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Research Article

Evaluation of the Effects of Fermentation with Natural Sourdough on the Phytic Acid Content of Corn Flour Produced in Guinea

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Abstract: In Guinea, iron and zinc deficiencies represent a major public health problem, exacerbated by the consumption of cereal-based foods rich in phytic acid, which reduces the bioavailability of these minerals. Natural fermentation of flours offers a simple approach to improving this availability. This study aimed to evaluate the effects of corn flour fermentation with a natural starter on phytic acid levels and fermentation parameters. The resulting flour was mixed with distilled water (1 : 1, 1 : 2, and 1 : 3 (w/v)). These suspensions were used to perform three fermentation conditions: fermentation with starter consisting of sterilized corn flour suspension and natural sourdough (2, 5, and 10%) (FFL); spontaneously fermented flour (FFS), composed of an unsterilized corn flour suspension without a starter; and a control composed solely of sterilized corn flour suspension without the addition of sourdough (FT). The results obtained show that after 48 h of fermentation, the pH varied from 5.71 ± 0.0071 to 3.41 ± 0.057, and the acidity evolved from 4.68 ± 0.062 to 13.63 ± 0.035 g/L in the samples (FFL, 1 : 3, 10%). A similar trend was observed in the samples (FFS, 1 : 3), where the pH went from 5.96 ± 0.0035 to 3.53 ± 0.0245 and the acidity from 2.04 ± 0.057 g/L to 11.37 ± 0.014 g/L. In FFS and FFL, a significant variation ($p < 0.05$) in phytic acid contents was observed; it went from 0.369 g/100 g in raw flour to 0.249 ± 0.057, 0.230 ± 0.071, and 0.196 ± 0.106 g/100 g in FFS samples with flour/water ratios of 1 : 1, 1 : 2, and 1 : 3 after 48 h of fermentation, respectively. FFL samples showed a variation, reaching 0.102 ± 0.028 g/100 g in the sample (FFL, 1 : 3, 10%). The present study shows that natural fermentation is significantly more effective than spontaneous fermentation in reducing phytic acid.

***Keywords*:** evaluation, fermentation, natural sourdough, phytic acid, corn flour

1. Introduction

Maize (*Zea mays* L.) is one of the main starchy foods consumed in East and West Africa, particularly in Guinea, where it represents a staple food. Its richness in carbohydrates makes it a significant source of energy in the daily diet. In these regions, maize consumption varies between 157 and 267 g per person per day [1]. However, approximately 80% of the minerals present in maize grains are found in the germinal part, which also serves as a reservoir for phytic acids. These phytic acids significantly limit the bioavailability of essential minerals such as iron, zinc, and calcium [2]. According to recent analyses, the Phytate-Zinc (Phy/Zn) and Phytate-iron (Phy/Fe) molar ratios should be less than 15 and 1, respectively, to ensure optimal absorption of these minerals [3]. However, maize displays molar

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ratios of 40.76 for Phy/Zn and 41.42 for Phy/Fe, as reported by Nsabimana et al. [4] in their study on improving the bioavailability of iron and zinc in maize by reducing phytates. This situation could increase the risk of iron and zinc deficiencies in regular consumers of maize and its derivatives [5]. In Guinea, malnutrition remains a critical challenge. The Standardized Monitoring and Assessment of Relief and Transitions (SMART) survey and Global Nutrition Report reported that approximately 750,000 (26%) children aged 6-59 months are chronically malnourished, and 48% of women of reproductive age are affected by anemia [6-7]. Similarly, chronic food insecurity, exacerbated by dependence on local grains such as maize, contributes to persistent deficiencies in iron, zinc, and vitamin A. These data highlight the importance of improving the nutritional quality of staple foods, particularly maize, in order to combat child malnutrition and micronutrient deficiencies in Guinea.

