



Effect of Fermentation on the Microbial, Proximate and Mineral Composition of Mung Bean (*Vigna radiata*)

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KAJ and OVO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author KAJ managed the literature searches and analyses of the study performed the spectroscopy analysis. Author OVO managed the experimental process and author KAJ identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To examine the effect of fermentation on the microbial, proximate and mineral composition of mung bean.

Study Design: Mung bean seed were obtained from local farmers in Ondo State, Nigeria. Unfermented sample was used as positive control. The study was carried out in the research laboratory, Federal University of Technology, Akure, Nigeria between December, 2012 and March, 2013.

Methodology: The bean was divided into two portions A and B. Portion A was fermented for 7 days and B was analyzed raw. The physiochemical, microbial, proximate and mineral properties of the fermented sample were monitored during the period of fermentation.

Results: The pH decreased significantly from 6.87 at 0 hour to 4.73 at 96 hours of fermentation and increased to 6.10 at 168 hours. There was also significant increase in total titratable acidity (TTA) value. There was a progressive significant increase in the bacteria count from 2.0×10^8 cfu/g

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to 2.5×10^8 cfu/g and significant decrease to 5.5×10^7 cfu/g on the last day. The fungi populations also increased from 2.8×10^4 sfu/g to 9.6×10^5 cfu/g as fermentation progressed. Seven bacteria viz; *Staphylococcus aureus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum* were isolated. Four fungi namely: *Aspergillus niger*, *Aspergillus flavus*, *Penicillium italicum* and *Saccharomyces cerevisiae* were also isolated. The proximate analysis revealed that there was significant increase in the moisture content from 4.81 to 8.94%, a slight increase in protein content from 25.45 to 25.63% and a significant reduction in the carbohydrate content from 51.82 to 48.95%. The mineral composition (mg/g) such as potassium increased significantly from 17.35 to 25.59%, zinc from 0.74 to 0.79% and calcium from 2.49 to 3.27%.

Conclusion: The proximate and mineral compositions as observed in the results suggest that fermentation may significantly improve the nutritional value of mung bean.

Keywords: Mung beans; fermentation; physicochemical property.

1. INTRODUCTION

Mung bean also known as Mung, Mungo, green gram or golden gram is the seed of *Vigna radiata* [1,2]. It is native to the Indian subcontinent [3]. It is a leguminous plant – an erect or climbing bean [4]. Mung bean is cultivated in many tropical African countries; in certain areas of Kenya as their principal cash crop [5,6]. It has been successfully introduced and grown in some Southern part of Nigeria being an ecological analogue of tropical African countries [7]. The seeds are small, ovoid in shape and mostly green, but are also seen in other colours as well. Mung bean are high in carbohydrate (>45%) and protein (>21%); fair source of calcium, iron, vitamin C, tonic and aperients [8].

Its sprouts are good source of vitamin B [1]. However, mung bean is still underutilized as food due to its tough texture, long cooking time and lack of education on its nutritional composition [9,10,11]. Its consumption is occasional. The dried matured seed is cooked and consumed either alone or in combination with starchy staples.

Fermentation is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions without net oxidation [12]. Fermentation is a processing method that is cost effective and affordable by the poor [13]. Fermented foods and condiments are one of the important constituents throughout many parts of Asia and Africa [14]. It often results in enhanced nutrition, stabilization of the original raw materials, detoxification of antinutritional factors [13]. Fermentation can also increase the soluble phenolic content of legumes like pigeon pea and consequently enhance its antioxidant activities.

Legume fermentation in Nigeria is usually done to produce condiments like 'iru' or 'dawadawa' [15]. In this study, the effect of fermentation on the microbial, proximate and mineral composition was examined.

2. MATERIALS AND METHODS

2.1 Sample Collection

Mung bean (*Vigna radiata* L.) seed used for this study were obtained from a local farmer raising them for food at Igasi Akoko, in Akoko North West Local Government Area of Ondo State, Nigeria. The seed were harvested from the previous season and sundried. This material were identified and authenticated at the Department of Crop Soil and Pest Management of the Federal University of Technology, Akure and were taken to the microbiology laboratory (FUTA) for analysis. The seed were thoroughly cleaned and sorted to remove extraneous matters and were sundried to reduce its moisture content. The sample was divided into two portions (A and B) of 500g each using electronic weighing balance (Electronic Balance, MT-301 Model) and were washed in clean tap water before processing. A 500 g of sample A was left to ferment for seven days at ambient temperature in a covered plastic bowl and about 1.5 liters of sterile distilled water was added. Sample B (500 g) which serves as the control was analyzed raw. The processing flow chart developed for this research is shown in Fig. 1.

