

Microbiological and physico-chemical characteristics of fermented bambara nut (*Vigna subterranea*) seeds

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Abstract

The microbial and chemical properties of fermented bambara nut seeds was carried out. *Vigna subterranea* seeds were steeped, and boiled and naturally fermented for 96 h at 28±2°C. The temperature, pH and titratable acidity were monitored at 24 h interval. The proximate composition of protein, oil, fibre, ash, moisture and carbohydrate as well as the mineral content of the fermented and unfermented samples were determined using standard methods. The predominant microflora of bambara nut fermentation were *Bacillus pumilis*, *B. licheniformis*, *B. cereus*, *B. subtilis* and the yeast *Saccharomyces cerevisiae*. The pH, titratable acidity and temperature during fermentation ranged from 6.80±0.03 to 5.30±0.09, 0.26±0.01% to 0.57±0.01% and 26.00±0.02°C to 36.00±0.06°C at 0 h to 96 h respectively. The moisture (42.77±0.01%), fibre (2.04±0.01%), oil (0.34±0.00 %) and protein (28.70±0.00%) content of the fermented product were higher than the corresponding values of 41.84±0.00%, 1.44±0.03%, 0.22±0.02% and 21.33±0.01% respectively for the unfermented samples. However, the carbohydrate (25.32±0.01%) and ash (0.78±0.01%) content of the fermented product were lower compared to (33.63±0.06%) and (1.52±0.01%) respectively of the unfermented bambara nut sample. Potassium was the most predominant mineral with values of 1.53±0.04 mg/100g and 1.01±0.01 mg/100g for fermented and unfermented samples.

Keywords: Bambara Nut, Fermented, Characteristics, Nutritional, Microorganisms

1. Introduction

Bambara groundnut botanically known as *Voandzeia subterranea* (L.) thosours, synonyms of *Vigna subterranea* is a legume species of African origin (Borget, 1992) with a subterranean fruit set which is widespread south of the Sahara (Ocran *et al.*, 1998). It is cultivated by small holders over much of semi arid Africa and it serves as an important source of protein in the diets of a large percentage of the population in Africa, particularly to poorer people who cannot afford expensive animal protein (Linnemann, 1992).

Despite the relatively low oil content, some tribes in Congo reportedly roast the seeds and pound them for oil extraction (Karikari *et al.*, 1995). The gross energy value of bambara groundnut seed is greater than that of other common pulses such as cowpea, lentil and pigeon pea (FAO,1982). Ihekoronye and Ngoddy,1985 reported that it is richer than groundnuts

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in essential amino acids. Bambara groundnut is a non-oily leguminous seed which contains only about 6% of ether extract therefore could not give a cash crop status, a great importance in food industry. In addition, the fatty acid content is predominantly linoleic, palmitic and linolenic acids (Minka and Bruneteau, 2000). The carbohydrate fraction of it is predominantly composed of starch and non-starch polysaccharides with lesser amount of reducing and non reducing sugar (Addo and Oyeleke, 1986).

Fermentation is one of the oldest methods of food preservation known to man. In Africa, the art of fermentation is widespread including the processing of fruits and other carbohydrate sources to yield alcoholic and non-alcoholic beverages as well as the production of sour-tasting 'ogi' the fermented cereal product which provide instant energy in breakfast and covalent diets (Adewusi *et al.*, 1991). Oil seeds such as African locust beans, melon, castor oil seed and legumes are also fermented to give condiments. Legumes are members of the leguminosae. Their seeds are rich in protein and they are fermented in various parts of the world for flavouring condiments or as major meals.

Seeds of legumes may account for up to 80% of dietary protein and may be the only source of protein for some groups. Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavors of foods (Odunfa and Adewuyi, 1985; Aidoo, 1986; Oniofiok *et al.*, 1996). With high contents of protein, legume condiments can serve as a tasty complement to sauces and soups and can substitute for fish or meat.

The food flavouring condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate components (Fetuga *et al.*, 1975; Eka, 1980). Apart from increasing the shelf life, and a reduction in the anti-nutritional factors (Odunfa and Adewuyi, 1985; Barimalaa *et al.*, 2005; Achi, 2005), fermentation markedly improves the digestibility, nutritive value and flavours of the raw seeds. Although fermented food condiments have constituted a significant proportion of the diet of many people, Nigerians have exhibited an ambivalent attitude in terms of consumer tastes and preferences for such foods (Achi, 2005). The introduction of foreign high technology products especially processed ones because of globalization and liberalization of the economy has radically changed the Nigerian food culture into a mixed grill of both foreign and local dishes (Ojo, 1991).

