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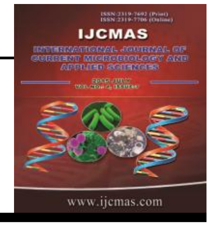

Original Research Article

The Amino Acid and Mineral Composition of Mono-Culture Fungal Fermented Baobab (*Adansonia digitata*) Seed

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A B S T R A C T

The effects of fungal fermentation on the amino acid profile and mineral composition of the pulverized seed of baobab (*Adansonia digitata*) were investigated in this study. *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizopus stolonifer, Mucor hiemalis, Mucor racemosus, Alternaria tenuis, Scopuloriopsis brevicaulis*, *Aspergillus niger* and *Aspergillus flavus* were isolated from naturally decomposed seed kernel and used singly to ferment the seed kernel flour of Baobab for five days (120 hours). The fermented samples were oven dried at 60^0 C and analyzed for their amino acid and mineral content standard methods. The amino acid content was obtained by using the Technicon sequential multi-sample amino acid analyzer (TSM) while the mineral content was done with the Atomic Absorption Spectrometry (AAS). The amino acid values of the fermented samples were higher (1.2-8.7mg/100g protein) compared to the unfermented sample except for phenylalanine (5.2-4.7mg/100gprotein). Essential amino acids particularly methionine, histidine, isoleucine, arginine, leucine, valine threonine and lysine content of fermented samples increased significantly. The chemical score of histidine (168-255%), leucine (127-129%) and threonine (107-109%) in *M. hiemalis* fermented sample, valine (117-133%) and arginine (102-106%) in *P. chrysogenum* were found to meet up with the daily requirement reference of the FAO/WHO/UNU standard for livestock, children and adults. The result of the analysis of the mineral composition of the fermented samples; Calcium (257-559mg/kg) Potassium (258-263mg/kg), Magnesium (60-243mg/kg) and iron (130-188mg/kg) showed that they had significantly increased compared to the unfermented. It is concluded that fermented baobab seed can be a potential replacement of protein in livestock feed formulation.

Keywords

Baobab, Fermentation, Increase, Fungal Mono-Culture, Minerals, Amino acids

Introduction

In the livestock industry, feed has been reported to account for more than 70% of the total cost of production thus seriously reducing the return and marginal profit (Saina, 2003; Saina *et al.*, 2005). According to various authors the increase in the cost of ingredients used in compounding livestock feeds is unprecedented hence the importance of identifying and proper utilization of unconventional feeds by livestock will not only benefit the animal industry but will also increase the economic return of many cash crops and small scale livestock farmers. Utilizing cheaper non-conventional feeds has been emphasized time and again (Ndams *et al.*, 2011).

Part of the contributing factors to this high cost is the competition between man and his livestock for the available conventional food materials. Earlier reports have shown in Nigeria, like other developing countries of the world, that protein of animal origin is quite expensive and often not within the reach of the ordinary man. Thus, efforts to alleviate the inadequacy in protein supply have been directed towards the utilization of readily available and inexpensive feed stuffs, which are plant proteins (Yussuf *et al.*, 2008). Among such non-conventional feed ingredients as reported by Islam *et al.*, (1994) are: agro-based industrial by-products like cola nut cake meal, cotton seed cake, palm kernel cake, cassava peel, yam and potato peels. Others include maize bran, rice bran and brewers dried grains (Dairo, 1999). Some of the limiting factors associated with using crop residues and agro industrial by products as animal feeds include; procurement, storage, poor feed intake, high fibre content, toxic substances, low digestibility, low nutrient contents and low minerals and amino acid profile (Oluokun and Olalokun, 1995). It has also been reported that the search for alternative source of plant protein due to high cost and scarcity of feed which militate against increased commercial livestock production requires the need to explore the potentials of lesser-known, ignored and underutilized trees and shrubs that are native to West Africa and Africa (Igboeli *et al.*, 1997).

