Studies on the Microbiological, Proximate Composition, and Anti-nutritional Content of Fermented Groundnut and Plantain Blends.

Anthony Okhonlaye Ojokoh*; Modupe Khadijat Daramola; and Agbi Deborah Ochukwa

Department of Microbiology, Federal University of Technology, PMB 704, Akure, Nigeria.

E-mail: tonyojokoh@yahoo.com
Telephone: +08129497120

ABSTRACT

The effects of fermentation on chemical composition of four combinations (100:0, 90:10, 80:20, and 70:30) of groundnut - plantain blend were investigated. A total number of twelve (12) microorganisms were isolated during the fermentation of groundnut-plantain blend; these comprise of seven bacteria, one yeast, and four molds. They include *Lactobacillus species, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Klebsiella aerogenes, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Aspergillus niger, Aspergillus spp., Rhizopus spp., and Mucor spp.* The pH and the total titratable acidity (TTA) significantly varied during fermentation. The proximate composition and anti-nutrient content of the unfermented and fermented flour blends were assessed. The crude protein and moisture contents increased in the fermented samples whereas the ash, carbohydrate, crude fiber and fat contents varied among all the samples. There was a decrease in anti-nutrient content in the fermented samples.

(Keywords: fermentation, groundnut flour, plantain flour, proximate analysis, anti-nutrient)

INTRODUCTION

Food crops have occupied an important place in human nutrition as they remain the major sources of calories and proteins for a large proportion of the world population particularly in the developing countries. The direct consumption of vegetable proteins in food products have been increasing over the years because of animal diseases, global shortage of animal proteins and economic reasons (Edema *et al.*, 2005). Hence the development of nutritionally balanced protein foods to feed the growing population in the world is receiving increasing attention. Amongst the legumes widely cultivated in Nigeria, Bambara groundnut (*Vigna subterranae*) is among the underutilized. It is a rich source of protein, carbohydrate and phosphorus and it is grown mainly for its oil protein, plant residue and seed cake.

Defatted groundnut floor (DGF) produced from cake blends enhances or enriches the nutritive value of wheat and other flour (Purohit and Rajyalakshme, 2011). Despite the fact that DGF has an excellent potential in food formulations because of the high protein content, its uses remain limited. Hence groundnut processors are seeking ways to add value to this by- product through novel utilization.

Plantain belongs to the genus Musa and is quite firmer than bananas. Plantain which looks like banana are starchy and not sweet like bananas and are majorly used as vegetables. They have thicker skin as compared to bananas (Osagie, 1998). Plantain contains about 65% moisture and an average plantain has about 220 calories and is a good source of potassium and dietary fibre. Though work on groundnut and plantain blends is limited as it is in the case for many other traditionally fermented beverages in Nigeria.

Fermentations have been used by various workers to both remove the anti-nutritional factors as well as improve the nutritional value of foods (Odetokun, 2000). Many food products owe their production and characteristics to the fermentative activities of micro-organisms. Foods like cheese, yoghourt and fermented sausages are preserved products in that their shelf life is considerably extended over that of the raw materials from which they are made. Therefore, this work is aimed at investigating the microbiological population, nutrient composition and nutritional factors of unfermented and fermented groundnut and plantain blends.

MATERIALS AND METHODS

Samples of raw fresh ripe plantain and groundnut were gotten from Uchi market in Auchi, Estako West in Edo State of Nigeria. Prior to further use, the groundnut sample was sorted, cleaned dried and ground into fine flour while the plantain sample was cleaned with water and then peeled. The pulp was cut into small slices, sundried and ground into fine flour to pass a 0.4mm screen also.