As with any other fermentation process the understanding of the microbial ecology of vegetable fermentations requires the knowledge of the fermentation substrates, i.e. the seeds of the various plants as well as the products obtained thereof. The use of these condiments, it is suggested, could be extended as a food ingredient included into most fabricated foods in order to further increase their versatility and utility. This because fermentation has been

found to improve the quality of protein-containing oil seeds (Giami and Bekebain, 1992). Moreover fermentation also can result in increased digestibility and increased nutritional content (Achi, 2005). The purpose of this study was to evaluate difference in composition of the fermented and unfermented bambara nut and the microorganisms responsible for the fermentation.

2. Materials and Methods

2.1 Collection of sample.

Cream coloured seeds of *Vigna subterranea* (Bambara groundnut) were obtained from New Benin market, Benin City. Healthy seeds were manually sorted, stored inside a clean nylon bag and refrigerated at 4°C until required for use.

2.2 Preparation of sample.

The method of Fadahunsi and Olubunmi (2009) was adopted with some modifications. One hundred and Twenty (120) grams of *Vigna subterranea* seeds was weighed using a weighing balance (Model search Tech KLS 10001, Germany). The nuts were divided into three (3) portions of 40g each, rinsed and steeped in 500ml of distilled water for 18 h. The water was drained and the nuts were transferred into a hot plate pan containing 400 ml distilled water. The nuts were boiled using a hot plate (Model, Stuart SB160, Germany) for 90 min until soft. The boiled nuts were sieved, allowed to cool for 15 min and then transferred to a sterile mortar followed by mashing into a pulp.

2.3 Spontaneous fermentation.

The mashed nuts were aseptically rolled into 3 balls and each wrapped in banana leaves. The set ups were labeled SF₁, SF₂ and SF₃ and kept in air-tight containers covered with a few sheets of banana leaves. The set ups were then kept on a surface sterilized laboratory bench to ferment naturally at room temperature (28±2°C) for 96 h.

2.4 Isolation of microorganisms.

Sampling was carried out at 24 h interval. 1gram of the fermenting bambara nut mash was aseptically transferred into a test tube containing 9ml of sterile water and shaken vigorously to dislodge the associated microorganisms, making a stock suspension. The 10⁻¹ suspension was subsequently serially diluted using ten-fold serial dilution up to 10⁻¹⁰. 1 ml aliquots of various dilutions were added to sterile petri dishes (duplicate for each dilution) to which approximately 15 ml of sterile cool molten (45°C) media was added. The dilution 10⁻³ was selected for enumeration of bacteria and fungi using nutrient agar and potato dextrose agar supplemented with 0.6 ml Chloramphenicol respectively. The plates were swirled to mix and the agar was allowed to solidify. Upon solidification, the plates were incubated in an

inverted position. Nutrient agar plates were incubated at 37°C for 24 h while the potato dextrose agar plates were incubated at room temperature (28±2°C) for 72 h to 96 h.

2.5 Enumeration of microorganisms

Discrete colonies on the Nutrient agar and Potato dextrose agar were selected and counted. The mean colony count on the nutrient agar and potato dextrose plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (cfu/g) Holt *et al.*, (2000).

2.6 Characterization and Identification of bacterial Isolates

Cultural characteristics of the bacterial isolates were observed on nutrient agar plates. The cultural characteristics include; colony size, shape, surface appearance, opacity, texture, elevation and pigmentation. These were determined by visual observation. Morphological and biochemical tests for the bacterial isolates were carried out according to the method of Cheesbrough (2000) and Holt *et al.*, (2004).

2.7 Characterization/Identification of fungal isolates

A drop of lactophenol blue stain was dropped on a clean grease- free sterilized glass slide and a sterile inoculating wire loop was used to pick the mycelium unto the glass slide from the mold culture. The mycelium was spread evenly on the slide. Teasing was carried out to separate the mycelium in order to get a homogenous mixture and the mixture was then covered with cover slips gently and then allowed to stay for some seconds before observing under the microscope using the X40 objective. The microscopic examination of actively growing mold was on the basis of structures bearing spores, presence or absence of septate on hyphae (Barnett and Hunter, 1998).

2.8 Determination of proximate composition

The proximate composition of moisture, crude fibre, protein, ash and carbohydrate of both fermented and unfermented samples were analysed by the standard methods of Association of Official Analytical Chemists (AOAC, 2000).

