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## Effect of Lactic Acid Bacteria Consortium Fermentation on the Proximate Composition and *in-Vitro* Starch/Protein Digestibility of Maize (*Zea mays*) Flour

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### Abstract

Maize provides carbohydrates, proteins, dietary fibers, vitamins and also serves as staple food crop in many parts of the world. Lactic acid bacteria (LAB) previously isolated from fermenting maize and sorghum were combined to obtain consortium from maize and consortium from sorghum respectively and used to ferment processed maize flour to determine the effect of fermentation on the proximate composition and *in-vitro* starch/protein digestibility using standard techniques at 12 h intervals. The result shows significant ( $p < 0.05$ ) increase in the moisture content as the fermentation time increases from  $9.66 \pm 0.02\%$  to  $10.82 \pm 0.03\%$ . The ash content increased from  $1.88 \pm 0.11\%$  to  $3.14 \pm 0.04\%$ . The lipid content decreased significantly ( $p < 0.05$ ) from  $4.58 \pm 0.05\%$  to  $4.08 \pm 0.09\%$ . Protein content increased significantly ( $p < 0.05$ ) from  $9.44 \pm 0.87\%$  to  $12.97 \pm 0.07\%$  while the crude fibre and carbohydrate contents decreased significantly ( $p < 0.05$ ) with the increasing fermentation time in all the samples from  $3.62 \pm 0.04\%$  to  $0.93 \pm 0.09\%$  and from  $70.82 \pm 1.11\%$  to  $68.01 \pm 0.09\%$  respectively. The result showed a significant ( $p < 0.05$ ) increase in the *in-vitro* starch digestibility (IVSD) with increasing fermentation periods from initial value of  $20.10 \pm 1.28\%$  to  $49.45 \pm 2.16\%$ ,  $10.68 \pm 0.92\%$  to  $49.32 \pm 0.58\%$ , and  $10.68 \pm 0.92\%$  to  $58.00 \pm 0.97\%$  for naturally fermented and LAB-consortium from maize and sorghum fermented samples respectively. The *in-vitro* protein digestibility (IVPD) increased significantly ( $p < 0.05$ ) from  $61.28 \pm 0.96\%$  to  $82.06 \pm 2.01\%$ ,  $61.28 \pm 0.96\%$  to  $84.62 \pm 1.26\%$  and  $61.28 \pm 0.96\%$  to  $88.70 \pm 1.36\%$  for naturally, LAB-consortium from maize and LAB-consortium from sorghum fermented samples respectively. This study has shown the effectiveness of LAB-consortium fermentation in improving the nutritional quality as well as increasing the IVSD and IVPD of flour from maize.

## 1. Introduction

Cereals such as rice, wheat, sorghum and maize are important because of their role as staple food crops in many areas of the world. They serve as important components of the daily diet, providing carbohydrates, proteins, dietary fibers and vitamins [1]. Epidemiological studies have indicated that whole grain plays foods protective role against several diseases such as type 2 diabetes [1-3], cardiovascular diseases and certain cancers [4].

Maize or corn (*Zea mays*) is cultivated globally as an important cereal crop which is believed to have originated in central Mexico 7000 years ago from a wild grass [5-6]. Maize is grown throughout the world and report indicated that the United States, China, and Brazil are the top three maize-producing countries in the world [5, 7]. Maize is a good source of carbohydrate, protein and calorie [7]. Maize is also utilized as basic element in formulation of animal feed as well as in manufacture of industrial products such as corn starch, maltodextrins, corn oil, corn syrup and can be precessed into a wide range of foods and beverages [8-9].

Natural fermentation has been reported to induce phytate hydrolysis through the action of microbial phytase from the microflora of cereals and legumes, leading to the reduction of phytic acids [10]. Also, [11] reported that fermentation increases the starch and protein digestibility as well as nutritive value of food, increase protein content enhances carbohydrate accessibility, improves amino acid balance and decreases antinutritional factors. Lactic acid bacteria fermentation is a common way of preparing fermented foods such as maize porridge, alcoholic beverages and dairy products traditionally in some parts of Africa [12]. Some of the main reasons for the fermentation practice using LAB are to increase food palatability and improve the quality of food by increasing the availability of proteins and vitamins as well as confers preservative and detoxifying effects on food [6, 12-13]. According to the reports of [10] and [14], lactic acid bacteria fermentation is not only important for economic values but also promotes human health as the fermented foods boost immune system.

