Effect of Solid State Fungal Fermentation on the Proximate and Antinutritive Composition of *Adansonia digitata* Seed

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Abstract: The seed of *Adansonia digitata* was fermented with the aim of producing additional plant protein food and feed. The seed was subjected to natural fermentation for 120 h under laboratory condition. Nine moulds and two yeasts were isolated and characterized macroscopically and microscopically as *Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Penicillium chrysogenum, Mucor racemosus, Mucor hiemalis, Rhizopus stolonifer, Alternaria tenuis, Scopuloriopsis brevicaulis, Saccharomyces cerevisiae and Schizosaccharomyces pombe*. Spores of isolated fungi were used as starter cultures in the fermentation of the seed using solid state fermentation method for 120 h. The fermented products were analyzed for proximate and antinutrient content using standard methods. The result showed a significant increase ($p < 0.05$) in crude protein, total ash and carbohydrate but decreased significantly ($p > 0.05$) in crude fat and crude fibre. The antinutrients in form of total tannins, saponins, oxalate and phytate content were significantly decreased after fermentation. From the findings in this research it was concluded that fermented *A. digitata* seed can serve as additional plant protein food source in food and feed formulation for livestock.

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INTRODUCTION

The growing concern for the acute food shortages for the world's expanding population has led to the exploitation of non-conventional food sources as potential alternatives[1]. The solution to the food problem must be sought through a combination of all available sources. Due to the increasing demand for protein and energy to support the ever increasing world population, efforts are been directed at exploring new and nonconventional sources of food that grow in the arid and semiarid land regions of the world. Food and agricultural scientists are now beginning to screen wild and under-exploited native plants for possible potential sources of food in an attempt to widen the narrow food base[2, 3]. Several reports have also indicated that lots of lesser-known native crop species are high in nutrients and could possibly relieve critical food shortages if given adequate promotion and

research attention[4, 5]. However, prior to utilization of such unconventional resources, data indicating the nutrient composition and toxic factors should be available. Toxicological evaluation of possible epidemiological response to the ingestion of novel food sources and the methods of processing that will enhance their utility as food or feed ingredient are all necessary in order to achieve optimal utilization[6]. Competition between man and his livestocks for food sources has been recognized as a major cause of the increase in the cost of ingredients used in compounding livestock feed. This has been recognized to account for >70% of the total cost of animal production thus seriously reducing the return and marginal profit in the developing countries essentially in Africa and particularly in Nigeria. Several methods have been employed to improve the nutritional quality of legumes, cereals and other form of seeds, essentially fermentation. Fermentation is one of the oldest methods and widely used process of producing and preserving food on local and industrial levels[7]. Fermentation for food production may be either anaerobic or aerobic or both. Fermentation changes the characteristics of the food by the action of the enzymes produced by the fermenting microbes whether bacteria, mould or yeasts. The term Solid State Fermentation (SSF) has been variously defined as the cultivation of microorganisms on solid, moist substrates in the absence of free aqueous phase[8] or cultivation of microorganisms in the presence of a liquid phase at maximal solid substrate concentrations or on inert carriers supporting moist substrate[9]. It has several advantages over liquid state or submerged fermentation. *Adansonia digitata*, the baobab tree is a member of the Bombacaceae family which consists of around 20 genera and around 180 species. Osman[10] reported that the acceptability and optimal utilization of *A. digitata* seed as a protein source is limited by the presence of inherent anti-nutrients such as protease inhibitors, tannins, phytic acid and amylase inhibitors. Phytochemicals (antinutrients) have been reported to be toxic at 5g per serving[11]. The acceptability and optimal utilization of *A. digitata* seed as a protein source has been reported to be limited by the presence of anti-nutritional factors such as trypsin inhibitors, protease inhibitors, tannins, phytic acid, oxalate, alkaloids, phytate and amylase inhibitors[12, 10]. De Caluwe *et al.*[13] suggested that though processing techniques may 'rob' a food item of some nutrients, processing systems may also enhance food nutritional quality by reducing or destroying the anti-nutrients present. The aim of this research, therefore, is to evaluate the proximate and antinutrient content of *A. digitata* seed fermented with mono-culture fungi under solid state techniques with a view to determining their nutritive potentials.

