Effect of Microbial Fermentation on the Nutritional and Anti-Nutritional Composition of Fermented Mung Bean (*Vigna radiata*)

[1]Lawal, R. T. and [2]Awe, S.

[1]Department of Science Laboratory Technology, Faculty of Science, Osun State Polytechnic, Iree, Nigeria

[2]Department of Biosciences and Biotechnology, College of Pure and Applied Sciences Kwara State University, Malete, Nigeria

Correspondence author email: lawalrtunde@gmail.com +2348034618105

sunday.awe@kwasu.edu.ng

Abstract: Adequate nutrition is a prerequisite for active and healthy living. However, getting the required nutrients from foods that are free from potential toxic constituents and readily available for human consumption are among the major concerns. This study investigated the effect of microbial fermentation on the nutritional and anti-nutritional composition of fermented mung bean (*Vigna radiata*). Grains of *V. radiata* were sorted, washed and sun-dried. About 10.5 kg of *V. radiata* were aseptically pulverized into powder using a blender. Seven portions of 500 g of pulverized samples in triplicate were subjected to both natural and induced fermentation in plastic containers for 4 days at 35^0C while the raw sample served as control. Nutrient agar and De Man Rogosa and Sharpe (MRS) agar were used for isolation and enumeration of microorganisms. Parameters assayed from each of the samples were antinutrients and proximate composition. *Streptococcus* sp, *Lysinibacillus* sp, *Brevundomonas* sp, *Lactobacillus* sp, and *Bacillus* sp were the predominant bacterial group isolated from fermented samples. Molecular confirmed the identity of the following microorganisms: *Lysinibacillus mangiferihumi, Lysinibacillus sphaericus, Lysinibacillus boronitolerans, Bacillus* sp, *Lactobacillus* sp, *Xanthobacter agilis, Brevundimonas diminuta, Brevundimonas olei* and *Streptococcus thermophilus*. Microbial counts ranged from $1.47 \times 10^7 \pm 0.00$ to $1.48 \times 10^7 \pm 0.02$ CFU/g for bacteria; $1.80 \times 10^6 \pm 0.01$ to $2.00 \times 10^6 \pm 0.01$ CFU/g for yeast; and $8 \times 10^4 \pm 0.15$ to $9 \times 10^4 \pm 0.10$ CFU/g for fungi. Increase in ash content values was recorded for samples C, D, E, F, and G compared to control which is an indication of the increase in mineral composition. Crude fibre were observed to reduce in value in D, F and G. The protein contents were observed to increase except in samples C and D where decreases in protein contents were observed. Having observed the reduction in antinutrient contents and improvement in nutrients composition of fermented *V. radiata,* this study has shown that fermentation enhanced the nutritional composition of mung beans.

Keywords: Chemical analysis, fermentation, microbial, mungbeans, sequencing

INTRODUCTION

Legumes are probably the second most important source of food next to cereal grains and are consumed worldwide as a major source of protein especially in developing countries. Thus, legumes are good supplements in areas where the staple food is high in carbohydrates but low in protein (Mensah and Olukoya, 2007). Mung bean (*Vigna radiata*) is one of the lesser-known and least used legumes in Nigeria. It has high nutritional potentials and was introduced to Nigeria (Mensah and Olukoya, 2007). It has high lysine content which makes it a good complimentary food for rice-based diets, in which lysine is usually the first limiting amino acid (Chen *et al.*, 2007). The male bean has good digestion and is free from satiety; And it can be used in creating value-added products for infants, recovery patients and the elderly. Traditionally, beans can be eaten alone or combined with rice or vegetables to make soup. However, Antioxidant factors determine the nutritional performance of such soybean. The cereals which are usually consumed in combination with legumes include; rice, maize, millet, and sorghum. Legume flour has been processed and used in many other food preparations such as baby foods and baked products (Uwaegbute, 1990; Nnam, 1994). Legumes contain antinutrients that are known to exert a deleterious effect on man and animals when ingested (Enwere, 1998).

In general, beans are excellent sources of dietary fibre, serving provides 2-4g of a mix of soluble and insoluble fibre (Messina, 1999). Fermentation is employed in preservation techniques to create lactic acid in sour foods such as sauerkraut, dry sausages, kimchi and yogurt, or vinegar (acetic acid) for use in pickling foods. In contrast to the solid (liquid) fermentation, the growth of microorganisms under controlled conditions in the absence of free water. For example, solid combustion products include enzymes and fuels (Biesebeke *et al*., 2002). Soaking involves adding water and/or salt solution to the legumes and discarding the water after some time or cooking with the soak water. Sodium chloride, acetic acid, and sodium bicarbonate solutions have been used in the soaking of legumes (Huma *et al*., 2008). Different soaking times have also been reported (Huma *et al*., 2008; Xu and Chang, 2008), but in most cases, the soaking is done overnight. Soaking of legumes can be done either in boiling water or water at room temperature. In addition to its important role in reducing the cooking time of legumes, soaking water reduces the amount of phytotoxic acids in the crop (Toledo and Canniatti-Brazaca, 2008). This was observed when legumes were not cooked with the soaking water. The flatulence factors in legumes are also reduced by soaking, as a result of the leaching out of stachyose and raffinose (George *et al*., 2014). The objectives of this study are to ferment mung beans using a different fermentation process, microbial and sequencing analysis as well as proximate and antinutrients compositions.

