

Effect of Fermentation on the Proximate Composition of Ripe and Unripe Plantain Flour

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAE designed the study, performed the statistical analysis and managed literature searches. Author AOO, wrote the protocol and the first draft of the manuscript. Authors OE, LE and AAC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to investigate the effect of fermentation when ripe and unripe plantain flour was fermented for 72 hours at room temperature (32°C±2°C).

Methodology: The effect of fermentation on the flours was accessed by determining the microbiological quality, proximate composition as well as the anti-nutritional content. Mixed flora of bacteria and fungi were isolated. Different species of fungi (*Aspergillus niger*, *Penicillium* spp, *Rhizopus* spp and *Fusarium* spp) and bacteria (*Staphylococcus aureus*, *Streptococcus* spp, *Lactobacillus* spp, *Leuconostoc* spp, *Bacillus subtilis*, *Bacillus cereus*) were identified.

Results: The result of the proximate analysis showed that there was an increase in the crude fibre, protein and carbohydrate contents of both ripe and unripe fermented samples. The contents of protein, crude fibre and carbohydrate of the fermented ripe plantain flour increased from 0.86 ±0.03

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to 2.91 ± 0.04 , from 1.68 ± 0.03 to 2.81 ± 0.11 and from 79.25 ± 0.01 to 85.43 ± 0.01 while that of the unripe fermented plantain increased from 1.75 ± 0.03 to 2.86 ± 0.12 , from 1.10 ± 0.07 to 1.61 ± 0.05 and from 79.78 ± 0.01 to 88.28 ± 0.08 respectively. Anti-nutrients properties such as tannin, alkaloids, glycosides and flavonoids of the fermented samples decreased.

Conclusion: This research work has proven that fermentation increases the nutritional value of food and helps to reduce the anti-nutrient content of food samples that can be hazardous to health.

Keywords: Fermentation; proximate analysis; anti-nutritional; plantain flour.

1. INTRODUCTION

There is an increased interest in the production of flours from locally available and abundantly grown dietary staple food. Fermentation is one of the methods employed by so many people or organization in the production of these flours. Traditional food crop play an important role in the diet of the people of African particularly in cereal production zones, flours from various cereals is one of the main raw materials used in the production of popular food products with high acceptability, good storage characteristic and affordable cost [1].

Fermentation is a metabolic process that converts sugar to acid and alcohol through the actions of molds, yeasts and bacteria. Fermentation is an age long method of processing cereals and legumes [2]. According to [3], fermentation of grains and oil seeds results in increased nutritional value and wholesomeness over the starting material and it may also lead to changes in vitamin levels. Food fermentation, especially lactic acid fermentation is an important technology in Africa. The technology is indigenous and is adaptable to the culture of the people. The fermentation process meets the requirements of being low-cost, preventing food spoilage and food-borne diseases with respect to consumers living in a climate, which favours the rapid deterioration of food. In addition, fermented foods are of particular importance in ensuring adequate intake of protein and/or calories in the diet [4]. Fermentation actually holds promise as a food processing method that can be used to diversify the food uses of some under exploited plant foods like plantain. The traditional processing of these foods needs to be changed or modified to improve their nutritional status. Fermentation has been viewed as a dynamic process during which several catabolic and anabolic reactions proceed simultaneously depending on several conditions, including substrate, micro flora and environmental factors. However, to the microbiologist, the term 'fermentation' describes a form of energy yielding

microbial metabolism in which an organic substrate, usually a carbohydrate, is completely oxidized, and an organic carbohydrate acts as an electron acceptor [4]. Fermented foods play an important role in providing food security, enhancing livelihoods and improving the nutrition and social well-being of millions of people around the world. Fermentation leads to improved food preservation, increasing the range of raw materials that can be used to produce edible food products. Food processing is probably the most important source of income and employment in Africa, Asia and Latin America. The Food and Agriculture Organization of the United Nations has stated that value added through marketing and processing raw products can be much greater than the value of primary production [5]. Generally, a significant increase in the soluble fraction of a food is observed during fermentation. The quantity as well as quality of the food proteins as expressed by biological value, and often the content of water soluble vitamins is generally increased, while the anti-nutritional factors show a decline during fermentation. Fermentation results in a lower proportion of dry matter in the food and the concentrations of vitamins, minerals and protein appear to increase when measured on a dry weight basis [6]. Some studies have been carried out in Nigeria on the effect of fermentation of some food materials. The findings of [7], showed that in some cases, there may be an enhancement of the nutritive value by an increase in the level of essential nutrients or reduction in the levels of toxicants in the food. Useful metabolites such as essential amino acids, vitamins and other essential nutrients may arise from the fermentation by microorganisms. Over fermentation may give cereals an irritating odour due to the production of ethanol and carbon dioxide. The benefits of fermented flour are many, apart from being enriched nutritionally, it is also easy to digest and the iron in cereal is better absorbed after fermentation. Furthermore, the lactic acid (sour substance produced during fermentation) in fermented flours prevent harmful microorganisms from growing fast and this