In response to these challenges, several strategies have been implemented to reduce phytic acid levels in corn. These methods are being explored as effective alternatives to increase the nutritional value and bioavailability of cereals [8]. Fermentation, a traditional food preservation technique, is one such method used to improve the nutritional quality and digestibility of food products through the action of enzymes, vitamins, and other compounds produced by microorganisms (bacteria, yeasts) [9]. Several recent studies support this approach. For example, a recent study showed that the fermentation process with yeasts can reduce the phytic acid content of whole wheat flour bread by more than 50% [10]. Moreover, Ojha et al. [11] reported that inoculating sorghum flour suspension with a strain of *Lactobacillus plantarum* reduced the phytic acid content by 77% during 48 hours of fermentation. Furthermore, another study has shown that certain strains of lactic acid bacteria and yeasts, frequently used in the sourdough fermentation process, have the ability to synthesize water-soluble B vitamins and reduce phytic acid content [12]. Natural sourdough is a stable symbiotic culture of lactic acid bacteria and yeasts, maintained by successive refreshments of a flour and water mixture. Unlike pure industrial yeasts, natural sourdough harbors a complex microbial flora whose composition varies depending on geographical origin, environmental conditions, and local practices [13]. The microbial diversity of sourdough is a determining factor in its unique functional properties, inducing high phytase activity and production of beneficial bioactive metabolites. In Guinea, despite the widespread practice of artisanal fermentation, to our knowledge, there are no studies in the scientific literature highlighting the impact of this practice on the nutritional composition of locally produced maize, particularly with regard to the reduction of phytic acid. This data gap limits the optimization of processing techniques likely to enrich local maize while improving the bioavailability of micronutrients. Faced with this problem, this study consists of evaluating how fermentation with natural sourdough influences the reduction of the phytic acid content of maize flour in Guinea. Thus, the hypothesis formulated is that fermentation of maize with natural sourdough significantly reduces the phytic acid content, which improves the nutritional value of fermented maize flour. The overall objective is to evaluate the impact of fermentation with natural sourdough on the phytic acid content of maize flour produced locally in Guinea. More specifically, it involves analyzing the phytic acid content of the flour before fermentation, establishing a fermentation protocol with natural sourdough, and monitoring the evolution of pH, acidity, and phytic acid content during fermentation.

2. Materials and methods

2.1 *Collection and preparation of materials*

The yellow corn kernels were purchased from the Dalaba market (Republic of Guinea). The kernels were manually sorted to remove bad kernels and foreign matter. Subsequently, they were ground in a grain mill (28,000 RPM 850 W), then sieved using a 0.3 mm mesh stainless steel sieve (Pujadas P 350.730). The flour obtained was packaged in airtight plastic and stored at 4 °C. Type 65 wheat flour (Bavo) produced by the Grand Moulin factory in Conakry, Guinea was collected from the Dalaba market. It was used to prepare the natural sourdough from a flour-water mixture (1 : 1), fermented at 30 °C for seven (7) days with daily refreshment. The Phytic Acid Assay Kit (K-PHYT; 700004327 made in Ireland), containing: alkanine phosphatase, phytase, phytase assay buffer (pH = 5.5), alkanine phosphatase assay buffer (pH = 10.4), and phosphorus standard was purchased from Megazyme International. Various chemicals (ammonium molybdate, ascorbic acid, concentrated sulfuric acid, trichloroacetic acid, hydrochloric acid, sodium hydroxide) were used.

