IJABR Vol. 5(1): 82 - 89 (2013)

Effect of Natural and Combined Fungal Fermentation on Phytate, Tannin and Some Mineral Contents of Corn cobs

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Submitted: March 7; Accepted: December 16, 2013; Published: January 14, 2014.

ABSTRACT

In an effort to reduce competition for our various grains between highly populated mankind and animals; an alternative food source is needed to be sought from agricultural waste for animal feeds. As such, the antinutrients; Phytate, Tannin and some mineral contents of the fermented corn cobs sample were evaluated. Three samples of corn cobs A, B and C were considered; sample A was unfermented , sample B was naturally fermented and sample C was fermented with a mixture of four fungi species namely, *Aspergillus niger*, *Penicillium*, *Trychoderma* and *Aspergillus flavus*, for 72hours. The phytate and tannin contents of the naturally fermented samples decreased significantly ($p<0.05$) compared to that of Control. However, only phytate contents of the combined fungal fermented sample showed significant decrease ($p<0.05$) contrary to its tannin contents with high value. There was also decrease in K content of the sample that was fermented with combined fungi contrary to other mineral contents of both fermented samples that showed significant increase compared to that of unfermented sample. However, Cu and Co were not detected in all the samples. It can be concluded from the study that natural and combined fungi fermentation can be used to reduce phytate while combined fungi species fermentation shows negative effect on tannin contents of the corn cobs waste. It can also be deduced from the study that both fermentation process are capable of improving some mineral contents of the corn cobs maize wastes. The reduction of some of these antinutrients and improvement of some of these mineral contents can make the corn cobs waste to be a better candidate for animal feeds.

Keywords: Anti-nutrient, fermentation, fungi, corn cobs, minerals

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INTRODUCTION

In most developing countries, majority of the people depend mainly on cereal grains as their staple food, due to limited income and high prices of animal feeds. Maize or corn (*Zea mays* L.) is an important cereal in Nigeria and other parts of West and Central Africa (Oladeebo and Ezekiel, 2006). According to FAO data, as reported by International Institute of Tropical Agriculture (2009), the area planted to maize in West and Central Africa alone increased from 3.2 million hectares in 1961 to 8.9 million in 2005. This phenomenal expansion of land area devoted to maize cultivation resulted in increased production from 2.4 million metric tonnes in 1961 to 10.6 million metric tonnes in 2005.

Maize cob is the central rachis to which the grains are attached and which remains as an agro-industrial waste after threshing. About 180.0kg of corn cobs are obtained from each tone of maize (Kent, 1990). Maize cob residue is an organic material consisting primarily of lignocelluloses and other extractives (Singh *et al.*, 1983) and hence subject to microbial degradation like other organic plant wastes. Therefore, its residues constitute major health and aesthetic environmental problems because of its disposal by dumping and burning. The dumps may serve as breeding sites for small animals of medical and non-medical importance while burning might produce ozone-depleting gases. Therefore, bioconversion is an alternative for productive utilization of discarded maize cobs. Recently, fermentation of the agricultural wastes such as maize cobs with fungi has gained considerable attention because it is necessary to increase the world food and feed supplies especially those high in protein and most of the nutritionally valuable metals with small amount of anti-nutrient content. This study thus aimed at useful microbial conversion of agricultural wastes to energy rich animal feeds and subsequent pollution Control (Zucker, 1978).

The presence of anti-nutrients is equally a major problem facing proper utilization of the nutrients in many plant seeds. Insoluble oxalate, phytate and tannins (particularly the condensed tannins) have been reported to be heat stable (Reddy and Sathe, 2002) and are therefore not eliminated completely by heat during processing. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. The astringency from tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine (McGee, 2004). Generally, if ingested in excessive quantities, tannins inhibit the absorption of minerals such as iron which may, if prolonged, lead to anemia (Brune*et al.*, 1989). This is because tannins are metal ion chelators, and tannin-chelated metal ions are not bioavailable. Many plants employ tannins to deter herbivores. Animals that consume excessive