Functional and nutritional aspects of fermentation have been widely studied in commonly consumed cereals and legumes [11, 15]. However, there is limited information on the proximate composition of maize flours fermented with lactic acid bacteria (LAB) consortium. Therefore the objective of this study is to evaluate the proximate composition and the *in-vitro* starch/protein digestibility of maize flour fermented with lactic acid bacteria consortium.

## 2. Materials and Methods

### 2.1. Source of Materials

White variety of maize (*Zea mays*) was bought from Yaba

market of Lagos, Lagos State, Nigeria and transported to the laboratory in a clean polythene bags for analysis at Federal Institute of Industrial Research Oshodi (FIRO). Lactic acid bacteria used were previously isolated from fermenting maize and sorghum and were selected based on their tolerance to acid, tolerance to salt, lowering of pH in a fermentation broth, level of acid production during fermentation and growth on nutrient depleted medium after a pre-fermentation study. All the chemicals used were of analytical grade (AR).

### 2.2. Sample Preparation

The sample was prepared according to the method used by [6]. The raw grains the maize were freed of foreign materials, washed with clean tap water and rinsed with distilled water and then dried with hot air oven at 60°C for 8 h. This was milled into powder using milling machine disinfected with 70% ethanol and stored in clean air tight containers at 4°C for further use.

### 2.3. Inoculum Development/Preparation

The inoculum was developed following the method of [6] with slight modification. Each of the lactic acid bacteria was grown at 37°C for 24 h on autoclaved MRS broth distributed in test-tubes in 10 ml aliquots. After 24 h of incubation the cells in the combination of *Lactobacillus plantarum* WCFS1, *Lactobacillus rhamnosus* GG, ATCC 53/03, *Lactobacillus nantensis* LP33, *Lactobacillus fermentum* CIP 102980, and *Lactobacillus reuteri* DSM 20016, for consortium from maize; and *Pediococcus acidilactici* DSM 20284, *Lactobacillus fermentum* CIP 102980, *Lactobacillus brevis* ATCC 14869, *Lactobacillus nantensis* LP33, and *Lactobacillus plantarum* WCFS1, for consortium from sorghum where transferred to a 250 ml conical flask containing 210 ml MRS broth respectively. These were incubated for 48 h in an orbital shaker incubator (REMI/396LAG) at 37°C for the inoculum to build-up. The cells were harvested by centrifugation at 5000 g for 10 min and washed with sterile distilled water.

### 2.4. Fermentation of Maize Flour

Fermentation was carried out following the method of [6]. 500 g each of the flours was mixed with 1000 mL of distilled water and 0.5 g/L potassium sorbate was added to inhibit fungal growth and other contaminating organisms. The mixture was inoculated with 10 mL of  $10^8$  cells/mL of the mixture of the lactic acid bacteria suspension and allowed to ferment. Samples were withdrawn at 12 h intervals, dried and ground for analysis. Moreover, a sample was allowed to ferment naturally without addition of potassium sorbate and starter organisms. The flow charts for the production of fermented maize flour was presented in figures 1 be.

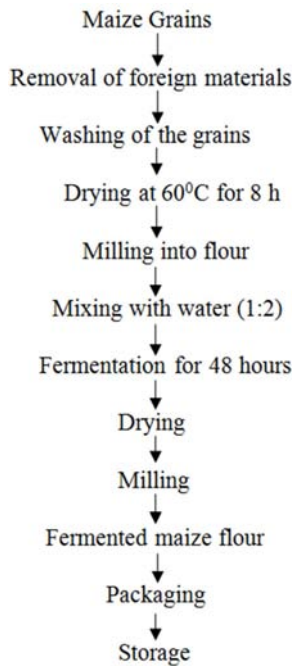


Figure 1. Flow chart for the production of fermented maize flour.