MATERIALS AND METHODS

Sample collection

Mung beans were purchased from *Irepodun* market, *Ekinrinade, Ijumu* Local Government, Kogi State, Nigeria and processed immediately. The mung bean was sorted, washed, and sun dried.

Sample pretreatment and preparation

The dried samples were pulverized using a blender. Pulverized mung bean was divided into seven (7) portions of 500g each and designated from A – G. Two (2) fermentation methods were employed; traditional and induced culture fermentation using plastic containers as fermentors. Sample A was the raw sample and served as a control for nutrients and antinutrients determination. Sample B was fermented by submerging the pulverized sample in 1500ml of distilled water in the cleaned plastics fermentor and covered for 4 days at 25^0C to ferment with indigenous microfloral spontaneous. Samples C, D, E, and F were allowed to undergo control fermentation in which each pure culture of *Lysinibacillus* sp*, Streptococcus* sp*, Brevundomonas* sp *and bacillus* sp were isolated from sample B, were used to inoculate the four samples. Sample G was allowed to ferment using standard strain of *Saccharomyces cerevisiae* to serve as a control for fermented samples from Ilesha brewery, Nigeria. All the fermented samples were dried using an electric oven at 50^0C for 18 hours and were used for the further analysis (Agbaje *et al*., 2015).

Isolation and characterization of microorganisms

Nutrient agar (NA) and De Man Rogosa and Sharpe Agar (MRS), were used for total viable bacteria count, lactic acid bacteria, and mould respectively. Bacteria isolated were further characterized and identified based on their cultural, morphological, physiological, and biochemical properties using Bergey's Manual of Systematic Bacteriology (Holt *et al*., 2000).

Total bacterial DNA extraction

Total bacterial DNA was extracted directly from fermented mung bean (sample B), using the DNeasy Blood and Tissue Extraction Kit (Qiagen, USA) according to the manufacturer's instructions.

Cell pellet already washed in TE buffer was lysed in enzymatic lysis buffer (containing 2 mg/ml lysozyme, 25 Mm Tris HCl pH 8, 10 Mm EDTA, 25% sucrose) and incubated at 37 °C for 30 min in an incubator (Uniscope SM9052, Surgifriend Medicals, England). Proteinase K and extraction buffer were added, mixed by vortexing and incubated at 56 °C in a water-bath (Uniscope SM101 Shaking Water bath, Surgifriend Medicals, England) for 30 min. The DNA was precipitated with ethanol (96 - 100%, v/v) and transferred into the DNeasy Mini spin column for binding of DNA to the column, washed with two different 500 µl washing buffers and eluted with 200 µl elution buffer. The resulting DNA was stored at – 20 °C (Elijah *et al*., 2014).

Bacterial isolate genomic DNA extraction

Genomic DNA extraction from bacterial isolates was also carried out using the DNeasy Blood and Tissue Extraction Kit (Qiagen, USA) following the protocol provided by the manufacturer. Overnight cultures grown in tryptone-soy broth (TSB) were centrifuged for 10 min at 5000 × g, to harvest cells. The pellet was washed 3 times in TE buffer. The procedure reported earlier for total bacterial fermented mung bean (sample B) DNA extraction was followed subsequently to obtain the DNA using the Elijah *et al*. (2014).

Amplification of the 16S rRNA genes

The 16S rRNA gene from total bacterial fermented mung bean (sample B) and genomic DNA respectively, was amplified by Polymerase Chain Reaction (PCR) using bacteria universal primers (27 F - AGAGTTTGATCCTGGCTCAG and 1492R - GGTTACCTTGTTACGACTT). The PCR amplification was carried out in a Techne TC-412 Thermal Cycler (Model FTC41H2D, Bibby Scientific Ltd, UK) in a 50 µl reactions containing 25 µl of 2 × PCR Master Mix (Norgen Biotek, Canada), 1.5 µl of template DNA (0.5 Mg), 1 µl of both forward and reverse primers (2.5 µM of each) and 21.5 µl of nuclease free water in a PCR tube added in that order. PCR was carried out at an initial denaturation step at 94 °C for 2 min, followed by 30 cycles at 94 °C for 30 sec, 52 °C for 30 sec and 72 °C for 2 min, and a final extension step at 72 °C for 5 min. PCR products (amplicons) were separated by electrophoresis on a 1% agarose TAE gel containing ethidium bromide and visualized by UV transillumination (Foto/UV 15, Model 33017, Fotodyne, USA) using the Altschul (1997) method.