amounts of these plants fall ill or die. The lethal dose is said to be around 6% of the animal's body weight. Humans would usually find the bitter taste of foods containing high amounts of tannins unpalatable. Phytate is the principal storage form of Phosphorus in many plant tissues, especially bran and seeds (Klopfenstein *et al*., 2002). Phosphorus in phytate form is, in general, not bioavailable to non-ruminant animals because they lack the digestive enzyme phytase, which is required to separate phosphorus from the phytate molecule. Phytate in plants are usually chelated with cations, protein and (or) starch and this chelated form called phytin constitutes between 1 and 3% by weight of many of the cereals and oilseeds used in animal feeds (Cheryan, 1980). It also acts as an acid, chelating the vitamin niacin, which is basic, causing the condition known as pellagra. Fermentation gradually changes the characteristics of the food by the action of enzymes, produced by some bacteria, moulds and yeasts. There are a number of roles that micro-organisms play in food processing, which can be either positive or negative. The negative effects include spoilage of food products and contamination by pathogenic micro-organisms (Bray, 1999). The positive effects are generally regarded as part of the fermentation process namely product preservation, flavour development and reduction of antinutrients (Deacon, 2005).

Due to the environmental hazards usually associated with the disposal of maize wastes in agrarian communities. It is therefore necessary to convert some of this corn wastes to useful materials such as animal feeds, which will also go a long way in reducing competition for maize grains and other cereals by highly populated mankind and animals.

MATERIALS AND METHODS

Source of Corn Cobs

Two kilogram (2kg) corn cobs were collected from Oba Ile in Akure North Local Government, Ondo State, Nigeria in the month of June 2004. After threshing of the maize grains, the cobs were washed, dried and pulverized with pestle and mortar. The fungi used for the study (*Aspergillus niger*, *Penicillium*, *Trychoderma* and *Aspergillus flavus*) were collected from Department of Microbiology, Federal University of Technology Akure (FUTA), while the experimental procedures were carried out in the Department of Biochemistry, Federal University of Technology Akure (FUTA), Ondo State Nigeria.

Preparation of Nutrient Solution

The nutrient solution for proper growing of fungi was prepared by weighing the following salts: 27g of Ammonium sulphate, 15g of Potassium hydrogen phosphate and 8.1g of urea. The salt mixtures were dissolved in little quantity of deionized water and made up to 600ml solution.

Preparation and Fermentation of the Corn Cobs

The preparation and fermentation of the corn cobs were carried out as was reported by Oboh and Akindahinsi (2003). The powdered corn cobs were first boiled for 45minutes with deionized water, sieved and dried in a drying cabinet 35g of each sample A, B and C was weighed. Samples B and C were placed in fermentation tank, 200ml of deionized water and 20ml of nutrient solution were added. The Fungi *Aspergillus niger*, Penicillium, *Trychoderma* and *Aspergillus flavus*, grown in petri dish cultured media were washed to sample B while sample C was left to be naturally fermented. After 72hours of fermentation, the fermented samples were washed with deionized water and dried .

Determination of Phytate

The Wheeler and Ferrel (1974)method was used to determine the phytate content. This method relies on the solubilization of phytate by dilute acid and the subsequent precipitation of the phytate with ferric ion (Fe^{3+}). Four grams (4g) of the sample was soaked in 100ml of 2% HCl for 3 hours, and then filtered. 25ml of the filtrate was dispensed into a conical flask and 5ml of 0.3 ml ammonium thiocyanate solution was added as indicator. Thereafter, 53.5ml distilled water was added to the mixture to give it a proper acidity and this was titrated with standard iron III chloride solution, which contained about 0.00195g of iron per millilitre (ml), until a brownish-yellow colour persisted for 5min.

Determination of Tannin and Minerals

The tannin content of fermented and unfermented samples were determined using the method of Makkar*et al.* (1993). This is based on the ability of tannin-like compounds to reduce phosphotungstomolybdic acid in alkaline solution to produce a highly blue colour solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in Spectrophotometer 725nm. The mineral (Ca, Fe, Cu, Zn, Mg and Co) contents were determined on aliquots of the solutions of the ash by established flame atomic absorption spectrophotometer procedures, using a Perkin-Elmer atomic absorption spectrophotometer (model 372), while Na and K were analyzed with flame photometer.

