Journal of Experimental Research

MARCH 2024, Vol 12 No 1

Email: editorinchief.erjournal@gmail.com
editorialsecretary.erjournal@gmail.com

Received: January, 2024
Accepted for Publication: March, 2024

STUDIES ON THE EFFECTS OF LACTIC ACID BACTERIA FERMENTATION ON THE PROXIMATE AND MINERAL COMPOSITIONS of *Digitaria exilis*

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ABSTRACT

Acha (*Digitaria exilis*) is a cereal crop that is highly nutritious and contains carbohydrates, dietary fiber, minerals, and amino acids.It also contains antinutritional factors such as oxalate, phytate, tannin, and saponin, which can reduce the bioavailability of nutrients. The aim of this study was to evaluate the effect of lactic acid bacteria (LAB) fermentation on the proximate and mineral composition of acha flour. Acha was processed into flour and then subjected to spontaneous fermentation and fermentation using a combination of *Streptococcus infantarius* FDAARGOS_1019 and *Limosilac tobacillus fermentum* SCB0035) previously isolated from cow milk. Exactly 250g of the flour was mixed with 500mL of distilled water followed by the addition of 0.02% sodium metabisulphate to inhibit the growth of microflora and other contaminating microorganisms. The same set-up was used for the spontaneous fermentation, except the addition of starter organisms and sodium metabisulphite. The proximate and mineral contents of the flours were determined using the association of official analytical chemistry (AOAC) and atomic absorbance spectroscopy (AAS) methods respectively. The result shows a significant ($p < 0.05$) increase in the moisture, ash, lipid and protein contents of the fermented flour when compared to the unfermented flour with the higher values occurring in the samples fermented with the LAB consortium. The carbohydrate and fiber content decreased significantly ($p < 0.05$) after fermentation. The result of the mineral contents of both unfermented and fermented acha flours demonstrated that magnesium, copper, iron, zinc, sodium, phosphorus, and calcium levels significantly increased ($p \leq 0.05$) through LAB fermentation, while potassium levels remained unchanged. This observation shows that LAB fermentation has the potential to enhance nutritional quality of acha flour more than spontaneous fermentation and can be applied in microbial food fortification.

Keywords: Acha flour, fermentation, Lactic Acid Bacteria, Proximate Composition, mineral analysis

INTRODUCTION

Locally fermented foods are typically produced through the activity of microorganisms such as yeasts and lactic acid bacteria (LAB). This fermentation process can occur either spontaneously or under controlled conditions, involving cereals as the substrate (Chelule et al. 2010; Oyewole and Isah, 2012; Sharma et al. 2013). Lactic acid bacteria are widely distributed in nature and naturally occur as part of the microflora in fermented foods (Wang et al. 2021). These bacteria are gram-positive, non-sporulating, anaerobic or facultative aerobic cocci or rods that produce lactic acid as a major fermentation byproduct during carbohydrate metabolism (Axelsson, 2004; Hayek and Ibrahim, 2013).

Grains serve as fundamental sources of macronutrients and energy for humans. Acha (*Digitaria exilis*), a traditional African cereal belonging to the Gramineae family, is one of the oldest known cereals, cultivated in West Africa for over 5000 years. It is commonly grown in regions with low rainfall, particularly in the plateau and savannah areas (Wakil and Olorode, 2018). This grain is also known by various names such as hungry rice, petit mil, fundi grain, fonio rice, and "grain of life" (Jideani and Jideani, 2011; Karasu et al. 2015). The term "grain of life" stems from its ability to mature and be harvested within 3-4 months after sowing, providing food during the growing season when many other crops are still immature (Temple and Bassa, 1991). The two common varieties found in Africa are *D. exilis* (white acha) and *D. iburu* (black acha), with *D. exilis* being more prevalent in West Africa, specifically Nigeria, Ghana, and Benin (Wakil and Olorode, 2018).

In Nigeria, *D. exilis*is extensively cultivated in the cool region of Plateau State, as well as parts of Bauchi, Kebbi, Taraba, Kaduna, and Niger States. Acha is highly regarded for its nutritional value due to its composition and high concentration of macronutrients. It is rich in carbohydrates, dietary fiber, B-vitamins, and essential amino acids such as phenylalanine, tyrosine, methionine, and cystine (Ayo et al. 2007), in addition to trace amounts of minerals like zinc, magnesium, manganese, iron, and potassium (Chukwu and Abdul-Kadir, 2008). Acha is consumed either as a staple food or as a significant part of the diet. Being a high-energy, gluten-free cereal, it is beneficial for promoting hair growth and weight loss. Approximately 3-4 million people rely on acha for sustenance (Wakil and Olorode, 2018). Its easy digestion and low sugar content make it suitable for consumption by children, the elderly, and individuals with diabetes or stomach disorders, as it helps stabilize blood glucose and insulin levels (Balde et al. 2008). Acha can also be ground and combined with other flours to produce bread, pastries, and weaning food, as suggested by Philip and Itodo (2006).

Fermented foods undergo significant biochemical changes through the action of microorganisms or enzymes, resulting in desirable modifications to the food (Halder et al. 2017; Mohammed et al. 2017). Lactic acid bacteria (LAB) have been identified as the predominant microorganisms found in fermented foods, particularly in relation to lactic acid fermentation, which contributes significantly to the beneficial characteristics observed in these foods (Holzapfel, 2002; Ayo et al. 2007;Adeleke et al.2010). The extensive enzymatic activities of a diverse microbial pool are responsible for producing a wide range of volatile compounds, bioactive peptides, and prebiotic oligosac charides, leading to substantial molecular changes in macronutrients during fermentation and resulting in the functional properties observed in foods fermented with lactic acid bacteria (Petrova and Petrov, 2020). Regular consumption of lactic acid bacteria fermented foods enhances the immune system and strengthens the body's defense against pathogenic bacterial infections. Thus, lactic acid bacteria fermentation not only holds economic significance but also promotes human health in Africa (Chelule, 2010).The objective of this research is to assess the impact of lactic acid bacteria fermentation on the proximate and mineral composition of acha flour.

MATERIALS AND METHOD

Sample Collection and Preparation

Acha (*Digitaria exilis*) grains were purchased new market in Wukari, Nigeria. The acha was sent to Biological Science Laboratory Federal University Wukari, for identification and processing. The grains were carefully sorted by hand to eliminate any foreign materials and then thoroughly washed to remove any sand or impurities. Afterward, the grains were air-dried and subsequently milled into flour.

Inoculum/ Starter culture preparation

The starter culture was prepared following the procedure described by Ogodo et al. (2017). Previously isolated *Streptococcus infantarius* FDAARGOS_1019 and *Limosilacto bacillus fermentum* SCB0035 were grown as co-culture in Erlenmeyer flasks containing de-Manne Rogosa and Sharpe (MRS) broth. The flasks were then incubated for 48 hours at 37°C to allow the inoculum to build up. The lactic acid bacteria (LAB) culture was harvested by centrifugation (NEW LIFE MODEL 800D) at 4000 rpm for 15 minutes. The harvested cells were washed with distilled water to remove any residual MRS broth. The washed LAB cells were diluted using sterile distilled water. The starter cultures were standardized to 0.5 McFarland standard before being used for fermentation purposes.