The baobab (*Adansonia digitata)* is a tree that grows well in the savannah region and belongs to the Malvaceae family (De Caluwé *et al.,* 2010). The plant has been described as a very massive tree with a very large trunk which can grow up to 25 m in height and may live for hundreds of years and it is widespread throughout the hot and drier regions of tropical Africa (FAO, 1988). The seed has been reported not only to be rich in protein and energy but also provides some necessary fiber, vitamins, minerals and amino acids, particularly, lysine and methionine which are limited in most cereals but essential for livestock growth and development (Glew *et al.*, 1997; Murray *et al.*,2001; Nnams and Obiakor, 2003; Magdi, 2004).

The reports of Longe (1988); Dierick (1989) supported the use of non conventional feed in livestock management after simple inexpensive processing techniques which will not unnecessarily add to the cost but will be justifiable in terms of bioavailability and biodigestibility of nutrient. Essential amino acids and minerals are not only required in proper functioning of life but in maintenance of well-being and adequate metabolism of the body for better output and yield (Saif, 2003). Deficiency in essential vitamins and minerals have been reported to have various negative effects which may be severe in most cases: hence this study is aimed at investigating the effect of mono culture fungal fermentation on the amino acids and mineral composition of baobab seed for utilization as protein source in the livestock industry.

Materials and Methods

Collection and preparation of Baobab seed: Baobab pods were collected in November 2013 within the premises of University of Ilorin after the bridge by the power house junction. This was taken to the herbarium section of the Department of Plant Biology, University of Ilorin where it was identified as *Adansonia digitata* with the voucher UIH 1048. The pods were cracked manually to release the seeds embedded within the pulp. They were washed with plenty of clean water to remove the pulp from the seeds which were later dried to safe moisture content. The seeds were pulverized with an electrical grinder to rough particles before they were stored in air tight container for further use.

Isolation of fungi: twenty grams (20g) of sample was mixed thoroughly with twenty millilitres (20ml) of distilled water and left for fermentation at ambient temperature (28^0 C±2) for 120 hours. Nine fungal isolates were obtained which were thereafter identified macroscopically and microscopically by comparing the observed characteristics (colour, shape, type, thickness and texture of hypha and spores) with literature (Harrow, 1968; Samson and Von Reen Hoekstra, 1988).

Preparation of inoculums: fungal spore suspension were prepared from actively growing mid log phase culture of the isolates obtained from the naturally fermented baobab seed and were individually adjusted to about $5x10^4$ spore/ml as described by Sani *et al.* (1992).

Fermentation: 50g of the pulverized seed was sterilized in the autoclave at 121^0C for 15 minutes. The sterile seed was mixed with 50ml of sterile distilled water in nine different glass fermentors and stirred properly until uniform mash was obtained. Five millilitres (5ml) from each of the mono-culture spore suspension of each of the fungus containing about 5×10^4 spore/ml was used as fermentation starter culture to inoculate each of the samples in the fermentors. The mixtures were allowed to ferment for 120hrs at ambient temperature, (28^0 C±2), (Lawal *et al*., 2005; Kayode and Sani, 2008). Fermented samples were oven dried at 60^0C and used for further analyses.

Mineral analysis: The mineral composition was measured using the "Buck Scientific Atomic Absorption Spectrophotometer Model 210A"AOAC, (2000) after the samples have been digested using concentrated Trioxo-nitrate (V) acid. Four macro minerals (Ca, Mg, K and Na) and six micro minerals (Mn, Cu, Zn, Fe, Cd and Pb) were analyzed.

Determination of amino acid profile: this was determined following the methods of Sparkman *et al. (*1958) and AOAC (2000) using the Technicon Sequential Multi-sample amino acid analyser (TSM) to separate and analyse for free acidic, neutral and basic amino acids.

Statistical analysis: all the data obtained were subjected to one way Analysis of Variance (ANOVA). Duncan's Multiple Range Test was used to determine significant differences among means as described by Obi, (2002). Means were significantly different at P≤0.05.