## 2.5. Proximate Analysis

The proximate composition of the sample was determined according to [16] methods.

## 2.6. In Vitro Starch and Protein Digestibility (IVSD)

The method described by [11] was used for the determination of *in-vitro* starch digestibility (IVSD) while the *in-vitro* protein digestibility was determined by enzymatic method as described by [17].

## 2.7. Statistical Analysis

The data obtained were analyzed using the one way analysis of variance for repeated measurement. Mean separation and comparison was done using SPSS version 20.0. Significance was accepted at  $p < 0.05$  and values expressed as mean  $\pm$  standard deviation.

## 3. Results

The moisture content of the maize flour showed an increase as the fermentation time increased. It increased from  $9.66 \pm 0.02\%$  to  $10.82 \pm 0.03\%$ . There was significant difference ( $p < 0.05$ ) when compared between naturally fermented, fermented with LAB-consortium from maize and LAB-consortium from sorghum (Figure 2).

Figure 3 presented the effect of fermentation on the ash content of the sample which showed an increase as the fermentation progressed. It ranged from  $1.88 \pm 0.11\%$  to  $3.14 \pm 0.04\%$ . The variations in the ash content did not differ significantly ( $p > 0.05$ ) when compared with LAB-consortium from maize and LAB-consortium from sorghum fermented samples.

Figure 4 showed the effect of fermentation on the percentage lipid compositions maize flour. The result shows a decrease in the lipid content of the samples with increasing time of fermentation. The lipid content ranged from  $4.08 \pm 0.09\%$  to  $4.58 \pm 0.05\%$ .

The protein content increased with increasing fermentation time. It ranged from  $9.44 \pm 0.87\%$  in the unfermented sample to  $12.97 \pm 0.07\%$  in 48 h sample fermented with LAB-consortium from sorghum. There was a significant difference ( $p < 0.05$ ) in the increase in the protein content of the fermented maize and the unfermented (Figure 5).

Figure 6 presented the percentage crude fibre composition of maize. The crude fibre decreased from  $3.62 \pm 0.04\%$  in the unfermented sample to  $0.93 \pm 0.09\%$  in the sample fermented with LAB-consortium from sorghum. There was significant difference ( $p < 0.05$ ) between the fibre content of fermented and unfermented samples.

The carbohydrate content of maize decreased from  $70.82 \pm 1.11\%$  to  $68.01 \pm 0.09\%$  in the unfermented and LAB-consortium from sorghum fermented samples respectively (Figure 7).

The percentage *in-vitro* starch digestibility of the maize flours was presented in figure 8. The result showed a significant increase ( $p < 0.05$ ) with increasing fermentation periods. It increased from  $20.10 \pm 1.28\%$  to  $43.02 \pm 1.10\%$ ,  $20.10 \pm 1.28\%$  to  $47.23 \pm 1.18\%$  and  $20.10 \pm 1.28\%$  to  $49.45 \pm 2.16\%$  in naturally fermented, LAB-consortium from maize fermented and LAB-consortium from sorghum fermented samples respectively. The changes in the *in-vitro* starch digestibility of LAB-consortium from maize and LAB-consortium from sorghum fermented samples do not differ significantly ( $p > 0.05$ ).

The percentage *in-vitro* protein digestibility of the maize flours under study was presented in Figure 9. There was significant increase ( $p < 0.05$ ) with increasing fermentation time. The values increased from  $61.28 \pm 0.96\%$  to  $82.06 \pm 2.01\%$ ,  $61.28 \pm 0.96\%$  to  $84.62 \pm 1.26\%$  and  $61.28 \pm 0.96\%$  to  $88.70 \pm 1.36\%$  in naturally fermented, LAB-consortium from maize fermented and LAB-consortium from sorghum fermented samples respectively. The changes in the LAB-consortium from maize and LAB-consortium from sorghum fermented samples do not differ significantly ( $p > 0.05$ ).