MATERIALS AND METHODS

Collection and authentication of seed: Mature, dried *Adansonia digitata* pods were collected from the premises of University of Ilorin, Ilorin, Kwara State, Nigeria was authenticated at the Department of Plant Biology of the same University as *Adansonia digitata* (UIH 1048).

Preparation of seed: The pods were cracked manually to release the seeds. The seeds ebbed in pulp were washed with plenty of clean water to remove the pulp before drying to remove the wetness. The seeds were pulverized with an electrical grinder to rough particles sizes of about 2 mm in diameter using an electrical grinder before they were stored in air tight container for further use.

Isolation of organisms from naturally fermented *A. digitata* seed: The pulverized seeds were subjected to natural fermentation as follows. The 250 g of each of the seeds was mixed with 250 mL of sterile distilled water plastic fermentors of two litre capacity. The mixtures was stirred properly until a uniform mash was obtained, covered and allowed to ferment at room temperature ($28\pm2°C$) in the laboratory for seven days[14]. Fungi were isolated from the naturally fermented seeds through serial dilution and pour plate method using Potato-Dextrose agar into which 10% Streptomycin has been added to inhibit bacteria growth. Culturing was done in duplicates.

Identification of the isolates: The fungi isolated from the fermenting mixture in were sub cultured until pure isolates were obtained. Morphological and microscopical analyses for the identification of the isolates were carried out and result obtained compared with literature to identify the organisms as described by Samson and Von-Reen Hoekstra[15]. Pure cultures of the fungal isolates were preserved on agar slant at 4°C for further use.

Fungal spore preparation and mono-culture fermentation of seed: Fungal spore suspension of actively growing mid log phase culture of the fungal isolates were prepared according to the method described by Sani *et al.*[16]. An agar slant of four day old pure culture of each of the organisms was used. The 10 mL of sterile distilled water was added to the slant and shook well to wash the spores. The spore suspension was counted using the Neubauer counting chamber. A spore suspension of about 5×10^4 spore mL^{-1} was used in each case for inoculation. The 20 g of the seed samples were measured separately into 250 mL Erlenmeyer flasks, plugged with

cotton wool, wrapped with aluminium foil and sterilized in the autoclave at 121°C for 15 min. The sterile samples were mixed with 20 mL of sterile distilled water and stirred properly until uniform mashes were obtained in each case. The 2 mL from each of the mono-culture suspension was used as fermentation starter to inoculate each of the samples in the fermentors. The mixtures were allowed to ferment for 120 h at room or ambient temperature (28±2°C)[14]. Fermented samples were taken daily, dried at 60°C in the oven for 4 h to safe moisture content and used for analysis of proximate (moisture content, crude fibre, crude protein, crude fat, ash content, carbohydrate and glucose), phytochemical (saponins, tannins, phytates and oxalates) analysis were done on fermented products after 120 h.

Determination of proximate content of the seed: The crude protein content of the samples was determined following the Kjeldahl method.

The moisture content, total ash, crude fibre, crude fat (Soxhlet extraction method) content of the seed were determined following AOAC[17]. The total carbohydrate or Nitrogen Free Extract (NFE) was determined by the difference method. The reducing sugar content of the seed was determine quantitatively by the 3,5-Dinitrosalicylic Acid (DNSA) as described by AOAC[17]. The water holding capacity of the seed was determined according to the method described by Zanzueta-Murale, etc.

Determination of the some antinutritional factors in the seed: The anti nutrient content of the seed was determined quantitatively. Total saponins was determined by the method of Makkar *et al.*[18], oxalates by the method of Iwuoha and Kalu[19], total soluble tannin by the method described by Padmaja[20] and the phytate content was determined by the method of Ma *et al.*[21].

RESULTS AND DISCUSSION

Fungi isolated: Nine moulds were isolated and identified during fermentation as *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Penicillium citrinum, Rhizopus stolonifer, Mucor racemosus, Mucor hiemalis, Alternaria tenuis, Scopulariopsis brevicaulis, Saccharomyces cervisiae* and *Schizosaccharomyces pombe* (Table 1).

Proximate content of fermented products: the effect of mono-culture fungal fermentation on *A. digitata* seed at ambient temperature for 72 h is presented in Table 2. Fermentation with each of the fungus has various effects on the proximate composition of the seed. The results obtained are significantly ($p < 0.05$) different from the unfermented sample. Crude protein, carbohydrate and total ash were significantly ($p < 0.05$) increased after fermentation while the crude fat and crude fibre were significantly ($p < 0.05$) decreased. The highest increase in protein (31.29%) was recorded in sample fermented with *Penicillium citrinum* while the lowest (28.80%) was recorded in sample fermented with *Mucor racemosus* (Table 2).