2.2 Isolation and Characterization

Nutrient Agar (NA), De Man Rogosa and Sharpe agar (MRS), Plate Count Agar (PCA) and Sabouraud Dextrose Agar (SDA) were used for the isolation of bacteria and fungi respectively on

the samples. Isolation of the microbial flora of the fermenting seeds was done every 24 hours at days 1-7 using the method described by [16]. Isolates were randomly selected from the plates used for viable counts and were subcultured before further investigations on their morphological and biochemical characteristics. Each of the representative colonies of the randomly selected isolates were further purified by subculturing them onto sterile plates by streaking method until pure cultures were obtained. All pure cultures were kept in triplicates in culture agar slants as working and stock cultures preserved in a refrigerator (Haier thermocool HR-137 Quinda, China) at 4°C.

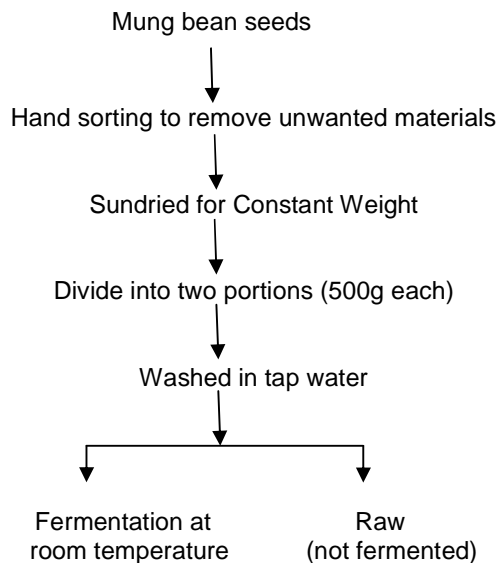


Fig. 1. Mung bean processing flow chart

Identification and taxonomic studies were carried out on the (bacteria and fungi) isolates based on their cultural, morphological, biochemical and physiological characteristics. The cultural characteristics of the purified isolates were done as described by [16,17]. Gram's reaction and shape of cells were determined using 18-24 hours old culture [18]. Biochemical characteristics of the bacteria were done as described by [18]. Identification was done according to the characteristics described by the Bergey's Manual of Determinative Bacteriology [19] and the ones modified by [20]. Fungi isolates were identified by their morphological and hyphae structures in accordance with the characteristics described by Illustrated Genera of Imperct Fungi [21].

2.3 Physiochemical Analysis

The following physiochemical properties such as temperature, pH, and total titratable acidity of fermenting mung beans were carried out on daily basis. Temperature determination was done carefully, by placing a scientific thermometer previously cleaned with cotton soaked in ethanol into the ferment and allowed to stay for a minute and the temperature was read. The pH of the sample was determined according to the method of [22]. 5 g of sample was crushed and mixed in 20 mL of distilled water and was filtered with Whatman filtered paper. The pH of the filtrate was measured using digital pH-meter (HANNA-instrument PK05 USA, Clarkson Laboratory and supply). 5 g of sample was crushed and mixed in 20 mL of distilled water and was filtered with Whatman filter paper. The mixture was titrated against 0.1 M NaOH according to the method of [23].

2.4 Chemical Composition

Proximate composition such as moisture, ash, crude fibre, fat, protein content and calculated carbohydrate were carried out according to the standard methods of [24]. Minerals were determined after wet-ashing by concentrated nitric acid and perchloric acid. Atomic absorption spectrophotometer was used in determining the mineral content [25].

2.5 Statistical Analysis

All data were statistically analyzed by one way analysis of variance (ANOVA) and Means were separated by Duncan's Multiple Range Test (SPSS 21.0 version). Differences were considered significant at 0.05%.

3. RESULTS

3.1 Total Microbial Counts of Fermenting Mung Bean

Table 1 showed the total viable counts of fermenting Mung bean. There was no bacteria growth at the beginning of fermentation on the entire bacteriological media (at 24 hour of fermentation). The highest microbial growth was observed on nutrient agar (NA) medium with significant increase from 1.96×10^8 cfu/g to 5.5×10^7 cfu/g at the seventh day of fermentation. The colonies on PCA significantly increased from 1.50×10^8 cfu/g to 5.0×10^7 cfu/g and colonies on MRS increased significantly from 2.9×10^7 to

3.4x10⁷ cfu/g, which is the lowest bacteria count observed. Fungal count was observed at the initial stage of fermentation and significantly increased from 2.8x10⁴ sfu/g to 5.1x10⁴ sfu/g on the last day of fermentation.

Penicillium italicum and *Saccharomyces cerevisiae*.

3.2 Colonial, Morphological and Biochemical Characteristics of Bacteria and Fungi Isolated from Processed and Unprocessed Mung Bean

Tables 2, 3, and 4 showed the colonial, morphological and biochemical characteristics of fermenting mung bean isolates. Bacterial species, 7 in number were isolated from the samples. These are: *Staphylococcus aureus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. Four fungi species were also isolated which are; *Aspergillus niger*, *Aspergillus flavus*,

3.3 Table 5: Occurrence of Microorganisms during Fermentation

Table 5 showed the occurrence of microorganisms isolated from the fermenting and raw mung bean. No bacterial growth was observed at the initial stage (0 hrs) of fermentation. *Staphylococcus aureus* was isolated on the first day (24 hrs) of fermentation. *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus megaterium* and *Lactobacillus plantarum* persisted throughout the period (seven days) of fermentation. *Bacillus licheniformis* was not isolated on the first day of fermentation but was isolated on the second day (48 hrs) and persisted till last day of fermentation. *Leuconostoc mesenteroides* was isolated on the first day but was not found on the sixth day of fermentation.