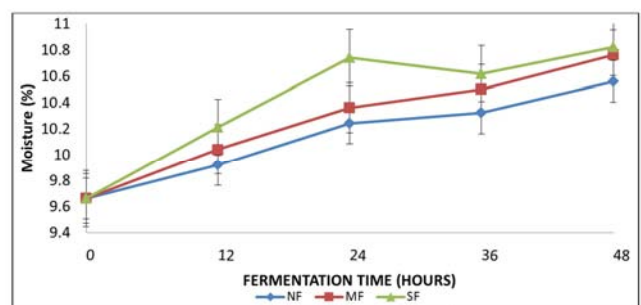


Figure 2. Effect of fermentation on the moisture content of maize flours.

NF = Naturally fermented, MF = LAB-consortium from maize fermented; SF = LAB-consortium from sorghum fermented; Values are mean of triplicate determination

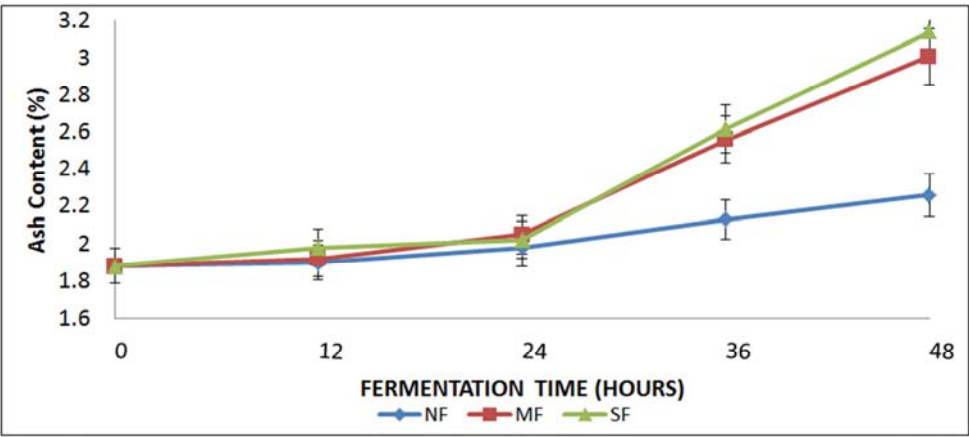


Figure 3. Effect of fermentation on the Ash Content of Maize flours.

NF = Naturally fermented, MF = LAB-consortium from maize fermented; SF = LAB-consortium from sorghum fermented, Values are mean of triplicate determination

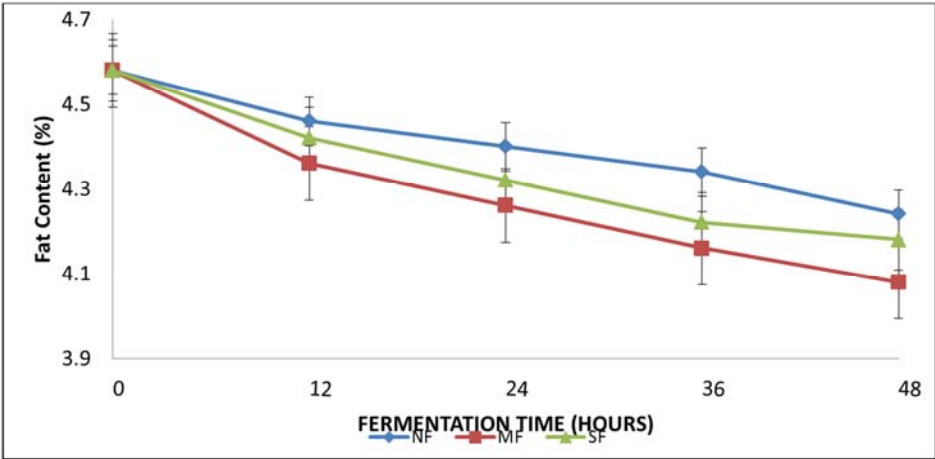


Figure 4. Effect of fermentation on the Fat Content of Maize flours.