Reducing sugar (glucose) content of fermented seed: The effect of fermentation on the reducing sugar content of mono-culture fermented seed is as presented in Fig. 1. The value of reducing sugar increased significantly ($p < 0.05$) in all the fermented products compared to the unfermented sample. The highest increase (16.5 mg g^{-1}) was recorded in sample fermented with *Shizosaccharomyces pombe* while the value was insignificantly reduced ($p < 0.05$) to 2.5 mg g^{-1} recorded in sample fermented with Rhizopus stolonifer.

Effect of mono-culture fermentation on some antinutrients in the fermented seed: The effect of mono-culture fermentation on saponins, oxalates, tannins and phytates content of *A. digitata* seed is presented in Fig. 2-5. The values of the phytochemicals significantly ($p < 0.05$) reduce after fermentation.

The valuable nutrients in the fermented seed such as crude protein, mineral ash and total carbohydrate were increased after fermentation. The increase recorded in some nutrients during this fermentation can be attributed

Table 1: Fungal isolates from naturally fermented pulverized *A. digitata* seed

Macroscopy and microscopy description of isolates	Fungal isolates
Filaments were white fast spreading with brown-black spores at the tip	*Aspergillus niger*
Colonies were greenish yellow with rough edges, flat and fast spreading	*Aspergillus flavus*
The filaments were bluish green or pale with broad white margin	*Penicillium chrysogenum*
Filaments were whitish on top but leathery and orange yellow underneath	*Penicillium citrinum*
Colony appeared fluffy white but turned black with age	*Rhizopus stolonifer*
Filaments appeared gray, fluffy white but turn dirty white with age	*Mucor racemosus*
Mycelia were grayish or slightly yellowish turning grayish brown with age	*Mucor hiemalis*
Colony has blackish aerial mycelium; spores were dark, brown borne in chains	*Alternaria tenuis*
Colony has brownish loose hyphae carrying lemon shaped spores	*Scopulariopsis brevicaulis*
Colonies were creamy with rough edges; cells were ovoid or ellipsoidal	*Saccharomyces cervisiae*
Colony was round and smooth at the edge; cells were cylindrical Schizosaccharomyces	Pombe

Table 2: Proximate composition of *Adansonia digitata* fermented with mono-culture of fungal isolates

Organisms	MC	TA	PRO	FAT	FIB	CHO
BTe	6.98±1.39[ab]	4.32±0.22[ab]	31.50±2.06[c]	11.92±0.64[a]	4.46±0.7[ab]	40.84±0.88[ab]
BSt	5.87±0.00[a]	3.96±0.00[a]	29.43±0.00[ab]	12.57±0.00[ab]	4.96±0.00[ab]	43.21±0.00[bc]
BRa	7.43±1.28[bc]	4.45±0.40[ab]	28.80±1.12[ab]	11.85±0.13[a]	4.88±0.21[ab]	42.6±0.24[bc]
BHe	6.29±0.05[ab]	4.44±0.05[ab]	28.92±0.33[ab]	11.91±0.60[a]	4.48±0.62[ab]	43.98±1.55[bc]
BCh	8.20±3.34[c]	6.75±2.02[b]	30.83±0.34[bc]	11.77±0.46[a]	3.74±1.90[a]	38.76±7.40[a]
BCt	8.27±3.29[c]	6.61±2.25[ab]	31.29±2.60[c]	12.25±0.41[a]	3.45±1.34[a]	38.14±7.21[a]
BNi	7.25±1.89[bc]	5.52±0.97[ab]	30.36±2.04[bc]	13.31±0.89[ab]	3.71±1.91[a]	39.87±1.95[a]
BFl	7.91±3,10[bc]	3.89±0.74[a]	30.60±1.68[bc]	12.59±0.21[ab]	3.65±1.91[a]	41.36±3.39[bc]
BCe	7.50±0.00[bc]	5.00±0.00[ab]	31.00±0.00[c]	13.50±0.00[ab]	3.50±0.00[a]	40.20±0.00[ab]
BPo	8.00±0.00[c]	5.00±0.00[ab]	30.00±0.00[bc]	13.00±0.00[ab]	3.00±0.00[a]	40.10±0.00[ab]
BAo	7.64±2.11[bc]	4.39±1.61[ab]	19.86±1.30[a]	18.60±8.46[b]	8.87±5.31[b]	40.01±1.35[ab]