Cloning

Cloning was carried out using the the PCR TRAP Cloning System (GenHunter Corporation, USA), following the manufacturer's protocol. Amplicons from total bacterial DNA were spliced into the PCR TRAP Cloning Vector using the T4 DNA ligase. Competent cells were transformed with the recombinant DNA and inoculated in Luria-Bertani (LB)-Tet agar (containing 20 µg/ml of tertacycline).

DNA sequencing and analysis

PCR products from the total bacterial and genomic DNAs were sequenced with 518F and 800R primers using ABI PRISM Big Dye Terminator cycle sequencer (Macrogen, USA). The gene sequences obtained were compared by aligning the result with the sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) search program at the National Centre for Biotech Information (NCBI) using the Altschul (1997) method. Phylogenetic tree to show evolutionary relatedness was constructed using the MEGA7.

Proximate composition of unfermented and fermented mung bean

Mung beans samples were analyzed for humidity, raw protein, ash, fat, and Crude fiber material by conventional process (AOAC, 2012), although, the carbohydrate content of the samples were calculated by the following difference: percentage point Carbohydrate = 100 – (percent moisture +% protein +% fat +% crude fibre +% ash) (Onwurafor *et al*., 2014).

Antinutrient analysis of unfermented and fermented mung bean

Oxalate (titrimetric), phytate, and tannin were determined using standard method AOAC (2012) while phenol was done as described by Anjum *et al*. (2012).

Statistical analysis

The experiments were conducted in a completely randomized design with three replications. All data were first subjected to analysis of variance (ANOVA), after checking their normality and later to Duncan's multiple range test to determine significant differences among the significant treatments at $p<0.05$. Statistical analyses were carried out using SPSS 24.0 (SPSS Science, Chicago, IL, USA).

RESULTS

Isolation and characterization of microorganisms

The lactic acid bacteria and bacteria were isolated and identified from fermented mung bean were *Streptococcus* sp, *Lactobacillus* sp, *Lysinibacillus* sp, *Brevundimonas* sp, and *Bacillus* sp in table 1.

The results obtained for a total viable count of the fermented sample B showed an increase when related with different hours of incubation ranges from 1.47×10^7 ±0.00 to 1.48×10^7 ±0.02 CFU/g for bacteria and ranges between 1.80×10^6 ±0.01 to 2.00×10^6 ±0.01 CFU/g for yeast while fungi ranges from 8×10^4 ±0.15 to 9×10^4 ±0.01 CFU/g respectively in table 2 the data are mean ±SD values of three determinations with different superscript in a column are significantly different (P< 0.05).

Molecular identification of isolates

The isolates share the same node with *Streptococcus thermophilus* which indicate that the same evolutionary relatedness while *Streptococcus thermophilus* are lactic acid bacteria and shares the same tree with all these bacteria. The *Bacillus* sp were reclassified as *Lysinibacillus* sp the results of sequences analysis were as follows: *Lysinibacillus mangiferihumi, Lysinbacillus sphaericus, Lysinibacillus boronitolerans, Bacillus* sp, *Lactobacillus* sp, *Xanthobacter agilis, Brevundimonas diminuta, Brevundimonas olei* and *Streptococcus thermophilus* showed in figure 1.

Proximate composition of mung bean

The moisture content of an unfermented sample A with value of 7.14 ±0.14 % and fermented samples B-G had values of 6.02 ±0.15 to 10.30 ±0.03 %. The ash content of the unfermented sample A had a value of 2.26 ±0.32 % while fermented samples B-G ranges from 1.64 ±0.58 to 3.66 ±0.00 %. The fermented samples values were higher than the unfermented sample. The protein content of the unfermented sample A was 12.70 ±0.03 % and fermented samples B-G ranges from 12.45 ±0.11 to 20.01 ±0.12 %. The unfermented sample A (12.70 ±0.03) had a lower value than fermented samples B-G (17.67 ±0.12, 13.30 ±0.03, 12.45 ±0.11, 18.36 ±0.06, 18.30 ±0.02 and 20.01 ±0.12 %). The crude fat content of the unfermented sample A value of 6.55 ±0.15 % while fermented samples B-G ranges between 4.80 ±0.15 to 7.76 ±0.06 %. It showed that fermented samples of sample B, C, D and F had the highest value of (7.76 ±0.06, 7.08 ±0.35, 7.18 ±0.10, 6.40 ±0.06 and 7.01 ±0.10 %) compared with the unfermented sample A, E and G (6.40 ±0.06, 4.80 ±0.15 and 6.55 ±0.15 %). The crude fibre content of the unfermented sample A had a value of 9.27 ±0.04 % while fermented samples B-G ranges from 5.23 ±0.02 to 22.15 ±0.39 %. The carbohydrate content of the unfermented sample A had a value of 64.13 ±0.07 % while fermented samples B-G ranges between 39.60 ±0.11 to 64.13 ±0.07 %. The fermented samples had the highest values.