2.9 Determination of mineral contents

Mineral such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and phosphorous were determined. Samples were digested following the procedure described by Salami and Non (2002). 1.0 g each of oven dried samples were digested with 5 ml concentrated nitric acid (HNO₃) and 1 ml each of concentrated sulphuric acid (H₂SO₄) and 60 % perchloric acid (HClO₄) were heated until white fumes of perchloric acid formed. The volume of the digest was reduced by heating but not to dryness. The flask was set aside to cool, after which the content was diluted with distilled deionized water and then filtered into a 50 ml volumetric flask. The content was made up to mark with deionized water and stored until

analyzed for mineral contents using Atomic Absorption spectrophotometer (AAS), phosphorous content of the digest was determined spectrophotometrically according to method described by Nahapetain and Bassiri (1975). To 0.5 ml of the diluted digest, 4 ml of distilled water, 3 ml of 0.75 M H_2SO_4 , 0.4 ml of 10 % Ammonium molybdate tetrahydrate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, (molar mass is 1236.0127 g/mol) and 0.4 ml of 2 % (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and absorbance readings were recorded at 660nm. The content of phosphorus in the sample was determined.

2.10 Physicochemical properties of the fermenting mash.

2.10.1 Determination of pH

Ten (10 g) grams of the fermenting mash from each set up (SF_1 , SF_2 and SF_3) was mixed in 50 ml of distilled water in 3 separate 100ml beaker. The mixture was stirred with a glass stirrer. The pH meter (Hana pH meter 107) was standardized with 4.6 ± 0.2 acetate buffer solution, then placed in SF_1 mixture and rinsed with distilled water before taking readings for SF_2 and SF_3 respectively. An average of the 3 readings gave daily reading. This was done at 24h interval (Kalra and Maynard, 1991).

2.10.2 Temperature

A multi thermometer H9269 was used to take the temperature readings of the fermenting mash at 24 h interval. Temperature reading of SF_1 , SF_2 and SF_3 were taken and an average of the 3 readings was obtained.

2.10.3 Titratable Acidity

This was estimated by weighing 10 grams of the beans into 90 ml of sterile distilled water to make slurry. 10ml of the filtered slurry was titrated against 0.1M sodium hydroxide (NaOH) using 3 drops of phenolphthalein indicator.

$$\% \text{ Titrated Acidity} = \frac{\text{Titre value} \times \text{normality of alkali} \times 9}{\text{Volume of sample}}$$

Where Normality of alkali = 0.1

9 = mol equivalent of lactic acid

Vol of sample = 10 ml

2.11 Statistical Analysis

Data obtained were statistically analysed using Statistical package for social software (SPSS) (version 16). This was carried out by using the student's unpaired t-test at 5% level of significance. Multiple comparison of mean was carried out by correlation and two ways ANOVA. A probability level of less than 5% was considered significant.

3. Results and Discussion

In Table 1 is shown the occurrence of microbial isolates in fermenting bambara nuts. Of the four *Bacillus* species two: *Bacillus cereus* and *Bacillus pumilus* occurred the most through-out the fermentation period. *Escherichia coli* and *Staphylococcus aureus* were transient flora. The moulds *Aspergillus* and *Rhizopus* species were not isolated for the first two days but were isolated thereafter as fermentation progressed. In Table 2 is shown the changes in pH and titratable acidity of the fermenting mash. The pH decreased from 6.80 ± 0.03 at 0 h to 5.3 ± 0.09 at 96 h while the titratable acidity increased from $0.26 \pm 0.01\%$ at 0 h to $0.57 \pm 0.01\%$ at 96 h. In Figure 1 is shown the change in temperature during the spontaneous fermentation. The initial temperature recorded at the start of the fermentation was $26.00 \pm 0.00^\circ\text{C}$ but increased to $42.4 \pm 0.00^\circ\text{C}$ at 72 h. This value however decreased to $36.00 \pm 0.02^\circ\text{C}$ at the end of fermentation after 96 h.

Figure 2 shows the proximate composition of both fermented and unfermented samples. The moisture content was higher in the fermented product ($42.77 \pm 0.00\%$) than in the unfermented sample ($41.84 \pm 0.00\%$). Similarly, the fibre content ($2.04 \pm 0.01\%$), fat content ($0.34 \pm 0.00\%$) and protein content ($28.70 \pm 0.00\%$) of the fermented product were higher than the corresponding values of the unfermented sample. However, the carbohydrate content ($25.32 \pm 0.01\%$) and ash content ($0.78 \pm 0.01\%$) of the fermented product were lower than the corresponding values of the unfermented sample of $33.63 \pm 0.06\%$ and $1.52 \pm 0.01\%$ respectively.