Results and Discussion

Nine fungi were isolated and identified as *Aspergillus niger, Aspergillus flavus, Alternaria tenuis, Rhizopus stolonifer, Penicillium chrysogenum, Penicillium citrinum, Scopuloriopsis brevicaulis, Mucor racemosus and Mucor hiemalis.* The effects of fermentation on the essential and non-essential amino acid in baobab seed is as presented in Table 1 and Figure1 respectively. The values obtained for some of the essential amino acids in the fermented samples were higher than those in the unfermented sample. Methionine (1.29 to 3.00 g/100g protein), isoleucine (3.71 to

4.91g/100g protein, valine (5.18 to 5.90g/100g protein) and histidine (2.86 to 4.35g/100g protein) content of the fermented samples increased considerably in samples fermented by *Mucor racemosus, Alternaria tenuis, penicillum citrinum* and *Mucor hiemalis* respectively compared with unfermented sample. The chemical score ranged between 111.45 to 116.71% (Tables 2 and 3)

The mineral composition of the fermented samples was significantly higher at P≤ 0.05 than unfermented sample as shown in Tables 4 and 5. The values of magnesium (60 to 247mg/kg), potassium (258 to 263mg/kg),calcium (257 to 555mg/kg), sodium (25 to 110mg/kg), iron(130 to 191mg/kg) and zinc(70 to 221mg/kg) increase significantly after fermentation while the values of heavy metals like cadmium (2.9 to 0.7mg/kg) and lead (2.4 to 0.0mg/kg) were significantly decreased or totally disappeared in some of the fermented samples.

Fermentation of the seed of *A. digitata* by mono-culture fungi led to increase in the amino acid and mineral composition. The chemical score (the score obtained when the quantity in the fermented seed was compared with the daily intake requirement) of all the essential amino acids in the various samples compared favourably with DRI (2002/2005) references value of daily amino acid requirement including lysine and methionine that are essentially required for proper growth, maintenance of body metabolism and proper development.

The increase in the amino acid content could be attributed to the activity of fungal proteases during fermentation. The growth and subsequent metabolism of the fungi on the organic complexes present in the seed and their production of mycoproteins may probably account for the increase in amino acids. The decrease noticed in some of the amino acid in some of the fermented samples could be as a result of the protein utilized for growth by the fermenting fungi. Amino acids are the building blocks of all proteins in the body and some hormones.

The value of calcium, magnesium, potassium, and iron in the mono culture fermented samples significantly increased after fermentation and they were found to have added values to the mineral nutritional quality of the samples and compared favorably with DRI (2002/2005) reference value of daily requirement. The increase in minerals noticed in these fermented seed can be attributed to the breaking down of the organic complexes in the seeds by the fermenting fungi. During growth, the fungi metabolized the complex organic compounds to simpler substances which are released into the fermenting medium and as a result of which the minerals are released and their bioavailability increased. The breakdown of carbohydrate-protein complexes by various enzymes such as amylases, endoglucamylases and other amylolytic enzymes, proteases and others secreted by fungi could lead to the release of these metals (Obizoba and Atti, 1991). Thus the metals are now in the free state and are biologically available for use if such fermented seeds are used for food. The reasons for the reduction in values of the heavy metals or their non detectable level in the fermented seed however could not be ascertained since fungi do not utilize them but it could probably have been converted to another substance and thus no longer available in the free or detectable state.

Mineral deficiency is usually caused by a low mineral content in the diet when rapid body growth is occurring and/or when there is poor absorption of minerals from the diet (Favier, 1993).

Table.1 Amino acid composition of mono culture fungal fermented Baobab seed

Amino acid profile	Amino acid concentration (g/100g)					
	BAO	BAS	BAA	BAP	BAR	BAH
Glycine	3.29	3.201	3.23	3.19	3.34	3.53
Alanine	5.82	4.781	5.61	5.13	4.91	4.76
Serine	4.96	4.631	4.50	4.76	4.64	4.69
Proline	4.46	4.08	4.59	4.11	4.22	4.32
Valine	5.18	5.68	5.12	5.87	5.45	5.22
Threonine	3.53	3.17	3.43	2.98	3.07	3.60
Isoleucine	3.71	3.55	4.92	3.77	3.75	3.78
Leucine	8.55	7.97	7.91	8.24	8.00	8.69
Aspartate	10.72	8.30	8.06	8.46	8.76	8.77
Lysine	7.69	7.69	7.34	8.22	8.66	7.95
Methionine	1.29	2.69	2.22	2.75	3.00	2.64
Glutamate	16.14	16.30	16.64	16.21	15.61	16.45
Phenyialanine	5.52	5.45	4.73	5.11	5.21	5.14
Histidine	2.86	3.82	3.25	3.72	4.05	4.35
Arginine	6.28	5.51	6.50	5.58	5.79	6.02
Tyrosine	2.94	3.26	3.50	3.29	3.58	3.16
Tryptophan	1.95	2.19	2.10	2.17	2.05	2.14
Cystine	8.17	6.51	7.25	5.49	5.70	5.69

