Proximate Compostion, Anti-Nutritional Factors, Mineral Composition and Amino Acid profile of Biochemically Treated Jatropha curcas Kernel Cake

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Article

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Proximate Compostion, Anti-Nutritional Factors, Mineral Composition and Amino Acid profile of Bio-chemically Treated *Jatropha curcas* Kernel Cake.

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ABSTRACT

This study evaluates the effect of biochemical treatment on the chemical composition and some antinutritional factors (ANF's) of *Jatropha curcas*. The biochemical treatment resulted in significant (P<0.05) increases in the crude protein, ether extract, and the ash content of the treated cakes compared to the untreated cake sample. There was a concurrent decrease in the crude fibre in the treated samples compared to the untreated sample. The antinutritional factors (Tannin, oxalate, phytate and saponin) had significant (P<0.05) decreases in the treated samples compared to the untreated sample. Phorbolester significantly (P<0.05) reduced in the treated samples. The sodium, calcium and manganese fraction in the treated samples were significantly lower (P<0.05) than the untreated sample, while iron, phosphorus, potassium and magnesium showed a significant (P<0.05) increase.The treated *Jatropha* kernel cake in this study showed a good amino acid enhancement compared to the untreated sample, soyabean and the WHO/FAO reference protein. The results of this study show that the biochemical treatment of *Jatropha curcas* kernel cake can be used to improve the nutritional quality of the cake by reducing the antinutrients and the toxic compound phorbolester, thereby making it a good feedstuff for use in ruminant industry

KEYWORDS: *Jatropha curcas,* Antinutritional factors, Phorbolester, Mineral, Amino Acid

INTRODUCTION

The aim of a farmer is not just to provide adequate protein in the diet of his animals, but the quality of the protein is a major theme in whether the protein would be available for nutritive value addition to farm animals. The quality of protein and its availability are affected by the quality of the amino acids in the protein source. Amino acids are the building blocks of life and have long played an important role in both human and animal nutrition and health maintenance (Bercovici and Fuller, 1995). According to Wolfgang *et al.,* 2005 Of the four production methods for amino acids—extraction, synthesis, fermentation, and enzymatic catalysis—it is particularly the last two biotechnological processes, their economic and ecological advantages, that are responsible for this spectacular growth of the amino acid production industry in the world. Advances in fermentation technology and strain improvement of amino acid producing microorganisms (Ikeda, 2003) have enabled industrial-scale production of some amino acids like lysine as well as glutamate (de Graaf *et al*. 2001; Pfefferle *et al*., 2003). Enzyme catalysis will remain the preferred production method for nonproteinogenic amino acids and amino acid derivatives. Therefore, with controlled

fermentation, the biological and nutritive values of feedstuffs can be improved upon without much drudgery to the farmers finance. The problem of antinutrients and toxins present in some can be solved with fermentation process, as the array of enzymes can adequately deal with the menace of toxicity in feedstuffs and the problem of nutrient binding by some antinutrients. Safari *et al.,* 2012 reported both elimination and reduction of antinutritional factors and improvement of taste, smell and nutritive values of fermented feedstuffs. The use of fermentation processing methods for soyabean as means of nutritional improvement and removal of anti nutritional factors of feedstuff have been reported by Matsui, 1996; Ayanwale and Kolo, 2001;Ayanwale and Ari, 2002; Dawodu, 2009. Oladele and Oshodi (2008) also reported that fermentation can produces important nutrients or eliminate antinutrients. That Hence the thrust of this study was to explore the biochemical pathway to making *Jatropha curcas* kernel cake a premium feedstuff of nutritional quality for extensive use in the feed industry.

OBJECTIVES

1. To determine the proximate composition of the *Jatropha curcas* kernel cake samples cake
2. To evaluate the anit-nutritional factors of the treated and untreated *Jatropha curcas* kernel cake samples
3. To analyse the mineral and amino acid profile of treated and untreated Jatropha curcas kernel cake

MATERIALS AND METHODS

Seed Collection

Matured and sundried seeds of *Jatropha curcas* were purchased around Ilorin metropolis. The seed coat was singly removed manually to obtain the kernel. The kernel was milled using mechanical grinder, and the oil extracted using the mechanical hydraulic oil press after which further oil extraction was carried out using petroleum ether. The cake was then packed in polythene bags for autoclaving at 121^{o}C for 15minutes so as to get rid of any microbes that could be present in the cake. The autoclaved cake was allowed to cool and then used as substrate for fungal growth.