Fermentation of Acha Flour

The fermentation of acha flour was conducted with a modified version of the method described by Ogodo et al.. (2019).Exactly 250g of acha flour was mixed with 500 mL of sterile distilled water in a 1000mL cornical flask. The content of the flask was mixed thoroughly to ensure uniform distribution. Exactly 0.02% of sodium metabisulphite ($Na_2S_2O_5$) was added to the mixture to inhibit the growth of fungi and other contaminating microorganisms. The sterility of the mixture was confirmed by observing no visible growth on plate count agar after 18-24 hours of incubation. The mixture in the conical flask was inoculated with 10 mL of the standardized starter culture, which consisted of the lactic acid bacteria (*Streptococcus infantarius* FDAARGOS_1019 and *Limosilacto bacillus fermentum* SCB0035) suspension. The inoculated mixture was allowed to undergo fermentation. The same procedure was followed for spontaneous fermentation except the addition of starter organisms and sodium metabisulphite ($Na_2S_2O_5$). Samples were withdrawn from the mixture at 24-hour intervals to monitor the pH and viable count. After 72 hours of fermentation, the liquid broth resulting from the lactic acid bacteria fermentation was decanted into a sterile flask. The fermented acha sample was then squeezed using a clean, sterile white mush in cloth to separate the liquid from the solid residue. The solid residue (fermented acha flour) was subsequently dried in a thermostat hot air oven (DHG-9101-1SA PEC MEDICAL USA) at 60°C for 8 hours.

Proximate Analysis

Moisture, ash, protein, lipid, fiber, and carbohydrate content were determined according to association of official analytical chemistry methods (AOAC, 2019).

Mineral analysis

The standard wet digestion method as described by Tasie and Gebreyes (2020) was utilized. In this approach, 0.5 g of acha flour was subjected to digestion using a mixture of 5 ml concentrated nitric acid (HNO_3) and 1 ml concentrated perchloric acid ($HClO_4$). After digestion, the sample was filtered and then brought up to a final volume of 100 ml in a standard flask. The atomic absorption spectrophotometer (DW-AA320NR) was employed to analyze all the minerals using the suitable lamp.

Statistical Analysis

The result analysis was done in triplicate and the mean data ± SD (standard deviation) was reported. A one-way analysis of variance (ANOVA) was employed in analysis and comparison of the parameters between unfermented and lactic acid bacteria fermented acha flour using statistical package for the social sciences (SPSS) version 20.0 software. Significance was accepted at $p \leq 0.05$.

RESULTS

Table 1 presents the microbial load of the acha flour during fermentation. The initial microbial load at the beginning of the spontaneous fermentation process was 3.7 x 10^5CFU/g, and this count significantly increased to 2.67 x 10^{10}CFU/g by the end of the fermentation period. The peak microbial load occurred at the 48h (1.34 x 10^{11}CFU/g). This trend was consistent with the lactic acid bacteria fermentation, wherein the microbial load progressively increased from 1.5 x 10^8CFU/g at 0 h to 2.97 x 10^{11}CFU/g at 48 hours. However, at 72 hours, the microbial load decreased slightly to 1.9 x 10^{11}CFU/g.

There were variations in the pH values of the acha flour during fermentation as presented in Figure 1. The pH values of the fermentation medium decreased throughout the fermentation process for both spontaneous and lactic acid bacteria fermentations. The decrease ranged from 5.80 (spontaneous fermentation) to 2.41 (lactic acid bacteria fermentation).

The results of the proximate analysis of nutritional factors in fermented and non-fermented acha flour is shown in Figures 2 to 7.In both LAB-fermented and spontaneously-fermented acha flour, the levels of moisture content, ash, lipid, and protein increased compared to non-fermented acha flour. However, the fibre and carbohydrate content of both LAB-fermented and spontaneously-fermented acha flour were lower compared to non-fermented acha flour. The fermentation process appears to have reduced the fibre and carbohydrate composition of the acha flour.

The variations in the mineral concentrations of non-fermented, spontaneously-fermented, and LAB-fermented acha flour is illustrated in Figure 8 and 9.Among the minerals analyzed, lactic acid bacteria fermented acha flour exhibited the highest concentrations for magnesium, copper, iron, manganese, zinc, sodium, and calcium. The fermentation process with LAB seems to have significantly increased the levels of these essential minerals in the final product. On the other hand, non-fermented acha flour had the highest concentration of phosphorus, while spontaneous-fermented acha flour showed the highest concentration of potassium. The presence of higher phosphorus in non-fermented acha flour and increased potassium in spontaneously-fermented acha flour suggests that the fermentation process might have different effects on the mineral composition.

Table 1: Microbial load (CFU/g) of the acha flour during fermentation.

Fermentation Time(h)	Spontaneous Fermentation	Lactic acid bacteria fermentation
0	$3.7x10^5$	$1.5x10^8$
24	$2.9x10^8$	$1.4x10^{10}$
48	$1.34x10^{11}$	$2.97x10^{11}$
72	$2.67x10^{10}$	$1.9x10^{11}$

CFU/g: Colony Forming Unit per gram

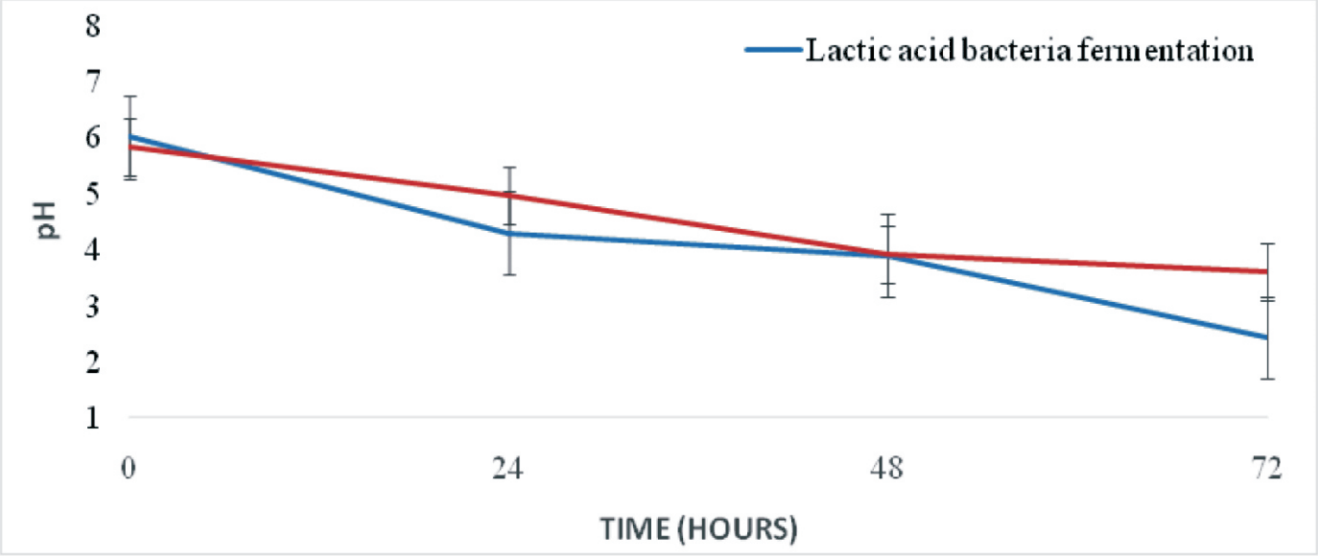

Fig. 1 Variations in the pH values of acha flour during fermentation; Values are mean of triplicate determination; error bars represent standard error.