BAO: unfermented baobab, BAS: baobab fermented with *Rhizopus stolonifer*, BAA: baobab fermented with *Alternaria terreus*, BAP: baobab fermented with *Penicillium chrysogenum*, BAR: baobab fermented with *Mucor racemosus*, BAH: baobab fermented with *Mucor hiemalis*.

Table.2 Chemical score of essential amino acid in the mono culture fermented samples compared with recommended value of DRI- Macronutrient

	Values obtained (g/100g)				Recommended Value(g/100g)	Chemical score (%)			
Amino acid	BAO	BAS	BAA	BAP		BAO	BAS	BAA	BAP
Isoleucine	3.71	3.55	4.91	3.77	3.20	116.25	110.94	153.75	117.81
Leucine	8.55	7.97	7.91	8.24	7.00	122.14	113.86	113.00	117.67
Lysine	7.69	7.69	7.34	8.22	6.50	118.31	118.29	112.97	126.51
Methionine	1.29	2.69	2.22	2.75	3.20	40.31	83.97	69.31	85.94
Phenylalanine	5.52	5.45	4.73	5.11	6.00	92.06	90.78	78.85	85.13
Valine	5.18	5.68	5.12	5.87	4.10	126.34	138.54	124.95	143.15
Histidine	2.86	3.82	3.25	3.72	2.30	124.43	165.87	141.26	161.74
Threonine	3.54	3.17	3.43	2.98	3.50	100.97	90.57	97.97	85.03
Arginine	6.28	5.11	6.50	5.58	6.10	102.87	90.26	106.55	91.41
Total	44.60	45.50	45.40	46.20	41.90	104.85	111.45	110.96	112.71

BAO: unfermented baobab, BAS: baobab fermented by *Rhizopus stolonifer*, BAA: baobab fermented with *Alternaria terreus*, BAP: baobab fermented with *Penicillium chrysogenum*, BAR: baobab fermented with *Mucor racemosus*, BAH: baobab fermented with *Mucor hiemalis*.
DRI= Dietary Reference Intake- Macronutrients (2002/2005), Institute of Medicine

Table.3 Chemical score of essential amino acid in the mono culture fermented samples compared with recommended value of DRI- Macronutrient.

	Values obtained (g/100g)			Recommended values(g/100)	Chemical score (%)		
Amino acid	BAO	BAR	BAH		BAO	BAR	BAH
Isoleucine	3.71	3.75	3.78	3.2	116.25	117.18	118.13
Leucine	8.55	8.00	8.69	7.0	122.14	114.28	124.10
Lysine	7.69	8.66	7.95	6.5	118.31	133.15	122.25
Methionine	1.29	2.99	2.64	3.2	40.31	93.59	82.50
Phenylalanine	5.52	5.21	5.14	6.0	92.06	86.88	85.65
Valine	5.18	5.45	5.22	4.1	126.34	132.90	127.22
Histidine	2.86	4.05	4.35	2.3	124.43	175.91	188.91
Threonine	3.53	3.07	3.60	3.5	100.97	87.80	102.94
Arginine	6.28	5.78	6.02	6.1	102.87	94.77	98.74
Total	104.85	46.96	47.37	41.9	104.85	115.16	116.71

BAO: unfermented baobab, BAS: baobab fermented by *Rhizopus stolonifer*, BAA: baobab fermented with *Alternaria terreus*, BAP: baobab fermented with *Penicillium chrysogenum*, BAR: baobab fermented with *Mucor racemosus*, BAH: baobab fermented with *Mucor hiemalis.*
DRI= Dietary Reference Intake- Macronutrients (2002/2005), Institute of Medicine