Chemical Treatment

This was done according to the methods described by Devappa *et al.* (2012). The milled defatted *Jatropha curcas* kernel cake was mixed with with ferric chloride solution ($FeCl_3.6H_2O$; 4ml, w/v 53µg/ml). The kernel cake was mixed until it absorbed the ferric solution and then incubated in a waterbath for 15minutes at 60^{o}C. The resulting ferric chloride treated kernel cake was mixed in an equilvalent solution of H_2O_2 at 60, 120 and 180mg/kg kernel cake to get a homogenous paste-like texture and then incubated in a waterbath for 15minutes at 60^{o}C. The treated kernel cake was dried at 50^{o}C prior to fermentation.

Fungus

Fungus (*Penicillum expansum)* was collected from the Department of Microbiology, Obafemi Awolowo University, Ile Ife, Nigeria and maintained on Potato Dextrose Agar contained in petridishes.

Inoculation and Incubation of Substrate

Suspension of actively growing mid-log phased culture of the *Penicillum expansum* was individually adjusted to $5x10^4$spore/ml with sterile distilled water. 20ml from the suspension ($5x10^4$spore/ml) was used to inoculate 1kg of the autoclaved and hydrogen peroxide treated kernel cake arranged in layers in a container as described by Belewu and Ogunsola (2010). The whole content was incubated in an incubator at room temperature for 7 days.

The autoclaved kernel cake was incubated separately with the fungus and later incubated at room temperature (21^{o}C) for 14days. The growth was terminated by oven drying at 80^{o}C for 24 hours.

Amino Acid Profile

The treated and the untreated *Jatropha curcas* kernel cake samples were dried to constant weight defatted, hydrolyzed and evaporated in a rotary evaporator and then loaded into the technicon sequential multisampling amino acid analyzer (TSM). The amino acid composition of the treated and untreated *Jatropha curcas* kernel cake was determined using Technicon Amino Acid Analyzer (TSM–1 Technicon Instrument, Basingstoke, Hampshire, UK) using Norleucine as internal standard. This procedure follows the method described by Bassler and Buchholz (1993) and Steer *et al.* (2003). The contents of different amino acids recovered were presented in g/100 g of protein. The amino acid contents of the total kernel cake proteins were compared with the FAO/WHO reference pattern, soyabean and reported literature values for *J. curcas* (FAO/WHO, 1990; Makkar and Becker, 1997; Bau *et al*).

Mineral Analysis

Two-grammes of pre-dried biochemically treated *Jatropha curcas* 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 (4ml) 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 50 seconds and then allowed to cool. 50ml of distilled water was added and then boiled for 50 seconds at medium heat. The solution was allowed to cool and filtered completely into a100ml 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 five macro minerals (Ca, Na, K, P Mg) and two trace minerals (Fe and Mn) were determined with "Buck scientific atomic absorption spectrophotometer Model 210A" (AOAC, 2000).

Tannin

The Tannin content was determined as described by Swain (1979). 0.20g of the sample was measured into a 50ml beaker, and 20ml of 50% methanol was added and covered with paraffin and then placed into waterbath at 77^{o}C to 80^{o}C for 1hour. The mixture was the shaken thoroughly to ensure homologous mixing. Whatman No.1 filter paper was double layered into a 100ml volumetric flask using 50% methanol for rinsing it. 20ml distilled water, 2.5ml folin-dennis reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stay for 20 minutes when the bluish-green colour was observed. Standard Tannic acid solutions of range 0 to 10ppm were treated similarly as 1ml of previous sample. The absorbance of the tannic

acid standard solutions as well as samples was read after colour development on a Spectronic 21D spectrophotometer, at a wavelength of 760nm. Percentage of tannin was calculated using the formula:

Tannin (%) = Absorbance of sample x gradient x dilution factor/weight of sample x 100

Phytate

The phytate content was determined using the methods of Lucas and Markakes (1975). 0.2g of the sample was weighed into different 250ml conical flasks. Samples were soaked in 100ml of 2% concentrated Hydrochloric acid for 3 hours. The samples were filtered, 50ml each of the filtrate was placed in 250ml beaker and 100ml of distilled water added to each sample. 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per ml. The percentage phytic acid was calculated using the formula:

Phytic acid (%) = Titre value x 0.00195 x 1.19 x 100/2

Saponin

The spectrophotometric method of Brunner (1984) was used in analysing saponin. 1g of finely ground sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure a uniform mixing. The mixture was then filtered using a Whatman No.1 filter paper into a 100ml beaker, and then 20ml of 40% saturated magnesium carbonate solution was added. The filtration was then repeated to obtain a colourless solution. 1ml of this colourless solution was pipette into 50ml volumetric flask after which 2ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 minutes for a blood red colour to be observed. 0 to 10ppm standard saponin solutions were prepared from saponin stock solution. The standard solution was treated with 2ml of 5% $FeCl_3$. The absorbance of the sample as well as the standard saponin solution were read after colour development in a Jenway V6300 Spectrophotometer at a wavelength of 380nm. Percentage saponin was calculated using the formula:

Saponin (%) = Absorbance of sample x gradient x dilution factor/weight of sample x 100

Oxalate

Oxalate determination was as described by Alabi *et al.* (2005). 2g of the sample was digested with 10ml of 6M Hydrochloric acid for one hour and made up to 250ml in a volumetric flask. The pH of the filtrate was adjusted with conc NH_4OH solution until the colour of the solution change from salmon pink to a faint yellow colour. The filtrate was then treated with 10ml of 5% $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500rpm after which the supernatant was decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4. The total filtrate resulting from the dissolution in H_2SO_4 was made up to 300ml. An aliquot of 125ml of the filtrate was heated until near boiling point and then titrated against 0.05M of standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 seconds, after which the burette reading was taken. The oxalate content

was calculated from the titre value.

Phorbolester

The phorbolester was determined as described by Makkar and Becker (2010). The dried ground kernel was extracted with methanol in a sealed container at a ratio 1:3 (w/v) at room temperature for 15min. the sample was then centrifuged for 8 min at 3200xg to collect the supernatant. Methanol extraction was repeated three times. The solvent was removed by using a rotary evaporator at 65^{o}C to recover the oil. The oily fraction containing phorbolester was re-extracted with a mixture of diethyl ether and water (1:1) at a ratio of 1:3 (w/v) in a separating funnel at room temperature. The upper solvent layer containing phorbolester was separated from the lower phase containing the oil. The phorbolester rich fraction was concentrated using a rotary evaporator to remove all the solvents. The weight of the phorbolester rich fraction was recorded.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) of a Completely Randomized Design (CRD) model using SPSS Model 16; the treatment means were separated using the same package.

RESULTS AND DISCUSSION

TABLE 1: PROXIMATE COMPOSITION OF TREATED AND UNTREATED CAKES

Parameters%	Untreated cake	Treatment one	Treatment two	Treatment three	±SEM
Dry Matter	91.10^{a}	93.73^{b}	94.95^{c}	93.92^{d}	0.011
Crude Protein	45.25^{a}	55.34^{b}	57.09^{c}	57.31^{b}	2.0x10^{-4}
Ether Extract	15.34^{a}	22.16^{b}	22.32^{c}	20.83^{d}	1.9x10^{-4}
Crude Fibre	7.13^{c}	3.76^{b}	3.74^{b}	3.69^{a}	0.9x10^{-4}
Ash	12.35^{a}	13.63^{b}	13.74^{c}	13.69^{d}	1.4x10^{-4}
NFE	19.93^{a}	5.61^{b}	6.78^{c}	5.78^{d}	2.2x10^{-4}

abc means on the same row with different superscripts are significantly different ($p<0.05$)