Table 1. Total microbial counts of fermenting mung bean

Time (hours)	Medium type/Total count Cfug; Sfu/g			
	NA (cfu/g)	PCA (cfu/g)	MRS (cfu/g)	SDA (sfu/g)
0	Nil	Nil	Nil	2.8X10 ⁴
24	1.96X10 ⁸	1.50X10 ⁸	2.9X10 ⁷	3.2X10 ⁴
48	2.28X10 ⁸	1.98X10 ⁸	8.3X10 ⁷	4.0X10 ⁴
72	2.46X10 ⁸	2.34X10 ⁸	1.54X10 ⁸	5.7X10 ⁴
96	1.86X10 ⁸	1.85X10 ⁸	1.82X10 ⁸	7.2X10 ⁴
120	1.64X10 ⁸	1.63X10 ⁸	1.45X10 ⁸	8.3X10 ⁴
144	8.6X10 ⁷	7.8X10 ⁷	4.6X10 ⁷	9.0X10 ⁴
168	5.5X10 ⁷	5.0X10 ⁷	3.4X10 ⁷	5.1X10 ⁴

Values are presented as means of replicate (n=3)

Key: NA = Nutrient Agar, PCA = Plate Count Agar, MRS= de Man Rogasa Sharpe Agar, SDA = Sabouraud Dextrose Agar, cfu/g = Colony forming unit per gram, sfu/g = Spore forming unit per gram

Table 2. Cultural and morphological characteristics of mung bean isolates

Isolate	Colony shape	Cell Shape	Gram's reaction	Endospore formation	Motility test
FMB 01	Small, non spreading glistening yellowish colonies with entire edges	Cocci in clusters	+	-	-
FMB 02	Rhizoid flat, pale whitish colony	Short rods	+	+	+
FMB 03	Large flat creamy, wide spreading and glistening surfaced colonies	Short rods	+	+	+
FMB 04	Large irregular creamy white colonies	Short rods	+	+	+
FMB 05	Creamy yellow irregular glistening surfaced colonies	Short rods	+	+	+
FMB 06	Circular-large colonies	Cocci in pairs	+	+	+
FMB 07	Large wide spreading fluffy creamy colonies	Long rods	+	-	+

Key: + = Positive, - = Negative, ND = Not determined; FMB: Fermenting mung bean

Table 3. Cultural and morphological characteristics of fungi colonies and their tentative identity

Cultural characteristics	Morphological characteristics	Identification
Fluffy cream colonies	Purple oval shape	<i>Saccharomyces cerevisiae</i>
Whitish to yellow cottony fungal colonies with powdery textures	Septate and hyaline hyphae. Conidiophores are rough and colourless. Phialides are both uniseriate (arranged in one row) and biseriate	<i>Aspergillus flavus</i>
Green fungal colonies	Black conidia, non-septate mycelia. Phialides are borne on metulae. Conidiophores are long.	<i>Aspergillus niger</i>
Whitish to light blue colonies	Round conidia with branched conidiophores	<i>Penicillium italicum</i>

Table 4. Biochemical characterization of mung bean isolates

Tests	FMB 01	FMB 02	FMB 03	FMB 04	FMB 05	FMB 06	FMB 07	FMB 08
Catalase	+	+	-	-	-	-	-	-
Coagulase	+	-	-	-	-	-	-	-
Methyl red	+	+	+	+	-	-	+	+
Voges-proskaur	+	+	+	+	-	-	-	-
Indole	+	+	+	+	+	-	+	+
Starch hydrolysis	+	+	+	+	+	+	-	-
Citrate	+	+	+	+	+	+	-	-
Ornithine	+	+	+	+	+	-	-	-
Casein	+	+	+	+	+	-	-	-
H ₂ S production	-	-	-	-	+	+	-	-
Arabinose	Ag	Ag	Ag	A	A	Ag	Ag	Ag
D-mannitol	Ag	Ag	Ag	Ag	A	A	Ag	Ag
Galactose	Ag	Ag	Ag	Ag	A	A	Ag	Ag
Sucrose	Ag	A	Ag	A	A	Ag	Ag	Ag
Fructose	Ag	A	Ag	Ag	A	Ag	A	Ag
Glucose	Ag	A	A	Ag	A	Ag	A	Ag
Maltose	Ag	A	Ag	Ag	A	Ag	A	Ag
Lactose	Ag	-	Ag	-	-	Ag	-	Ag
Dextrose	Ag	Ag	Ag	Ag	Ag	Ag	A	Ag
Sorbitol	Ag	Ag	Ag	Ag	A	Ag	A	Ag
Suspected organisms	<i>Staph. Aureus</i>	<i>Bacillus coagulans</i>	<i>Bacillus licheniformis</i>	<i>Bacillus subtilis</i>	<i>Bacillus megaterium</i>	<i>Leuconostoc mesenteroides</i>	<i>Lactobacillus plantarum</i>	<i>Sacch. cerevisiae</i>