Table 1 Occurrence of microorganisms in fermenting bambara nut

Microbial Isolates	Fermenting Time (H)				
	0	24	48	72	96
<i>Bacillus cereus</i>	+	+	+	+	+
<i>Bacillus pumilus</i>	+	+	+	+	+
<i>Bacillus licheniformis</i>	-	+	+	+	+
<i>Bacillus subtilis</i>	-	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	-	-	-
<i>Escherichia coli</i>	+	+	-	-	-
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+
<i>Aspergillus niger</i>	-	-	+	+	+
<i>Rhizopus</i> spp	-	-	-	+	+
<i>Penicillium</i> spp	+	-	-	-	-

Note : + = present, - = absent

Table 2 Changes in pH and titratable acidity in fermenting bambara nut seed.

Time (h)	pH	Titratable acidity (%)
0	6.8±0.03	0.26±0.01
24	6.2±0.09	0.34±0.00
48	5.8±0.23	0.48±0.01
72	5.7±0.12	0.53±0.01
96	5.3±0.09	0.57±0.01

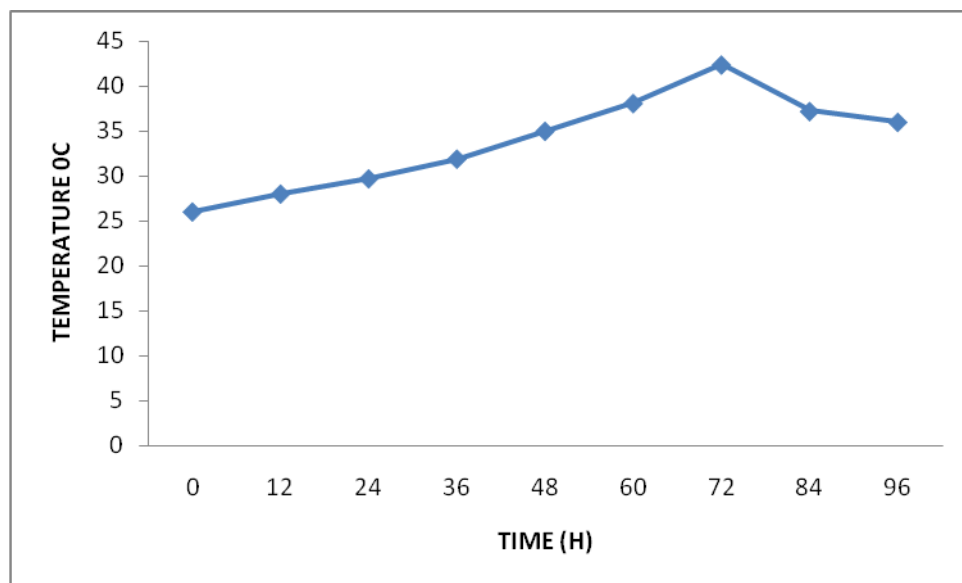
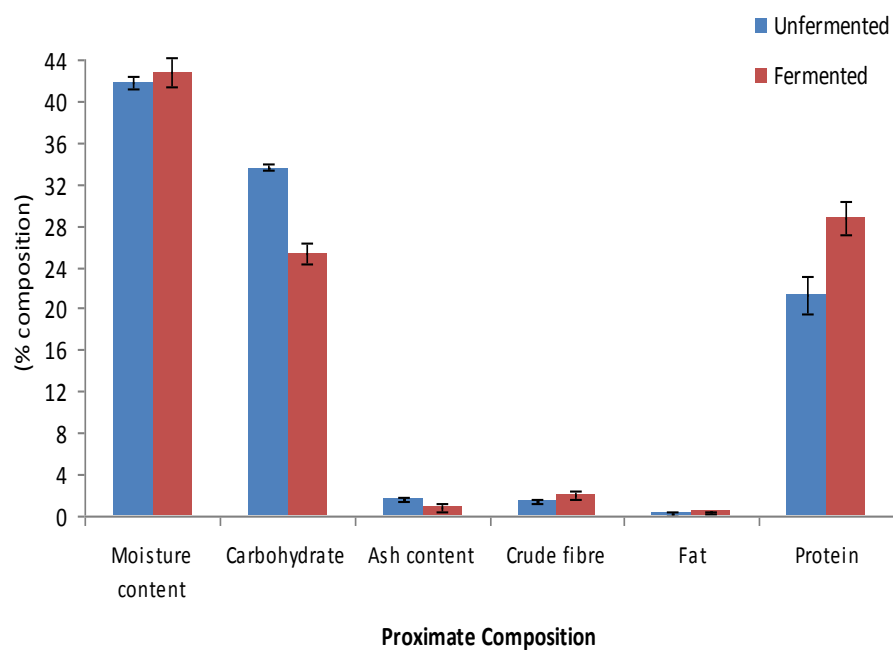
**Figure 1** Changes in temperature during spontaneous fermentation of bambara nut seeds.**Figure 2** Proximate composition of fermented and unfermented bambara nut seeds