Data are mean of two replicate ± SEM. Mean within the same row carrying different superscripts are significantly different at (p<0.05). (MC = Moisture Content, TA = Total Ash, CPRO = Crude Protein, CFAT = Crude Fat, CFIB = Crude Fibre and CHO = total Carbohydrate) (BSt = Baobab fermented with *R.stolonifer,* BTe = Baobab fermented with *A. tenuis,* Bra = baobab fermented with *Mucor racemosus,* BHe = baobab fermented with *M. hiemalis,* BCh = baobab fermented with *P. chrysogenum,* BCt = baobab fermented with *P. citrinum,* BNi = baobab fermented with *A. niger,* Bfl = baobab fermented with *A. flavus,* BPo = baobab fermented with *S. pombe,* Bce = baobab fermented with *S. cerevisiae* and Bro = unfermented baobab seed)
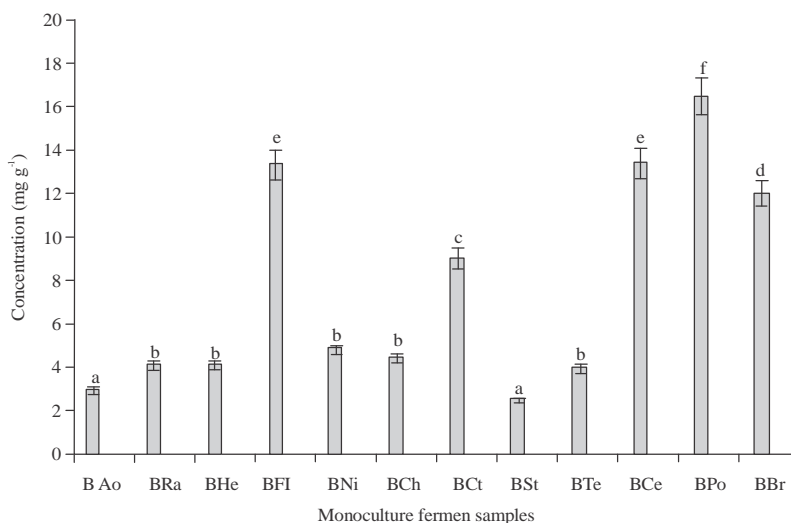

Fig. 1: Glucose content of mono-culture fungal fermented *Adansonia digitata*; Data are mean of two replicate±SEM; (BAo = Unfermented baobab; Bte = Moringa fermented with *Alternaria tenuis*; BSt = Baobab fermented with *Rhizopus stolonifer;* BRA = Baobab fermented with *Mucor hiemalis*; BCh = Baobab fermented with *Penicillium chrysogenum*; BCt = Baobab fermented with *Penicillium citrinum*; BNi = Baobab fermented with *Aspergillus niger*; BFl = Baobab fermented with *Aspergillus flavus*; BBr = Baobab fermented with *Scopuloriopsis brevicaulis*; BPo = Baobab fermented with *Schizosaccharomyces pombe*; BCe = Baobab fermented with *Saccharomyces cerevisiae*)

to the amylolytic and proteolytic activities of the fungi involved. This was made possible by the enzymes released by the fungi during growth in the process of fermentation. These enzymes were involved in breaking down complex organic molecules into simpler ones such as glucose and amino acids. The product of the enzymes is also utilized by the organisms for their own metabolic activities[22]. Moreover, the noticeable increase in crude protein in the mono-culture fermented seed could also be attributed to the addition of mycoprotein (single cell protein), non-protein nitrogen amide and nucleic acid synthesized by fungal cells during growth[14]. The increase in carbohydrate particularly glucose content, decrease in crude fat and crude fibre recorded could be attributed to