Key: + = Positive, - = Negative, Ag = Acid and gas production, A = Acid production, Staph. = Staphylococcus, Sacch. = Saccharomyces, FMB = Fermenting mung bean

Table 5. Occurrence of microorganisms isolated during fermentation

Organisms	Time (Hours)							
	0	24	48	72	96	120	144	168
<i>Staphylococcus aureus</i>	-	+	-	-	-	-	-	-
<i>Bacillus coagulans</i>	-	+	+	+	+	+	+	+
<i>Bacillus licheniformis</i>	-	-	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	+	+	+	+	+	+	+
<i>Bacillus megaterium</i>	-	+	+	+	+	+	+	+
<i>Leuconostoc mesenteroides</i>	-	+	+	+	+	+	-	-
<i>Lactobacillus platarum</i>	-	+	+	+	+	+	+	+
<i>Saccharomyces cerevisiae</i>	-	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	+	+	+	+	+	+	-	-
<i>Aspergillus flavus</i>	+	+	+	+	+	+	-	-
<i>Penicillium italicum</i>	+	+	+	+	+	+	-	-

Key: + = Present, - = Not present

Saccharomyces cerevisiae was not isolated at the initial stage of fermentation but was found on the first day (24 hrs) of fermentation till the last day. *Aspergillus niger* and *Aspergillus flavus* were isolated at the initial stage (0 hrs) and persisted till the sixth day but were not found on the seventh day of fermentation. *Penicillium italicum* was present at the initial stage of fermentation till the fifth day of fermentation but was not found on the sixth and seventh day of fermentation.

Aspergillus flavus, *Aspergillus niger*, and *Penicillium italicum* were isolated from the raw seeds. Table 6 shows the percentage occurrence of microorganisms associated with the fermentation of bean. It was observed that *Bacillus* spp. had the highest percentage occurrence.

Table 6. Percentage occurrence of microorganisms during fermentation

Organisms	Percentage occurrence
<i>Bacillus</i> spp.	36%
<i>Staphylococcus aureus</i>	9%
<i>Leuconostoc mesenteroides</i>	9%
<i>Lactobacillus platarum</i>	9%
<i>Saccharomyces cerevisiae</i>	9%
<i>Aspergillus</i> spp.	18%
<i>Penicillium italicum</i>	9%

3.4 The Physiochemical Parameters of Fermenting Mung Bean

Figs. 2, 3, and 4 showed the pH, Total Titratable Acidity and Temperature of fermenting mung bean. The pH of the fermenting mung bean were determined as well as the free water (distilled)

used for the fermentation for the seven days period. There was a significant reduction in the pH of the water in the fermenting medium from 6.93 at the initial stage (0hrs) to 4.73 on the 3rd day and a significant increase from 4.73 to 6.13 on the 7th day (168th hour).

Similarly, the pH of the fermenting mung bean was 6.87 at (0hrs), it decreased steadily to 4.73 on the 3rd and 4th day of fermentation. The increase and decrease in pH of the liquid and the substrate (beans) in the fermenting medium were in the same trend.

The TTA of the liquid and the substrate in the fermenting medium were determined on a daily basis. There was significant increase in the TTA value of the liquid in the fermenting medium from 1.00 to 18.00 on the 5th day and a reduction from 18.00 to 12.07 on the 7th day. The TTA value of the fermenting bean was observed from 0.20 to 5.27 on the 2nd day and later decreased significantly to 3.60 on the 7th day of fermentation. The increase and decrease in the TTA of the liquid and solid (bean) in the fermenting medium were in the same trend.

There was a significant increase in the temperature of the fermenting mung bean from 28°C (time 0) to 31°C at the 3rd day, a steady decline in the temperature to 29°C was later observed at 7th day.

3.5 Effect of Fermentation on the Proximate Composition of Mung Bean Samples

Table 7 showed the proximate composition of processed and unprocessed mung bean. The moisture content of the raw sample was 4.81%

and the fermented sample had the highest moisture content of 8.94%. The crude fibre of the unprocessed sample was 2.06% and the fermented sample had the lowest crude fibre of 1.61%. The ash content of the raw sample was 3.59% while the fermented sample was found to have the lowest ash content of 2.02%. The crude

fat of the raw sample was 12.13%. There was significant increase in the fat content of the fermented sample from 12.13 % to 12.77 %. The protein content of the raw sample was 25.45%. Fermentation slightly increased the protein content from 25.45 to 25.63%.

