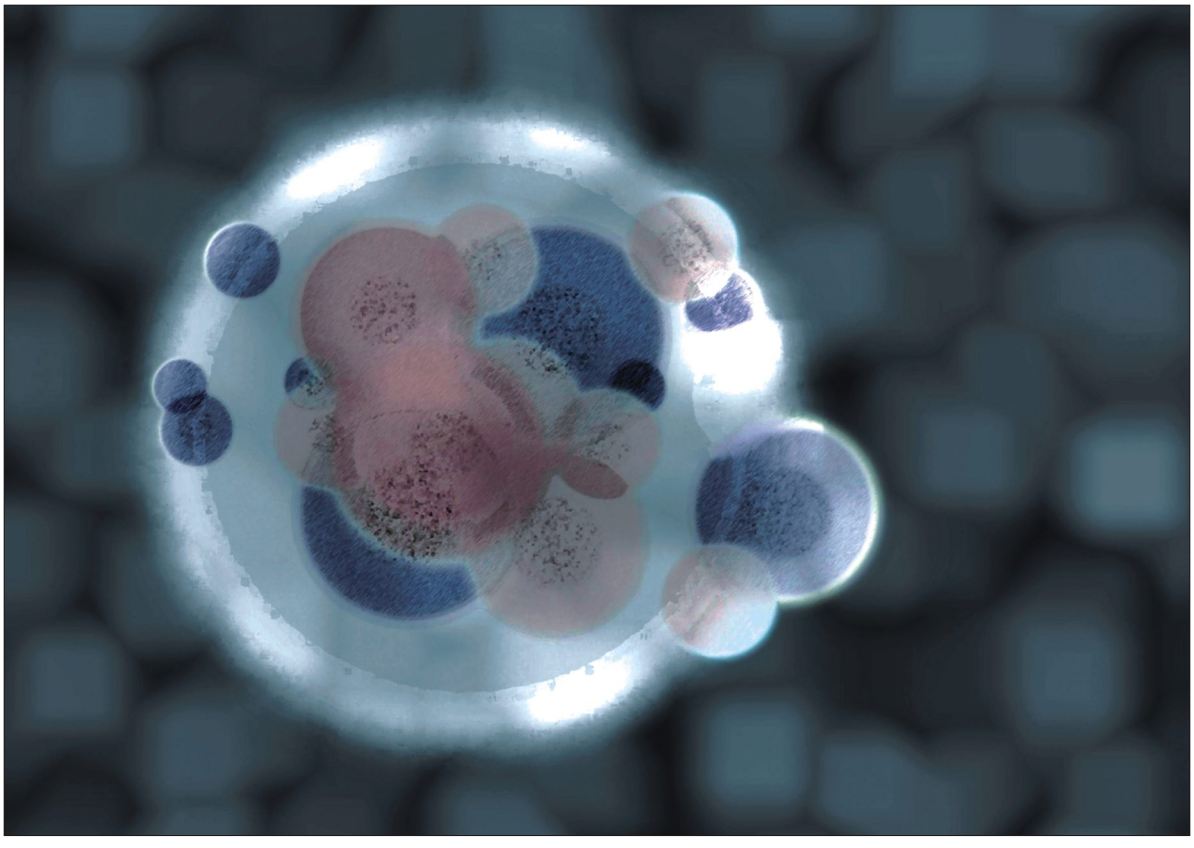

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Research Article
Fermentation by Lactic Acid Bacteria Consortium and its Effect on Anti-nutritional Factors in Maize Flour

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Abstract

Background and Objective: This study investigated the effect of fermentation with lactic acid bacteria (LAB)-consortium on the anti-nutritional factors of maize flour. **Materials and Methods:** Maize was processed into flour, fermented spontaneously and with LAB-consortium (*Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus nantensis, Lactobacillus fermentum, Lactobacillus reuteri*, *Pediococcus acidilactici* and *Lactobacillus brevis*) previously isolated from maize and sorghum to evaluate their effects on tannins, phytate, polyphenol and trypsin inhibitor activity at 12 h intervals using standard techniques. **Results:** The result showed significant ($p<0.05$) decrease in the contents tannin, phytate, polyphenol and trypsin inhibitor activity with increasing fermentation period. The reductions in the anti-nutritional factors were more in the fermentations set-ups by LAB-consortium than spontaneous fermentation. **Conclusion:** This suggested that altering the natural microflora of maize flour by LAB-consortia during fermentation has potential to decreasing the anti-nutritional factors and improve nutrient bioavailability.