Table 3: Effects of Bio-chemical Treatment on the Mineral Composition on Untreated and Graded Levels of Treated *Jatropha curcas* Kernel Cake

PARAMETER	Untreated**	60mg/kg	120mg/kg	180mg/kg	±SEM
Calcium (ppm)	493.19^{d}	374.13^{b}	385.51^{c}	365.15^{a}	0.02
Manganese (%)	15.20^{d}	13.19^{b}	13.73^{c}	12.63^{a}	0.01
Sodium (ppm)	242.13^{d}	205.40^{b}	228.99^{c}	184.88^{a}	0.04
Phosphorus (ppm)	118.72^{b}	122.01^{c}	109.86^{a}	142.21^{d}	0.06
Potassium (ppm)	156.26^{a}	173.70^{c}	168.47^{b}	184.50^{d}	0.04
Magnesium (ppm)	104.47^{a}	126.66^{c}	118.46^{b}	132.57^{d}	0.15
Iron (ppm)	107.03^{a}	117.17^{b}	121.99^{c}	129.95^{d}	0.22

abc means on the same row with different superscripts are significantly different ($p<0.05$)

values of mean of triplicate analysis

****Untreated: Deffated and unfermented**

Table 4: Effects of Bio-chemical Treatment on the Amino Acid Profile of Untreated and Graded Levels of Treated *Jatropha curcas* Kernel Cake (g/100g)

PARAMETERS	Untreated	60mg H_2O_2	120mg H_2O_2	180mg H_2O_2	WHO/ FAO req FAO (1990)	Soyabean (Bau *et al,* 1994)	Untreated Jatropha curcas (Makkar *et al*., 1994)
Glycine	5.98	5.31	4.57	3.59	ND	4.01	4.92
Alanine	2.64	3.51	3.86	4.68	ND	4.23	5.21
Serine	4.23	3.99	3.56	3.31	ND	5.67	4.80
Proline	5.75	3.24	3.28	5.45	ND	4.86	4.96
Valine	3.84	4.01	4.68	4.22	4.2	4.59	5.19
Threonine	4.68	3.24	4.54	2.98	2.8	3.76	3.96
Isoleucine	3.33	3.67	2.84	3.96	4.2	4.62	4.53
Leucine	7.48	8.34	7.54	8.17	4.2	7.72	6.94
Aspartate	7.62	8.48	9.18	8.33	ND	11.30	9.49
Lysine	4.21	7.65	7.60	6.87	4.2	6.08	4.28
Glutamate	11.93	14.76	14.59	14.42	ND	16.90	14.68
Methionine	9.38	9.73	9.55	9.20	2.2	1.22	1.91
Phenylalanine	3.99	4.31	4.05	5.44	2.8	4.84	4.34
Histidine	3.25	3.44	3.33	3.42	ND	2.50	3.30
Arginine	11.29	12.43	12.65	12.29	ND	7.13	11.9
Tyrosine	4.43	4.27	3.16	3.30	2.8	3.39	2.99
Cystine	1.52	1.04	1.44	1.37	2.0	1.70	2.24

Table 5: Effects of Bio-chemical Treatment on Some Antinutritional Factors in Untreated and Graded Levels of Treated *Jatropha curcas* Kernel Cake

PARAMETERS (%)	Untreated *	60mg/kg	120mg/kg	180mg/kg	±SEM
Tannin	103.84^{b}	39.36^{a}	33.32^{a}	36.28^{a}	2.02
Phytate	6.28^{c}	4.37^{b}	2.34^{a}	3.01^{a}	0.24
Saponin (%)	39.00^{c}	17.66^{a}	19.91^{b}	20.27^{b}	0.52
Oxalate (%)	69.55^{b}	51.13^{a}	51.73^{a}	53.39^{a}	0.79
Phorbolester (mg/g)	0.22^{d}	0.09^{c}	0.05^{b}	0.02^{a}	0.01

^{abc} **means on the same row with different superscripts are significantly different ($p<0.05$), values of mean of triplicate analysis**

