Mineral Composition and Amino Acid Profile of Mono-Culture Fungal Fermented Mango (*Mangifera Indica*) Kernel Cake

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Abstract

The effect of mono-culture fungal fermentation on mineral composition and amino acid profile of mango (*Mangifera indica*) kernel cake was investigated. *Rhizopus oligosporus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum* were isolated from decomposed mango kernels and then used to ferment mango kernel cake (MKC) for 168hrs. After fermentation the resulted MKC was dried at 60^0C before analysis for minerals and amino acid profiles. The values of calcium (119.3mg/kg), potassium (457.1mg/kg) and iron (187.1mg/kg) of the *R. stolonifer* fermented MKC were higher ($p<0.05$) than MKCs that were fermented with *R. oligosporus*, *A. niger*, *P. chrysogenum* and unfermented MKC. Threonine (1.36 - 2.03 g/100g protein) and isoleucine (2.62 - 3.00 g/100g protein) content of the fermented MKC increased appreciably compared with the unfermented MKC, while, methionine (0.40 - 0.60 g/100g protein), phenylalanine (2.73 - 3.30 g/100g protein) and valine (2.26 - 2.73 g/100g protein) were decreased ($p<0.05$) compared with unfermented MKC. The chemical score of isoleucine and histidine of the *R. oligosporus* fermented MKC (67.50 and 72.35%), *R. stolonifer* fermented MKC (75.00 and 130.59%) and *P. chrysogenum* fermented MKCs (65.75 and 62.94%) respectively were comparable with FAO/Maynard reference for growing chicks. Total score of the essential amino acids of the fermented MKC ranged between 44.42 and 55.68%. It is concluded that fungal fermented mango (*Mangifera indica*) kernel cake may be a potential supplement in feedstuff formulations.

Keywords: Mango kernel meal, Mango kernel cake, Mono-culture fermentation, Mineral composition, Amino acid profile

1. Introduction

The cake obtained after fat extraction of some tropical seeds play a role as an energy and protein storage materials. However, the role of these unconventional tropical seeds in monogastric diets has attracted much attention in recent years due to the fact that they elicit anti-nutritive effects which results in poor nutrient bioavailability and utilization (Kayode and Sani, 2008). Longe (1988) and Dierick (1989) advocated the increase utilization of non-conventional feed resources in non-ruminant feed. They suggested processing techniques, which are simple and inexpensive and do not significantly increase costs but still make it worthwhile in terms of nutrient availability. The use of microorganisms (fungi and bacteria); which serve as probiotics, antibiotics, enzymes and ensiling have constituted methods of enrichment of non-digestible feedstuffs and those imbued with anti-nutritive factors.

In poultry, small quantities of tannins in the diet were found to have caused adverse effects, while the inclusion of additional proteins or amino acids into the diets may alleviate the effects of tannins (Reed, 1995 and Cannas, 2007). Decrease methionine availability could increase the toxicity of cyanogenic glycosides; because methionine is involved in the detoxification of cyanide via methylation to thiocyanate (Reed, 1995; Giner-Chavez, 1996; Cannas, 2007).

Essential mineral elements are as important as amino acids and vitamins in maintenance of life, well-being and production (Saif, 2003). Minerals are essential for regulating and building the living and aid in fighting depression (Kolawole *et al*., 2007). Phosphorus and calcium are closely associated in metabolism, particularly in bone formation; phosphorus plays a critical role in the maintenance of acid-base balance, while calcium is essential mineral for building living cells that make up the body; their deficiencies often resulted in soft, weak and abnormal bones formation in poultry (Saif, 2003). Magnesium has been found to help keeps the muscles relaxed and formation of strong bones and teeth; it was said to be involved in the control of blood pressure and nerve transmitter (Kolawole *et al*., 2007). Iron has been reported an important element that is necessary in the heamoglobin of the red blood cells and myoglobin in the muscle (Thomas, 2002). Iron deficiency in poultry diet resulted in a hypochromic microcytic anaemia, reduced hatchability and reduced concentration of non-haem iron in plasma and feather pigmentation (Saif, 2003) and decreased synthesis of niacin from tryptophan in chicken (Oduwo *et al*., 1994). (Saif, 2003) also reported that reduced intake of cupper in the diets of coloured breeds of fowl was characterized by reduced number of circulatory erythrocytes and impaired feather pigmentation. Zinc deficiency in poultry diet resulted in retarded growth, poor feathering, enlarged hocks, scaling of the skin, dermatosis, awkward arthritic gait, thickened long bones and that the deficiencies of iodine in chickens diet has caused an enlarged thyroid and in some cases lower body weight gain in growing chicks (Saif, 2003). Therefore, this study was designed to investigate the effect of mono-culture fungal fermentation on mineral composition and amino acid profile of “Oori” mango kernel cake.