Reagents /Chemicals

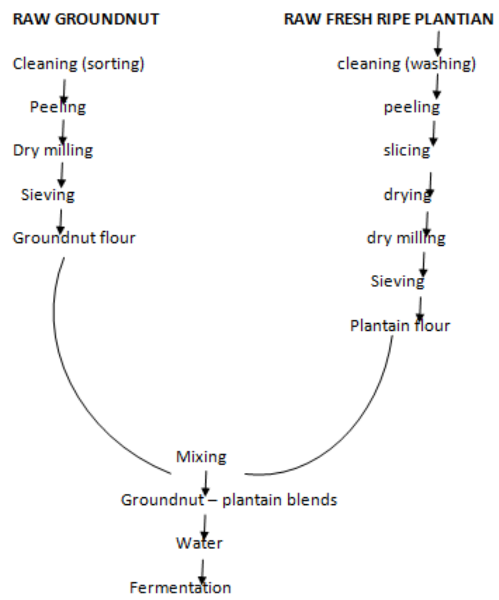
All reagents and chemicals were obtained from the Department of Microbiology, Federal University of Technology, Akure, Ondo State Nigeria.

Experimental Design

Preparation of composite flour for fermentation: The groundnut (G) – plantain (p) was formulated in ratios 100:0, 90-10, 80:20, and 70:30 and were labeled appropriately to avoid mixed up sample i.e.:

GpA	-	G 100g + p 0g
GpB	-	G 90g + p 10g
GpC	-	G 80g + p 20g
GpD	-	G 70g + p 30g

Four unfermented samples and four fermented samples giving total of 12 samples.



Fermentation of Samples: 40ml of sterile water was added to each of the groundnut (G) – plantain (p) formulated samples and properly mixed to give a damp or pastry mixture. This was allowed to ferment for 4days at 25±2^{o}C (room temperature).

Microbiological Analysis

The raw and fermenting blend samples were subjected to microbiological analysis using serial dilution and pour plated in duplicates on the following media (1) Nutrient agar for estimation of bacteria (2) PDA agar for estimation of fungi (3) MRS agar for total lactic acid bacteria (LAB) incubated anaerobically at 35^{0}C for 48 hours in anaerobic jars and that of PDA and NA were incubated at 25^{0}C and 35^{0}C for 72 hrs.

Physicochemical Analyses

pH and Total Titrable Acidity: A mixture of 10g of each of the raw and fermented samples was used for pH determination as described in AOAC (1995). Total titratable acidity (TTA) was determined by titrating 20ml of the same sample against 0.1M NaOH.

Proximate Composition: The moisture, crude fibre, fat, protein (N×6.25), ash and carbohydrate contents of both the fermented and raw samples were determined using relevant methods described previously (AOAC, 1995).

Anti-Nutrient Content Analysis: Phytate, hydrogen cyanide and tannin were determined using AOAC (1995) methods while oxalate content was by the titrimetric method (AOAC, 1995).

Statistical Analysis

Unless otherwise indicated results are expressed as means ± SD of three replicates. Data were subjected to one –way analysis of Variance (ANOVA) using SPSS version 14.0. The Duncan's Multiple Range test was used to separate the means at the 5% level of probability.

RESULTS

In this study seven bacteria, one yeast, and four molds were isolated. Table 1a and b below shows the microorganism isolated from the samples before and after fermentation. The predominant organism isolated was *Lactobacillus plantarum.*

Table1a: Showing the Bacteria Isolates and Total Count from the Fermentation of Groundnut-Plantain Blend.

Organisms	Bacterial count Cfu/ml
Bacillus subtilis	3.0×10^3
Staphylococcus aureus	6.2×10^3
Escherichia coli	6.5×10^3
Klebsiella aerogenes	5.8×10^3
Lactobacillus species	7.36×10^3
Pseudomonas aeruginosa	3.5×10^3
Proteus vulgaris	2.0×10^3

Table 2b: The Morphological Characteristics and Microscopic Observation of the Fungi Isolates from the Fermentation of Groundnut and Ripe Plantain Blend.