Key words: Nutrient bioavailability, anti-nutritional factors, maize flour, microbial ecology, fermentation, LAB-consortium

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize as an important cereal is a major source of carbohydrate, protein and energy. Reports indicated that maize contains 72% starch and 10% protein with energy density[1,2] of 365 Kcal/100 g. Moreover, maize also serves as a source of raw materials for food and manufacturing industries and can be processed into different foods, snacks and beverages[3,4]. Also, the digestibility and bioavailability of the nutrients in maize are affected by the phytochemical contents, plant variety, processing and storage conditions as well as anti-nutrients (such as phytic acid, tannins and polyphenols) composition[5-7].

Fermentation converts sugar and other carbohydrates to usable end products. During traditional fermentation of foods diets are enhanced through development of flavour, aroma and texture in food substrates and as well leads to food preservation through lactic acid, alcohol, acetic acid and alkaline fermentation. Fermentation also enhances food quality with essential amino acids, essential fatty acids, protein, minerals and vitamins. It leads to increased digestibility of food and improved bioavailability of nutrients. Moreover, anti-nutrients are detoxified through food fermentation processes, cooking time and fuel requirements are also reduced[8-12].

Lactic acid bacteria fermentation has been reported to reduce the level of anti-nutritional factors and toxins in food substrates[13]. Also, mycotoxins in food substrates are degraded irreversibly without effect on the nutritional value of food and without leaving and toxic residue during fermentation[14]. For instance, plants like cassava, cereals and legumes may contain substances such as cyanogenic glycosides, oxalic acids, lectins and other anti-nutritional factors including phytic acid and tannins which interfere with protein and carbohydrate digestibility as well as reduce mineral bioavailability are converted to various edible products by fermentation[8]. This study therefore, evaluated the effect of lactic acid bacteria consortium fermentation on the anti-nutritional factor associated with maize flour and compared them with natural fermentation. This is novel since there is little or no report of lactic acid bacteria consortium fermentation on the anti-nutritional factors of maize flour which holds potential for application in food industries during food fortification.

MATERIALS AND METHODS

Source of materials and preparation of sample: Maize (*Zea mays*) was obtained from Yaba market in Lagos, Nigeria and transported to Federal Institute of Industrial Research Oshodi (FIIRO) in a clean polythene bag for identification and analysis. The raw grains were sorted to remove foreign materials and then washed with distilled water. The grains were dried at 60°C for 8 h in hot air oven (GL, England) and then milled into powder using laboratory disc mill. The flour was stored in clean air tight containers for further use. Lactic acid bacteria were previously isolated from fermenting maize and sorghum.

Selection of starter and preparation of starter culture: The starter culture (inoculum) were selected with reference to their reported benefits[15,16] as well as their tolerance to acid, salt, lowering of pH, level of acid production and growth on medium with reduced nutrient after a pre-fermentation study. The starter culture was prepared and combined following the method of Ogodo *et al.*[17] with slight modification. Five lactic acid bacteria were combined relative to their source (maize and sorghum) as follows, *Lactobacillus plantarum* WCFS1, *Lactobacillus rhamnosus* GGATCC53/03, *Lactobacillus nantensis* LP33, *Lactobacillus fermentum* CIP 102980, *Lactobacillus reuteri* DSM 20016 (consortium from maize), *Pediococcus acidilactici* DSM 20284, *Lactobacillus fermentum* CIP102980, *Lactobacillus brevis* ATCC14869, *Lactobacillus nantensis* LP33, *Lactobacillus plantarum* WCFS1(consortium from sorghum). The combined LAB were respectively grown in an Erlenmeyer flasks containing 210 mL de-Man Rogosa and Sharpe broth and incubated for 48 h in an orbital shaker incubator (REMI/396LAG) at 37°C for the inoculum to build-up in a co-culture. The inocula were thereafter harvested, washed with sterile distilled by centrifugation at 5000 rpm for 10 min and then diluted with sterile distilled water to obtain 0.5 McFarland standards.