The energy value of the unfermented sample A was 1514.21 ±2.72 kj/g while fermented samples B-G ranges from 1222.72 ±1.80 to 1567.97 ±0.90 kj/g in table 3 the data are mean ±SD values of three determinations with different superscript in across the row are significantly different (P< 0.05).

Antinutrient compositions of mung bean

The phytate content of the unfermented sample A had value of 8.200 ±0.20 mg/g while the fermented samples B-G ranges from 6.871 ±0.04 to 8.652 ±0.06 mg/g. Oxalates of fermented samples B-G ranges between 1.171 ±0.01 to 3.512 ±0.00 mg/g while the unfermented sample A had value of 2.555 ±0.03 mg/g. The tannins content of the unfermented sample A had a value of 1.025 ±0.06 mg/g and fermented samples B-G ranges from 0.696 ±0.01 to 2.011 ±0.03 mg/g. Phenols' contents of fermented samples B-G were between 7.483 ±0.04 to 22.595 ±0.46 % while the unfermented sample A value of 11.010 ±0.01 %. The phytic acid content of the unfermented sample A was 2.309 ±0.05 mg/g and fermented samples B-G ranges from 1.935 ±0.15 to 2.403 ±0.02 mg/g in table 4 the data are mean ±SD values of three determinations with different superscript in across the row are significantly different (P< 0.05).

.

Table 1: Morphological and biochemical characteristics of bacteria isolates of mung bean from sample B

Isolate ID	**C**	**D**	**E**	**F**
Cultural Characteristic				
Colour	Cream	Dirty white	Dirty white	Dirty white
Surface	Rough	Smooth	Smooth	Smooth
Edge	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Raised
Biochemical Test				
Gram's Reaction	+ve	+ve	+ve	+ve
Shape	Rod	Rod	Rod	Rod
Catalase	+	-	+	+
Spore	+	-	-	+
Motility	+	-	+	+
Capsule	-	-	-	-
Oxidase	+	-	-	+
Mellibiose	+	+	-	+
Cellibiose	-	-	-	+
Lactose	+	+	+	+
Sucrose	+	+	+	+
Raffinose	+	+	-	-
Gluconate	+	+	-	-
Salicine	-	-	+	+
Arabinose	+	+	+	+
Probable Organisms	*Lysinibacillus* sp	*Streptococcus* sp	*Brevundomonas* sp	*Lysinibacillus* sp

Keywords: Inoculation of starter culture after sterilization of each sample C – F
C – *Lysinibacillus* sp D - *Streptococcus* sp
E – *Brevundomonas* sp F – *Lysinibacillus* sp

Table 2: Microbial Count Population of Fermented Mung Bean Sample B

Time (hrs)	Bacteria CFU/g	Yeast CFU/g	Fungi CFU/g
24	$1.48\times10^{7}\pm0.02^{a}$		$9.00\times10^{4}\pm0.01^{a}$
		$2.00\times10^{6}\pm0.01^{a}$	
48	$1.47\times10^{7}\pm0.00^{a}$		
		$1.80\times10^{6}\pm0.01^{b}$	$8.00\times10^{4}\pm0.01^{b}$
72	$1.47\times10^{7}\pm0.00^{a}$		
		$1.85\times10^{6}\pm0.01^{c}$	$8.00\times10^{4}\pm0.15^{b}$

Results are expressed as mean ± standard deviation. Data having different superscripts along the column are significantly different ($p < 0.05$).