Table.4 Macro-mineral composition of mono culture fungal fermented Baobab seed

Fermented Samples	Macro-mineral composition (mg/kg)			
	K	Na	Ca	Mg
BAA	188.9±0.02^{a}	67.4±0.70^{b}	330.9±0.06^{b}	206.7±0.20^{b}
BAS	221.3±0.03^{c}	67.1±0.25^{b}	557.2±2.47^{d}	220.4±1.00^{c}
BAF	256.0±0.01^{e}	80.3±0.00^{d}	938.4±9.34^{e}	239.5±0.90^{d}
BAPCT	263.0±0.03^{g}	110.2±0.33^{e}	505.9±0.00^{c}	243.6±0.00^{e}
BAN	251.9±0.02^{d}	74.7±0.37^{c}	555.8±4.92^{d}	247.1±0.25^{f}
BAB	206.2±0.01^{b}	66.6±0.35^{b}	261.2± 4.66^{a}	205.9±0.25^{b}
BAO	258.5±0.00^{f}	25.0±0.00^{a}	257.0±0.00^{a}	60.6±0.00^{a}
SEM	0.095	2.05	21.39	1.61

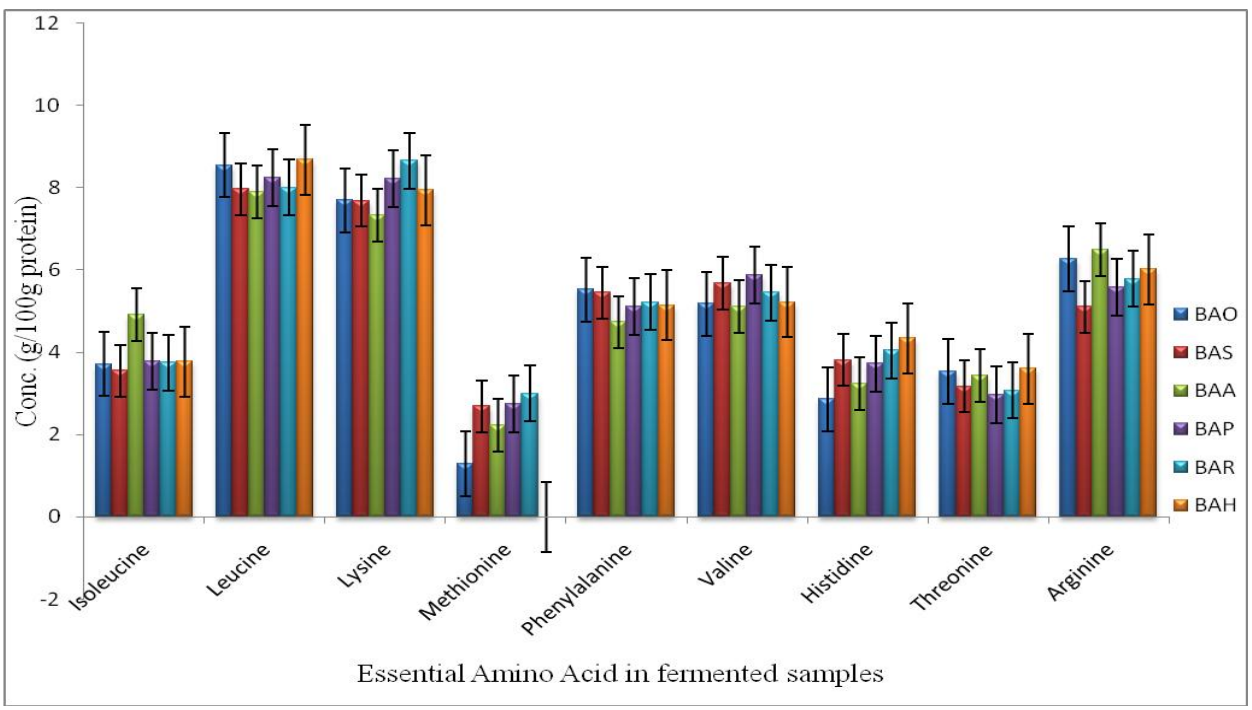
Values within the same column carrying different superscript are significantly different at P≤ 0.05.BAA: baobab fermented with *Alternaria teneus*, BAS: baobab fermented with *Rhizopus stolonifer*, BAF: baobab fermented with *Aspergillus flavus*, BAPCT: baobab fermented with *Penicillium citrinum*, BAN: baobab fermented with *Aspergillus niger*, BAB: baobab fermented with *Scopuloriopsis brevicaulis*, BAO: unfermented baobab and SEM standard Error of Mean.