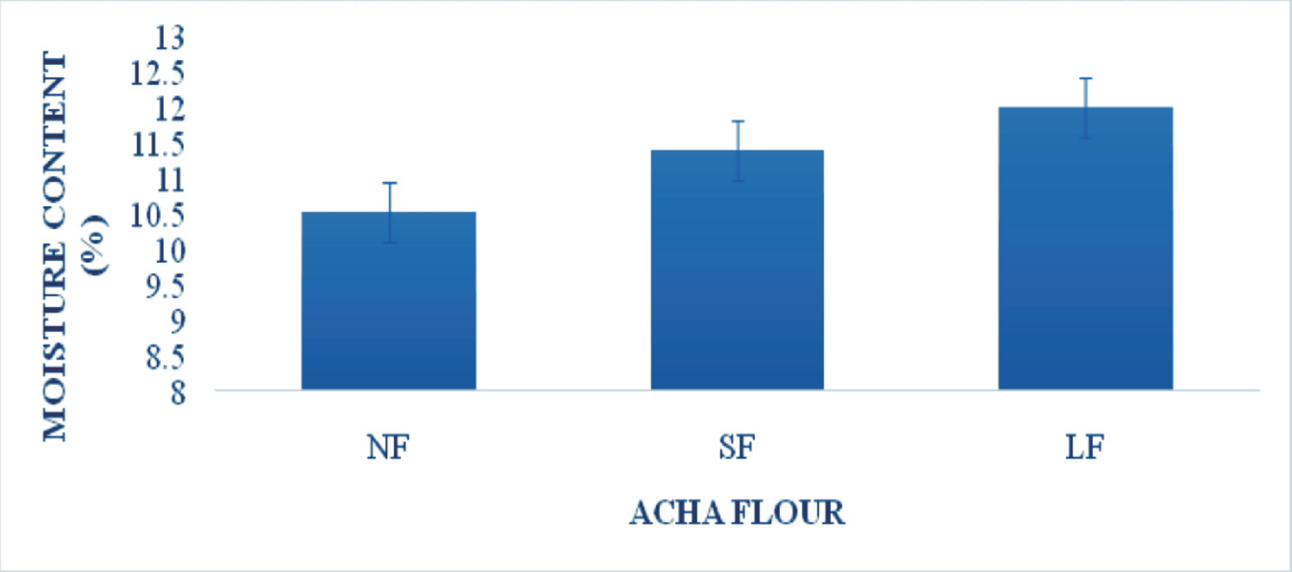

Fig.2 Moisture content of fermented and non-fermented Acha flour.
Values are mean of triplicate determination; error bars represent percentage error.
NF: non-fermented; SF: Spontaneously Fermented; LF: lactic acid bacteria fermented

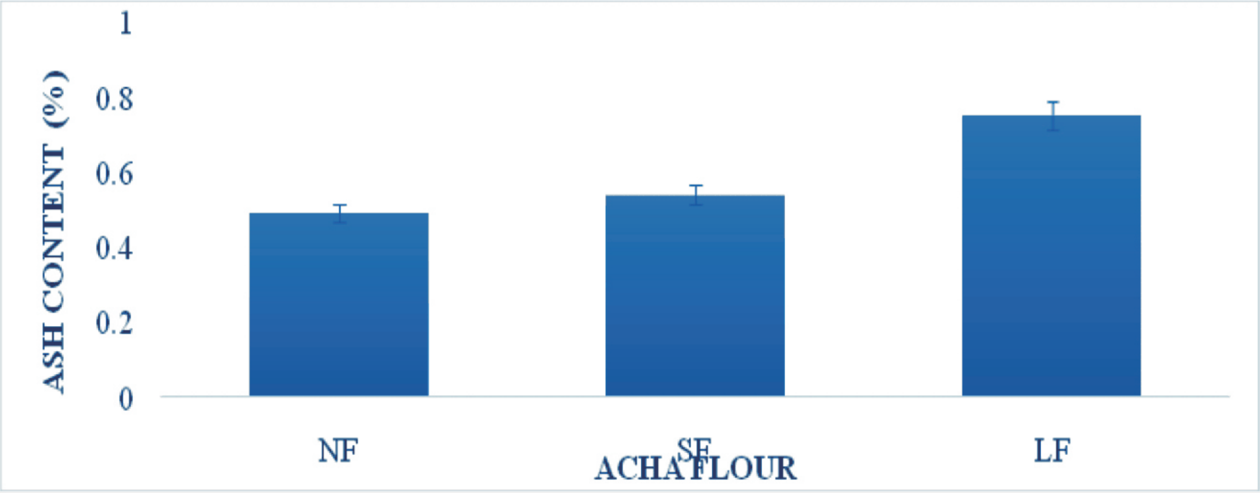

Fig.3 Ash content of fermented and non-fermented acha flour.
Values are mean of triplicate determination; error bars represent percentage error.
NF: non-fermented; SF: Spontaneously Fermented; LF: lactic acid bacteria fermented

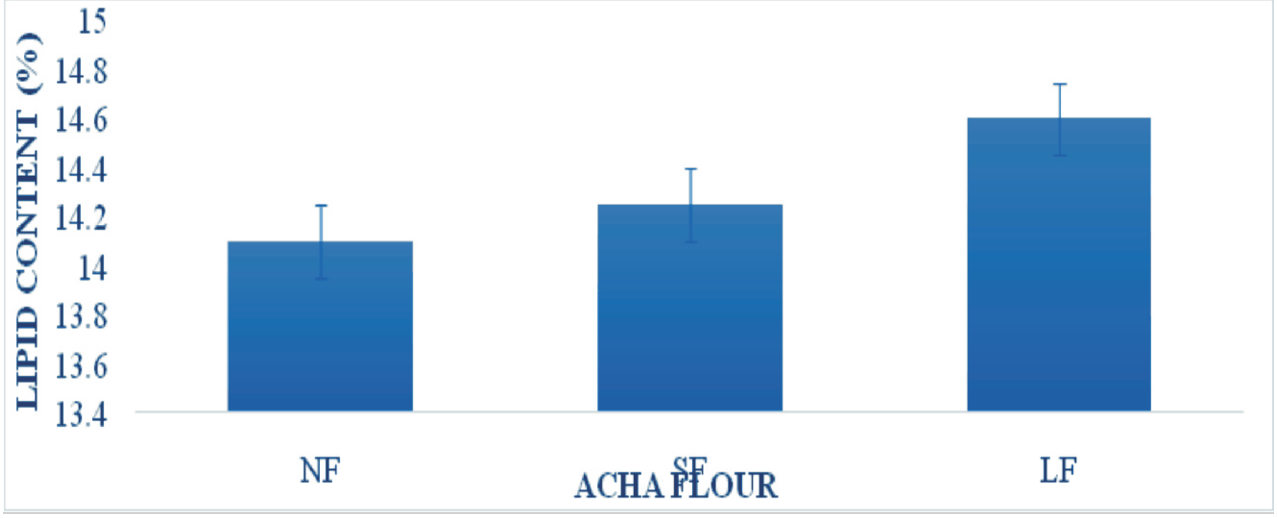

Fig.4 Lipid content of fermented and non-fermented acha flour.
Values are mean of triplicate determination; error bars represent percentage error.
NF: non-fermented; SF: Spontaneously Fermented; LF: lactic acid bacteria fermented