Microscopic characteristic	Morphological characteristic	Suspected organism
Upright conidiophores with stripes smooth walled. Conidia are one-celled vesicles globose. Septate and collumella.	White base with black conidiophores which makes appear black in color.	*Aspergillus niger*
Non-septate hyphae, thin sporangiophore with a sporangium in umbrella-like form.	Dark brown colonies with mycelluim	*Rhizopus spp.*
An upright conidiophores that terminates in a davate swelling, bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose.	Yellow mycelia	*Aspergillus spp.*
Septate hyphae no stolon sporangiosphore arise at a point in the branched aerial mycelium	White, creamish fluffy mycelium	*Mucor spp.*
Cream color, umbonate and smooth surface with entire edge. Budding cells with formation of ascospores but no pseudomycelium.	Dark pinkish foully color	*Saccharomyces cerevisiae*

On the basis of pH and Total titratable acidity(tta), the four fermented samples studied showed high similarity (Figures 1-2).There was significant decrease ($p \le 0.05$) in pH at the end of 72 hrs fermentation while titratable acidity increased significantly ($p \le 0.05$) for all the fermented samples.

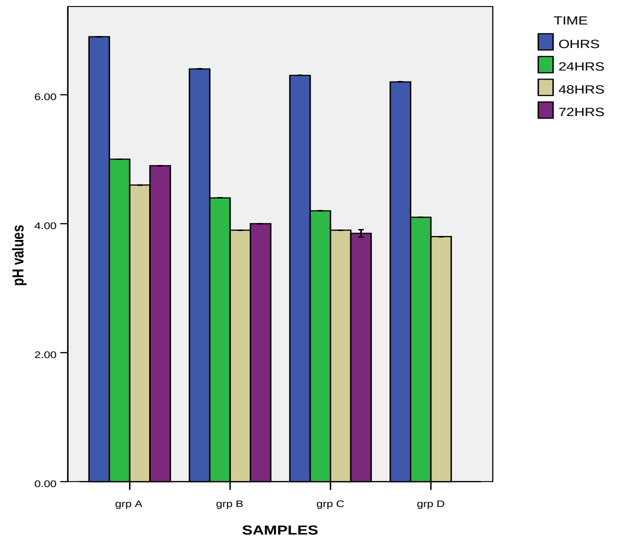


Figure 1: pH of Samples during 72 hr Fermentation.

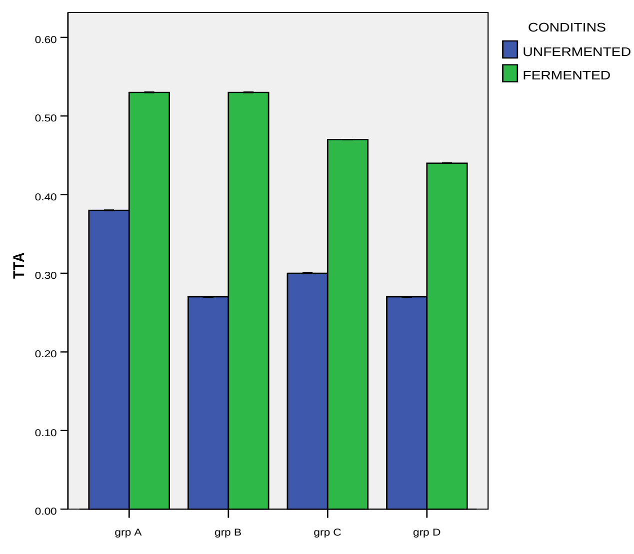


Figure 2: Total Titratable Acidity of the Unfermented and Fermented Samples.

The proximate analysis showed that there was general increase in protein and moisture contents in all the fermented samples except in the third sample were there was a decrease in protein.

The mean crude protein content for the unfermented and fermented samples were 27.59 % and 44.40 % while the mean moisture content were 2.24 % and 3.18 %.