***Untreated: Deffated and unfermented**

The untreated cake used in this study had a crude protein content of 45.25% which was similar to that of the soyabean meal (49-54%); while the treatment groups had a higher crude protein (55.34%, 57.09%, and 57.31%) than soyabean meal which is often referred to as the standard among plant protein sources (Devappa *et al.,*2012). This showed that biochemically treated *Jatropha curcas* kernel cake can adequately replace/compliment soyabean in the diets of ruminant animals. The crude protein of the treated *Jatropha curcas* kernel cakes was higher ($p<0.05$) than that of the untreated cake, this can be attributed to the microbial protein that was added to the cake during fermentation, this finding agreed with the works of Aro (2008) who reported an increase in the crude protein content of fermented cassava. Research works of Barkie *et al* (1995); Oboh and Akindahunsi (2003); Vijayakumar (2003); Bhatnagar (2004); Nwafor and Ejukonemu (2004); Martinez-Herrera *et al* (2006); Ekpe *et al.* (2007) and Belewu (2008) gave clear evidences of crude protein increase in fermented substrates. The improvement in the crude protein content could also be partially due to pre-treatment (de-shelling, cold extraction and hydrogen peroxide treatment) of *Jatropha curcas* kernel cake, and this was in line with the reports of Devappa *et al.* (2012) where an increase in protein content after de-shelling and defatting was reported.

The crude fibre of the treated kernel cake also reduced ($p<0.05$) when compared to the untreated cake, this corroborates the reports of Belewu and Belewu (2005); Oseni and Ekpegirin (2007); Aro (2008); Belewu and Ogunsola (2010). The reduction observed in the crude fibre could be attributed to the catalytic enzymatic ability of the fungus used. The fungus utilizes the substrates' crude fibre for its own metabolism, thereby reducing it; this confirmed the assertions of Belewu and Adeniyi (2001); Bennet *et al.* (2002); Belewu and

Belewu (2005) and Aro (2008) that reduction in crude fibre content of a fermented substrate is due to their catalytic power in biotechnological processes. The hyphal growth of the fungus allowed a close contact between the hyphae and substrate surface, hence the fungal mycelium synthesize and excrete high quantities of hydrolytic enzymes which helps breakdown the substrate for fungal metabolism. The ease with which the organism reduced the crude fibre could also be due to the pre-treatment's (milling, cold extraction, hydrogen peroxide treatment), as pre treatment was reported to be required for accessibility of the chemical constituents of the substrate to the organism; it also makes the physical structure more susceptible to mycelia penetration (Bettache *et al.,*2014).

The ether extract content was higher in the treated samples and this agreed with the works of Belewu and Ogunsola (2010) that the fungus *Penicillum expansum* possessed lipogenic properties; Conversely, Aro (2008) reported a non significant difference in the ether extract of fermented cassava peels. The difference observed could be due to the different organisms used. There was significant increase in the ash content of the treated samples, and this was in line with the reports of Aro, 2008. The observed increased ash content in the fermented kernel cake could be a product of crude fibre breakdown by *Penicillum expansum*. The increase in the ash content corroborates the assertions of Aro and Aletor (2012) that lower crude fibre content of fermented substrate led to a higher ash deposition within the fermented substrates. The fungus *Penicillum expansum* possess enzymes (catalase, lipase and esterase) that could have chelating properties which could have led to the increase in the ash content. The reduction in NFE could be due to available carbohydrate (NFE) of the substrate decreasing with the increase in protein and fat contents of the substrate during solid state fermentation suggesting that the carbohydrates in the substrate could have been converted into microbial protein and other compounds (Imelda-Joseph *et al.,* 2008).