the effect of amylolytic and lipolytic enzymes secreted by the fungi during fermentation which brought about the breaking down of the complex organic carbon compound in the seed hence the increase in glucose and decrease in crude fibre and fat content[23]. However the available carbohydrate and lipid in the fermented seed was still sufficient to meet the daily requirement and are now in a readily accessible form upon consumption by animals when incorporated into food and feeds items. The increase in carbohydrate content may also be attributed to the reduction in the crude fibre content. During fermentation polysaccharides including cellulose, pectin, lignocellulose and starch are broken down by microorganisms thereby reducing the fibre content of such seed. This result was in
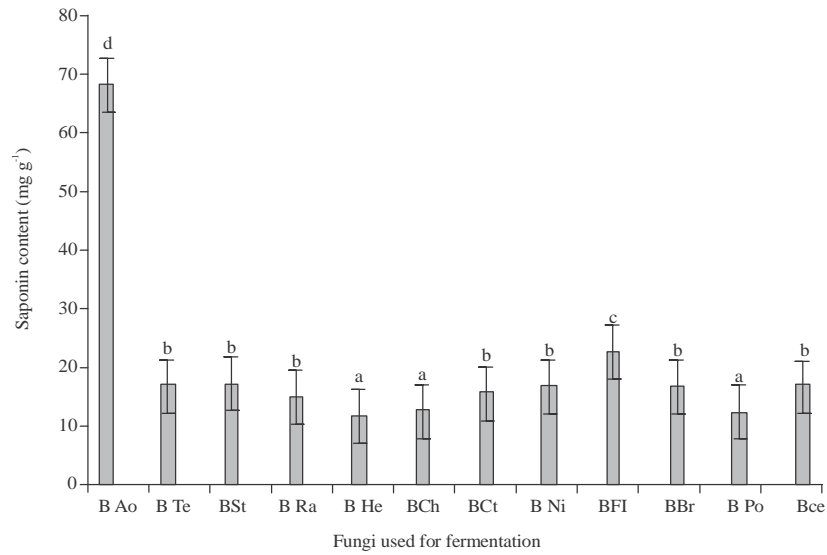
Fig. 2: Saponin content of mono-culture fungal fermented *Adansonia digitata;* Data are mean of two replicate±SEM; (BAo = Unfermented baobab; BTe = Moringa fermented with *Alternaria tenuis*; BSt = Baobab fermented with *Rhizopus stolonifer*; BRA = Baobab fermented with *Mucor racemosus;* BHi = baobab fermented with *Mucor hiemalis*; BCh = Baobab fermented with *Penicillium chrysogenum*; BCt = Baobab fermented with *Penicillium citrinum*; BNi = Baobab fermented with *Aspergillus niger*; BFl = baobab fermented with *Aspergillus flavus*; BBr = Baobab fermented with *Scopuloriopsis brevicaulis*; BPo = Baobab fermented with *Schizosaccharomyces pombe*; BCe = Baobab fermented with *Saccharomyces cerevisiae*)
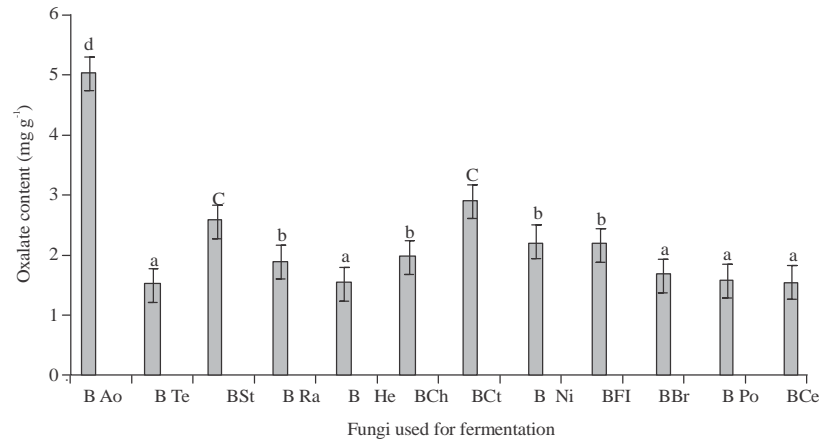