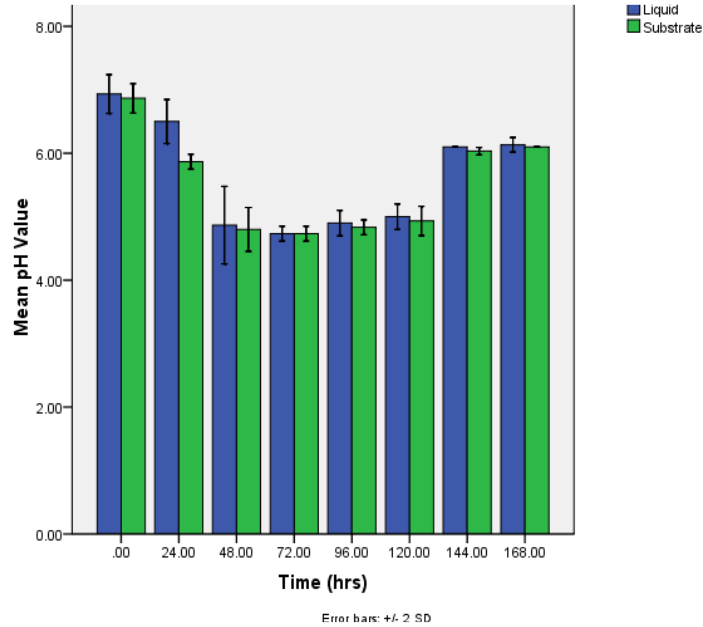


Fig. 2. Changes in pH of fermenting mung bean
 Key: Liquid = distilled water, Substrate = Mung bean seed

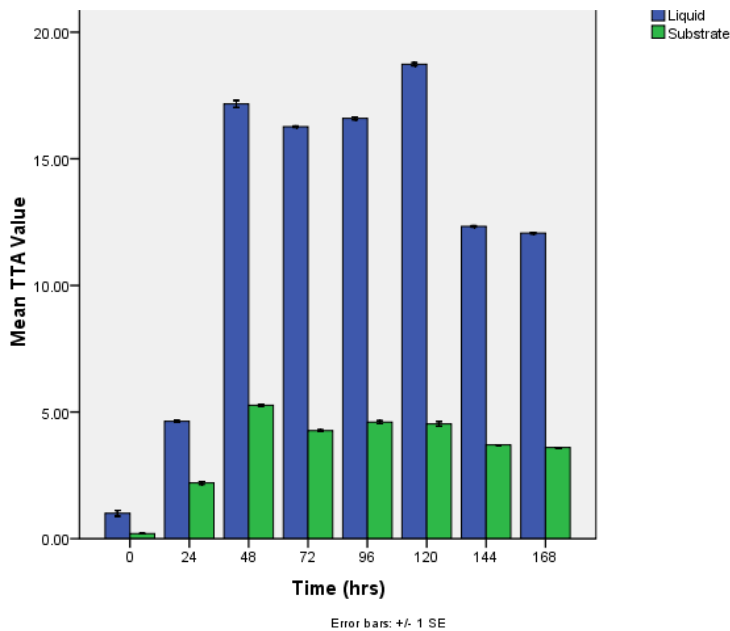


Fig. 3. Changes in total titratable acidity (TTA) of fermenting mung bean
 Key: Liquid = distilled water, Substrate = Mung bean seed

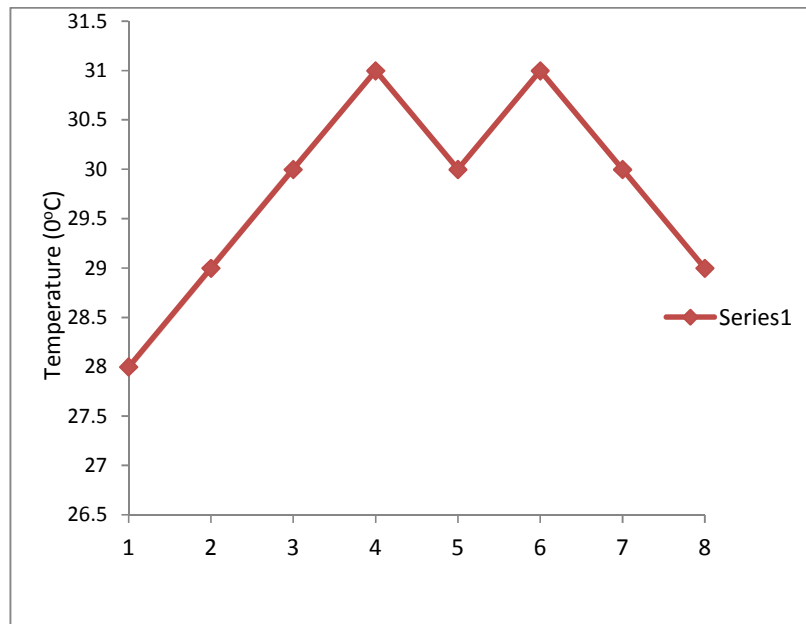


Fig. 4. Changes in the temperature of fermenting mung bean

Table 7. Proximate composition of fermented and unfermented (raw) mung bean

% Proximate composition	Fermented	Raw
Moisture content	8.94±0.45 ^a	4.81±0.01 ^a
Crude fibre	1.61±0.01 ^b	2.06±0.11 ^b
Ash content	2.02±0.45 ^c	3.59±0.01 ^c
Crude fat	12.77±0.01 ^d	12.13±0.26 ^d
Crude protein	25.70±0.40 ^e	25.45±0.01 ^e
Carbohydrate content	49.48±0.01 ^f	51.96±0.01 ^f