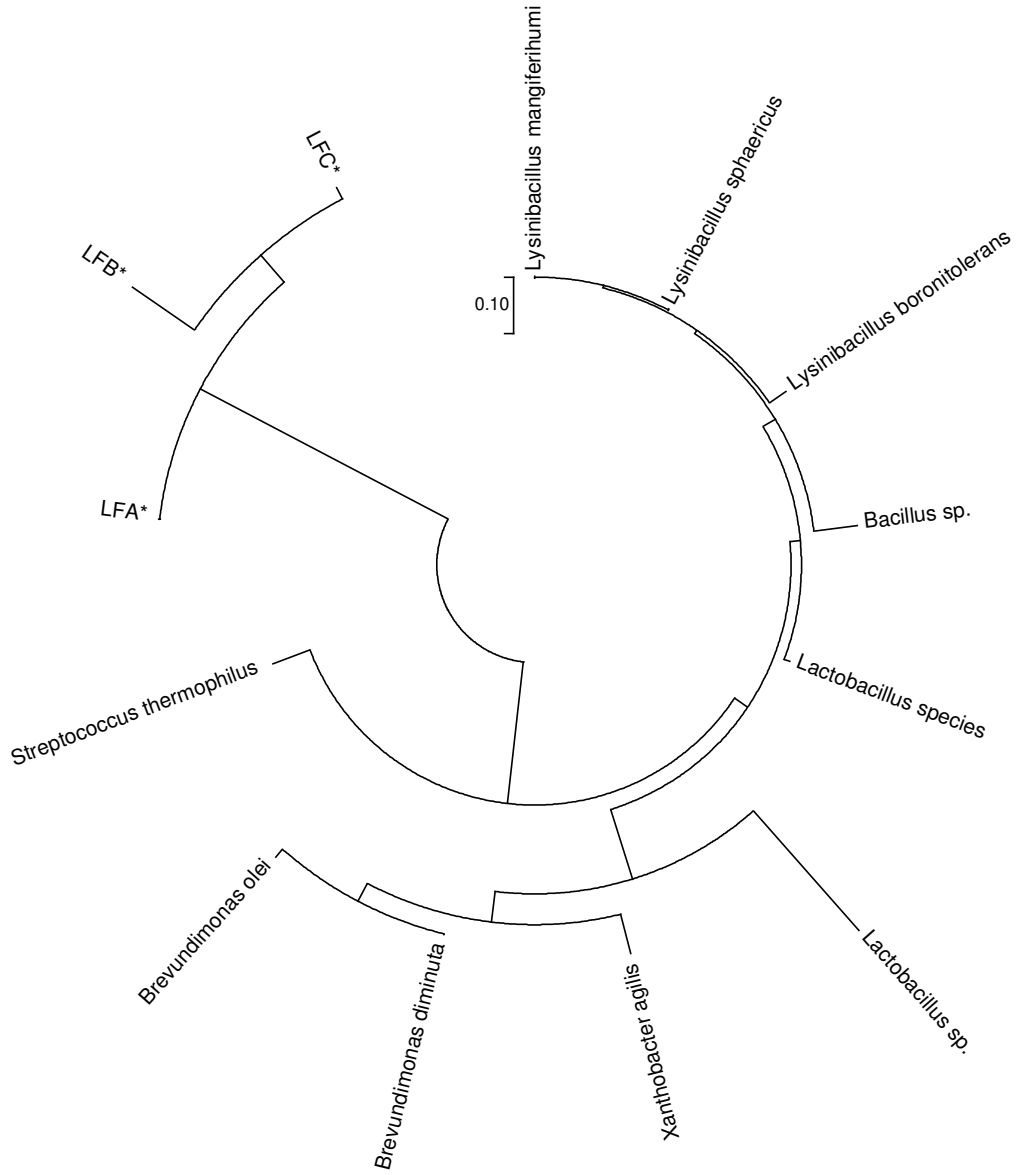

Figure 1: Evolutionary relationships of taxa

Table 3: Proximate Composition of Unfermented and Fermented *Vigna radiata*

Parameter(s)	A	B	C	D	E	F	G
Moisture Content (%)	7.14±0.14^{a}	10.30±0.03^{b}	7.58±0.13^{a}	7.43±0.02^{a}	7.27±0.15^{a}	8.28±0.10^{b}	6.02±0.15^{c}
Ash content (%)	2.26±0.32^{a}	1.64±0.58^{b}	3.11±0.58^{c}	3.56±0.00^{c}	3.44±0.00^{c}	3.64±0.06^{c}	3.66±0.00^{c}
Protein Content (%)	12.70±0.03^{a}	17.67±0.12^{b}	13.30±0.03^{a}	12.45±0.11^{a}	18.36±0.06^{b}	18.30±0.02^{bc}	20.01±0.12^{c}
Crude Fat Content (%)	6.55±0.15^{a}	7.76±0.06^{b}	7.08±0.35^{ab}	7.18±0.10^{ab}	6.40±0.06^{a}	7.01±0.10^{ab}	4.80±0.15^{c}
Crude Fibre Content (%)	9.27±0.04^{a}	12.00±0.25^{b}	22.15±0.39^{c}	5.23±0.02^{d}	24.46±1.63^{c}	7.59±0.31^{e}	6.08±0.04^{de}
Carbohydrate Content (%)	62.11±0.17^{a}	55.98±0.13^{b}	46.38±0.19^{a}	64.13±0.07^{a}	39.60±0.11^{d}	55.18±0.06^{b}	59.44±0.12^{ab}
Energy Value (kj/g)	1514.21±2.72^{a}	1353.94±0.04^{b}	1275.83±4.86^{c}	1567.97±0.90^{a}	1222.72±1.80^{c}	1508.10±0.56^{a}	1528.10±0.56^{a}

Results are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different ($p < 0.05$).

Keywords: Sample A- unfermented mung beean, B- fermented mung bean self inoculation, C - fermented mung bean with *Lysinibacillus* sp, D - fermented mung bean with *Streptococcus* sp, E - fermented mung bean with *Brevundomonas* sp, F - fermented mung bean with *Lysinibacillus* sp, G - fermented mung bean with *Saccharomyces cerevisiae*