Fig. 3: Oxalate content of mono-culture fungal fermented *Adansonia digitata*; Data are mean of two replicate±SEM*;* (BAo = Unfermented baobab; BTe = Moringa fermented with *Alternaria tenuis*; BSt = Baobab fermented with *Rhizopus stolonifer*; BRA = Baobab fermented with *Mucor racemosus;* BHi = baobab fermented with *Mucor hiemalis*; BCh = Baobab fermented with *Penicillium chrysogenum*; BCt = Baobab fermented with *Penicillium citrinum*; BNi = Baobab fermented with *Aspergillus niger*; BFl = baobab fermented with *Aspergillus flavus*; BBr = Baobab fermented with *Scopuloriopsis brevicaulis*; BPo = Baobab fermented with *Schizosaccharomyces pombe*; BCe = Baobab fermented with *Saccharomyces cerevisiae*)

contrast to that obtained by earlier author on carbohydrate content of fermented *A. digitata* seed[24, 25] but was similar to that obtained by Kayode and Sani[14], Kayode *et al.*[26] and Adebowale and Maliki[27] on fermentation of *Mangifera indica* seed and pigeon pea seed. Fermentation

significantly ($p<0.05$) increased the ash content of the seed. The high level of total ash recorded can be due to the breakdown of organic complexes in the seed to release the minerals and make them bioavailable[28]. The inherent antinutrients in the seed decreased significantly ($p<0.05$)
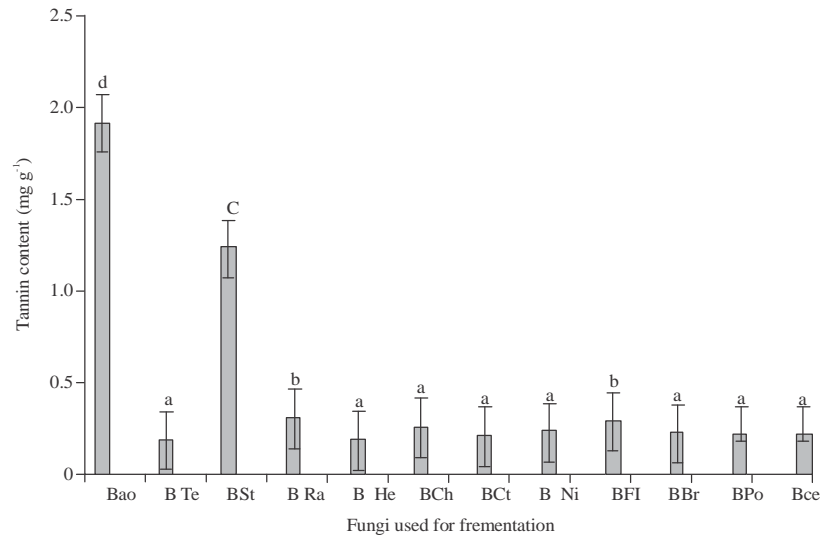
Fig. 4: Tanin content of mono-culture fungal fermented *Adansonia digitata*; Data are mean of two replicate±SEM*;* (BAo = Unfermented baobab; BTe = Moringa fermented with *Alternaria tenuis*; BSt = Baobab fermented with *Rhizopus stolonifer*; BRA = Baobab fermented with *Mucor racemosus;* BHi = baobab fermented with *Mucor hiemalis*; BCh = Baobab fermented with *Penicillium chrysogenum*; BCt = Baobab fermented with *Penicillium citrinum*; BNi = Baobab fermented with *Aspergillus niger*; BFl = baobab fermented with *Aspergillus flavus*; BBr = Baobab fermented with *Scopuloriopsis brevicaulis*; BPo = Baobab fermented with *Schizosaccharomyces pombe*; BCe = Baobab fermented with *Saccharomyces cerevisiae*)
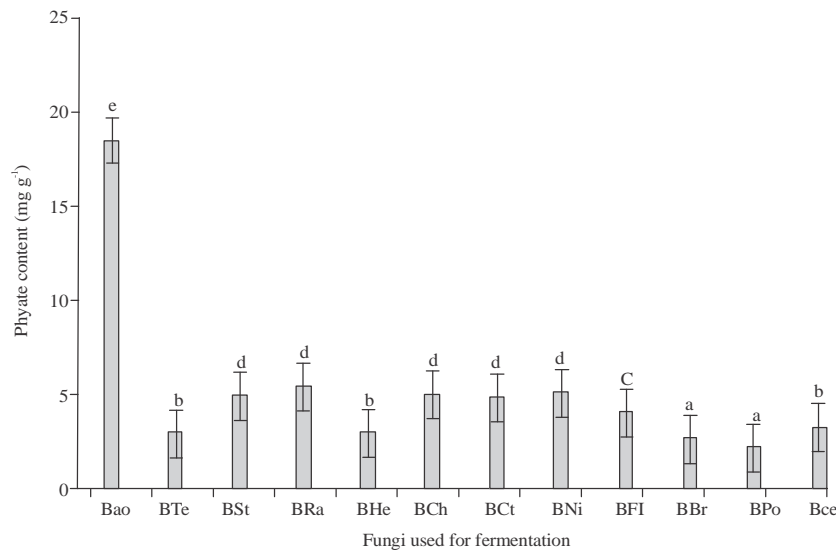