NF = Naturally fermented, MF = LAB-consortium from maize fermented; SF = LAB-consortium from sorghum fermented, Values are mean of triplicate determination

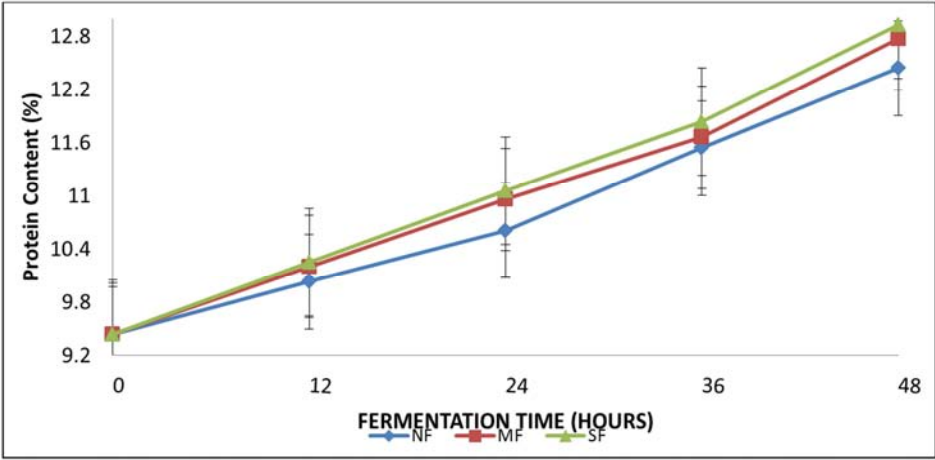
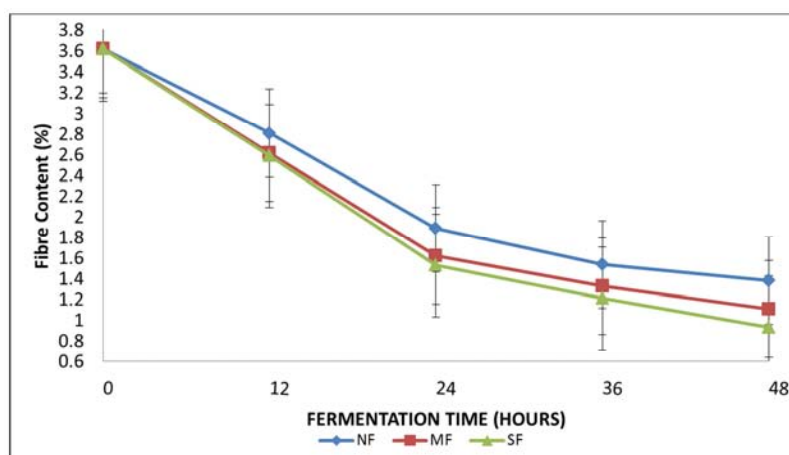


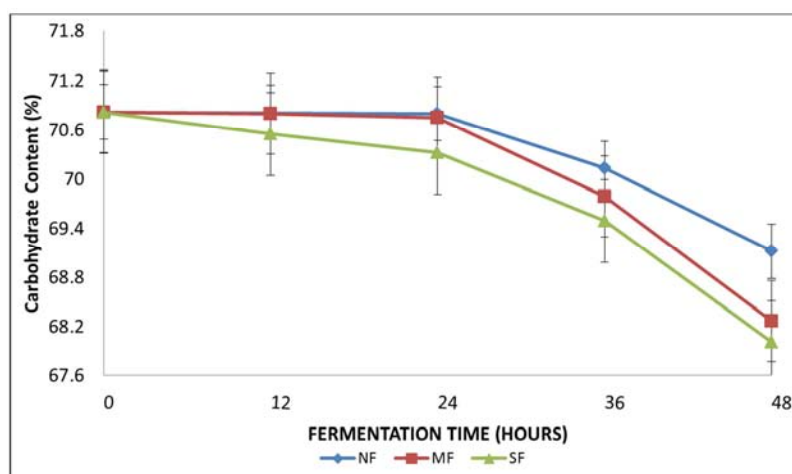
Figure 5. Effect of fermentation on the Protein Content of Maize flours.

NF = Naturally fermented, MF = LAB-consortium from maize fermented; SF = LAB-consortium from sorghum fermented, Values are mean of triplicate determination.