makes porridge from fermented flour much safer to eat and nutrient dense compared with porridge from unfermented flour [8]. In lactic acid fermentation, the high acidity and low pH attained is responsible for inhibiting other microbes and other undesirable chemical changes. The bioconversions caused by fermentation contribute very much to the character and organoleptic properties of the fermented products.

On the other hand, Plantain (*Musa* spp) is an important dietary source of carbohydrate in the humid tropical zones of Africa, Asia and South America [9]. Plantain is rich in vitamin A, C and B groups as well as minerals such as calcium and iron [10]. *Musa* spp is useful as food to be consumed by human either as flour to be used in confectionaries or as jams and jellies, in chips etc. The peel can be used as animal feed and other parts of plantain plant have been discovered to possess medical applications. The flour can be used in bronchitis, dysentery and in ulcer conditions, while cooked flours are given to diabetic patients. The astringent plant sap is used in cases of hysteria, epilepsy, leprosy, fevers, haemorrhages, acute dysentery and diarrhoea, and it is applied on haemorrhoids, insect and other stings and bites [11]. Young plantain leaves are placed as poultices on burns and other skin infections. The astringent ashes of the unripe plantain peels and leaves are taken in cases of dysentery and diarrhoea, and also used for treating malignant ulcers. The roots are administered in digestive disorders, dysentery and other ailments. Banana seed mucilage is given in cases of catarrh and diarrhoea in India, while Plantain leaves are widely used as plates and for lining cooking pots for wrapping food that are cooked for storage purpose [11]. The objective of this work is to determine the effect of fermentation on flour blends from ripe and unripe plantain in terms of their nutritional and anti-nutritional qualities and also to isolate and identify the microorganisms associated with the fermentation of ripe and unripe plantain flour.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Matured fresh unripe plantain was bought from Oja Oba Market in Akure, Ondo State Nigeria. Part of the plantain bought was allowed to ripen naturally. The samples were washed and then peeled using a clean knife. The back was

discarded and the fruits were used for analysis. The fruits were dried in an oven at 80°C±2°C for 12 hours and then milled.

2.1.1 Preparation of plantain flour

Ripe plantain and unripe plantains were collected, cut into small sizes and sun dried for four days. The sizes of the peels were further reduced by grinding using a grinding machine (SM-1 Retsch GmbH 5667 HAAN, West-Germany).

2.2 Fermentation of Samples

Spontaneous fermentation was carried out on the Plantain flour which was mixed with water to form slurries. Each sample was fermented in a transparent, sterile container for 72 hours at room temperature and chemical analysis like (pH, temperature, titratable acidity, proximate analysis and anti-nutritional factors analysis) were carried out on the ripe and unripe fermented samples on daily basis for 72 hours.

2.2.1 pH

This was done by the method of [12] with some modifications. Before the pH measurements were taken, the sample slurry was thoroughly stirred to homogenize the mixture and achieve uniformity. The pH electrode was dipped in each of the slurries and measurement was taken when the readings on the pH meter was stable using a HANNA pH Meter 209.

2.2.2 Total titratable acidity (TTA)

The TTA analysis was done using [13] method. 10 ml of sample was pipetted into a conical flask and 3 drop of phenolphthalein indicator was added. Titration was done using 0.1 M NaOH to a faint pink colour for at least 1 minute compared against a white background. The titre volume was noted and used to calculate TTA which was expressed as Percentage Lactic Acid.

TTA was determined and expressed as follows:

$$\% \text{ Lactic acid} = A \times 0.009 \times 100/V$$

Where A = ml of 0.1 NaOH required for the titration, and V = ml of sample taken for the test.