Fermentation: The maize flour was fermented spontaneously and with the consortia of LAB according to the method described by Ogodo *et al.*[18]. The uncooked flour was mixed with distilled water in the ratio of 1:2 w/v in sterile fermentation container. Exactly 0.5 g L^{-1} of potassium sorbate was added to the mixture to inhibit fungi and other contaminating microorganisms which was verified by no observable growth on plate count agar. The mixture was inoculated with 10 mL of 1.0×10^8 cells mL^{-1} of the mixture of the lactic acid bacteria suspension and allowed to ferment. The set-up for control (spontaneous) fermentation was prepared using the same procedure except the addition of potassium sorbate and the starter organisms. Samples were withdrawn at 12 h intervals for analysis of the anti-nutritional factors.

Determination of tannin, phytate, polyphenol and trypsin inhibitor activity: Tannin was determined following the method of Onyango *et al.*[19] with slight modification. In Erlenmeyer flasks, 10 mL of 4% HCl in methanol was added to 0.25 g of the flour, closed with paraffin and then shaken gently for 20 min in a shaker. The resulting extract was centrifuged for 10 min at 4500 rpm. To obtain the second extraction, exactly 5 mL of 1% HCl in methanol was added to the residue from the first extraction. The first and second extracts were combined and made up to 25 mL. Catechin standard solutions were prepared from 100-1000 ppm using methanol. About 1 mL of the extract and 1 mL of each respective standard were combined with 5 mL of vanillin-HCl reagent. The preparation of the blank was done by adding 5 mL of 4% HCl in methanol to 1 mL of the aliquot extracts. The absorbencies of the standard solutions, sample extracts and blanks were read at 500 nm in UV754 spectrophotometer (HospiBrand, USA). The catechin equivalents (CE) (%) were determined using the formula:

$$CE\ (\%) = \frac{CC \times VM}{VE \times Wt} \times 100$$

Where:

CC = Catechin concentration
VM = Volume
VE = Volume of extract
Wt = Weight of sample

The method described by Onyango *et al.*[19] was used to determine the phytate composition of the flour.

Polyphenol content of the maize flour was determined as described by Akond *et al.*[20] with slight modification. Five milliliter of 50% methanol/water and 5 mL of 1.2 M HCl in 50% methanol/water was used for extracting the flour followed by heating at 90°C for 3 h, allowed to cool, diluted to 10 mL with methanol and then centrifuged for 5 min at 5000 rpm. Exactly 1 mL of Folin-Ciocalteu reagent was added to 50 µL of the extract solution in a test tube, mixed thoroughly followed by addition of 1 mL 10 % Na_2CO_3 after 3 min and then allowed to stand for 1 h in the dark. The absorbance read at 760 nm in UV spectrophotometer (UV754, HospiBrand, USA). The total phenolic compound was determined as micrograms of gallic acid equivalent and expressed as mg/100 g gallic acid equivalent of dry mass.