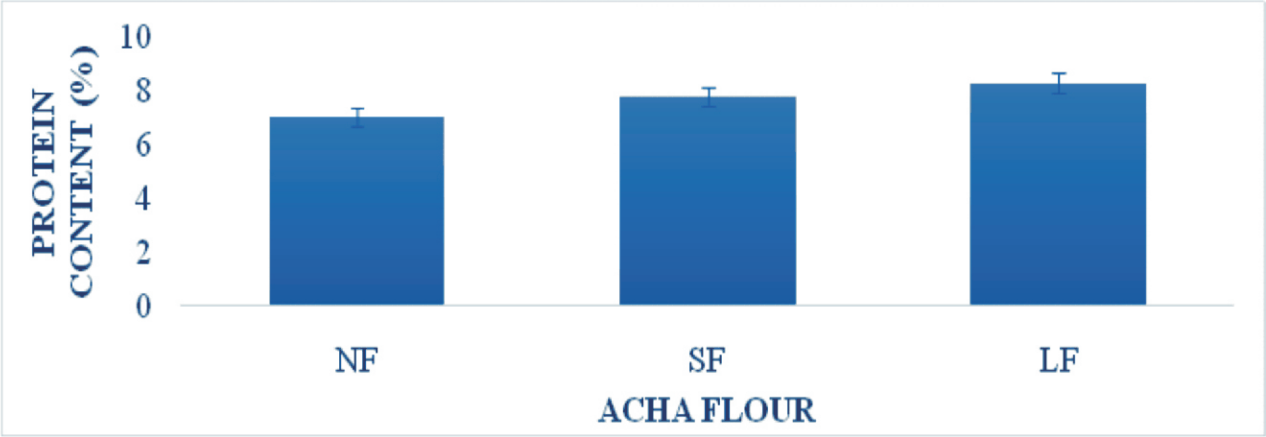

Fig.5: Protein content of fermented and non-fermented acha flour.
Values are mean of triplicate determination; error bars represent percentage error.
NF: non-fermented; SF: Spontaneously Fermented; LF: lactic acid bacteria fermented

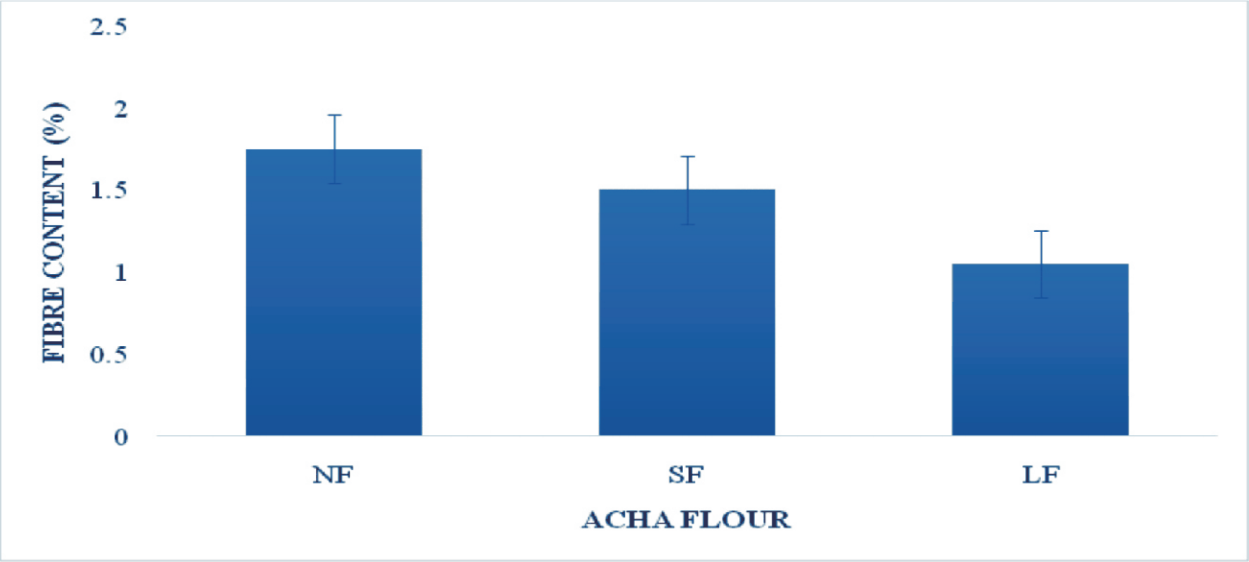

Fig. 6 Fibre content of fermented and non-fermented acha flour.
Values are mean of triplicate determination; error bars represent percentage error.
NF: non-fermented; SF: Spontaneously Fermented; LF: lactic acid bacteria fermented

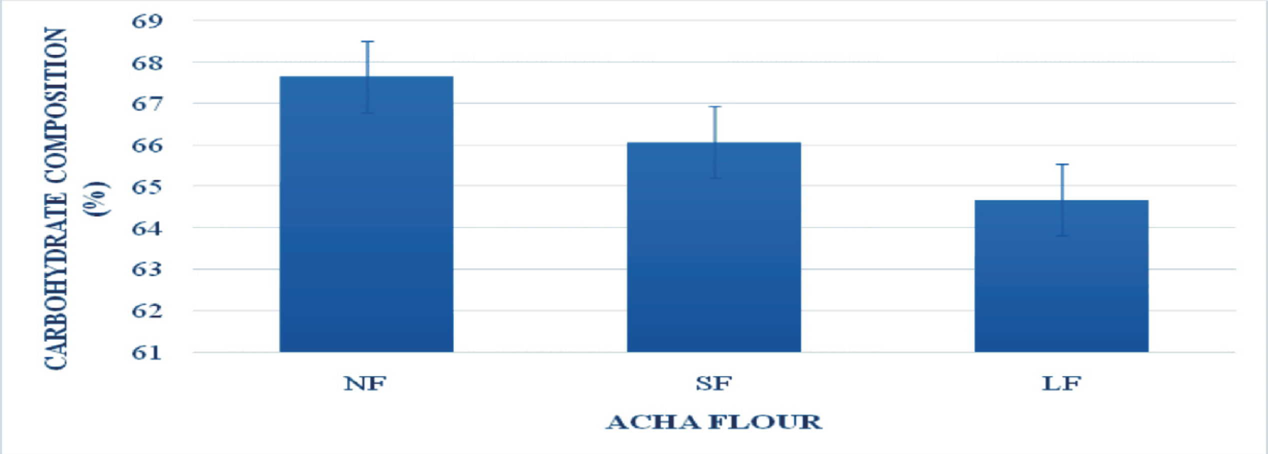

Fig.7 Carbohydrate content of fermented and non-fermented acha flour
Values are mean of triplicate determination; error bars represent percentage error.
NF: non-fermented; SF: Spontaneously Fermented; LF: lactic acid bacteria fermented