The acidity was calculated as lactic acid was used as the relationship:

$$\frac{\text{Volume of base used} \times \text{Normality of NaOH (N)} \times 9}{\text{Volume of Sample used (average titre)}}$$

2.2.3 Microbiological analysis of ripe and unripe plantain flour

Daily changes in the microbial population (cfu/ml) of the total viable count of fungi and lactic acid bacteria were determined. The samples of the fermenting flour were collected at 0, 24, 48 and 72 hours interval respectively and serially diluted using sterile distilled water. Appropriate dilutions were plated on Nutrient Agar (Total viable count), Potato Dextrose Agar (fungi/mold count) and De Man Rogosa Sharpe, MRS (*Lactobacillus* spp). The nutrient agar plates were incubated at 35°C±2°C for 24 hours while MRS and PDA plates were incubated at 28°C±2°C for 72 hours according to manufacturer's instructions. MRS plates were incubated under anaerobic condition. Classification of isolates was based on established method using important biochemical and morphological observations and test.

2.3 Preparation of Sample and Culture Media

Serial dilution of each sample was carried out by pipetting 9ml of distilled water in test tubes. One milliliter (1 ml) of the fermented samples was pipetted aseptically into the tube containing 9 ml of sterile distilled water and mixed thoroughly. The culture media used in this study was prepared according to the manufacturer's instructions. Nutrient agar, PDA is a general purpose based medium, which is used for the isolation of bacteria and fungi respectively and was prepared by weighing appropriate grams in the appropriate ml of distilled water according to the volume needed. It was dissolved in conical flask and sterilized at 121°C for 15 minutes in an autoclave. This was used for the enumeration of total viable count. Selective media, MRS and Mannitol salt agar, which is selective for *Bacillus* spp and *Lactobacillus* spp respectively. The media were prepared according to manufacturer's specification.

2.4 Proximate Analysis

2.4.1 Moisture content

The moisture content was determined by using dry-oven method. Clean and dry petri-dishes were weighed by using meter balance and their respective weights were recorded (W1). 5 g of the sample was weighed into pre-weighed dried dishes (W2) spreading as much as possible. The dishes containing the sample were

transferred into an oven maintained at 105 ±2°C and dried for three hours. After three hours they were transferred to the desiccators to cool and then weighed. This process was continued until a constant weight (W3) was taken as the percentage moisture content.

$$\% \text{ moisture} = \left[\frac{\text{loss in weight due to drying (W2-W3)}}{\text{Weight of sample taken (W2-W1)}} \right] \times 100 / 1$$

2.4.2 Ash content

1 g of each sample was weighed into clean dried pre-weighed crucibles with lid (W1). The organic matter was burnt off using flame (lid removed) until the sample became charred. The crucibles were then transferred to the muffle furnace set at 550°C (lid removed). Ashing was continued until a light grey or white ash was obtained. The crucible was then cooled in desiccators and weighed (W2).

$$\% \text{ Ash} = \frac{W2 - W1}{\text{Weight of sample}} \times 100 / 1$$

2.4.3 Fat Content determined by soxhlet extraction method

Clean and dry thimbles were weighed as (W1) and 5 g oven dried sample was added and reweighed (W2). Round bottom flask was filled with petroleum ether (40-60°C) up to ¾ of the flask. Soxhlet extractor was fixed with a reflux condenser and adjusted to the heat source so that the solvent boils gently. The sample was placed inside the thimble and inserted into the soxhlet apparatus and extraction under reflux was carried out with petroleum ether (40-60°C). The barrel of the extractor was emptied and the condenser removed. The thimble was also removed, and thereafter taken into the oven at 100°C for one hour and later cooled in the desiccators and weighed again (W3).

$$= \left[\frac{\text{weight loss of sample (extracted fat)(W2-W3)}}{\text{Original weight of the sample (W2-W1)}} \right] \times 100 / 1$$

