eventually leads to aid in the degradation of recalcitrant compounds and an increase in the utilizable compounds. (20). A 17 - 30% reduction was reported by Kuo et al, (22) in crude fiber, following fermentation with Lathyrus sativas. A higher percentage observed with L. flavidum in this study could be as a result of the higher lignolytic activity of the basidiomycetes group of white rot fungi as compared to the other group of hydrolytic fungi. Fermentation accounts for close to 12.5% loss of substrate in dry matter after utilization of the cellulose, hemicellulose and other carbon sources for the growth and metabolism with the end results being protein and carbon dioxide. Maize cob ash has been previously reported (23) to contain nutrients such as phosphorous, calcium and magnesium, potassium. The ash content in the present study agreed with the findings of Enyisi et al. (24), Increase in ash content following fermentation, could be a result of increase of mineral composition of substrates. These increments could possibly be as a result of the enrichment of mycelia of fungi. (25). The increase in mineral contents support the crude ash values, and supports the claim on the ability of fermentation to improve the mineral and thus content bio-availability of fermented samples. (26).

Among the nutritional benefits of fungal fermentation of agricultural biomass the production of amino acids and other primary nutrients (27). Mono and co-culture Fungal treatment of corn cobs in the current study led to slight increase in most amino acids with most of the significant changes observed in the essential amino acids; histidine, leucine, phenylalanine, valine and isoleucine credited to L. flavidum and its co-cultures with T. reesei. Alanine, aspartic acid and glutamic acid are among the noticeable increases in non-essential amino acids attributed mostly to mono-culture of L. flavidum. The different variations in the changes of essential amino acids during the fermentation process might be due to variability in the fungal inoculum itself (28). Increased microbial growth during fermentation process often leads to increase of own amino acid content, which possibly might have reflected in the fermented samples. Furthermore, the changes in other nutrients and dry matter loss could also be a possible reason for the increase of amino acids observed in the present study. Specific amino acids could be utilized by certain fungi for growth and the production of enzymes which could result in the variation of individual amino acid contents (29). As reported by Malomo et al. (30), total free amino acid of corn and acha increased during fermentation and was strongly attributed to the proteolytic activities of the fermenting organisms.

Agro-residues are generally rich in anti-nutritional factors, particularly tannins (31). The composition of the solid substrate medium is usually modified by the fermentation process through the production of enzymes and several metabolites. (33). The effect of fermentation on some antinutrient composition of maize cobs was investigated in the current study. Tannin was not significantly affected at $P>0.05$ while significant changes were observed in cyanide, oxalate, saponin and phytate after 8days of fermentation. Most agroresidues from cereals and legumes are known to naturally contain phytate. When above a certain level, phytates reduce the availability of minerals and solubility, as well as the functionality and digestibility of proteins (34). There was significant reduction in the phytate level in most samples fermented in this study. Phytase which degrades phytic acid is known to be produced by a wide range of microflora (35) which may also contribute partly to the reduction in phytic acid content in the fermented cobs. (36). The fungal treatment reduced the level of cyanide in the corn cobs to almost non-detectable level. The activity of the cellulosome components which include hydrolase, -glucosidase could be responsible for this reduction. -glucosidase is known to catalyze the hydrolysis of dhurrin, a major cyanogenic glycoside in sorghum and related cereal products. However, corn cobs have not been reported to contain high amount of cyanogenic glycoside (37).

CONCLUSION

The improvement in nutritional quality and compositional changes associated with the SSF fermentation of maize cobs using single and mixed cultures of several lignocellulolytic fungi has also been demonstrated. Significant increase in the crude protein and ash contents and a decrease in the fiber content. The antinutrients phytate, saponin and flavonoids were significantly reduced. The total amino acid content increased Amino acid analysis revealed different percentage increases in some essential and non-essential amino acids.

Recommendation

With further studies, on formulated feeds and animal trials, fungal fermented maize cobs can serve as a good potential energy substitute addition to complementing other limiting nutrients in animal feed production.

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