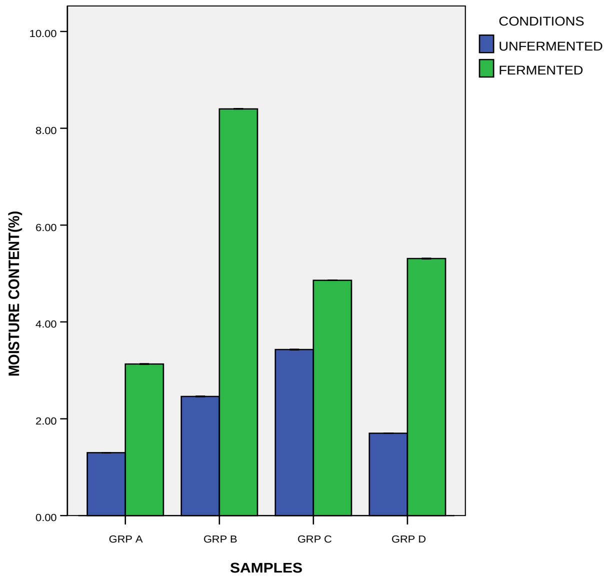


Figure 3: Moisture Content (%) of the Unfermented and Fermented Samples.

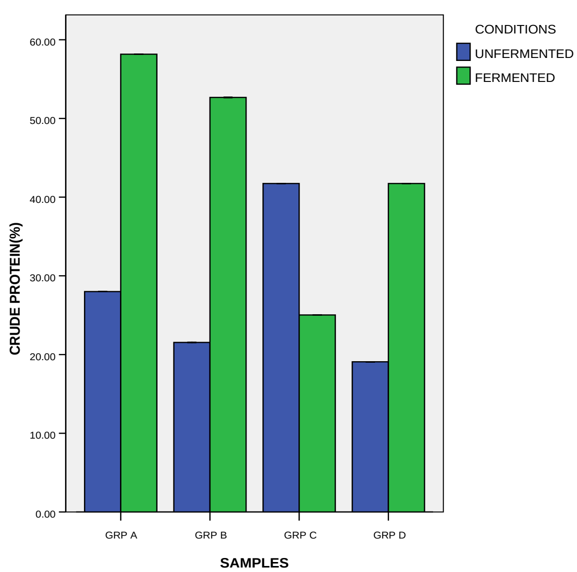


Figure 4: Crude Protein Content (%) of the Unfermented and Fermented Samples.

There was a decrease in carbohydrate content of the second (GpB) and fourth (GpD) samples. The mean carbohydrate content for the unfermented and fermented samples was 24.03% and 13.83%.

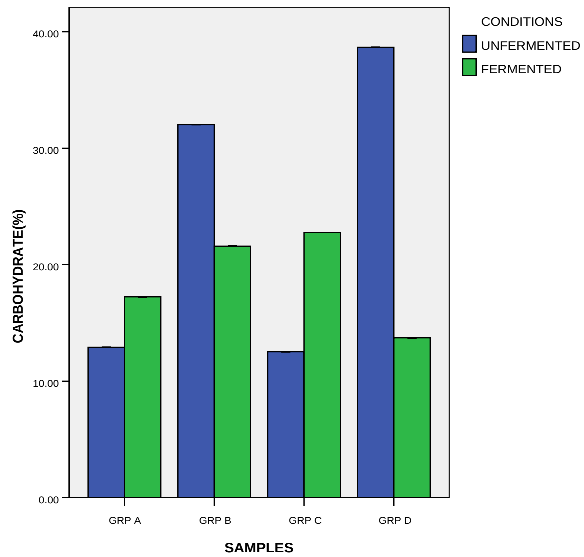


Figure 5: Carbohydrate Content of the Unfermented and Fermented Samples.

There was a decrease in ash content of the third sample (GpC). The mean ash content for the unfermented sample was 1.91 as compared to 2.02 in the fermented samples. There was decrease in crude fiber with a slight increase in the second (GpB) and third (GpD) samples while the fat content decreased in the first (GpA) and fourth (GpD) samples. The mean values of crude fiber were 4.45% and 3.98% and fat 3.98% and 40.69% for the unfermented and fermented samples.