2.2 *Experimental methodology*

The experimental approach (Figure 1) was carried out following the methods described by Sokrab et al. [14] and Nsabimana et al. [4] with a slight modification. The experimental design was based on three formulations, namely: a control fermentation consisting of sterilized corn flour suspension without the addition of sourdough (FT), spontaneous fermentation consisting only of spontaneously fermented flour (FFS), and fermentation with starter containing sterilized corn flour suspension and natural sourdough (FFL). The suspensions were prepared according to three flour-water ratios (w/v): 1 : 1, 1 : 2, and 1 : 3, representing three levels of substrate hydration. Also, three inoculation proportions (2, 5 and 10%; w/v) were carried out for the FFL. Then, four-time conditions (fermentation for 12, 24, 36, and 48 h) were applied. Each experimental condition was tightly covered with tissue and performed in triplicate. Indeed, the inoculum used was prepared from wheat flour, chosen for its richness in natural fermentative microflora, primarily composed of lactic acid bacteria and endogenous yeasts. This choice could promote rapid fermentation kinetics and efficient acidification of the medium, consistent with the observations of De Vuyst [13] and Gänzle [15], who described the high microbial diversity of wheat sourdough. The use of wheat flour as an inoculum carries a potential risk of transferring allergens, particularly gluten proteins (gliadins and glutenins), to the fermented substrate.

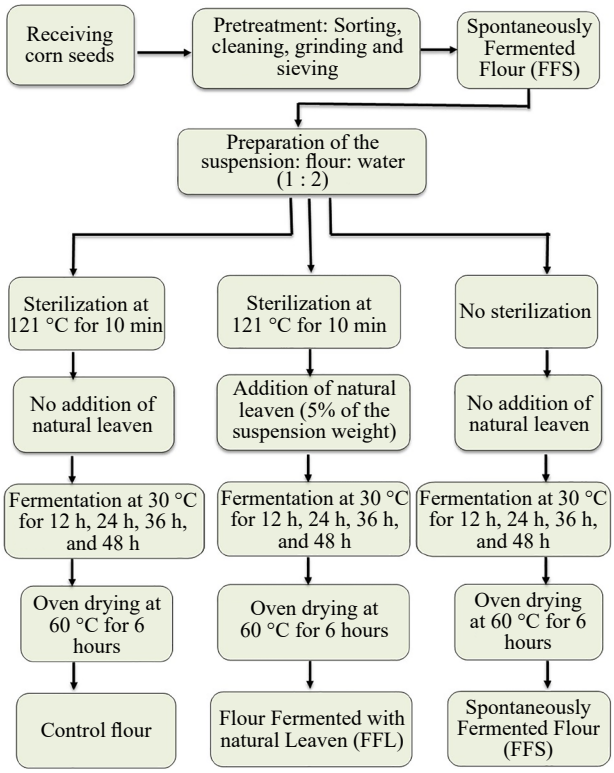


Figure 1. Schematic presentation of the experimental approach

The suspensions for FT and FFL were prepared by separately sterilizing corn flour and distilled water in an autoclave at 121 °C for 10 min and cooling for 30 min at room temperature before mixing them aseptically under the hood to avoid gelatinization of the substrate starch. The sterilized FFL suspension was aseptically inoculated with 2, 5, and 10% natural starter culture (containing a microbial population of 1×10^7 CFU/mL). All suspension containers were incubated in a Memmert incubator (model INB 200) at 30 °C for 48 h, while sampling every 12 h. The acidity, pH, and phytic acid content for each of these samples were determined. At the end of fermentation, decantation was carried out to separate the fermentation water and the fermented flour. The samples were transferred onto aluminum foil and then dried in an oven at 60 °C for 6 h. The dried samples were then ground in a grain mill (28,000 RPM 850 W) and stored at 4 °C for phytic acid content analysis.

2.3 *Chemical analyses*

All chemical analyses were performed in triplicate.

2.3.1 *Determination of moisture content*

The moisture content of corn flour was determined following the method described by Castro-Alba et al. [16]. Thus, Moisture content was determined using 5 g of maize flour placed in pre-dried and pre-weighed glass Petri dishes. Samples were dried in a hot-air oven (Daihan Scientific Wig 155 L) at 105 °C for 24 h until constant weight. The formula below was used to calculate the moisture content.

$$H\ (\%) = \frac{P1\ (\mathrm{g}) - P0\ (\mathrm{g})}{P\ (\mathrm{g})} \times 100.$$

- $P0$ (g) = weight of the dried Petri dish.
- $P1$ (g) = weight of the sample and Petri dish after drying.
- P (g) = weight of the sample.