Table 4: Antinutrient Composition of Unfermented and Fermented *Vigna radiata*

Parameter(s)	A	B	C	D	E	F	G
Phytates (mg/g)	8.200±0.20^{a}	7.423±0.00^{ab}	6.871±0.04^{b}	7.210±0.06^{ab}	7.416±0.01^{ab}	8.072±0.21^{a}	8.652±0.06^{a}
Oxalate (mg/g)	2.555±0.03^{a}	1.171±0.01^{b}	2.712±0.5^{a}	3.038±0.01^{ac}	3.512±0.00^{c}	2.802±0.00^{a}	2.881±0.05^{a}
Tannins (mg/g)	1.025±0.06^{bc}	2.011±0.03^{d}	1.696±0.10^{a}	1.316±0.15^{ac}	0.696±0.01^{b}	1.451±0.06^{a}	1.124±0.08^{c}
Phenols (%)	11.010±0.01^{a}	22.595±0.46^{b}	18.065±0.91^{c}	13.882±0.14^{ac}	7.483±0.04^{d}	15.630±0.16^{ac}	11.957±0.13^{a}
Phytic Acid (mg/g)	2.309±0.05^{ac}	2.091±0.00^{ab}	1.935±0.15^{b}	2.028±0.00^{ab}	2.089±0.01^{ab}	2.274±0.02^{ac}	2.403±0.02^{c}

Results are expressed as mean ± standard deviation. Data having different superscripts across the row are significantly different ($p < 0.05$).

Keywords: Sample A- unfermented mung beean, B- fermented mung bean self inoculation, C - fermented mung bean with *Lysinibacillus* sp, D - fermented mung bean with *Streptococcus* sp, E - fermented mung bean with *Brevundomonas* sp, F - fermented mung bean with *Lysinibacillus* sp, G - fermented mung bean with *Saccharomyces cerevisiae*

DISCUSSION

The results showed that *Streptococcus* sp and *Bacillus* sp were predominant in which was isolated from the fermented sample B. This may be because these organisms can invade and proliferate in many types of food materials. *Bacillus* sp are known to ferment most sugars, i.e. involve in fermentation (Prescott *et al*., 1999). *Streptococcus* sp and *Lactobacillus* sp isolated from fermented samples belong to the group of lactic acid bacteria which are highly responsible for the uncontrolled fermentation process (Wakil *et al*., 2014). *Bacillus* sp are often associated with the fermentation of food plant origin (Afolabi *et al.*, 2016). The *Bacillus* sp have been isolated and identified in various studies of fermented products such as fufu, burukutu alcoholic beverages (Ogbona *et al*., 1983) as well as legumes and cereals (Tucker, 2003).

The microbial count after 24 hrs fermentation had the highest number than 48 and 72 hrs fermentation. This is similar to the report of other researchers (Agbaje *et al*., 2015; Kayode and Oyetayo, 2016). The phylogenetic tree of evolutionary relatedness. The *Bacillus* sp were reclassified as *Lysinibacillus* sp and the results of sequences analysis were as follows: *Lysinibacillus mangiferihumi, Lysinibacillus sphaericus, Lysinibacillus boronitolerans, Bacillus* sp, *Lactobacillus* sp, *Xanthobacter agilis, Brevundimonas diminuta, Brevundimonas olei* and *Streptococcus thermophilus* the results of sequences analysis were in line with the work of Liu *et al*. (2013).

The moisture content of sample B of fermented mung bean had higher value compared with the value obtained for jackbean reported by Odedeji *et al*. (2018). It was also higher than the field bean (Myrene, 2013). High moisture content may encourage microbial proliferation and spoilage of food (Ajayi and Oyetayo, 2009). The ash content of fermented sample B was lower to the laboratory co-fermented millet/cowpea reported by Oyarekua (2011). The ash content of unfermented sample A was lower than that of an unfermented mixture of millet/cowpea mixture as reported by Oyarekua (2011). This may be due to the leaching of some minerals into soaking water. Nwosu, (2013) have made a tandem observation for yam bean. The ash content of samples C, D, E, F, and G fermented mung bean were higher than fermented laboratory co-fermented millet/cowpea and laboratory co-fermented sorghum/cowpea reported by Oyarekua (2011). The protein content of unfermented and fermented mung bean flour of sample A-G was lower compared to the unfermented and fermented soya bean 'iru' reported by Afolabi *et al*., (2016). This may be because microorganisms responsible for fermentation must have secreted extracellular enzymes which increases the protein content (Agbaje *et al*., 2015). Proteins that protect the body from viruses and bacteria promote proper growth and development in children, adolescents, and pregnant women. The fat content of sample A-G had higher value compared with the value obtained for jack bean (Odedeji *et al*., 2018). The low values obtained for the samples could be utilized in food formulation for obese people that require low-fat food products. The crude fibre content of unfermented and fermented sample A-G of this study were higher than that of raw and roasted jack bean reported by Odedeji *et al*. (2018). Increased fiber composition can reduce the risk of many diseases including heart diseases, diabetes, obesity, and some forms of cancer (Marlett *et al*., 2002). Carbohydrate content in unsaturated and fermented mango flour of sample A-G recorded the highest value than that of the value obtained for unfermented and fermented soya bean 'iru' Afolabi *et al*. (2016).This may be due to the ability of the fermented microorganisms to utilize carbohydrate Adejuyitan *et al*. (2009). Higher carbohydrate may result in higher energy supply to the body (Ibironke *et al*., 2017). Energy value of unfermented and fermented samples A-G of mung bean flour was higher

than that of *Olax subscorpoidea* and *Daniella oliveri* seeds (Otori and Mann, 2014).