Values are presented as Mean ± S.D (n=3). Means with the same superscript letters along the same column are not significantly different (P<0.05)

Table 8. Mineral composition of fermented and unfermented (raw) mung bean

Minerals (mg/g)	Fermented	Raw
Potassium	25.77±0.01 ^a	17.24±0.01 ^a
Sodium	1.95±0.45 ^b	2.68±0.01 ^a
Calcium	3.27±0.27 ^c	2.37±0.01 ^b
Magnesium	0.60±0.10 ^d	0.59±0.95 ^c
Zinc	0.79±0.90 ^e	0.73±0.01 ^d
Iron	0.72±0.20 ^f	0.65±0.01 ^e
Phosphorus	2.66±0.66 ^g	3.83±0.01 ^f
Copper	0.02±0.01 ^h	0.01±0.01 ^g
Manganese	0.12±0.10 ⁱ	0.23±0.00 ^h

Values are presented as Mean ± S.D (n=3). Means with the same superscript letters along the same column are not significantly different (P<0.05)

3.6 Effect of Processing Method on the Mineral Composition of Fermented and Unfermented Mung Bean Samples

Table 8 showed the mineral composition of processed and unprocessed mung bean.

Potassium had the highest value among the entire mineral assayed for in all the samples. The potassium content of the raw sample was 17.24 mg/g. Fermented sample had the highest value of 25.77 mg/g. The phosphorus value of the raw sample was 3.83 mg/g. 2.66 mg/g was observed in the fermented sample. Copper had the least

value among the entire mineral analyzed in all the samples ranging between 0.00 mg/g and 0.02 mg/g. The raw sample had a value of 0.01 mg/g while the fermented sample had a value of 0.00 mg/g.

4. DISCUSSION

Seven bacteria species were isolated from the mung beans seeds during the seven days of fermentation. These are: *Staphylococcus aureus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*. Four fungi species Viz; *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium italicum* were also isolated. These organisms have been found to be responsible for the fermentation of most legumes and cereals [26,27]. The presence of these organisms during the fermentation periods confirms that they grow in close association with the food substrate and produce extracellular enzymes [28]. The authors [29,30] reported that yeasts, such as: *Saccharomyces cerevisiae*, *Kloeckera apis*, *Candida humicola* play important role in the early stages of fermentation, in paving way for further fermentation by bacteria such that they convert sugars into ethanol and CO₂, decrease the acidity of the medium giving rise to citric acid. Also, [30] reported that predominant lactic acid bacteria at the early stage of (in the first 36 to 48 hours) of fermentation include *Lactobacillus cellobiosus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides* and *Leuconostoc lactis*. The main function of lactic acid bacteria is to metabolize (glucose and fructose) and citrate to produce lactic acid, acetic acid, ethanol, and mannitol. These microorganisms have also been thought to contribute to yeast's ability to use citrate as carbon source [31]. Increase in pH and high temperatures leads to the development of *Bacillus* species i.e *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus subtilis*. These bacteria form chemical compound that cause acidity [30].

The isolation of *Bacillus* species as the most predominant bacterial flora in the fermenting mung beans is in accordance with the previous works done on fermented legume seeds that was reported in previous works on "anyi" condiment [32].

There was a steady increase in temperature of the fermenting mung beans observed from 1st day till the 4th day of fermentation and a

fluctuation was observed towards the end of the fermentation. This supports the fact that fermentation is an exothermic process and that the heat generated was due to metabolic activities of the microorganisms such as the *Bacillus* species converting ethanol previously produced by yeast to acetic acid thereby releasing heat [30,33].