2.4.4 Protein content determination

Kjedahl nitrogen method was employed for the determination of protein content of the sample 1.0 g of the sample was weighed into the digestion flask. Kjedahl catalyst (5 selenium tables) and 20 ml of concentrated H₂SO₄ was added to the sample and then fixed for 8 hours in the digestion unit (450±2°C) of the kjedahl apparatus in a fume cupboard. The digest which was pure yellow after cooling changed into a

colourless liquid that was transferred into 100 ml volumetric flask and made up to mark with distilled water. About 20 ml of 4% boric acid solution was pipetted into conical flask. A drop of methyl red was added to the flask as indicator. The sample was thereafter diluted with 75 ml of distilled water made alkaline with 20 ml of NaOH (20%) and distilled. The steam exit of the distillatory was closed and the change in colour of boric acid solution green was timed. The mixture was distilled for 15 min [14]. The filtrate was then titrated against 0.1 N HCl. The protein content was calculated from the relationship given below:

$$\text{Total protein} = \left[\frac{\text{Titre} \times \text{Normality of Acid} \times 0.014}{\text{Sample weight}} \right] \times 100/1$$

$$\begin{aligned} \text{Protein (\%)} &= \% \text{ nitrogen} \times 6.25, \\ \text{Normality of acid (HCl)} &= 0.1 \text{ N}, \\ \text{Sample weight} &= 1.0 \text{ g} \end{aligned}$$

2.4.5 Fibre content determination

2 g (W1) of the sample was weighed into one litre conical flask containing 200 ml of boiling and 1.25% of H₂SO₄ which was added and boiled gently for 30 minutes. The mixture was filtered through muslin cloth and the residue washed thoroughly with hot distilled water and then rinsed once with 10% HCl twice with ethanol and rinsed to drain dry, then the residue was scrapped into a crucible and allowed to dry in the oven at 105±2°C, cooled in a desiccators and weighed (W2). The residue was ashed at 55°C, for 90 minutes in a muffle furnace, cooled and weighed again (W3).

$$\% \text{ Fibre} = \left[\frac{W2 - W3}{W1} \times 100/1 \right]$$

2.4.6 Carbohydrate content determination

Carbohydrate content was determined according to the official method of AOAC, [13]. It was carried out by subtracting from the sum of the 100 percent moisture, ash, protein, fat and fibre. The remaining value gives the carbohydrate content of the sample.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Protein} + \% \text{ Fibre})$$

2.5 Anti-nutrient Analysis of the Sample

2.5.1 Determination of tannin

Tannin content was determined according to the official method of Makker et al, [15]. 2.2 g of

finely ground sample was weighed into a 50 ml sample bottle. 100 ml of 70% aqueous acetone was added and properly covered. It was put into an ice bath shaker for 2 hours at 30°C. Each solution was then centrifuged and the supernatant stored in ice (0.2 cm³) of each solution. It was then pipetted into test tubes and 0.8 cm³ of distilled water was added. The standard tannic acid solutions were prepared from a 0.5 mg/ml stock and the solution made up 1 ml with distilled water 0.5 cm³ Folin Ciocalteu reagent was added to both samples and the standard followed by 2-5 ml of 20% NA₂CO₃. The solutions were then vortexed and allowed to incubate for about 40 min at room temperature; its absorbance was read at 725 nm against the reagent black concentration of the samples from a standard tannic curve.

2.5.2 Determination of alkaloids

Alkaloids content was determined according to the method of Harborne [16] following the gravimetric method with some modification.

2.5.3 Determination of flavonoid

The total flavonoid content of both extracts was determined using a slightly modified method as reported by Meda [17]. 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol; 50 ml of (10% AlCl₃), 50 ml of (1 m potassium acetate) and 1.4 ml water, which was allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415m; The total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

2.6 Statistical Analysis

Unless otherwise indicated, results are expressed as means ± SEM of three replicates. Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 15.0.

3. RESULTS AND DISCUSSION

From the results obtained during this study, analysis on moisture content showed that ripe and unripe plantain before fermentation recorded 6.89% and 6.10% respectively, but there was a reduction in the moisture content of ripe-fermented plantain flour to about 6.5% while

unripe fermented moisture increased to 6.4%. For the fat content analysis for both ripe and unripe plantain samples, it recorded a reduction from 7.18 and 8.58 to 1.2 and 0.50% respectively after fermentation for 72 hours. Changes were observed in the crude fibre content of fermented plantain samples (ripe and unripe plantain) as shown in (Fig. 1) which recorded greater values of crude fibre of 2.81 and 1.61 than in the unfermented samples. It was observed that fermentation increased the protein content of both ripe and unripe plantain flour from 0.86 and 1.75 to 2.91 and 2.86 respectively. Furthermore, for the Carbohydrate content of fermented samples as shown in (Fig. 1) it recorded greater values before fermentation.