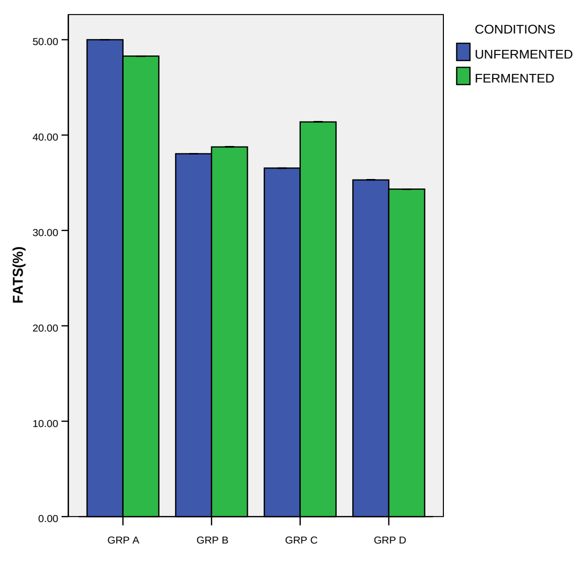


Figure 6: Fats Content (%) of the Unfermented and Fermented Samples.

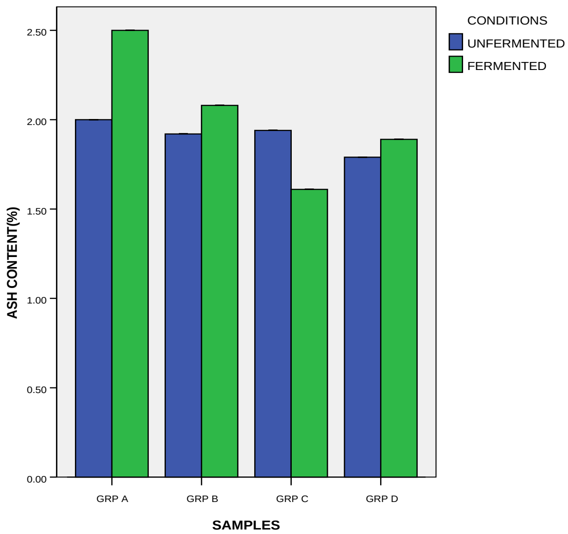


Figure 7: Ash Content (%) of the Unfermented and Fermented Samples.

The results of the anti-nutrient contents of the samples are shown in Tables 2 a-d. The cyanide, oxalate and phytic contents decreased in all the fermented analogues while the tannin content increased in samples A and B and later decreased in samples C and D.

Tables 2 a – d: Anti-Nutrient Content (mg/100g) of Samples (unfermented and fermented).

(a) Hydrogen Cyanide Content

Sample	Unfermented	Fermented
GpA	1.35	0.68
GpB	1.35	0.68
GpC	1.35	0.68
GpD	1.35	0.68

(b) Oxalate Content

Sample	Unfermented	Fermented
GpA	0.36	0.18
GpB	0.36	0.18
GpC	0.36	0.27
GpD	0.27	0.18

(c)Phytate

Sample	Unfermented	Fermented
GpA5.77	13.18	5.77
GpB	12.36	5.77
GpC	10.71	6.59
GpD	10.71	6.59

(d) Tanin

Sample	Unfermented	Fermented
GpA	0.16	0.39
GpB	0.80	0.86
GpC	0.55	0.47
GpD	0.48	0.31

DISCUSSION

The reduction in pH observed in this study has been reported to be due to the production of acids by fermenting microorganisms and the observed increase in titratable acidity could be due to the dominance of the environment by lactic acid bacteria which degrade carbohydrates resulting in acidification. The observation is in agreement with earlier studies by Nout et al., (1989) and Ariahu et al. (1999).

High titratable acidity has been reported to reduce incidence of diarrhea in infants consuming fermented legumes (Mensah et al., 1990). The groundnut-plantain blends will then have two attributes, such as antimicrobial properties and high protein content.