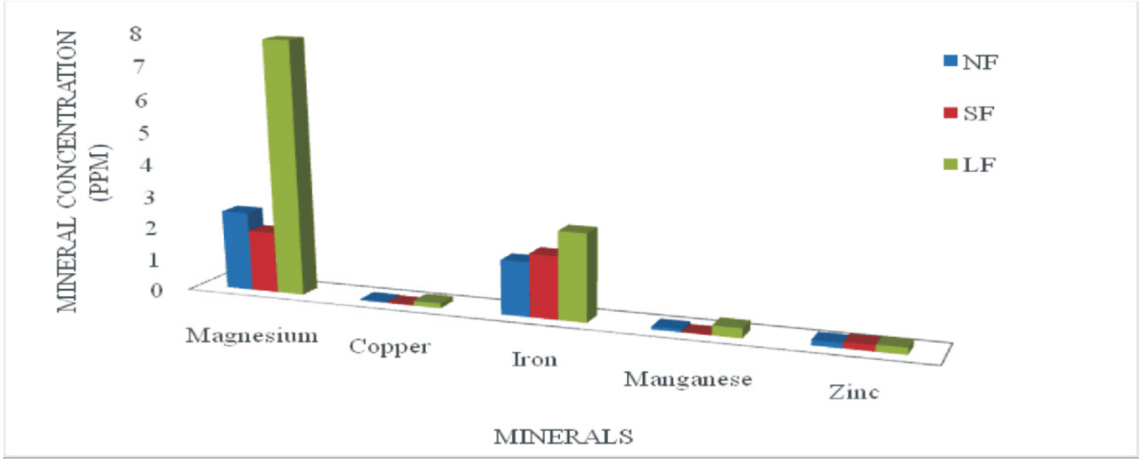

Fig. 8 Concentration of micronutrients in fermented and non-fermented Acha flour
Values are mean of triplicate determination
NF = non-fermented; SF = Spontaneously Fermented; LF = lactic acid bacteria fermented

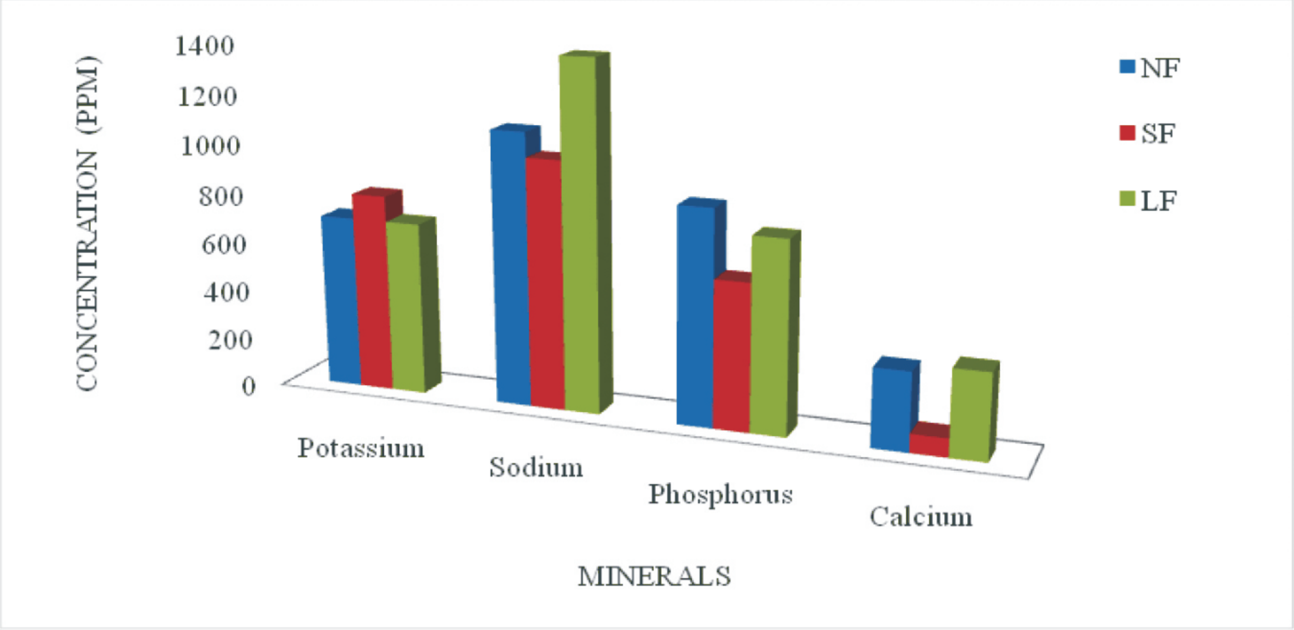

Fig.9 Concentration of macronutrients in fermented and non-fermented Acha flour.
Values are mean of triplicate determination
NF = non-fermented; SF = Spontaneously Fermented; LF = lactic acid bacteria fermented

DISCUSSION

The results indicate a noticeable trend in the microbial load during the fermentation process, where an increase in microbial count was observed between 24-48 hours, followed by a decrease at 72 hours. The initial increase in microbial load between 24-48 hours can be attributed to the favorable growth conditions prevailing during this period. Factors such as the availability of a nutrient-rich medium and minimal competition for resources may have contributed to the rapid proliferation of microorganisms. The depletion of nutrients as the fermentation continued could have limited the growth rate of microorganisms. Moreover, the accumulation of metabolic by-products or secondary metabolites within the fermenting medium might have reached inhibitory levels, hindering further microbial growth (Ogodo, et al. 2018). The fluctuations in microbial load during the fermentation process can be attributed to the interplay of various factors, including nutrient availability, pH changes, competition, and waste accumulation within the fermenting medium. These factors collectively influence the growth dynamics of microorganisms during fermentation. Similar observations have been reported by Nwachukwu et al. (2011) and Hwabejire et al. (2020). In their study, Hwabejire et al. (2020), observed an increase in lactic acid bacteria growth from 24 to 48 hours during the production of fermented weaning food from Digitariaexilis (acha) using lactic acid bacteria. These studies collectively reinforce the notion that microbial dynamics during fermentation processes, particularly those involving lactic acid bacteria, can exhibit common patterns. The initial increase in microbial load, followed by a subsequent decrease, reflects the complex interactions between microorganisms and their changing environment as the fermentation progresses. Such understanding is crucial for optimizing fermentation processes and ensuring the production of safe and high-quality fermented food products.

The result of the present study shows a decrease in the pH of the medium during fermentation. The decrease ranged from 5.80 (unfermented flour) to 2.41 (LAB- fermented flour). This observation could be due to metabolic activities of the fermenting organisms leading to production of organic acids which lowers the pH of the fermenting broth. Moreover, the dominance of lactic acid bacteria which produce lactic acid during the breakdown of sugars resulted to the acidic medium, hence the lowering of pH (Ojokohet al. 2015;Hwabejireet al. 2020).

The proximate composition of acha flour in the present study showed a significant increase

(P<0.05) in the moisture content when the non-fermented flour was compared to both spontaneously fermented flour and lactic acid bacteria fermented flour. This observation suggests that fermentation processes increase the moisture content. This is in agreement with research findings of Echendu et al. (2009), Uwagbale et al. (2016), and Mustapha et al. (2018)which could be attributed to several factors such as addition of water to the substrate to enable microbial growth and activity, temperature and duration of drying of the fermented acha flour. (Olagunju and Abiodun, 2017; Ajibola et al. 2020).Moisture content is an important parameter to consider for food quality, storage, and microbial safety. Proper drying and storage practices should be implemented to prevent microbial growth and maintain the quality of the fermented acha flour (Oluwamukomi et al. 2013; Olagunju and Abiodun, 2017).