2. Research Materials and Methods

2.1 Preparation of mono-culture starters and fermented “Oori” mango kernel cake

Rhizopus oligosporus, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum* were isolated from decomposed mango kernels in May, 2009 using potato dextrose agar. The fungi were identified according to Samson and Van Reen-Hoekstra (1988), while mango kernel cake was prepared as described by Kayode and Sani (2008). Suspensions of actively growing mid-log phase culture of the fungi were individually adjusted to $5x10^4$spore/ml with sterile distilled water according to the methods described by Sani *et al*. (1992). One-kilogramme of autoclaved (sterile) mango kernel cake was mixed with 1litre of sterile distilled water in four (4) different fermenters and stirred properly to obtain a uniform mash as described by Kayode and Sani (2008). Twenty millilitres from the suspension ($5x10^4$spore/ml) of each fungus was used as fermentation starter to inoculate 1kg of the cake in the fermenter before commencement of fermentation and incubation at ambient temperature (27 ± 5^0C) for 168 hrs (Lawal *et al*., 2005).

2.2 Mineral Analysis

Two-grammes of mono-culture fermented mango kernel cake (oven dried at 60^0C) were weighed into a 125ml Erlenmeyer flask which has been previously washed with acid and distilled water. Four millilitres of perchloric acid, 25ml of concentrated HNO_3 and 2ml concentrated H_2S0_4 were added under a fume hood. The contents were mixed and heated gently at low heat on a hot plate until dense white fumes appeared. It was finally heated strongly for half a minute and then allowed to cool. Fifty milliliters of distilled water was added and then boiled for half a minute at medium heat. The solution was allowed to cool and filtered completely with a wash bottle into a 100ml Pyrex volumetric flask, and then made-up to mark with distilled water before it was filtered with Whatman No 42 filter paper into a sample bottle before analysis (AOAC, 2000). Phosphorus was determined using ascorbic acid method, while four macro minerals (Ca, Na, K, Mg) and five trace minerals (Fe, Cu, Mn, Zn and Co) were determined with “Buck scientific atomic absorption spectrophotometer Model 210A” (AOAC, 2000).

2.3 Estimation of amino acid profiles

Two grammes of a pre-dried fermented mango kernel cake was properly defatted with chloroform/ethanol (2.1) mixture using soxhlet extraction methods as described by AOAC (2000) before it was hydrolyzed with 7ml of 6M HCl and then evaporated using a rotary evaporator. The hydrolysate was dissolved with 5ml of acetate buffer (pH 2.0) before 10µl of it was loaded into the cartridge of the technicon sequential multi-sample amino acid analyzer (TSM) to separate and analyze for free acidic, neutral and basic amino acids as described by Sparkman *et al.* (1958).

2.4 Experimental design and Statistical analysis

Completely Randomized Design (CRD) was used, while data obtained was analyzed by one-way ANOVA. Significant differences among the means were determined by using Duncan’s New Multiple Range Test as outlined by Obi (2002).

3. Analysis Results

The mineral composition of the mono-culture fermented MKC is shown in Table 1. The values of calcium (119.3mg/kg), potassium (457.1mg/kg) and iron (187.1mg/kg) of the *R. stolonifer* fermented MKC were higher ($p<0.05$) than other treatment cakes including the unfermented MKC. The range of values obtained for potassium (438.1 to 459.0mg/kg), calcium (112.5 to 122.2mg/kg) and iron (180.9 to 193.7mg/kg) in all the fermented MKCs were significantly higher ($p<0.05$) than the values of potassium (436.0mg/kg) calcium (103.1mg/kg) and iron (44.2mg/kg) of the unfermented MKC. While, on the contrary the range of values of sodium (75.0 to 85.4mg/kg), magnesium (10.2 to 37.5mg/kg), phosphorus (50.0 to 500.0mg/kg) and zinc (2.87 to 14.36mg/kg) were significantly lower ($p < 0.05$) compared with the value of sodium (102.7mg/kg) magnesium (47.5mg/kg) phosphorus (575.3mg/kg) and zinc (43.69mg/kg) of the unfermented MKC (Table 1).