The method of Mbata *et al.*[21] was used to determine the trypsin inhibitor activity with slight modification. One gram of the maize flour was extracted by soaking overnight at 4°C in 50 mL 0.01 sodium hydroxide (pH 8.4). Synthetic benzoyl DL arginine-p-nitroanilde (BAPNA) was used as substrate. Residual enzyme activity was determined using 2 mL of the sample extracts and the absorbance measured at 410 nm. Trypsin inhibitor activity (TIA) relative to milligrams of pure trypsin sample was calculated as:

$$TIA = \frac{2.632 \times D \times \Delta 1}{D}$$

Where:

1 = Change in absorbance due to trypsin inhibition/mL diluted sample extract
D = Dilution factor and S = weight of sample (g)

Statistical analysis: All analyses were carried out in triplicate and the Mean±SD were reported. Analysis of variance (ANOVA) for repeated measurements was used to compare the data obtained from spontaneous fermentation, LAB consortium from maize and LAB consortium from sorghum fermentations respectively using statistical package for the social sciences (SPSS) version 20.0 software. Significance was accepted at $p \leq 0.05$.

RESULTS

The tannins content of the maize flour after fermentation was presented in Fig. 1. The result showed that the tannin content decreased significantly ($p<0.05$) with increasing fermentation time. The decrease ranged from 43.64±0.04%

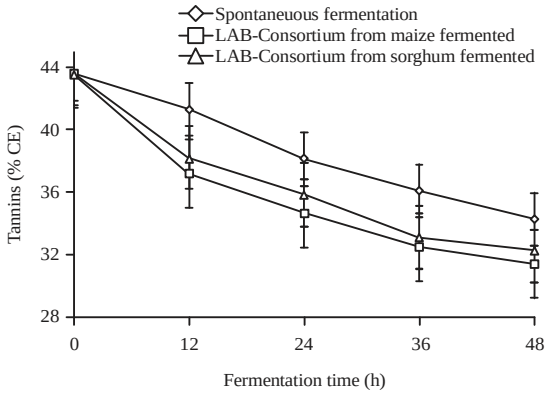

Fig. 1: Effect of LAB-consortium fermentation on the tannin content of maize flour. Each point represents the mean of three independent experiments and error bars indicate ±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

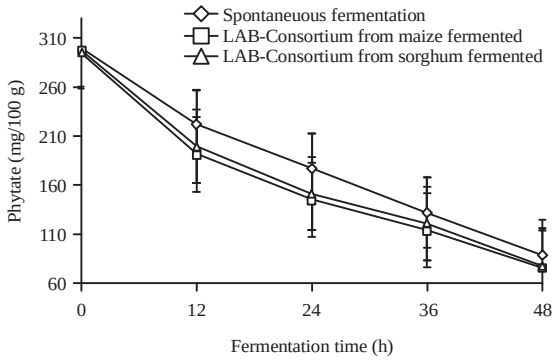

Fig. 2: Effect of LAB-consortium fermentation on the phytate content of maize flour. Each point represents the mean of three independent experiments and error bars indicate ±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

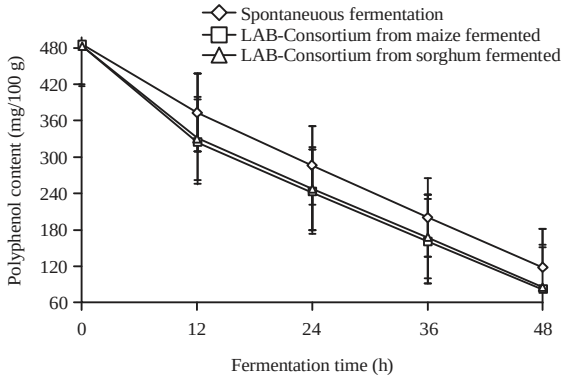

Fig. 3: Effect of LAB-consortium fermentation on the polyphenol content of maize flour. Each point represents the mean of three independent experiments and error bars indicate ±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

(0 h) to 31.38±0.08%. The highest decrease was observed in LAB-consortium from maize fermented sample (31.38±0.08%), followed by LAB-consortium from sorghum fermented sample (32.28±0.12%) while the spontaneous fermentation was the least (34.26±0.23%) after 48 h.