Fig. 5: Phytate content of mono-culture fungal fermented *Adansonia digitata*; Data are mean of two replicate±SEM*;* (BAo = Unfermented baobab; BTe = Moringa fermented with *Alternaria tenuis*; BSt = Baobab fermented with *Rhizopus stolonifer*; BRA = Baobab fermented with *Mucor racemosus;* BHi = baobab fermented with *Mucor hiemalis*; BCh = Baobab fermented with *Penicillium chrysogenum*; BCt = Baobab fermented with *Penicillium citrinum*; BNi = Baobab fermented with *Aspergillus niger*; BFl = baobab fermented with *Aspergillus flavus*; BBr = Baobab fermented with *Scopuloriopsis brevicaulis*; BPo = Baobab fermented with *Schizosaccharomyces pombe*; BCe = Baobab fermented with *Saccharomyces cerevisiae*)

after fermentation. The decrease in tannin, saponins, oxalates and phytate content of *A. digitata* seed could be due to degradation by microbial enzymes that are secreted during fermentation[25]. This decrease can be attributed to the enzymatic breakdown during the growth and metabolism of the fungi which invariably brought about

the fermentation. These antinutrients are known to occur in complex compounds (protein-tanin complexes, calcium- oxalate complex and other forms of complexes) in plants where they occur. The breaking down of these complexes by various amylolytic, proteolytic and lipolytic enzymes produced by these fungi during growth and metabolism led to the reduction in antinutrients. Tannins are known to reduce the availability of proteins, carbohydrates and minerals by forming indigestible complexes with the nutrients. The reduction in tannin level due to fermentation could improve the availability of nutrients in the seed[29, 25]. However Addy *et al.*[24] reported increase in tannin level after fermentation of *A. digitata* seed. Fermentation can be used to improve the nutrient quality and drastically reduced the anti-nutritional factors to safe level because the process produces enzymes that break down protein-tannin complexes to release free tannins. Similarly, the reduction in phytic acid is attributable to increase in the activities of phytase during fermentation. The action of the enzyme on successive steps terminating with the formation of inositol and phosphoric acid releases certain metals to increase their availability and cause subsequent decrease in phytate and increase in total ash[25, 30]. This agreed with previous reports on the reduction of phytic acid content of some plant products including *A. digitata* seed after processing[31, 32]. Phytic acid forms complexes with proteins (protein-phytate complex)[33] and chelates essential dietary minerals such as iron, zinc, calcium and magnesium, thus decreasing their utilization.

Saponins can affect nutrition animal performance and metabolism in a number of ways such as erythrocyte haemolysis, depression of growth rate, bloat (ruminants), inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption. Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effects when ingested[34]. Saponin content of *A. digitata* seed was significantly ($p < 0.05$) decreased after fermentation. The finding was similar the report of Umaru *et al.*[35] and Uchegbu *et al.*[30] on the reduction in saponin content of fermented seeds.

Oxalates, like phytates, bind minerals like calcium and magnesium and interfere with their metabolism. They also cause muscular weakness and paralysis, gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia[36]. Oxalates content of fermented *A. digitata* seeds reduced significantly after fermentation with the isolated fungi. Fermentation and other methods of processing have been reported to decrease the oxalate content of food[37]. Kayode and Sani[25] however reported an increase in oxalate content of mango kernel cake fermented by *A. niger*.

CONCLUSION

Mono-culture fungal fermentation has desirable effects on the seed of *A. digitata*. Fermented seed could therefore be considered as an alternative, supplement or additional protein source for propounding animal feed. This will tremendously reduce the cost of feeding in animal production and also reduce the competition between man and animal for the available staple food. However only fungi without history of poisoning or production of harmful metabolites should be employed for the fermentation.

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