The phytates samples B to E were lower than samples A, F, and G and it was lower than that of the value obtained for *Daniella oliveri* seed (Otori and Mann, 2014). Phytate is an essential ingredient in plant seeds that can oxidize silicic acid such as magnesium, zinc, and calcium.such chelates make the element nutritionally unavailable thereby reducing dietary it normally inhibits the activity of enzymes (Odedeji *et al.,* 2018). The oxalate content of fermented and unfermented mung bean were higher than the raw and roasted jackbean reported by Odedeji *et al.* (2018). These factors can be easily be reduced to tolerable limits by proper processing techniques such as handling, soaking, and cooking (Anhwange *et al*., 2006). Tannin values of fermented and unfermented mung bean were in line with the values obtained from field bean (Myrene, 2013). Odedeji *et al.,* (2018) recorded a decrease in tannin content of red peanut and small red kidney beans due to the roasting method. The phenols content of fermented and unfermented samples A-G of mung bean flour were higher. Phenolic compounds are capable of reducing oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, diabetes, arthritis, autoimmune diseases, aging, and brain dysfunction (Pietta *et al*., 1998). The phytic acid of fermented and unfermented mung bean flour recorded lower values compared with the value obtained for field bean (Myrene, 2013). The antinutritional content was investigated and was found to be significantly reduced during fermentation processing.

CONCLUSION

The study established the effect of fermentation on the microbial and nutrient compositions of mung bean (*Vigna radiata*). The microbial analysis showed that some of the isolated bacteria were beneficial and there pure strains were confirmed using sequencing analysis. The further work revealed that fermenting mung beans led to an increase in protein and lipid contents coupled with decrease in antinutrient composition which will make more nutrients available from the seed. This is of importance in solving protein energy malnutrition.

REFERENCES

Adejuyitan, J. A., Otunola, E. T., Akande, E. A., Bolarinwa, I. F. and Oladokun, F. M. (2009). Some physicochemical properties of flour obtained from fermentation of tigernut (*Cyperus esculentus*) sourced from market in Ogbomoso, Nigeria. *African Journal of Food Science*, 3:51-55.

Ajayi, O. B. and Oyetayo, F. L. (2009). Potential of *Kerstingiella geocarpa* as health food. *Journal of Medicinal Food*, 12: 184-187.

Afam, A. O. C., Agugo, U. A. and Anyaegbu, E. C. (2016). Effect of germination on the nutritional and anti-nutritional contents of mung bean (*Vigna radiata*). *African Journal of Agricultural Science* and *Technology,* 4(7): 801-805.

Afolabi, F. T., Abdulkadir, M. and Onilude, A. A. (2016). Isolation and screening of microorganisms associated with locust bean (Iru) for the ability to ferment soya bean to produce soy iru. *British Microbiology Research Journal,* 13(6): 1-10.

Agbaje, R. B., Oyetayo, V. O and Ojokoh, A. O. (2015). Assessment of the microbial and physio-chemical composition of tigernut subjected to different fermentation methods. *Parkistan Journal of Nutrition,* 14(11):742-748.

Altschul, S. F. (1997). Gapped BLAST and PSIBLAST: a new generation of protein database

search programs. *Nucleic Acids Res*, 25, 3389-3402.

AOAC (2012). *Official Methods of Analysis*. International 18th edn. Association of Official Analytical Chemists, Washington D.C., U.S.A.

Anhwange, B. A., Ajibola, V. O. and Okibe, F. G. (2006). Nutritive value and anti-nutritional factors in wild seeds. *Journal of Fisheries International,* 2: 73-76.

Anjum, F. M., Tufail, S., Hussain, S., Khan, M. I., Arshad, M. S. and Nadeem, M. (2012). Effect of bioprocesses on phenolic compound, phytic acid and HCI extractability of minerals in wheat cultivars. *Food Science Technology Research,* 18(4): 555-562.

Biesebeke, R., Ruijter, G., Rahardjo, Y.S.P., Hoogschagen, M.J., Heerikhuisen, M., Levin, A,; van Driel, K.G.A., Schutyser, M.A.I., Dijksterhuis, J., Zhu, Y., Weber, F.J., de Vos, W.M., van den Hondel, K.A.M.J.J., Rinzema, A. and Punt, P.J. (2002). "Aspergillus oryzae in solid-state and submerged fermentations progress report on a multi-disciplinary project, FEMS *Yeast Research,* 2: 245–248.

Chen, C. Y., Tsou, S. C. S. and Wang, H. H. (2007). Utilization pattern of mung bean in the Chinese diet. In: Shanmugasundaramm (ed.) *Mung bean: Proceeding of the second International Symposium*. Shanhua, Taiwan: *Asian Publication*. 88 – 304: 486 – 507.