2.3.2 *Determination of pH and acidity*

The pH and acidity of the samples (FFS, FT, and FFL) were determined following the method described by Castro-Alba et al. [16]. For pH measurement, 10 g of each sample was placed in a 250 mL beaker and mixed with 90 mL of distilled water. The mixture was thoroughly stirred using a magnetic stirrer for 10 minutes until complete dissolution. The pH of the suspension was then measured by immersing the electrode of a pH meter (Metrohm 744) into the homogenized mixture.

For acidity determination, 10 g of the sample was placed in a 250 mL beaker and mixed with 90 mL of distilled water, followed by 10 minutes of stirring. The resulting suspension was titrated with 0.1 N sodium hydroxide using 1% phenolphthalein as an indicator. Acidity was expressed as grams of lactic acid per liter (g/L).

The following formula was used to calculate acidity:

$$\text{Acidity}\ (\mathrm{g/L}) = \frac{V(\mathrm{NaOH}) \times C(\mathrm{NaOH}) \times M}{V}$$

- V(NaOH) poured = volume of NaOH poured.
- C(NaOH) = concentration of NaOH.
- M = Molar mass of lactic acid.

2.3.3 *Determination of phytic acid content*

The colorimetric method described by McKie and McCleary [17] was used. This technique was carried out in several stages:

▪ **Preparation of the color reagent**

The color reagent consisted of two solutions, A and B.

Solution A was prepared by dissolving 10 g of ascorbic acid in distilled water in a 100 mL volumetric flask, adding 5.35 mL of concentrated sulfuric acid, and then making up the volume to the mark with distilled water.

Solution B was prepared in a 25 mL volumetric flask by dissolving 1.25 g of ammonium molybdate in distilled water and bringing the volume to 25 mL with distilled water. Finally, the two solutions were combined by mixing 1 part of Solution B with 5 parts of Solution A to obtain the color reagent.

▪ **Extraction of phytic acid**

To extract phytic acid, 1 g of the sample was weighed into a 100 mL glass beaker, followed by the addition of 20 mL of hydrochloric acid (0.66 M). Then, the beaker was covered with aluminum foil and stirred using a magnetic

stirrer overnight. At the end of stirring, 1 mL of extract was transferred into a 1,500 µL Eppendorf tube and centrifuged at 13,000 rpm for 10 min. After centrifugation, 0.5 mL of the supernatant was immediately transferred into a new 1,500 µL Eppendorf tube and neutralized using 0.5 mL of sodium hydroxide solution (0.75 M). Thus, the neutralized sample extract was used in the enzymatic dephosphorylation reaction procedure.

▪ **Enzymatic dephosphorylation reaction**

On each neutralized sample extract, an enzymatic dephosphorylation reaction (total phosphorus and free phosphorus) was applied. For the total phosphorus reaction, the following were added in a 1,500 µL Eppendorf tube: 0.60 mL of distilled water, 0.20 mL of the phytase assay buffer, 0.05 mL of the sample extract, and 0.02 mL of the phytase. For the free phosphorus reaction, 0.62 mL of distilled water, 0.20 mL of the phytase assay buffer, and 0.05 mL of the sample extract were added in a second 1,500 µL Eppendorf tube. Then, the reaction solutions were thoroughly mixed by vortexing and incubated in a water bath at 40 °C for 10 min. After incubation, 0.02 mL of Alkaline Phosphatase (ALP) and 0.20 mL of ALP assay buffer were added to the total phosphorus reaction, and 0.02 mL of distilled water and 0.20 mL of ALP assay buffer were added to the free phosphorus reaction. The reaction solutions were again thoroughly vortexed and incubated at 40 °C for 15 min. All reactions were stopped by adding 0.3 mL of trichloroacetic acid (50%, w/v), then vigorously vortexed, followed by centrifugation at 13,000 rpm for 10 min. Finally, the supernatant was transferred to a new 1,500 µL Eppendorf tube and used for colorimetric phosphorus determination.