The fermented sample as shown in (Fig. 2) recorded lower value in tannin content having 0.28 and 0.08 than the values recorded in unfermented samples of 0.88 and 0.17. In (Fig. 2), the fermented samples recorded lower values of 14.28 and 7.19 in alkaloids content compared to the fermented samples. Also in (Fig. 2), the fermented sample recorded lower values of 36.02 and 27.23 in Glycoside content compared to the value in the unfermented sample which recorded 36.45 and 29.02. In (Fig. 2), the fermented samples recorded lower values of 20.18 and 11.20 in flavonoid content when compared to the unfermented samples having 30.50 and 16.39.

Changes were recorded in the pH of all the samples as shown in Fig. 3. The pH of the samples was acidic as fermentation progressed during the study.

The changes in temperature for all the samples are shown in (Fig. 4). The temperature range recorded was between 22°C to 28°C.

The change in total titrable acidity of all the samples is shown in (Fig. 5). The highest value of titrable acidity was (g/100 lactic acid) as observed within 24 hours for unripe plantain sample and the highest bacterial load were observed at 48 hours for ripe and unripe plantain.

3.2 Discussion

A wide variety of micro-organisms were found associated with the fermented plantain and these include; *Aspergillus niger*, *Penicillium* spp, *Rhizopus* spp, *Fusarium* spp, *Staphylococcus aureus*, *Streptococcus* spp, *Lactobacillus* spp, *Leuconostoc* spp, *Bacillus subtilis* and *Bacillus cereus*. The highest bacterial count for (*Streptococcus* spp) recorded value of 1.10×10^6 cfum⁻¹ which was observed at 48 hours for ripe and unripe plantain during fermentation. The next prevalent Gram-positive bacterium was *Lactobacillus* spp, which had a population of 7.0×10^5 cfum⁻¹.

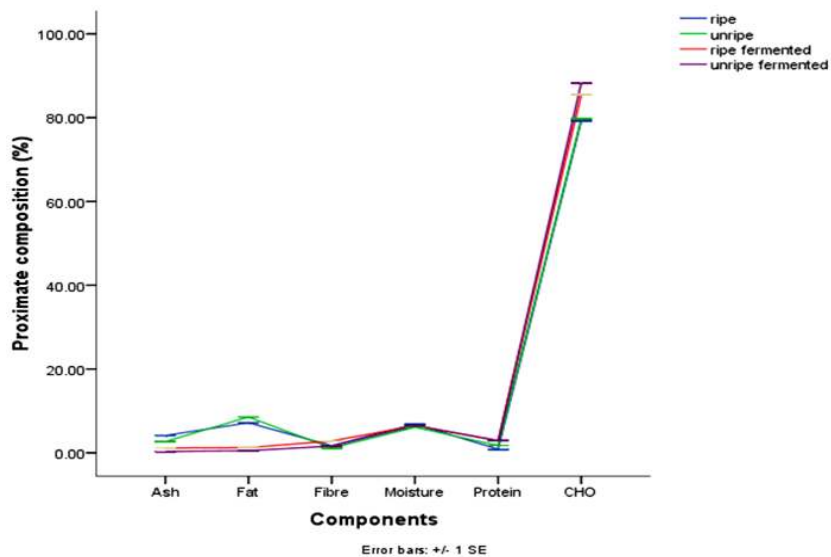


Fig. 1. Proximate composition (%) of ripe and unripe plantain before and after fermentation at (72hr)

Bars are presented as Mean ± S.E of replicates (n=3)