Elegbede, J. A. (1998). Legumes: In Nutritional quality of plant foods. Osagie, A. U. and Eka, O. U. (Ed). Post Harvest Research Unit, University of Benin, Benin. 53-83.

Elijah ,A. I.[1], Atanda, O. O.[2], Popoola, A. R.[3] and Uzochukwu, S. V. A.[4] (2014). Molecular characterization and potential of bacterial species associated with cassava waste. *Nigerian Food Journal* (32) 2: 56-57, 57a, 58-65.

Enwere, N. J. (1998). *Foods of plant origin*. Afi-o - Orbis Published Limited, Nsukka, Nigeria. 24-182.

Holt, J. G., N. G. Kreig, H. A. Peter, S. T. Sneath and S. T. Williams, (2000). *Bergeys manual of systematic bacteriology* 9th Edition, Lippincott Williams and Wilkins Publisher, 175-201, 527-528.

Ibironke, S. I., Ige, M. M. and Adepeju, A. B., (2017). Haematological and in-vivo study of *Moringa olifera* seed. *Moj Food Processing and Technology*, 4 (6): 1 - 6.

Kayode A. J. and Oyetayo, V. O. (2016). Effect of fermentation on the microbial, proximate and mineral composition of mung bean (*Vigna radiata*). *Journal of Applied Life Sciences International,* 5 (4): 1-12.

Lane, D.J. (1991). 16S/23S rRNA sequencing. In: E stackebrandt, MN goodfellow (Eds.). nucleic acid techniques in bacterial systematics, Wiley, Chichester. 115-147.

Liu, H., Song, Y., Chen, F., Zheng, S. and Wang, G. (2013). *Lysinibacillus manganicus* sp. nov., isolated from manganese mining soil. *International Journal of Systematic* and E*volutionary Microbiology,* 63, 1-6.

Marlett, J. A., McBurney, M. I. and Slavin, J. L. (2002). Position of the American dietetic association: health implications of dietary fiber. *Journal of the American Dietetic Association,* 102(7): 993–1000.

Mensa, J. K. and Olukoya, D. K. (2007). Performance of mung beans (*Vigna mungo* (L) Hepper) grown in mid-western Nigeria. *American-Eurasian Journal of Agriculture and Environmental Science* 2: 696 – 701.

Messina, M. J. (1999). Legumes and soybeans: overview of their nutrition profiles and health effects. *American Journal of Clinical Nutrition,* 70, 4395-50S.

Myrene, R. D'souza. (2013). Effect of traditional processing methods on nutritional quality of field bean. *Advanced Bioresearch*, 4 (3): 29-33.

Nnam. N. M. (1994). Nutritional and organoleptic evaluation of fermented products and composite flours developed from plant hods: Ph. D. Thesis, Department of Home Science and Nutrition, University of Nigeria Nsukka.

Nwosu, J. N. (2013). Evaluation of the proximate composition and antinutrition properties of African yam bean (*Sphenostylis sternocarpa*) using malting treatment. *International Journal of Basic and Applied Sciences*, 2(4):157-169.

Odedeji, J. O., Akande, E. A., Fapojuwo, O. O. and Ojo, A. (2018). Effect of roasting on proximate composition of jackbean (*Canavalia ensiformis*) flour. *7th National Conference of WITED* held at federal polytechnic, Nekede Owerri 13th - 16th March.

Ogbona, C. I. C., C. O. Akuesh and J. D. Yilzung, (1983). Studies on some Nigerian indigenous alcohol beverages. A laboratory production of burukutu. Nig*eria Journal of Biotechnology*, 1: 103-120.

Otori, A. A. and Mann, A. (2014). Determination of chemical composition, minerals and anti-nutritional factors of two wild seeds from Nupeland, north central Nigeria. *American Journal of Chemistry and Application,* 1(1): 20-26.

Oyarekua, M. A. (2011). Evaluation of the nutritional and microbiological status of co- fermented cereals/cowpea 'Ogi'. *Agriculture and Biology Journal North American,* 2(1):61-73.

Pietta, P., Simonetti, P. and Mauri, P. (1998). Antioxidant activity of selected medicainal plants. *Journal Agricultural and Food Chemistry,* 46: 4487-4490.

Prescott, L. M., J. P Harley and D. A. Klein, (1999). *Microbiology* 4th Edition WCB McGraw-Hill, Boston.

Tucker, S. C. (2003). Floral development in Legumes. *Plant Physiology*, 131: 911-926.

Uwaegbute, A. C. (1990). Soyabean in infant feeding. In Nigeria lnstitute of Food Science and Technology (N.1. F.S. T.). Easl symposium on baby foods . J. C. Okaka (Ed). 17-31.

Wakil, S. M., Ayenuro, O. T. and Oyinlola, K. A. (2014). Microbiological and nutritional assessment of starter-developed fermented tigernut milk. *Food and Nutrition Science*, 5: 495-506.