The fungus used (*Penicillum expansum*) helped decrease the phytate content of the *Jatropha curcas* kernel cake; this shows that the organism used has the ability to produce the enzyme phytase and it agreed with the works of Safari *et al.* (2012) who reported a reduction in the phytate content of canola treated with fungus. It is also in line with the reports of Ojokoh*,* (2005) that phytase activity is a characteristic that most microflora possess. Fagbemi *et al*. (2005) also reported that fermentation causes a reduction in the antinutritional factors in oil seeds. The reduction in the phytate could have also helped in the availability of the nutrients to the animal because phytate binds mineral and make them unavailable to the animal. This agreed with the reports of FAO (1990) who reported that Phytic acid bound some mineral (calcium, iron, and zinc) thereby making them unavailable to the animal. Safari *et al.* (2012) reported phosphorus bioavailability as a result of reduction of phytate in fermented substrate. The reduced tannin content of the treated cakes could have added quality to the protein, because it has been reported by Adeyeye and Fagbohun (2005) that increased tannin reduces protein availability to animals. Odetokun (2000); Enujiugha and Akanbi (2005) also reported a decrease in the tannin content of fermented Jack bean and African oil bean respectively. The use of solvent extraction on the pre-fermented kernel cake could have added to the reduction of the antinutrients in the *Jatropha curcas* kernel cake; this is in line with the reports of Brenes *et al* (2004) where a reduction in the antinutrients and inactivation of toxic gossypol in cotton seed meal extracted with expander solvent was reported. Mustafa *et al.* (2000) also reported that using solvent extraction processing method had significant impact on antinutrients. Solvent extraction used in this study was highly effective in the reduction of antinutrients in *Jatropha curcas* kernel cake. The reduction could be due to the use of the kernel instead of the whole seed; the seed coat is said to possess higher antinutrients level. The observed reduction in the phorbolester of the treated samples could also be due to the biochemical treatment. The level of 0.22mg/g reported for the untreated

cake in this study was lower than the 0.328mg/g reported for oil extracted *Jatropha curcas* seed cake by Vittaya *et al.* (2012). Hydrogen peroxide in the presence of Fe^{2+} (as a catalyst) produces free hydroxyl radicals (OH^-) which are volatile in nature and can penetrate the intricate parts of the cell. Furthermore, the fungus *Penicillum expansum* produces the enzyme esterase which could have helped in breaking the phorbolester which are esters in *Jatropha curcas* kernel cake. The mechanical and cold extraction of oil from the *Jatropha curcas* before the treatment could have also helped in reducing the phorbolester, as the seed cake with high content of oil was reported to contain the highest concentration of the phorbolester content (Vittaya *et al.,*2012).

Jatropha curcas is reported to be low in amino acids like lysine (Makkar *et al.,* 1997; Makkar *et al.,* 1998). Essential amino acids like alanine, phenylalanine, leucine, aspartate, arginine, glutamate, and lysine showed considerable increase. Marginal increases were observed in essential amino acids like methionine and isoleucine. Most of the essential amino acids were higher than the values reported for soyabean by FAO/WHO (1990). The observed increase in amino acids in the fermented *Jatropha curcas* kernel cake samples showed that microbial degradation are closely related to protein production. The reduction observed for certain amino acids (essential and non essential) could be due to the fact that the *Penicillum expansum* used them for growth and metabolism, this was in line with the works of Imelda - Joseph *et al.* (*2008*). It could also be due to the action of the hydrogen peroxide as a result of the action of the hydroxyl radicals which are volatile to cell constituents, and are non-selective reactors in this reaction. Devappa *et al.* (2012) reported a decrease in some essential amino acids of *Jatropha curcas* seed meal treated with H_2O_2 only.

The amino acid profile of the cake determines the nutritional quality of the protein in the sample, hence the biochemically treated *Jatropha curcas* showed increase in some of the amino acids compared to the untreated sample; this finding corroborate the works of Aro and Aletor (2012) who reported an improvement in the nutritive value of fermented cassava wastes. The treated samples in this study showed a good amino acid enhancement compared to the untreated sample, soyabean and the WHO/FAO (1990) reference protein.