Statistical analysis

To test the level of significance, data were subjected to Analysis of Variance (ANOVA). The means were separated using Duncan Multiple Range Test (DMRT) and $P<0.05$ value was considered significant.

Table 1: Tannins and Phytate contents of the corn cobs Samples	Tannins Quantity(µg/100g)	Phytate Quantity(mg/kg)
A	558.33 ± 38.18[b]	300.86 ± 32.56[c]
B	758.33 ± 14.43[c]	131.62 ± 32.57[a]
C	466.67 ± 14.43[a]	150.43 ± 32.57[b]

All values are mean ± SD, and when followed by same superscripts in the same column are not significantly different at $p > 0.05$.

NOTE: A=unfermented corn cobs; B= fermented corn cobs with combined fungi; C=naturally fermented corn cobs.

Table 2: Minerals contents in part per million (ppm) of the corn cobs

	Fe	Cu	Zn	Mg	Ca	Na	K	Co
A	71.58 ±1.4	N.D	275.00±1.6	163.61±1.8	558.60±2.3	725.04±2.7	402.00±2.9	N.D
B	107.92±1.3	N.D	394.77±1.5	289.41±2.2	653.92±1.8	797.13±3.8	377.26±2.1	N.D
C	89.26±2.1	N.D	417.16±2.1	278.88±3.0	810.42±2.6	673.28±3.8	721.41±5.7	N.D

All values are mean ± SD of the mineral contents.
NOTE: A, unfermented corn cobs; **B**, fermented corn cobs with combined fungi; **C**, naturally fermented corn cobs; N.D, Not detected

RESULTS AND DISCUSSION

A significant reduction ($p<0.05$) in Phytate was observed in both fermented samples compared to Control. The observation might be as a result of phytases enzymes produced by the fungi which are known to degrade phytate and hydrolyzes phosphate groups from the phytin molecule, potentially making the hydrolyzed Phosphorus from within the phytin available to the animals (Makkar*et al*., 1993); and *A. niger* had been reported to produce such an enzyme (Applegate *et al*., 2003). Phytic acid has a strong binding affinity to important minerals such as Calcium, Magnesium, Iron, and Zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be biologically unavailable. The mineral contents of the fermented sample increased significantly as compared to the unfermented sample while Cobalt and Copper were not detected in all the samples. This might be as a result of the availability of some metals in the fermented samples especially the divalent metals such as Ca, Fe, Mg and Zn as a result of reduction of phytate content which might have initially bound to them prior to fermentation. This action of binding of phytate to such metals can contribute to mineral deficiencies in organisms whose diets rely on these foods for their mineral intake (Brune *et al*., 1989). In addition, the mineral contents of the microbes used in fermentation might have contributed to the increment of some elements in the fermented samples.

Tannins are known to undergo hydrolysis by acids, bases or some hydrolytic enzymes. Therefore, the hydrolytic enzymes produced by *A. niger* that might be present in naturally fermented corn cobs might be responsible for degradation of the tannin content (especially, pectin which could be degraded by pectinases) of the fermented sample. Contrarily, significant increase of tannin contents in combined fungi fermented corn cobs was observed when compared to other samples despite the presence of *A. niger* among the fungi used. Nevertheless, the activities of *A. niger* hydrolytic enzymes might have been hindered by the metabolic activities of other fungi present in the combined fungi fermented samples. Tannins have been shown to precipitate proteins (Hurrell, 2003), which inhibits in some ruminant animals the absorption of nutrients from high-tannin grains such as sorghum. Tannins also interfere with iron absorption through a complex formation with Iron when it is in the gastrointestinal lumen and this decreases the bioavailability of Iron (Reed, 1995). In sensitive individuals, a large intake of tannins may cause bowel irritation, kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain. Hence, reduction in the level of tannins by the fermentation process is in agreement with earlier observations (Obiazoba, 1998; CFP, 1973). The significant increase of some minerals in the fermented samples compared to that of Control might be as a result of inactivation of the phytates and tannins as was earlier discussed.

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