The ash content of acha flour in the present study increased after 72 hours of fermentation in both spontaneously fermented and LAB-fermented flours. According to Ndabikunze et al. (2021) ash content represents the total amount of minerals present in the food, including essential minerals such as calcium, iron, and potassium. Lactic acid bacteria have the ability to release minerals from the substrate through enzymatic activities. These bacteria can break down organic compounds and complex forms of minerals, making them more bioavailable and increasing their concentration in the fermented product (Tamang et al. 2016; Dahiya et al. 2017; Nielson and Gibson, 2018). In addition, fermentation can result in the degradation of phytic acid (through the activities of phytase) which binds to minerals, preventing their absorption in the digestive system (Ogodo et al. 2017; Hefnawy et al. 2020). This can contribute to the higher ash content observed in the fermented acha flours. The increase in ash content during fermentation reported by Ogodo et al. (2017) in maize flour using a lactic acid bacteria consortium corroborates with the findings of the present study.

The significant increase in lipid content observed in fermented acha flours in the present study compared to non-fermented acha flour maybe attributed to the activity of lipases during fermentation (Ojokoh et al. 2015). During fermentation, the lactic acid bacteria present in the acha flour may produce lipases that break down the fat molecules present in the substrate by hydrolysis of to release fatty acids and glycerol, which can be utilized by microorganisms for energy and as building blocks for the synthesis of new lipids (Sanjukta et al. 2016). Similar findings from Okeke and Chikwendu (2015) have been reported, where they observed an increase in lipid content during the fermentation of African yam bean and acha flour blends. They attributed this increase to the hydrolytic activity of lipases during fermentation, which resulted in the release of fatty acids and glycerol. It is important to note that flours with higher lipid content have an increased likelihood of oxidative rancidity because lipids are susceptible to oxidation, especially when exposed to air and light as reported by Perera et al. (2017) and Zou et al. (2019). The presence of high levels of lipids in fermented acha flour may make it more prone to spoilage and development of off-flavors due to lipid oxidation. Therefore, proper storage conditions, such as using airtight containers and minimizing exposure to light, should be employed to prevent oxidative rancidity (Okeke and Chikwendu, 2015).

In the present study, fermentation of acha flour resulted in a significant increase (p<0.05) in protein content compared to non-fermented flour. Lactic acid bacteria fermented acha flour had the highest protein content, which was significantly different from both non-fermented and spontaneously fermented acha flours. This finding agreed with the assertions of Ejike and Nwachukwu (2017), Adegunwa and Aluko (2019) and Olasupo and Falade (2020) that fermentation can enhance the protein content of food products. Protein is an essential nutrient required for various physiological functions in the body, including growth, repair, and maintenance of tissues (Pasiakos et al. 2015; Wolfe, 2017). The increase in protein content during fermentation can be attributed to the ability of the fermenting organisms to synthesize proteins (Yarwood and Miller, 2016; Ogodo et al. 2017). Furthermore, the increase in microbial cell mass during fermentation can also contribute to the higher protein content (Ogodo et al. 2018; Rezac et al. 2018). Similar findings have been reported in other studies. For instance, Ojokoh and Onasanya (2017) reported an increase in protein content during the fermentation of extruded blends. Similarly, Jeff-Agboola and Oguntuase (2006) observed that microorganisms can enhance the protein content of pearl millet-acha blends. The higher protein content in fermented acha flour can contribute to its nutritional value and make it a more suitable ingredient for functional foods and nutraceuticals.

Crude fiber, as an indigestible component of food, plays an important role in providing bulk to the diet and promoting healthy digestion. It aids in regulating physiological functions and contributes to the overall health of the intestines (Yang et al. 2016; Saeed and Khalid 2018). In this present study, the fiber content of the fermented acha flours was found to be significantly lower compared to the non-fermented flours. Specifically, the fiber content decreased from 1.75% in non-fermented acha flour to 1.05% in LAB-fermented acha flour. Lactic acid bacteria, such as the strains used in this study, have the ability to utilize fiber as a carbon source for their growth and metabolic activities (Fillannino et al. 2016). During fermentation, these bacteria produce enzymes that can break down the complex fiber structures into simpler components, which they can then utilize for energy and growth. As a result, the fiber content of the fermented acha flour decreases (Tamang et al. 2016). This decrease in fiber content observed in the present study aligns with the findings of Ogodo et al. (2019), who reported a similar decrease in fiber content during the fermentation of Sorghum bicolor flour with a lactic acid bacteria consortium.

Carbohydrates are vital nutrients that play a crucial role in various physiological functions, including supporting the immune system and facilitating human development (Chatterjee et al. 2018). They are one of the main types of nutrients and serve as the primary source of energy for the body. Additionally, carbohydrates are a significant substrate for microbial fermentation (Ojokoh et al. 2015).In this research, a significant decrease ($p<0.05$) in the carbohydrate content was observed in fermented acha flours compared to the non-fermented flour. According to Elayaraja et al. (2019), the reduction in carbohydrate content can be attributed to the utilization of fermentable sugars by lactic acid bacteria during the fermentation process. Lactic acid bacteria metabolize these sugars for their own growth and other metabolic activities, which leads to a decrease in the overall carbohydrate content of the fermented product Elayaraja et al. 2019). Similar findings were reported by Ojokoh et al. (2013) during the fermentation of breadfruit and cowpea blend flours, where a decrease in carbohydrate content was observed. They also attributed the decrease to the metabolic activities of microorganisms during fermentation, as they consume and convert the carbohydrates present as substrate. Ray and Joshi (2017) and Elayaraja et al. (2019) agreed that utilization of fermentable sugars by lactic acid bacteria for growth and metabolic activities is a common phenomenon in fermentation processes.

In this study, fermentation was found to improve the mineral content of acha flour compared to non-fermented acha flour. This increase in mineral content is attributed to the breakdown of antinutritional factors, such as phytic acid and oxalate, during fermentation. Phytic acid and oxalate can bind with minerals and reduce their absorption and bioavailability in the body. By reducing these antinutrients, fermentation enhances the availability of minerals in the fermented acha flour. The study revealed that fermentation increased the quantity of most macro and trace minerals, except for phosphorus and calcium. The increase in other macro and trace minerals indicates that fermented acha is more desirable for meeting dietary mineral needs. Similar findings were reported by Echendu et al. (2009) in a study on fermented hungry rice, where they observed an increase in iron, sodium, zinc, magnesium, manganese, and potassium during the fermentation process. Additionally, Obadina et al. (2013) reported an increase in manganese, zinc, and iron during the fermentation of soy milk by lactic acid bacteria. The consistent results from these studies emphasize the effectiveness of fermentation in improving the mineral content of various food products. This has significant implications for improving the nutritional quality and overall health benefits of traditional staple foods like acha, making them more valuable dietary sources of essential minerals.