The initial pH of the free water in the fermentor was 6.93. There was a reduction in the pH of the free water from 6.93 to 4.73 on the 3rd day and an increase from 4.73 to 6.13 on the 7th day (168th hour). In the same vein, the pH of the fermenting mung bean was 6.87 at (0hrs). It decreased steadily to 4.73 on the 2nd day of fermentation. An increase from 4.73 to 6.10 was observed on the 7th day of fermentation. The increase and decrease in the pH of the liquid and the substrate (bean) in the fermenting medium were in the same trend. The decrease in pH observed could be attributed to the production of various organic acids from the utilization (hydrolysis) of the carbohydrate present in the substrate by the fermenting microorganisms [13,34]. There was an increase in the TTA value of the liquid in the fermenting medium from 1.00% to 18.00% on the 5th and a reduction from 18.00% to 12.07%. The TTA value of the fermenting bean increased from 0.20% to 5.27% on the third day and later decreased to 3.60% on the 7th day of fermentation. The increase and decrease in the TTA of the liquid and the substrate (bean) in the fermenting medium were in the same trend. The significant increase in pH observed and decrease in TTA at the end of the fermentation may be due to an increase of ammonia towards the end of the fermentation leading to alkalinity by hydrolysis of protein as illustrated by [35]. It could also be due to the deaminase and protease enzymes produced by *Bacillus* isolates [36].

The ash content of the raw sample was 3.59% while the fermented sample was found to have ash content of 2.02%. These values obtained are in accordance with the value of 3.6% obtained for melon seeds by [37]. There was significant increase in the fat content of the fermented sample from 12.13 to 12.77%.

The protein content of the raw sample was 25.45%. This close to 27.5% reported by [38]. Fermentation slightly increased the protein content from 25.45 to 25.63%. The slight increase in the protein content of the fermented sample may be as a result of the proteolytic

activity of the *Bacillus* species being able to breakdown the protein in the seeds and utilizing them for growth as well as the considerable loss of nutrient to the free water in the fermenting medium.

There was a significant increase in potassium, calcium, iron, copper and zinc in the fermented sample. These data agree with the report of [39] and the work of [40] on boiled fenugreek seeds.

5. CONCLUSION

This study established the effect of fermentation on the microbial and nutrient contents of mung bean (*Vigna radiata*). The result of the proximate analysis indicates that; natural fermentation slightly increases the protein with a reduction in the carbohydrate content of mung bean. The result of the mineral composition revealed that mung bean is rich in potassium, phosphorus, sodium and calcium but a poor source of copper, manganese, and magnesium. Mung beans is relatively high in protein (25.45%) and it can be a substitute for animal protein in Nigeria. More so, fermentation can better enhance the nutritional composition of mung beans (*Vigna radiata*).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wilczek LR. Brief introduction of mung bean (*Vigna radiata*) extract, green mung bean extract, powder (*Phaseolus aureus*), Roxb *Vigna radiata*, MD idea extract; 2008.
2. Guriqbal S, Sekhon HS, Gurdip SJS, Brar TSB, Shanmugasundram S. Effect of plant diversity on the growth and yield of mungbean [*Vigna radiata* (L.) Wilczek] genotypes under different environment in India and Taiwan. *International Journal of Agricultural Research*. 2011;6(7):573-583.
3. Walshaw SC. Converting to rice: Urbanization, islamization and on pembe, 700-1500 AD. *Journal of World Archeology*. 2010;42:137-154.
4. Fuller DQ. Contrasting pattern in crop domestication and domestication rates: Recent Archeological insights from The World *Annals of Botany*. 2007;100(5):903-924.
5. Lambrides CJ, Godwin ID, Chittarrajan K. Genome mapping and molecular breeding in plants. 2006;3:69-90.
6. Motgotsi KK. *Vigna radiata* (L) Wilczek R. In: M. and Belays, G. (Editor). PROTAL: Cereals, and pulses/cereals et legumes secs. [CD-ROM], PROTA, Wageningen, Netherlands; 2006. Available:www.prota4u.org
7. Agugo BAC. Introducing mung bean into the South Eastern Nigeria's humid forest: Overview of potentials. *Proceedings of the 37th annual conference of agricultural society of Nigeria*. University of Calabar; 2003. 16th -20th Nov; 2005.
8. Wiley BJ; 2008. Available:www.answers.com
9. Amaefule KU, Nwagbara NN. The effect of processing on the nutrient utilization of pigeon pea (*Cajanus cajan*) seed meal and pigeon pea seed meal based diets by pullets. *International Journal of Poultry Science*. 2004;3:543-546.
10. Fasoyiro SB, Obatolu VA, Asaye OA, Adejo EA, Ogunleti DO. Chemical and sensory qualities of pigeon pea (*Cajanus cajan*) developed into a local spice 'dawadawa'. *Food Journal*. 2009;27:150-158
11. Odeny DA. The potential of pigeon pea (*Cajanus cajan* L. Mill sp.) In: *African Natural Resource Forum*. 2007;31:297-284.
12. Steinkraus KH. *Handbook of indigenous fermented foods*. New York, Marcel Dekker, Inc; 1995.
13. Omezuruike IO. Microbiological studies on the production of anyi – a potential condiment made from the laboratory fermentation of *Samanea saman* (monkey pod) seeds (Jacq.) Merr. *Electronic Journal of Environmental, Agricultural and food Chemistry*; 2008. ISSN: 1579-4377.
14. Ogunshe AAO, Ayodele AE, Okonko IO. Microbial studies on Aisa: A potential indigenous laboratory fermented food condiment from *Albiza saman* (Jacq.) F. Mull. *Pakistan Journal of Nutrition*. 2006; 5(1):51-58.
15. Oboh G, Ademiluyi AO, Akindahunsi AA. Changes in polyphenol distribution and antioxidant activity during fermentation of some underutilised legumes. *Journal Food Science and Technology International*. 2009;15:41-46.