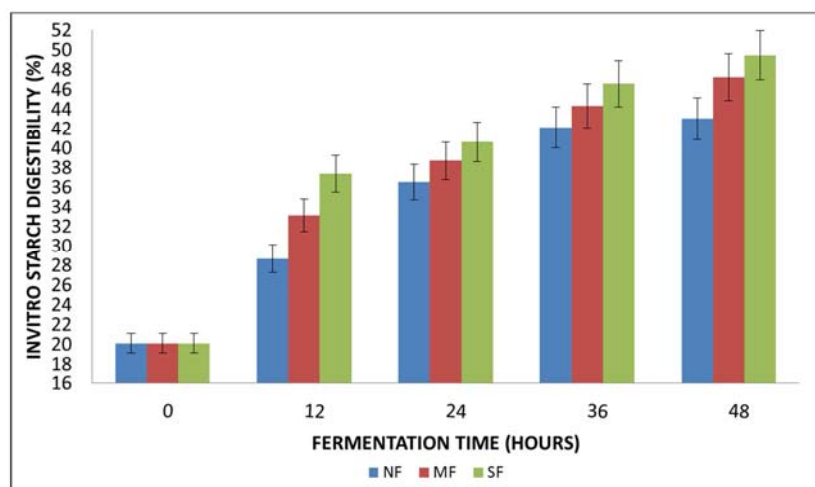
**Figure 6.** Effect of fermentation on the Fibre Content of Maize flours.

NF = Naturally fermented, MF = LAB-consortium from maize fermented; SF = LAB-consortium from sorghum fermented, Values are mean of triplicate determination.



**Figure 7.** Effect of fermentation on the Carbohydrate Content of Maize flours.

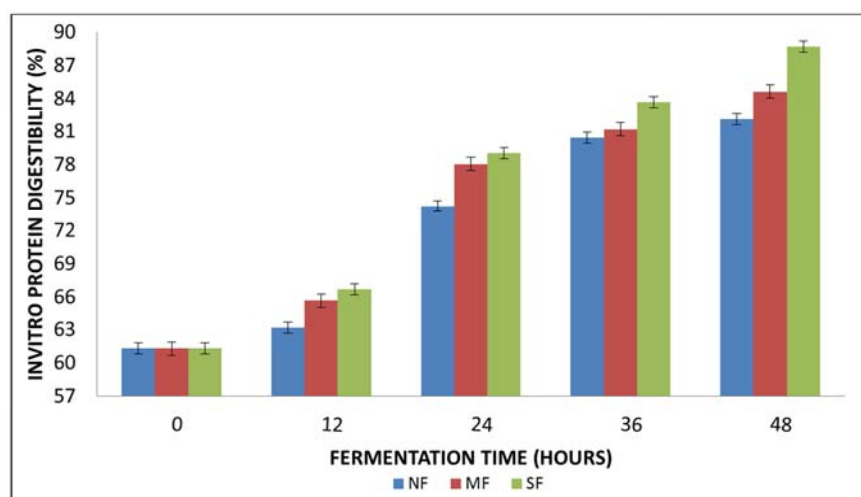
NF = Naturally fermented, MF = LAB-consortium from maize fermented; SF = LAB-consortium from sorghum fermented, Values are mean of triplicate determination.



**Figure 8.** Effect of fermentation on In-vitro starch digestibility of Maize flours under study at different time interval.

NF = Naturally fermented; MF = Fermented with LAB-consortium from maize; SF = Fermented with LAB-consortium from sorghum. Values are mean of triplicate determination.





**Figure 9.** Effect of fermentation on *In-vitro* protein digestibility of Maize flours.

NF = Naturally fermented; MF = Fermented with LAB-consortium from maize; SF = Fermented with LAB-consortium from sorghum. Values are mean of triplicate determination.