2.3.3.1 *Colorimetric determination of phosphorus*

In a 1,500 µL Eppendorf tube, 0.5 mL of the color reagent prepared above was added to 1 mL of supernatant, then mixed thoroughly by vortexing and incubated in a water bath set at 40 °C for 1 h. After incubation, mix again by vortexing, and then 1 mL was transferred to a microcuvette, and the absorbance was measured at 655 nm. A reagent blank (containing all reagents except the sample) was processed in parallel, and its absorbance was subtracted from all measurements to correct for background color. The absorbance values of the samples and phosphorus standard solutions were used for the calculation of total phosphorus and phytic acid.

2.3.3.2 *Preparation of the phosphorus calibration curve*

Phosphorus standard solutions STD0, STD1, STD2, STD3, and STD4, at concentrations of 0, 0.5, 2.5, 5, and 7.5 µg phosphorus/mL, respectively, were prepared in distilled water from the 50 µg/mL phosphorus standard solution. Each standard solution was used for the colorimetric determination of phosphorus, and the absorbance values of 655 nm measured using the spectrophotometer (model 4251/50) were used for the calculation of total phosphorus and phytic acid.

2.3.3.3 *Calculation*

▪ **Phosphorus calibration curve**

After measuring the absorbance at 655 nm of each prepared phosphorus Standard (STD 0-4), the absorbance of STD 0 was subtracted from that of the other Standards (STD 1-4) to obtain the absorbance variations ($\Delta A_{\text{phosphorus}}$). Then, the slope ($M$) for each standard and the mean (M) were calculated using the formulas below:

$$M = \frac{P\ (\mu\text{g})}{\Delta A_{\text{phosphore}}}$$

$$M = \frac{M_{\text{STD1}} + M_{\text{STD2}} + M_{\text{STD3}} + M_{\text{STD4}}}{4}$$

P (µg) = phosphorus concentration in micrograms.

▪ **Phosphorus/Phytic Acid Content**

The phosphorus content was determined using the spectrophotometer (model 4251/50), at an absorbance at 655 nm. The change in phosphorus absorbance ($\Delta A_{\text{phosphorus}}$) was calculated by subtracting the absorbance of the "free

phosphorus" sample from that of the "total phosphorus" sample, then calculating the phosphorus content according to the following formula:

$$P\ (\text{g}/100\ \text{g}) = \frac{M \times 20 \times 55.6}{1,000 \times 1 \times 1} \times \Delta_{\text{phosphorus}}$$

$$\text{Phytic acid}\ (\text{g}/100) = \frac{P\ (\text{g}/100\ \text{g})}{0.282}$$

P = phosphorus content;

M = average of the slopes of the standards.

For phytic acid, the following results are obtained: where 0.282 is the factor used to convert the measured phosphorus content to phytic acid content, since phytic acid contains 28.2% phosphorus.

The phytic acid calculation assumes that the measured amount of phosphorus is specifically released by phytic acid (IP 6) and not by other phosphoric esters, including lower myoinositol phosphates (InsP 1-5).