The essential and non-essential amino acids in the fermented and unfermented MKCs were shown in Table 2. Higher values of some amino acids were obtained in the fermented MKCs. Threonine (1.36 to 2.03 g/100g protein) and isoleucine (2.62 to 3.00 g/100g protein) content of the fermented MKCs increased appreciably compared with the unfermented MKC. On the other hand, there was general decrease in the values of methionine (0.40 to 0.60 g/100g protein), phenylalanine (2.73 to 3.30 g/100g protein) and valine (2.26 to 2.73 g/100g protein) compared with the values of unfermented MKC (Table 2).

The total score of the essential amino acids of the fermented MKCs ranged between 44.42 and 55.68% when compared with the reference pattern (Tables 3 and 4).

4. Discussion and Conclusion

The levels of calcium and iron content of the mono-culture fermented mango kernel cake has added values to the mineral nutritional quality, since calcium is essential factor for building living cells that make up an animal body.

The iron content may improve the health condition of animals that fed on the cake, since iron is an important substance in the heam group of oxygen-carrying protein and myoglobin in the muscles (Thomas, 2002). The quantity of magnesium recorded may still be adequate for animal nutrition since its help in keeping the muscles relaxed and used for the formation of strong bones and teeth in addition to controlling blood pressure and nerve transmitter (Thomas, 2002). The values of potassium in most of the fermented mango kernel cakes may be adequate for animal feeding; thereby preventing the danger of muscular paralysis, mental disorientation and cardiac irregularities often associated with a fall in the level of potassium in the plasma. The level of phosphorus of the fermented mango kernel cakes is generally lower than the content of maize (900.0mg/kg) as reported by Aduku (1993). Although the amount of calcium recorded in relation to the low levels of phosphorus in the MKCs fermented with mono-culture of *A. niger* and *P. chrysogenum* may be an advantage over the MKCs that were fermented with mono-culture of *R. oligosporus* and *R. stolonifer* for normal calcification of bone and reproduction.

The chemical score of isoleucine and histidine of the *Rhizopus oligosporus* fermented MKC (67.50 and 72.35%) in Table 3, *Rhizopus stolonifer* fermented MKC (75.00 and 130.59%) and *Penicillium chrysogenum* fermented MKCs (65.75 and 62.94%) in Table 4 respectively may favourably compared with FAO/Maynard reference value for growing chicks. However, the levels of the individual essential amino acid of the fermented MKC suggested that there is still need for adequate supplementation of lysine and methionine that are critically important for proper growth and development of poultry when the mono-culture fungal fermented MKC is to be used as chicken's feedstuff.

In conclusion mango kernels of the "Oori cultivar's" can be defatted and the resulted cake fermented with the mono-culture of fungal isolates to improve on mineral content and changes in amino acid profile of the fermented products. If the mono-culture fungal fermented mango kernel cake is fortified with the deficient amino acids, it may be used as a supplement for maize in a quest for non-conventional alternative feedstuff for poultry production. This may lead to the utilization of mango kernels which has constituted wastes in the tropics especially in Nigeria during harvest seasons and perhaps a reduction in the cost of poultry production.

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Table 1. Macro and trace minerals of "Oori" mango kernel cake after mono-culture fungal fermentation at ambient temperature (27$\pm$5^{0}C) for 168 hours

Mono-culture fermented MKC	**Mineral composition (mg/kg)**									
	Ca	Na	K	Mg	P	Fe	Cu	Mn	Zn	Co
R.oligosporus	113.9^{b}	85.4^{b}	438.1^{a}	37.5^{c}	550.0^{c}	193.7^{d}	0.0	0.0	10.74^{b}	0.0
A. niger	122.2^{d}	83.0^{b}	446.6^{b}	34.9^{c}	50.0^{a}	180.9^{b}	0.0	0.0	14.36^{c}	0.0
R.stolonifer	119.3^{c}	79.25^{ab}	457.1^{c}	20.0^{b}	365.0^{b}	187.1^{c}	0.0	0.0	2.87^{a}	0.0
R.chrysogenum	112.5^{b}	75.0^{a}	459.0^{c}	10.2^{a}	71.3^{a}	188.0^{c}	0.0	0.0	7.87^{b}	0.0
Unfermented cake	103.1^{a}	102.7^{c}	436.0^{a}	47.5^{d}	575.3^{d}	44.2^{a}	0.0	0.0	43.69^{d}	0.0
SEM	0.61	1.98	1.97	1.78	6.20	1.52	0.0	0.0	0.94	0.0