Figure 3 shows the mineral contents of calcium, magnesium, potassium, sodium and phosphorus in the fermented and unfermented samples of bambara nut. Values for the fermented samples were higher than the corresponding values of the unfermented samples, with potassium having the highest value recorded for both samples ($1.01 \pm 0.01 \text{ mg/100g}$ to $1.53 \pm 0.00 \text{ mg/100g}$ for unfermented and fermented samples respectively.)

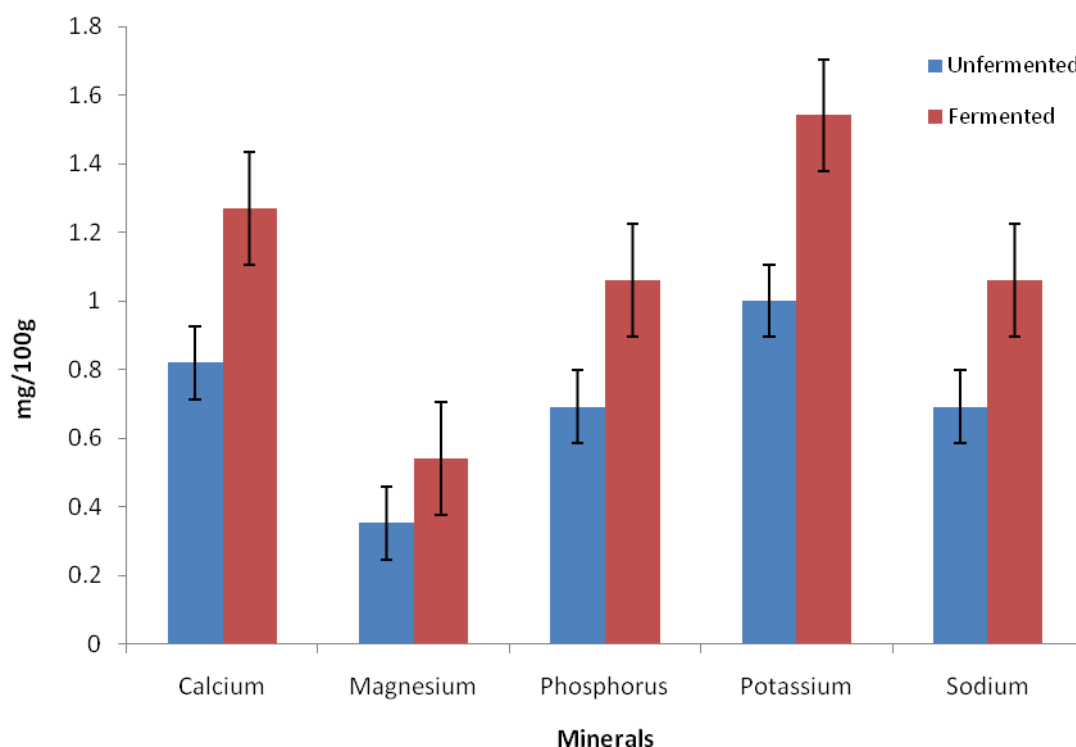


Figure 3 Mineral content of both fermented and unfermented bambara nut samples

The participation of a variety of microorganisms in spontaneous food fermentation is common and does not render the final product unsafe for human consumption, especially if they are non-pathogenic (Oyeyiola, 2002). The growth of microorganisms during the fermentation of bambara nut is likely to have had a significant influence on the quality and flavour of the final product. Some of the organisms involved in the fermentation may have been introduced by chance inoculation from the environment. However the initial boiling eliminated most of the surface microflora of the bambara nuts. Boiling before fermentation has the effect of eliminating the species responsible for an acid fermentation and encouraging a non-acid fermentation that is dominated by *Bacillus* species (Achi, 2005). The predominance of *Bacillus* species in the fermented nut samples is expected since they have been found to be associated with fermenting legume seeds for condiment production (Ogunshe *et al.*, 2007, Ouoba *et al.*, 2008). The presence of these organisms, which are proteolytic, may lead to an