16. Olutiola PO, Famurewa O, Sonntage HG. Introduction to general microbiology. Practical Approach; 2000. 2nd Edition. Publishing and Media Consultants, Nigeria.
17. Prescott LM, Harley JP, Klein DA. Microbiology, 6th Edition. 2005;501-502.
18. Seeley HWI, Van Denmark PJ. Microbes in action. A laboratory manual of microbiology, 2nd edition. Freeman WA and Co. San Francisco. 1972;361.
19. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. Ninth edition. Willisms and Wilkins Company. 1994; 1013-1209.
20. Cowan ST, Steel KJ. Manual for Identification of medical bacteria. Third Edition /edited and revised by Barrow GI, Fellhan RKA; 1993.
21. Barnett HL, Barry BH. Illustrated genera of imperfect fungi. Third edition by Barnett H. Division of Plant Sciences Wes, West Virginia University and Barry BH, Department of Biology, Carlifornia State College, Carlifonia, Pennsylvania. Burgess Publishing Company, 426 South six. Minnesopolis. Minnesota 55415; 1995. Printed in the United State of America, Library of Congress Catalog Card No: 71-163710. Standard Book No: 8007-0266-1.
22. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. Published by association of official analytical chemists; 2000. Washington D. C.
23. Valverde JM, Valero D, Martinez-Romero D, Guillen F, Castillo S, Serrano M. Novel edible coating based on Aloe vera gel maintain table grape quality and safety. Journal of Agricultural and Food Chemistry. 2005;53:7807-7813.
24. AOAC (b). Official methods of analysis of the Association of Official Analytical Chemists. 17th edn. AOAC, VA, USA. 2005;(chapter 4):57-66.
25. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. Published by association of official analytical chemists. Washington D. C; 2002.
26. Amusa NA, Ashaye OA, Oladipupo MO. Microbiological quality of 'ogi' and soy-ogi (a Nigerian fermented cereal porridge) widely consumed and notable weaning food in Southern Nigeria. Nigerian Journal of Food, Agriculture and Environment. 2005;3(2):81-83.
27. Oyarekua MA. Biochemical and morphological changes during the production of fermented pigeon pea (*Cajanus cajan*) flour. African Journal of Food Science and Technology. 2011; 2(10):223-131.
28. Aderibigbe EY. Characteristics of extracellular proteinase from strains of *Bacillus subtilis* group. Nigerian Journal of Microbiology. 1977;11:93-97.
29. Schwan RF, Wheals AE. The microbiology of cocoa fermentation and its role in chocolate quality. Critical Reviews in Food Science & Nutrition. 2004;44(4):205-21.
30. Ardhana MM, Fleet, GH. The microbial ecology of cocoa bean fermentations in Indonesia. International Journal of food Microbiology. 2003;86(1-2):87-99.
31. Ardhana MM, Fleet GH. The microbial ecology of cocoa bean fermentations in Indonesia. International Journal of food Microbiology. 2003;86(1-2):87-99.
32. Camu N, De Winter T, Verbrugghe K. Dynamics and biodiversity of populations of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. Applied and Environmental Microbiology. 2007;73(6):1809-24.
33. Okonko IO. Microbial studies on anyi – A fermented food condiment from *Samanea saman*. HND Thesis. Microbiology/Virology Unit, Laboratory Technology Training School, University of Ibadan, Ibadan, Nigeria. 2002;120.
34. Odunfa SA. African fermented foods. In: Wood BJB. (ed). Microbiology of fermented foods. Amsteram Elsevier Applied Science Publishers Ltd. Essex. U.K. 1985;11:155-191.
35. Adams MR, Moss MO. Food microbiology. Royal Society of Chemistry Cambridge. 1999;255-291.
36. Whitaker JR. Biochemical changes occurring during the fermentation of high protein foods. Journal of Food Technology. 1978;32:175.
37. Omafuvbe BO, Oyedapo OO. Observed biochemical changes during the fermentation of soybean of soy-dawadawa – Nigerian food condiment. Journal of Food Microbiology. 2000;17:469-474.
38. Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SRA. Chemical and changes

- biochemical changes in African locust bean (*Parkia biglobosa*) and Melon (*Citrullus vulgaris*) seeds during the fermentation to condiments. Pakistan Journal of Nutrition. 2004;3:140-145.
39. Mubarak KK. Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processing. Journal of Food Chemistry. 2005;89:489-495.
40. Masour EH, El-Adawy TA. Nutritional potential and functional properties of heat treated and germinated fenugreek seeds. Lebensm Wiss Technology. 1994;69: 133-136.

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