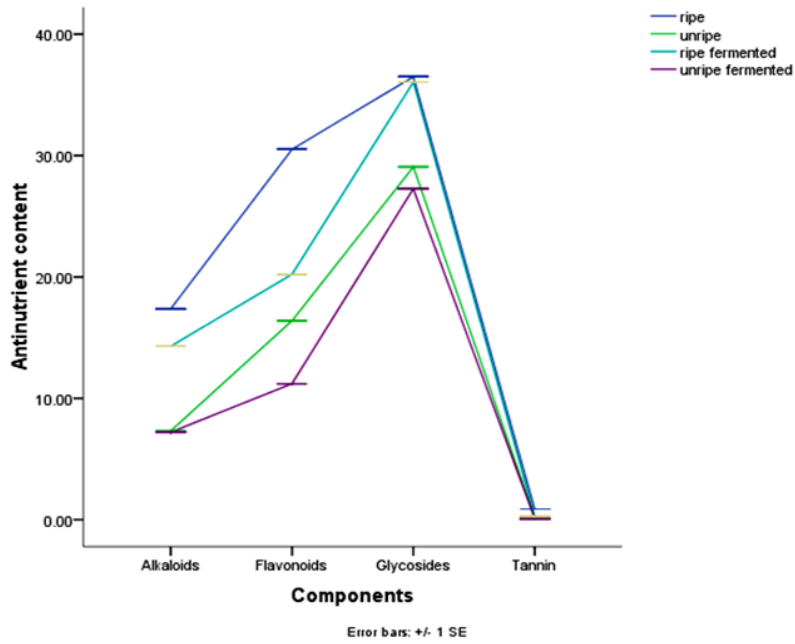


Fig 2. Anti-nutrient contents (mg/g) of ripe and unripe plantain before and after fermentation (72hrs)
 Bars are presented as Mean ± S.E of replicates (n=3)

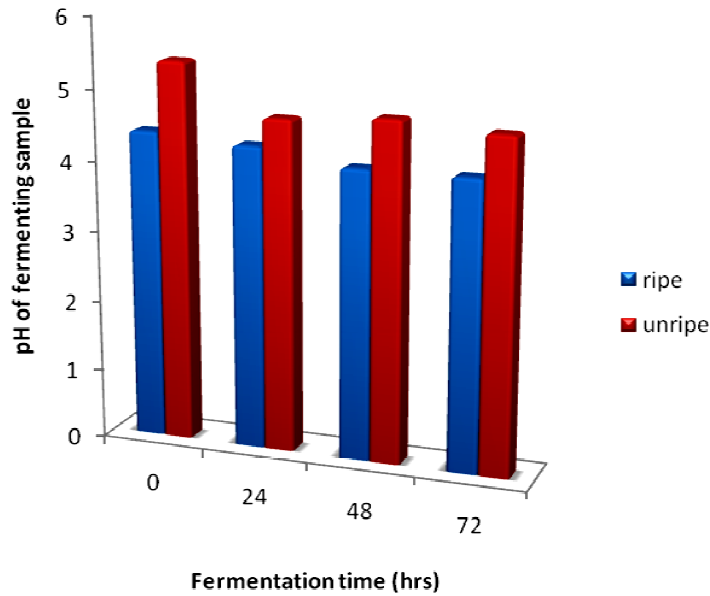


Fig. 3. Changes in pH of ripe and unripe plantain before and after fermentation (72hrs)

Fermentation had effect on the proximate composition of ripe and unripe plantain as observed during the study. The fermented unripe plantain recorded the highest moisture content than that of the ripe plantain. The increase in

protein content after fermentation for ripe and unripe plantain flour could be as a result of the metabolic activities of microorganisms present thereby resulting in the release of extracellular enzymes into the samples [18]. The increase

also agrees with the work of [19] who reported that fermentation process may prove a means of improving product functionality and protein contents. There was a decrease in ash content after fermentation for ripe plantain and unripe plantain. Since the ash content is a measure of the amount of minerals present in a food sample, an increase in its level during microbial fermentation could be as a result of incomplete utilization of minerals by fermenting organisms during their metabolism. There was also an increase in crude fibre content after fermentation of ripe and unripe plantain respectively. Fibre containing food are known to expand the inside

walls of the colon, easing the passage of waste, thus making it an effective anti-constipation; it lowers cholesterol level in the blood and reduce the risk of various cancers as reported by [20]. The carbohydrate (given as nitrogen free extract) levels of all fermented ripe and unripe plantain were higher than the ones observed in the raw samples.

There were changes in anti-nutrient composition of unfermented and fermented ripe and unripe plantain. Anti-nutrients are compounds which affect the nutritional value of food products such as flavonoids, alkaloids, glycosides and tannin.

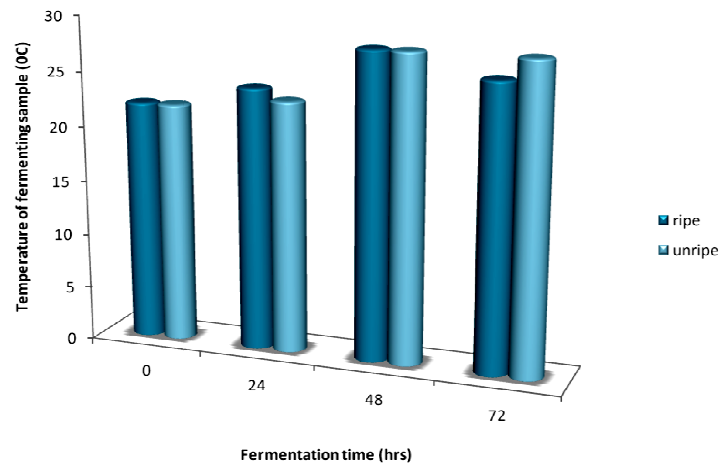


Fig. 4. Changes in Temperature of ripe and unripe plantain before and after fermentation (72hrs)

