**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 *Limosilactobacillus 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*≤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 a fundamental source 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 oligosaccharides, 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 *Limosilactobacillus 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 (Na2S2O5) 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 *Limosilactobacillus 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 (Na2S2O5). 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 mushin 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 (HNO3) and 1 ml concentrated perchloric acid (HCLO4). 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* ≤ 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 105 CFU/g, and this count significantly increased to 2.67 x 1010 CFU/g by the end of the fermentation period. The peak microbial load occurred at the 48 h (1.34 x 1011 CFU/mL). This trend was consistent with the lactic acid bacteria fermentation, wherein the microbial load progressively increased from 1.5 x 108 CFU/mL at 0 h to 2.97 x 1011 CFU/g at 48 hours. However, at 72 hours, the microbial load decreased slightly to 1.9 x 1011 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 | 3.7x105 | 1.5x108 |
| 24 | 2.9x108 | 1.4x1010 |
| 48 | 1.34x1011 | 2.97x1011 |
| 72 | 2.67x1010 | 1.9x1011 |

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 and Ijeoma (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 *Digitaria exilis* (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 (Ojokoh *et al.*, 2015; Hwabejire *et 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 may be 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 research, ~~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

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