CONCLUSION

Fermentation with LAB has a significant impact on the proximate composition of acha flour. It results in an increase in moisture, ash, lipid, and protein content, while fiber and carbohydrate content decrease. Fermentation also leads to a reduction in antinutritional factors. These changes contribute to the improved nutritional quality, safety, and potential health benefits of fermented acha flour. However, further studies are needed to assess the sensory attributes, functional properties, and shelf life of the fermented product to determine its suitability for consumption.

Conflicting Interest

The authors have declared no conflict of interest

REFERENCES

Adegunwa MO, Aluko RE. (2019). Fermentation and enzymatic treatments for improving protein content and quality of plant-based foods. *Critical Reviews in Food Science and Nutrition.* **59(16):** 2549-2562. doi: 10.1080/10408398.2018.1507287.

Adeleke RO, Abiodun OA. (2010). Physicochemical properties of commercial local beverages in Osun State, Nigeria. *Pakistan Journal of Nutrition*. **9(9):** 853-855.

Ajibola CF, Akinoso R, Betiku E. (2020). Comparative study on the effect of malting and fermentation on the functional and pasting properties of Digitariaexilis (Acha) flour. *Food Science & Nutrition***. 8(5):** 2662-2670. doi: 10.1002/fsn3.1520.

AOAC (2019). AOAC guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals. AOAC International. 21st edn. Gaithersburg, MD, USA. pp 5-13.

Axelsson L. (2004). Lactic acid bacteria: Classification and Physiology. Lactic Acid Bacteria: Microbiology and Functional Aspects. 1-66.

Ayo JA, Ayo VA, Nkama I,Adewori R. (2007). Physiochemical, in-vitro digestibility and organoleptic evaluation of "Acha" wheat biscuit supplemented with. Nigerian Food Journal. 25(1): 77-89.

Balde NM, Besancon S, Sidibe TA. (2008). P191 glycemic index fonio *(Digitaria exilis)* interest in feeding in diabetic subjects in West Africa. *Diabetes & Metabolism*. **34(3):** H93.

Chatterjee R, Yeh HC. (2018). Edible carbohydrates: A review of their digestion, absorption, and physiological utilization. *Critical Reviews in Food Science and Nutrition*. **58(14):** 2371-2378. doi: 10.1080/10408398.2017.1345389.

Chelule PK, Mokoena MP, Gqaleni N. (2010). Advantages of traditional lactic acid bacteria fermentation of food in Africa. In: Mendez-Vilas, A. (Ed.), Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology Series 2. vol. 2. Formatex, Spain, pp. 1160–1167.

Chukwu O, Abdul-Kadir JA. (2008). Proximate (Chemical) composition of Acha (Digitariaexilis and Digiritariaiburua) grains. Journal of Food Technology.6:214-216.

Dahiya DK, Puniya AK, Shandilya UK, Dhewa T, Kumar N. (2017). Gut microbiota modulation and its relationship with obesity using prebiotic fibers and probiotics: A review. *Frontiers in Microbiology.* **8:** 563. doi: 10.3389/fmicb.2017.00563

Echendu CA, Obizoba IC, Anyika JU,Ojimelukwe PC. (2009). Changes in chemical composition of treated and untreated hungry rice acha (Digitariaexilis). *Pakistan Journal of Nutrition*. **8(11):** 1779-1785.

Ejike CE, Nwachukwu ID. (2017). Optimization of fermentation conditions for enhanced protein content and antioxidant activities of sprouted brown rice (Oryza sativa) yogurt. *Food Science & Nutrition*.**5(1):** 33-42. doi: 10.1002/fsn3.484.

Elayaraja S, Annamalai N, Mayavu P. (2019). Fermentation and nutritional enrichment of green gram flour using lactic acid bacteria and yeast. *Journal of Food Processing and Preservation. 43(10):* e14134. doi: 10.1111/jfpp.14134

Filannino P, Di Cagno R, Addante R, Pontonio E, Gobbetti M, De Angelis M. (2016). Metabolic responses of lactic acid bacteria to polysaccharides from different sources: A comparison of exopolysaccharide biosynthesis traits. *International Journal of Food Microbiology.* **222:** 41-49. doi: 10.1016/j.ijfoodmicro.2016.02.021

Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. (2017). Indigenous probiotic Lactobacillus isolates presenting antibiotic like activity against human pathogenic bacteria. *Biomedicines.* **5(2):** 31.

Hayek SA, Ibrahim SA. (2012). Antimicrobial activity of xoconostle pears *(Opuntia matudae*) against Escherichia coli O157:H7 in laboratory medium. *International Journal of Microbiology*. **2012 (ID 368472):** 6. doi: 10.1155/2012/368472

Hefnawy T, Abdel-Latif MA, Mostafa MA. (2020). Fermentation: A strategy to improve the nutritional quality of cereals. *Food Science & Nutrition.* **8(6):** 2885-2901. doi: 10.1002/fsn3.1549

Holzapfel WH. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology.***75:** 197–212.

Hwabejire HO, Wakil SM, Akpoghelie PO. (2020). Production of fermented weaning food from Digitariaexilis (Acha) using lactic acid bacteria. *Nigerian Journal of Microbiology.***34(1):** 4920 – 4935.

Jeff-Agboola YA,Oguntuase OS. (2006). Effect of Bacillus sphaericus on proximate composition of soybean (*Glycine max*) for the production of soy iru. *Pakistan Journal of Nutrition*. **5(6):** 606-607.

Jideani IA, Jideani VA. (2011). Developments on the cereal grains Digitariaexilis (acha) and Digitariaiburua (iburu). *Journal of Food Science and Technology.* **48:** 251–259.

Karasu A, Alpsoy HC, Öz M. (2015). Effects of some natural origined growth regulating substances on the seed yield and some agronomical characteristics of common pea (Pisum sativum). *Journal of Food and Nutrition Research.* **47(3):** 60-67.

Mohammed SSD, Orukotan AA, Musa J. (2017). Effect of fermentation and malting on some cereal weaning foods enriched with african locust beans. *Journal of Applied Sciences & Environmental Management.* **21(5):** 911-921.

Mustapha KB, Zubairu HL, Adamu A. (2018). Comparison of nutritional values of wheat (Triticum aestivum) and acha (Digitariaexilis) grains. *Bayero Journal of Pure and Applied Sciences.* **11(1):** 133-138.

Ndabikunze BK, Sivakumar D, Meincken M. (2021). Proximate composition, minerals, and anti-nutrient contents of cereals and tubers commonly consumed in South Africa. *Food Science & Nutrition.* **9(4):** 2099-2111. doi: 10.1002/fsn3.2171.

Nielsen S, Gibson GR. (2018). The human gut as a potential source of antimicrobial resistance. *Trends in Microbiology*. **26(11):** 906-918. doi: 10.1016/j.tim.2018.05.005.

Nwachukwu E, Achi OK, Ijeoma IO. (2011). Lactic acid bacteria in fermentation of cereals for the production of indigenous Nigerian foods. *Global Journal of Food and Agribusiness Management.* **2(1):** 001-006.

Obadina AO, Akinola OJ, Shittu TA, Bakare HA. (2013). Effect of natural fermentation on the chemical and nutritional composition of fermented soymilk nono. *Nigerian Food Journal.* **31(2):** 91-97.