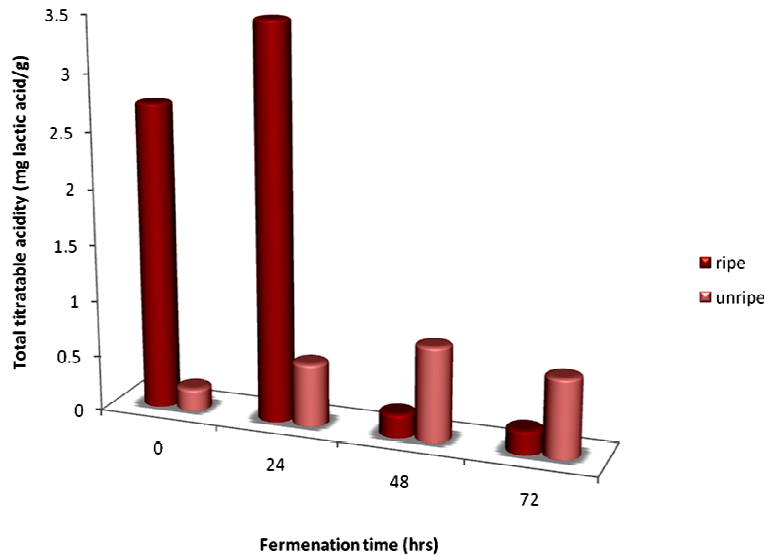


Fig. 5. Changes in total titratable acidity of ripe and unripe plantain before and after fermentation (72hrs)

Before fermentation the compositions of these anti-nutrients content were higher in all samples but after fermentation, the anti-nutrients content greatly reduced. Flavonoids in plantain, derivatives of the monomeric polyphenol flavan-4-ol, are called anthocyanidins [21]. The concentration of flavonoids was found to decrease for ripe and unripe plantain after fermentation. Tannins are polymers resulting from condensation of flavan-3-ols. Tannins bind to both exogenous and endogenous protein including enzymes of the digestive tract, affecting the utilization of proteins and iron absorption with high tannin food [22]. The concentration of Tannin was found to have decreased during the study for ripe and unripe sample respectively. The decrease in Tannin level after fermentation agrees with the report of [23] Odedokun and [24]. The decrease was attributed to the hydrolysis process during fermentation. [25] Reported that tannin – protein, tannic acid – starch and tannin-iron complex is broken down during fermentation to release free nutrients. Since tannins are known to reduce the availability of proteins, carbohydrates and minerals through the fermentation of indigestible complexes, breakdown of such complexes will invariably improve the availability of the nutrients. The results obtained from this study have proven that ripe and unripe plantain contains some naturally occurring toxins and anti-nutrients which were reduced during fermentation. The pH value determination carried out on all the samples showed that the pH of the ripe plantain was more acidic at (4.1) than the unripe plantain sample of (4.8). The total titratable acidity ranged from 0 to 72 hours of fermentation. When measuring titratable acidity, sodium hydroxide (NaOH) is added to the sample to deal with the free hydrogen responsible for the pH. There was an increase in total titratable acidity at 24 hours for ripe plantain sample than the unripe plantain sample but at the end of 72 hours the TTA of the ripe sample decreased while unripe sample increased, this findings is in accordance with the findings of [26], who reported that pH drops while total titratable acidity increases as the fermentation progressed. The increase in total titratable acidity could be as a result of the dominance of the environment by lactic acid bacteria (LAB) which degrades carbohydrate resulting in acidification. These observations are in agreement with earlier studies of [27].

4. CONCLUSION

In conclusion, fermentation as shown from this study increased protein, crude fibre and

carbohydrate contents of the fermented ripe plantain sample with corresponding decrease in anti-nutrient contents of the samples. Therefore the process involved in fermentation procedure as a means of improving food quality could be a promising method in the food industry during food processing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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