## 4. Discussion

In the present study, the moisture content differ significantly ( $P < 0.05$ ) when compared between naturally fermented, fermented with LAB-consortium from maize and fermented with LAB-consortium from sorghum samples at each successful fermentation time interval. The values obtained in the present study is higher than 7.52% obtained by [18] but is consistent with the report of [19] who reported increase in moisture content with increasing fermentation time. The increase in the moisture content can be attributed to the addition of water to the substrate prior to fermentation. The result in the present study also fall within the range reported for millet [20]. Moisture is an important parameter in the quality and acceptability of flour and flour products as it affects the shelf life and microbial growth during storage [21]. According to [22] as reported by [23], the moisture content of maize meal flour should not exceed 15.5%. The result of the present study was within the recommended limit. The moisture content of maize in the present study were lower than the  $22.92 \pm 0.60\%$  reported by [24] on white maize flour but compared favourably with the finding of [25] who reported 9.30% in maize. Also, the result in the present study is lower than the report of [26] who reported 13.20% for fermented maize flour and within the recommended limit of 15.5% [22]. The low moisture content obtained in the present study for all the samples in desirable because it will enhance the storability of the flours because food spoiling microorganisms thrives where there is adequate moisture [27].

The percentage ash content of maize flour showed an increase as the fermentation progresses although there was no significant difference ( $p > 0.05$ ) at 12 h and 24 h when compared with the unfermented sample in all set - up. The ash content of fermented products showed that the naturally fermented samples are significantly different ( $p < 0.05$ ) when

compared with the LAB-consortium from maize and LAB-consortium from sorghum fermented samples. Decrease in ash content of fermenting maize samples have been reported by [28] which did not correspond with the present study. However, the present study agreed with the reports of [29], [30] and [31] who reported increase in ash content with increasing fermentation in the range of 1.4-3.3%, 1.79 – 1.80% and 1.94-1.95% respectively. Ash content increment leads to increase in mineral content and an indication of the level of mineral composition of the substrates [23]. In all the samples in the present study, the ash content increased with increasing fermentation periods. The increment was highest in sample fermented with LAB-consortium from sorghum, followed by sample fermented with LAB-consortium from maize and then the naturally fermented sample in the cereals under study. This finding suggests the effectiveness of the consortia in improving the mineral composition of the substrates.

The result of the percentage fat composition of the samples in the present study shows a decrease with increasing time of fermentation. It decreased from  $4.58 \pm 0.05\%$  (unfermented sample) to  $4.08 \pm 0.09\%$  (48 h LAB-consortium from maize fermented sample). The decrease differed significantly ( $p < 0.05$ ) at all-time intervals in all the fermentation set-ups when compared with the unfermented sample. The observation in the present study agreed with the work of [28], who reported decrease in the fat content of maize from  $2.2 \pm 0.02\%$  to  $1.5 \pm 0.1\%$  in unfermented and fermented samples. Also, [31], reported decrease in fatty matter during fermentation of maize based food. The highest decrease in fat content of the cereal flours under study was recorded in samples fermented with LAB-consortium from maize followed by the LAB-consortium from sorghum fermented sample. Therefore fermentation with lactic acid bacteria consortium could serve as an effective way of reducing the fat composition of samples as demonstrated in the present study. The decrease in the fat composition during fermentation observed in the present study in all the samples could be attributed to the fact

that biochemical and physiological changes occurred during fermentation require energy and part of the lipids contained in the samples were utilized for the production of energy [32-34]. It could also be due to the metabolism of fatty acids and glycerol by the fermenting organisms which enhances aroma, taste and texture [19].

The protein content increased with increasing fermentation time from  $9.44 \pm 0.87\%$  in the unfermented sample to  $12.97 \pm 0.07\%$  in 48 h sample fermented with LAB-consortium from sorghum. This result is lower than 18.40% reported by [26] and is consistent with the report of [20] who reported increase in protein content of millet after fermentation from 8.00-9.52%. According to [22], the minimum requirement of protein in maize meal flour is 8.0%. Therefore, the protein content above 9.0% in the present study is an indication of improved quality due to fermentation with LAB-consortium. The increase in the protein contents of the samples with increasing fermentation time could be attributed to the activities and increase in the number of microorganisms during fermentation [35-36]. It can also be due to proteolytic enzymes produced by the fermenting organisms [20, 37]. It can also be as a result of synthesis of protein by fermenting substrates which could have resulted to increased production of amino acids [38]. The highest increase in the protein content was observed in the LAB-consortia fermented samples. The present report has demonstrated the more effectiveness of the LAB consortium from sorghum and LAB consortium from maize in improving the palatability and texture of the final product which also agreed with the report of [23] that protein content plays important role in texture and palatability of final product.