Values are means of three replicates readings; SEM = Standard error of mean abc…= Means within column having different superscripts differ significantly (p<0.05); MKC = Mango kernel cake

Table 2. Amino acid profile of "Oori" mango kernel cake after mono-culture fungal fermentation at ambient temperature for 168 hours

Amino Acids profile	**Amino acids concentration (g/100g protein) of the fermented mango kernel cake**				
	Rhizopus Oligosporus	*Aspergillus niger*	*Rhizopus stolonifer*	*Penicillium chrysogenum*	unfermented cake (umkc)
Lysine	1.92	1.80	2.32	2.41	2.16
Histidine	1.23	0.80	2.22	1.07	0.90
Arginine	2.50	3.00	3.61	2.80	3.21
Aspartic acid	3.76	4.08	5.11	4.02	4.41
Threonine	1.36	1.38	2.03	1.65	1.24
Serine	1.50	1.69	2.50	2.08	2.25
Glutamic acid	4.33	5.48	4.58	6.05	5.26
Prolein	0.70	1.12	0.90	1.03	0.84
Glycine	2.00	1.66	2.54	1.41	2.35
Alanine	1.97	2.21	2.00	2.19	2.27
Cystine	0.80	0.50	0.67	0.63	0.56
Valine	2.26	2.73	2.53	2.60	3.00
Methionine	0.58	0.40	0.60	0.50	0.66
Isoleucine	2.70	2.62	3.00	2.63	2.54
Leucine	3.02	2.86	3.56	3.80	3.44
Tyrosine	1.10	0.90	1.31	1.20	1.05
Phenylalanine	2.73	3.14	3.07	3.30	3.40

Table 3. Chemical score of essential amino acids in the cake fermented with mono-culture of *R. oligosporus* and *A. niger* as compared with recommended values for growing chicks

Essential amino acids profile	**[1]Recommended value (g/100g protein)**	**Mono-culture fermented mango kernel cake**			**Chemical score (%)**		
		umkc	Ro	An	umkc	Ro	An
Isoleucine	4.00	2.54	2.70	2.62	63.50	67.50	65.50
Leucine	6.70	3.44	3.02	2.86	51.34	45.07	42.69
Lysine	5.50	2.16	1.92	1.80	39.27	34.91	32.72
Methionine	3.50	0.66	0.58	0.40	18.86	16.57	11.43
Phenylalanine	6.00	3.40	2.73	3.14	56.67	45.50	52.33
Valine	4.40	3.00	2.26	2.73	68.18	51.36	62.05
Histidine	1.70	0.90	1.23	0.80	52.94	72.35	47.05
Threonine	3.30	1.24	1.36	1.38	37.58	34.00	35.50
Arginine	6.10	3.21	2.50	3.00	52.62	40.98	49.18
Total	41.20	20.55	18.30	18.73	49.88	44.42	45.46

[1]FAO (1973) and [1]Maynard *et al*. (1979)
Ro = *R. oligosporus*
An = *A. niger*
umkc = unfermented mango kernel cake

Table 4. Chemical score of essential amino acids in the cake fermented with mono-culture of *R. stolonifer* and *P. chrysogenum* as compared with recommended values for growing chicks

Essential amino acids profile	**[1]Recommended value (g/100g protein)**	**Mono-culture fermented mango kernel cake**			**Chemical score (%)**		
		umkc	Rs	Pc	umkc	Rs	Pc
Isoleucine	4.00	2.54	3.00	2.63	63.50	75.00	65.75
Leucine	6.70	3.44	3.56	3.80	51.34	53.13	56.71
Lysine	5.50	2.16	2.32	2.40	39.27	42.18	43.64
Methionine	3.50	0.66	0.60	0.50	18.86	17.14	14.29
Phenylalanine	6.00	3.40	3.07	3.30	56.67	51.17	55.00
Valine	4.40	3.00	2.53	2.60	68.18	57.50	59.09
Histidine	1.70	0.90	2.22	1.07	52.94	130.59	62.94
Threonine	3.30	1.24	2.03	1.67	37.58	61.52	50.00
Arginine	6.10	3.21	3.61	2.80	52.62	59.18	45.90
Total	41.20	20.55	22.94	20.75	49.88	55.68	50.36

[1]FAO (1973) and [1]Maynard *et al*. (1979)

Rs = *R. stolonifer*

Pc = *P. chrysogenum*

umkc = unfermented mango kernel cake