Ogodo AC, Ugbogu OC, Onyeagba RA, Okereke HC. (2017). Effect of lactic acid bacteria consortium fermentation on the proximate composition and in-vitro starch/protein digestibility of maize (Zea mays) flour. *American Journal of Microbiology and Biotechnology.* **4(4):** 35-43.

Ogodo AC, Ugbogu OC, Onyeagba RA, Okereke HC. (2018). In-vitro starch and protein digestibility and proximate composition of soybean flour fermented with lactic acid bacteria (LAB) consortia. *Agriculture and Natural Resources.* **52(5):** 503-509.

Ogodo AC, Agwaranze DI, Aliba NV, Kalu AC, Nwaneri C B. (2019). Fermentation by lactic acid bacteria consortium and its effect on anti-nutritional factors in maize flour. *Journal of Biological Sciences*. **19(1):** 17-23.

Ojokoh AO, Daramola MK, Oluoti OJ. (2013). Effect of fermentation on nutrient and anti-nutrient composition of breadfruit (Treculiaafricana) and cowpea (Vigna unguiculata) blend flours. *African Journal of Agricultural Research.* **8(27):** 3566-3570.

Ojokoh AO, Fayemi OE, Ocloo FCK, Nwokolo FI. (2015). Effect of fermentation on proximate composition, physicochemical and microbial characteristics of pearl millet (Pennisetum glaucum (L.) R. Br.) and Acha (Digitariaexilis (Kippist) Stapf) flour blends. *Journal of Agricultural Biotechnology and Sustainable Development.* **7(1):** 1-8.

Ojokoh AO, Onasanya TT. (2017). Effect of fermentation and extrusion on the nutrient and anti-nutrient composition of soybeans (Glycine max, L) and Acha (Digitariaexilis Stapf). *Microbiology Research Journal International.* **21(1):** 1-21.

Okeke PN, Chikwendu JN. (2016). Effects of Varying Fermentation Periods on the Chemical Composition of African Yam Bean (Sphenostylisstenocarpa) and Acha (Digitariaexilis) Flour Blends and Sensory Properties of Their Products. *International Journal of Nutrition and Food Engineering.* **9(4):** 431-438.

Olagunju AI, Abiodun OA. (2017). Effects of fermentation and drying methods on the chemical composition of acha (Digitariaexilis) flour. *Journal of Cereal Science.* **75:** 126-132. doi: 10.1016/j.jcs.2017.02.007.

Olasupo NA, Falade KO. (2020). Enhancement of protein content and protein digestibility of fermented soybean flour with starter cultures. *Journal of Food Science and Technology.* **57(2):** 501-510. doi: 10.1007/s13197-019-04169-2.

Oluwamukomi MO, Akinoso R, Oluwajuyitan OE. (2013). Quality attributes of fermented acha (Digitariaexilis) flour as influenced by germination and fermentation. *Nigerian Food Journal.* **31(1):** 62-70.

Oyewole OA, Isah P. (2012). Locally fermented foods in Nigeria and their significance to national economy: a review. *Journal of Recent Advances in Agriculture.* **1:** 92–102.

Pasiakos SM, McLellan TM, Lieberman HR. (2015). The effects of protein supplements on muscle mass, strength, and aerobic and anaerobic power in healthy adults: A systematic review. *Sports Medicine*. **45(1):** 111-131. doi: 10.1007/s40279-014-0242-2.

Perera CO, Jayawardena N, Gunaratne A. (2017). Oxidative stability of flour: A review. *Food Science & Nutrition.*

5(4): 658-666. doi: 10.1002/fsn3.438.

Petrova P, Petrov K. (2020). Lactic acid fermentation of cereals and pseudocereals: Ancient nutritional biotechnologies with modern applications. *Nutrients.* **12(4):** 11-18.

Philip TK, Itodo IN. (2006). Acha (Digitaria spp.) a "rediscovered" indigenous crop of West Africa. Agricultural Engineering International: CIGR Journal. purpose. *Journal of Food and Nutrition Research.* **46(2):** 51-57.

Ray RC, Joshi VK. (2017). Lactic acid fermentation: Microbiological, chemical, and technological aspects. In Ray RC., Montet D. (Eds.), Microorganisms and Fermentation of Traditional Foods (pp. 3-41). CRC Press.

Rezac S, Kok CR, Heermann M, Hutkins R. (2018). Fermented foods as a dietary source of live organisms. *Frontiers in Microbiology*.**9:** 1785. doi: 10.3389/fmicb.2018.01785.

Saeed F, Khalid N. (2018). Role of dietary fiber in human health: A review. *Food and Nutrition Sciences.***9(08):** 837-847. doi: 10.4236/fns.2018.98067.

Sanjukta S, Rai AK, Mandal N. (2016). Identification and characterization of lactic acid bacteria isolated from fermented rice bran product, chhurpi. *Indian Journal of Microbiology.* **56(2):** 189-198. doi: 10.1007/s12088-016-0592-x.

Sharma N, Handa S, Gupta AA. (2013). A comprehensive study of different traditional fermented foods/beverages of Himachal Pradesh to evaluate their nutrition impact on health and rich biodiversity of fermenting microorganisms. *International Journal of Research in Applied Natural and Social Sciences.* **1:**19-28.

Tamang JP, Shin DH, Jung SJ, Chae SW. (2016). Functional properties of microorganisms in fermented foods. *Frontiers in Microbiology.* **7:** 578. doi: 10.3389/fmicb.2016.00578.

Tasie MM, Gebreyes BG. (2020). Characterization of nutritional, antinutritional, and mineral contents of thirty-five sorghum varieties grown in Ethiopia. *International Journal of Food Science.* **2020:**1-10

Temple VJ, Bassa JD. (1991). Proximate chemical composition of Acha (Digitariaexilis) grain. *Journal of the Science of Food and Agriculture.* **56(4):** 561-563.

Uwagbale EED, Saratu AS, Akagwu OV, Stephen OO, Lilian AM. (2016). African Cereals and Non-African Cereals: *A Comparative Review of Their Nutritional Composition.* World. 1(2): 30-37.

Wakil SM, Olorode EM. (2018). Potential probiotic properties of lactic acid bacteria isolated from malted and spontaneously fermented Acha (Digitariaexilis) Flour. *Advances in Research*.**17(6):** 1-12.

Wang X, Chen S, Ma X, Yssel AE, Chaluvadi SR, Johnson MS, ... Deynze VA. (2021). Genome sequence and genetic diversity analysis of an under-domesticated orphan crop, white fonio (Digitariaexilis). *Giga Science.* **10(3):**13.

Wolfe RR. (2017). Branched-chain amino acids and muscle protein synthesis in humans: myth or reality? *Journal of the International Society of Sports Nutrition.* **14(1):** 30.

Yang J, Wang HP, Zhou L, Xu CF, Wei HK. (2016). Analysis of nutrient composition and bioactive components of ten edible flowers from China. *Journal of Food Science.* **81(3):** H646-H656. doi: 10.1111/1750-3841.13212.

Yarwood RR, Miller SL. (2016). The origin of metabolism. Proceedings of the National Academy of Sciences.**113(28):** 7466-7467.
doi: 10.1073/pnas.1609190113

Zou Y, Zhao L, Li B. (2019). Impact of lipid content on oxidative stability of wheat flour during storage. *Journal of Cereal Science.* **86:** 24-29. doi: 10.1016/j.jcs.2019.01.004