The fibre composition of the samples decreased with the increasing fermentation time in all the samples. The crude fibre composition of maize decreased from  $3.62 \pm 0.04\%$  in the unfermented sample to  $0.93 \pm 0.09\%$  in the sample fermented with LAB-consortium from sorghum. There was significant difference ( $p < 0.05$ ) between the fibre content of fermented and unfermented samples at all the time intervals in each fermentation set-up. The variations in the values of the fibre content of maize did not differ significantly ( $p > 0.05$ ) when LAB-consortium from maize fermented sample was compared to LAB-consortium from sorghum fermented sample while both are significantly different ( $p < 0.05$ ) when compared with the naturally fermented sample. This observation agrees with the report of [28] who reported decrease in fibre content from  $2.20 \pm 0.1$  to  $1.7 \pm 0.2$  in unfermented and fermented maize for *ogi*.

The general decrease in the fibre content could be due to the ability of the fermenting organisms to metabolize the fibre. It can also be attributed to the enzymatic breakdown of the fibre during fermentation by lactic acid bacteria which utilize the fibre as carbon source [19]. The highest decrease in fibre content was observed in LAB-consortium from sorghum fermented sample, followed by LAB-consortium from maize fermented sample, and then naturally fermented sample. The present investigation has also shown the effectiveness of the LAB consortia from maize and sorghum

in metabolizing the fibre more than natural fermentation.

The result of the effect of fermentation on the carbohydrate composition showed a decrease as the fermentation time increases. There was no significant difference ( $p > 0.05$ ) in the reductions when compared between LAB consortium from sorghum, LAB consortium from maize and naturally fermented sample. The reduction in the carbohydrate content in the present study with fermentation could be attributed to breakdown and utilization of fermentable sugars by lactic acid bacteria present for growth, energy and other metabolic activities [35]. Also, [28] reported a decrease in the carbohydrate content of fermented maize products. Report has also shown that LAB from maize have strong amylolytic activity [39].

The *in-vitro* starch digestibility (IVSD) of the samples in the present study were lowest in the unfermented sample. This could be attributed to the restriction of starch caused by the endosperm protein as reported by [11]. In the present study, fermentation resulted in significant increase ( $p < 0.05$ ) in the IVSD of the cereals. This is in agreement with the report of [11] who reported increase in the IVSD of sorghum, maize and millet with increasing fermentation time. The increase could be attributed to the role of fermentation in causing changes in the endosperm protein which makes starch more accessible to the digestive enzymes [11, 40].

In the present study, fermentation was found to cause significant increase ( $p < 0.05$ ) in the *in-vitro* protein digestibility of the cereals. The starter organisms and the microflora of the grains may have produced proteolytic enzymes which could be responsible for the increased protein digestibility [11]. These results also agree with the work of [11] and [41] who reported that fermentation improves the IVPD of maize cultivars which could be attributed to the partial degradation of storage proteins into simple and more soluble products. Also it could be as a result of the reduction in pH during fermentation which plays an important role in enhancing the proteolytic enzyme activity and in turn leads to the breakdown of proteins to smaller polypeptides which are easily digested [11]. The highest starch/protein digestibility were observed in samples fermented with the LAB consortia while the naturally fermented and the unfermented samples were the lowest. This shows that LAB consortia fermentation is more effective in improving the IVSD and IVPD of the sample more than the natural fermented and the unfermented sample.

## 5. Conclusion

The proximate properties, *in-vitro* protein and starch digestibility of maize and sorghum in the present study improved significantly ( $p < 0.05$ ) after natural, LAB-consortium from maize and LAB-consortium from sorghum fermentation. The highest improvement was observed in the samples fermented with the LAB-consortia in all the parameters analyzed except moisture. There was no significant difference between the parameters of LAB-consortium from maize and LAB-consortium from sorghum

fermented samples. This suggests effectiveness of lactic acid bacteria consortium fermentation in improving the nutritional qualities of maize flour as well as IVSD and IVPD more than the natural fermentation.

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