increased proteinase activity, causing the breakdown of proteins. The presence of *Staphylococcus* species in the samples was typical of the microflora of fermenting beans (Antai and Ibrahim, 1986). *Staphylococcus* species have been associated with fermenting foods of plant origin especially vegetable proteins (Jideani and Okeke, 1991). The coagulase-negative *Staphylococcus* species are non-pathogenic and safe organisms on vegetable proteins (Ouoba *et al.*, 2008). These organisms may contribute to the flavour of the fermenting bambara nut ogiri because of their lipolytic activity. *Escherichia coli* was also found to be present in the samples at the beginning of fermentation but absent after 24 h of fermentation. *E. coli* though fermentative and found in the air and soil, has been isolated from some fermentation products (Ogunshe *et al.*, 2007). *Saccharomyces cerevisiae* was found throughout the fermentation period. This organism is known to be able to grow well in the complete absence or presence of very little oxygen (Oyeyiola, 2002). Moulds such as *Aspergillus* spp and *Penicillium* spp are present as typical organisms associated with dried farm produce.

The temperature change of the fermenting mash could be attributed to increased metabolic activity of the microorganisms and an exothermic nature of the fermentation process whereby heat was evolved (Jeff-Agboola (2007). The observed increase in titratable acidity resulted from the amylolytic and lipolytic activities of the fermenting microorganisms causing the breakdown of carbohydrate and fat contents of the beans, thereby releasing acids and utilizable products of hydrolysis for growth. The pH of the processed beans decreased from 6.80 ± 0.03 to 5.30 ± 0.09 at the end of the fermenting process. However, most of the documented works on spontaneous fermentation of other vegetable proteins reported a steady increase in pH as fermentation proceeded as reported by Onawola *et al.* (2012), David and Aderibigbe (2010), Omafuvbe and Oyedapo (2004) in 'Okpa', 'iru' and 'ogiri' production respectively. The observed decrease in pH in this present study, as also observed in the study by Fadahunsi and Olubunmi (2009) during the fermentation of bambara nut into 'iru' (an indigenous condiment) could be due to the chemical composition of the beans which is higher in carbohydrate than other leguminous seed. The hydrolysis of carbohydrate led to acid production which lowered the pH.

The increase in moisture content may have resulted from the treatments such as soaking and boiling which the beans were previously subjected to before fermentation and the moist nature of the fermentation process which is in concordance with (Fadahunsi and Olubunmi, 2009). The hydrolytic decomposition of the fermenting beans cotyledons had earlier been reported to contribute to the increased moisture content as observed by David and Aderibigbe (2010) in their study.

The decrease in the carbohydrate content observed may have been as a result of the hydrolytic effect of microbial amylase, converting carbohydrate into sugars (Fadahunsi and Olubunmi, 2009). *Bacillus* spp have been reported as producers of enzymes such as amylase, galactase, galactosidase, glucosidase and fructofuranosidase which are involved in the degradation of carbohydrates (Omafuvbe and Oyedapo, 2000).

The increased crude protein might be attributed to the effect of fermentation process which improves the nutritional quality of fermented foods by increasing the protein content. A similar increase in fat content was also recorded. An increase in protein and fat content with a decrease in carbohydrate content after fermentation had been reported by Fadahunsi and Olubunmi (2009) in the fermentation studies of bambara nut into 'iru'; and by Eka (1980) in the study of the effect of fermentation on the nutritive content of locust beans.

The increase in crude fibre of the 'ogiri' produced has also been reported by Onawola *et al.* (2012), where the crude fibre content of the ogiri produced from undehulled melon seeds increased from 2.60% to 6.58%. An increase in the percentage composition of mineral contents in the fermented sampled can be attributed to the fact that fermentation helps to improve the nutritional and organoleptic quality of food. This was also reported by Ogbonna *et al.* (2001), where an increase in calcium, phosphorous and potassium content was observed during the fermentation of African yam bean for condiment production. In order to enhance the utilisation of bambara nut, it will be worthwhile to ferment it for condiment production.

4. Conclusion

Fermentation of bambara nut seeds led to an increase in the nutritive value of these seeds. This is both locally and industrially significant. Since the seeds cannot be consumed like cowpeas after boiling, increased usage can be by fermentation. Food products can also be fortified since fermentation enhances digestibility of legumens.

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