Table.5 Micro-mineral composition of mono culture fungal fermented Baobab seed

Fermented samples	Micro-mineral composition (mg/kg)					
	Mn	Fe	Zn	Cu	Cd	Pb
BAA	9.0 ±0.58^{a}	131.2±2.79^{a}	30.8±0.7^{a}	10.3±0.24^{a}	0.8±0.35^{a}	0.0±0.00^{a}
BAS	13.8±0.13^{b}	148.6±0.00^{b}	195.6±2.45^{c}	13.1±0.13^{c}	1.4±0.13^{a}	0.0±0.00^{a}
BAF	13.9±0.58^{b}	191.4±0.00^{e}	221.3±7.00^{d}	15.5±0.24^{d}	1.3±0.35^{a}	4.9±0.47^{c}
BAPCT	17.8±0.86^{c}	187.6±0.62^{d}	41.6±0.86^{a}	14.8±0.13^{d}	1.4±0.13^{a}	1.1±0.13^{b}
BAN	20.3±0.12^{d}	162.8±0.49^{c}	199.2±7.38^{c}	87.0±0.62^{e}	1.1±0.13^{a}	0.0±0.00^{a}
BAB	13.6±0.35^{b}	128.1±0.23^{a}	37.8±0.69^{a}	12.0±0.00^{b}	0.7±0.23^{a}	0.0±0.00^{a}
BAO	17.0±0.00^{c}	130.0±0.00^{a}	70.6±0.00^{b}	13.0±0.00^{c}	2.9±0.00^{b}	2.4±0.00^{a}
SEM	2.75	3.93	18.98	1.34	0.68	0.59

Values within the same column carrying different superscript are significantly different at P≤ 0.05.BAA: baobab fermented with *Alternaria teneus, BAS:* baobab fermented with *Rhizopus stolonifer, BAF:* baobab fermented with *Aspergillus flavus, BAPCT:* baobab fermented with *Penicillium citrinum, BAN:* baobab fermented with *Aspergillus niger*, *BAB:* baobab fermented with *Scopuloriopsis brevicaulis*, *BAO:* unfermented baobab and SEM standard Error of Mean

Fig.1 Essential amino acid composition of mono culture fungal fermented Baobab seed



BAO: unfermented baobab, BAS: baobab fermented by *Rhizopus stolonifer*, BAA: baobab fermented with *Alternaria terreus*, BAP: baobab fermented with *Penicillium chrysogenum*, BAR: baobab fermented with *Mucor racemosus*, BAH: baobab fermented with *Mucor hiemalis*

Hence, the absolute amounts of minerals must not only be increased in the edible portion of foods but these minerals must also be in forms that are bioavailable to the person consuming them. The value of magnesium, a major mineral required for muscles and bone formation, control of blood pressure and nerve transmitter in the fermented samples met the level requires for human and animal feed. Also the values of calcium and potassium in the samples met the requirement for animal feed (DRI 2002/2005). These two minerals are essential for bone calcification thereby preventing muscular paralysis and bone malady.

Iron deficiency anemia is one of the most prevalent nutritional deficiencies affecting the world's population today, especially among children and women in developing countries (Clark and Fox, 2009; Leung, 1998). The level of iron in the fermented samples is adequate for animal feeding and may improve the health of animal feeding on them. Iron is required in the haemoglobin and myoglobin in the muscles (Thomas, 2002). This result is similar to the result obtained by Kayode and Sani, (2010) on the effect of mono culture fungal fermentation on the amino acid and mineral of the seed kernel of *Mangifera indica*. The amino acid and mineral composition of the fermented samples are found to be adequate for the level required for animal feeding and may improve on their health status.

In conclusion, the fermented samples could be a suitable replacement for protein in the search for non conventional protein source in the livestock producing industries and this may lead to a more economic way of utilizing baobab seed and probably reduce the cost of animal feed production.

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