The Fig. 2 presented the effect of lactic acid bacteria consortium fermentation on the phytic acid compositions of maize flour. The result showed that the phytate composition decreased significantly ($p<0.05$) with increasing fermentation time and ranged from 296.10±0.15 to 88.84±0.22 mg/100 g (spontaneous), 296.10±0.15 to 76.76±0.49 mg/100 g (LAB-consortium from maize) and LAB-consortium from maize fermented sample from 296.10±0.15 to 79.25±0.08 mg/100 g (LAB-consortium from sorghum) fermentations. The variations differ significantly ($p<0.05$) when compared between spontaneous, LAB-consortium from maize and sorghum fermented samples, respectively.

The effect LAB-consortium on polyphenol composition of maize flour showed a significant ($p<0.05$) decrease with increasing fermentation time. The decrease ranged from 486.22±0.19 to 118.18±0.08 mg/100 g, 486.22±0.19 to 83.23±0.09 mg/100 g and 486.22±0.19 to 86.78±0.18 mg/100 g in spontaneous, LAB-consortium from maize and LAB-consortium from sorghum fermentations, respectively. The lowest value was obtained in the LAB-consortium from maize fermented sample (83.23±0.09 mg/100 g). The values obtained at the various time interval differ significantly when compared between the spontaneous fermentation and the LAB-consortia fermentations at all-time intervals (Fig. 3).

The effect of change in the microbial ecology of maize flour by LAB-consortium during fermentation on the trypsin inhibitor activity showed a significant ($p<0.05$) decrease with increasing fermentation time. The decrease ranged from 52.08±0.12 to 38.42±0.29 mg/100 g (spontaneous), 52.08±0.12 to 33.18±0.16 mg/100 g (LAB-consortium from maize) and from 52.08±0.12 to 34.20±0.00 mg/100 g (LAB-consortium from sorghum) fermentations. The decreases differ significantly ($p<0.05$) when compared between naturally fermented, LAB-consortium from maize and LAB-consortium from sorghum fermented samples. The lowest value polyphenol was observed in LAB-consortium from maize fermented samples at 48 h (Fig. 4).

DISCUSSION

In the present study, the percentage tannin content showed a significant ($p<0.05$) decrease with increasing fermentation time in all fermentation set-ups. The decrease was more in LAB- consortium from maize fermented flour at 48 h. This decrease could be that the organisms form synergy to degrade the anti-nutritional factors. Moreover, some lactic acid bacteria such as *Lactobacillus paraplantarum*, *Lactobacillus pentosus* and *Lactobacillus plantarum* are reported to be capable of degrading tannins as a result of their acetylhydrolase tannin activity[22]. The report of Onyango *et al.*[19] indicating significant reduction in tannin

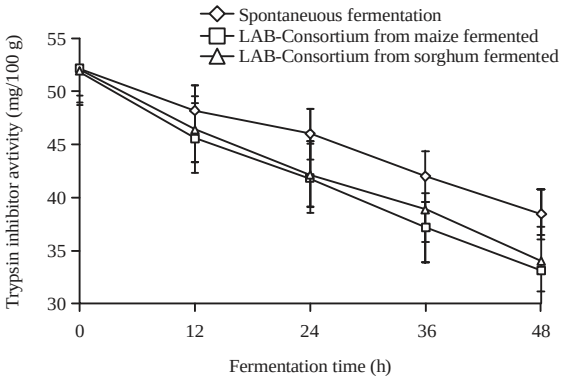

Fig. 4: Effect of LAB-consortium fermentation on the trypsin inhibitor activity of maize flour. Each point represents the mean of three independent experiments and error bars indicate ±SE. There were significant differences ($p<0.05$) at each successive time interval when natural fermentation is compared to LAB consortia fermentations

content of red sorghum, white sorghum and millet after 8 days of fermentation at 25°C is consistent with the present report. However, this observation did not agree with initial increase in tannins content during fermentation of pearl millet reported by Osman[23]. The decrease in tannin could be due to mobilization of enzymes as phenyl oxidase, α-galactosidase and other enzymes associated with seeds[24,25]. Tannins affect palatability of food due to its bitter and stringent taste and as well reduces digestibility by forming complex with proteins[10]. Therefore, fermentation in this study could improve nutritional value of maize flour especially when the natural fermenting organisms are replaced with lactic acid bacteria consortium. This is also in indication effectiveness of LAB-consortium in tannin content reduction more than natural microflora during fermentation of maize flour.