From Table 1a, total count from all the flour (4) samples on MRS medium and nutrient medium shows that lactic acid bacteria colonies increases in population after 24 hrs. of fermentation due to their acclimatization of the environment and their activities. There was decrease in population of other bacterial species as the fermentation process extends.

The presence of organisms like *Staphylococcus sp.*, *Pseudomonas sp., Escherichia coli and Proteus spp.* could have been as a result of contamination during handling and processing. Some of these bacterial are dangerous as can produce toxic metabolite which can affect the consumer. *Aspergillus spp*. and *Bacillus* are known to be associated with grains. The eventual disappearance of other microorganisms may not be unconnected with the increase in the acidity of the medium as a result of the fermentative activity of the lactobacillus.

The significant increase ($p \leq 0.05$) in protein content is probably due to increase in microbial cell mass during fermentation. Increase in protein content of food resulting from increase in microbial cell mass has been reported by other investigators during fermentation of various foods including jack beans (Onyango, *et al*., 2004a) and soya products (Ojokoh and Wei, 2011).

Supplementation level and interaction between the blends may have affected the protein content of the third sample hence there was a reduction in the protein content.

The significant decrease ($p \leq 0.05$) in carbohydrate content in some samples agrees with the findings of Odetokun *(*2000), who reported that fermentation decreased the carbohydrate content of cereal and legume blend when compared with unfermented samples. However the variations in the proximate composition could be associated with the processing techniques employed.

The decrease in dry matter in the fermented sample compared to the unfermented sample might be due to lactic acid accumulation with concomitant increase in acidity and a decrease of dry matter yields. The decrease in ash content may be due to the fact that some biological macromolecules were released into the solution from such structures (Yagoub and Abdalla, 2007).

Decrease in ash content has been reported in various foods including sieve maize mash (Antai and Nzeribe, 1992) and garri (Sanni,1991). Fermentation caused a reduction in the fiber content of the samples probably this could be associated with the activities of the fermenting microorganisms which would have synthesized cellulase enzyme there by breaking down the cellulose in the fermented sample. The low value of fiber in the fermented sample is desirable because possible undesirable aspects of high fiber levels in weaning period include; increased bulk and lower calorie density, irritation of the gut mucosa, reduced digestibility, reduced vitamin and mineral availability and local effects on intestinal mucosa (Odetokun, 2000).

Fat increased with ground nut level supplementation in the second and third samples. The decrease in fat in the other samples could be attributed to the hydrolysis of glyceride to free fatty acids and glycerol. However, the significantly($p \leq 0.05$) low moisture content of the fermented sample compared to the unfermented lima beans, indicates that the product will have a good keeping quality since spoilage microbes thrive better in the presence of adequate moisture.

Fermentation diminished the level of anti-nutrients in the samples. The reduction observed in the hydrogen cyanide (HCN) content of the samples after 72hours of fermentation and processing may be as a result of microbial enzymes activities during fermentation process (Kobawila *et al.* 2005).

A wide range of microflora has been known to possess phytase activity (Akindahunsi et al.,1999) which may be partly responsible for reduction in phytic acid content in the fermenting samples.

The reduction in tannin in some fermented samples may be due to the processing that the samples were subjected to, coupled with activities of the microbial enzymes involved in the fermentation. Tannins are capable of lowering available protein by antagonistic competition and can therefore elicit protein deficiency syndrome, kwashiorkor (Maynard, 1997). Ekop *et al.* (2004) reported that most popular toxicant can be reduced by processing method such as fermentation.

The oxalate content value of the samples was observed to be decreasing with increase in the days of fermentation. This achievement could be attributed to the combined effect of various organisms involved during the natural fermentation. This reduction is of great importance because oxalate can form complex with most essential trace metals thereby making them unavailable for enzymatic activities and other metabolic processes (Ekop *et al.,* 2004)

CONCLUSION

This study has revealed that groundnut and plantain blend can be fermented to produce food of enhanced and better nutritional value with low anti-nutrient content.

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