The reduction in the antinutritional factors observed could also be linked to the improvement of amino acid profile in the biochemically treated cakes, and it corroborates the fact that improvement in amino acids profile by fermentation reduces antinutritional factor contents and produces values product with the functional properties (Safari *et al*. (2012) and Wolfgang *et al.* (2005). The improvement in the amino acid profile confirmed the improvement in the protein quality of the biochemically treated *Jatropha curcas* kernel cake as shown by the increase in the amino acid content of the fermented cake compared to the untreated cake. The findings of this study also confirmed the assertations by Wolfgang *et al.* (2005) that fermentation and enzymatic catalysis are responsible for spectacular amino acid production, especially the production of the essential amino acids which cannot be synthesized by the animals. There was an increase in lysine, methionine and threonine, which are limiting amino acids; specifically, *Jatropha curcas* is said to be limiting in lysine. The findings on amino acids corroborate the reports of Aro and Aletor (2002); Wolfgang *et al.* (2005) and Ari *et al*. (2012) where the best results were reported for microbially fermented feed. The controlled nature of fermentation in this study may also be responsible for the improvement in the amino acid profile of the treated cake and it confirmed the report of Caprita and Caprita (2008) that controlled fermentation produced better amino acid profile and uncontrolled fermentation leads to putrefaction. Frias *et al.* (2009) also reported total improvement in the amino acid profile of fermented substrates.

The values of essential amino acids for some treatments (lysine, valine, threonine, histidine, isoleucine, lysine and phenylalanine) were higher than the values reported by Ari *et al.* (2012) who profiled the amino acids for Lactobacillus fermented soyabean. This could be due to the fact that fungi are better fermenters than bacteria. The improvement observed in the lysine of fermented *Jatropha curcas* kernel cake was comparable to the result of Devappa *et al.* (2012) where an improvement was reported in the lysine of fungal biomass protein of *Jatropha curcas* seed cake.

The amino acid profile in the treated *Jatropha curcas* kernel cake observed in this study showed an improvement in the nutritive value of the cake and it is an indication that the amino acid added during fermentation will enhance the quality of feedstuffs. The values obtained for amino acid profile of biochemically treated *Jatropha curcas* kernel cake compared with the profile for soyabean and FAO/WHO (1990) reference protein.

The result of the mineral composition obtained for both treated and untreated *Jatropha curcas* kernel cake showed significant differences. The higher values were observed in the magnesium, potassium, phosphorus and iron in the treated samples and this could be due to the ability of the enzymes produced by *Penicillum expansum* to chelate minerals. This result corroborates the improvement in the magnesium, potassium and phosphorus reported by Oladele and Oshodi (2008), the values obtained for calcium was not in line with their result on increase in calcium, the difference could be as a result of usage of different organism because organisms have different affinity for calcium. It could also be as a result of the hydroxyl radicals affecting the calcium, manganese and sodium level during pre-treatment. The result showed that the treated samples are good sources of Mg, K, P, Fe because their values are higher than that of the untreated sample. The minerals are vital in the regulation of the acid-base balance, transmission of nerve impulses and other metabolic activities.

The noted increase in some minerals was in line with the report of Oladele and Oshodi (2008); Ekundayo *et al.* (2013) that fermentation increased the content of vital minerals like P, K, Mg. The values they obtained for sodium, calcium and iron were lower and higher respectively than the values they reported. The lower value of iron is in line with the lower value reported by Ekundayo *et al* (2013). This could be that the organism is calcium, iron and/or sodium-specific (it uses calcium and/or iron/sodium for its growth and metabolism (Bello and Akinyele, 2007). The increase in some of the mineral sample corroborates the report of Akindahunsi *et al*. (1999) that fermented sample was rich in some minerals which perform various functions in the body. Amoo (2004) also reported a higher mineral content of fermented samples compared to raw samples of coconut cake.

Conclusion

This study supported the fact that fermentation improves the quality of *Jatropha curcas* kernel cake through increase in the concentration of available nutrients (minerals and protein), improvement in the flavour (chocolate flavour) observed in the fermented kernel cake, improve the essential amino acid profile, and reduction in the antinutritional factors especially phorbolester. It was also observed that pre-treatment with graded levels of H_2O_2 was effective and enhanced the quality of the kernel cake, with 120mg/fermentation having the best result in the nutrient quality, except in phorbolester where 180mgkg/fermentation has

a significantly reduced phorbolester value

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request

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