Phytate in the present study decreased significantly ($p<0.05$) with increasing fermentation time. The fermentation set-ups with the LAB consortium were observed to decrease phytic acid more than the spontaneous fermentation. Phytates in seeds and grains are the main storage form of phosphorus where they form complex minerals and proteins thereby reducing their digestibility and bioavailability[10]. The present observation is similar to the report of significant decrease in phytate content of pearl millet[23], millet and sorghum[19] and four corn cultivars[26] during fermentation. Moreover, Sokrab *et al.*[27] reported decrease in the phytate composition of two maize varieties after 14 h fermentation from 1047.00-155.00 mg/100 g and from 87.6-0.31 mg/100 g, respectively. The significant reduction in phytate content observed in this study could be attributed to the production phytases and phosphophytases by the fermenting organisms, especially LAB which hydrolysis phytate[22,28]. The major sources of phytate in diets are legumes and cereals. This phytates are not digested through the gastrointestinal tract but form complexes that restricts the absorption of essential elements and nutrients. This implies that the extent to which phytates are degraded can affect the nutritional value of a phytate-rich food or diet[27,29]. Moreover, phytate in its natural form decrease availability of manganese, zinc, copper, calcium, molybdenum, iron and magnesium, hence, affects digestibility of minerals and vitamins[27,30]. Therefore degrading phytate during fermentation, especially LAB-consortium fermentation as observed in the present study holds potential for increase in nutrient, vitamins and minerals bioavailability in diets.

Polyphenol content of maize flour decreased significantly ($p<0.05$) in this study as fermentation time increased. The decreases were observed to be more in fermentations by from maize and then LAB-consortium from sorghum fermented samples while spontaneous fermentation showed least reduction after 48 h fermentations respectively. Similar observations have been reported by other researchers in millet[31], sorghum[28] and maize[22]. The reduction in polyphenols content of the maize flour in this study during fermentation could be due to the action of certain enzymes such as tannase and polyphenol oxidase produced by the fermenting microorganisms, especially the LAB-consortium[22,27]. The observation in this study is an indication of the potential of LAB-consortium to effectively reduce polyphenol content of maize flour more than spontaneous fermentation.

Fermentation in this study decreased trypsin inhibitor activity (TIA) of maize flour significantly ($p<0.05$) in all set-ups. However, the decrease was observed to be more in LAB-consortia fermentations than spontaneous fermentation after 48 h. Similar observations have been reported by other researchers on different substrates after fermentation[10,14,23,24]. The TIA reduces protein digestibility and has been implicated in pancreatic hypertrophy and poor growth performance in rat, mice and chicks[23]. Therefore their reduction could improve growth performance and nutritional quality of maize flour.

CONCLUSION

The present study shows that tannin, phytate, polyphenol and trypsin inhibitor activity decreased significantly ($p<0.05$) during fermentations. The highest decreases were observed

in LAB-consortia fermentation set-ups. This suggests that change in microbial ecology by LAB-consortium during fermentation is an effective means of reducing tannin, phytate, polyphenol and trypsin inhibitor activity. This will improve nutrient bioavailability and digestibility. Therefore, this study suggests more research to optimize and scale up the process in order to be applied in food industries for commercialization.

SIGNIFICANCE STATEMENT

Lactic acid bacteria (LAB) consortia were used to significantly reduce the antinutritional factors of maize flour. This reduction will enhance the bioavailability of nutrients and minerals. Hence, this research has the potential to be applied in food and other industries to improve the nutrient quality of maize flour which could be useful in food fortification.

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