2.4 *Statistical analysis of data*

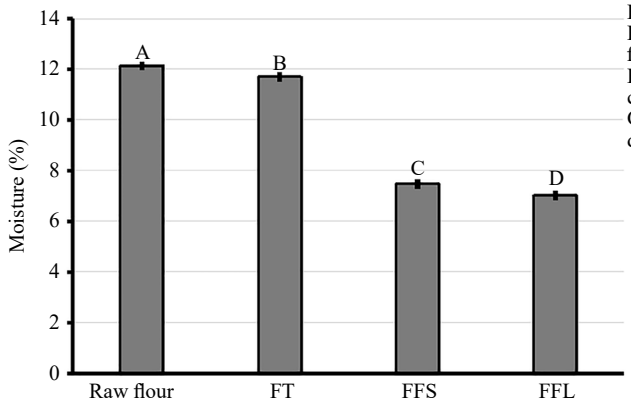
XLSTAT V2019 software was used for statistical tests. All fermentation trials were performed in triplicate. Data were reported as mean values ± standard deviation, and we applied parametric tests for statistical analysis. Analysis of Variance (ANOVA) was used to compare the influence of fermentation type, hydration level, starter culture rate used, and fermentation duration on pH, acidity, and phytic acid content. Following ANOVA, the Tukey test was considered to compare means at a threshold of 0.05.

3. Results and discussion

Moisture and phytic acid contents were determined in raw and fermented flour samples, while pH and acidity were measured in suspensions (FFL, FFS, and FT) at regular 12 h intervals (0, 12, 24, 36, and 48 h).

3.1 *Moisture content of flours*

The moisture contents found in raw flour and fermented flours, FFL, FFS and FT, are 12.13 ± 0.31%, 7.02 ± 0.27%, 7.47% and 11.7 ± 0.18% respectively (Figure 2). Following ANOVA, the Tukey test revealed that fermentation significantly influenced ($P < 0.05$) the moisture.



FFS: Spontaneously fermented flour;
FT: Control fermentation consisting of sterilized corn flour suspension without the addition of sourdough;
FFL: Fermentation with starter containing sterilized corn flour suspension and natural sourdough;
Capital letters on the histograms represent statistical differences obtained from analysis of variance

Figure 2. Moisture content value determined on samples

These results corroborate those of Nsabimana et al. [4], who observed a decrease in moisture from 10.6% in raw maize flour to 6.94% and 6.49% respectively, in spontaneously fermented maize flour and maize flour fermented with yogurt containing viable *L. Casei*. Furthermore, the results are supported by the work of Wei et al. [18] and Senanayake et al. [19], who demonstrated that microbial activity during fermentation modifies the structure of starch, protein, and fiber, releasing more bound water for evaporation during drying.

3.2 *pH measurement during fermentation*

In both naturally fermented (FFL) and spontaneously fermented (FFS) samples, a variation in pH value was recorded throughout the fermentation compared to the control samples (Figure 3).

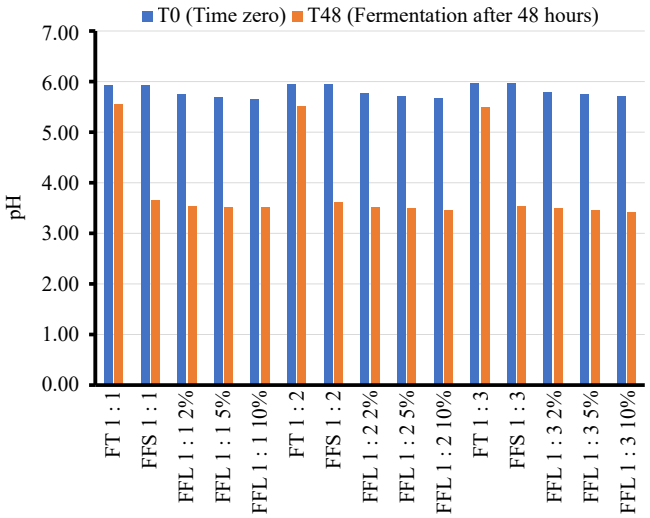


Figure 3. pH value obtained on samples during fermentation

The observed variation is more marked in samples fermented with natural sourdough (FFL), particularly at 10% sourdough addition with the hydration level 1 : 3, where the pH decreased from 5.71 ± 0.0071 to 3.41 ± 0.057 after 48 h of fermentation. A similar trend is observed in samples fermented spontaneously with the hydration level 1 : 3, decreasing from 5.96 ± 0.0035 to 3.53 ± 0.0245. As for the control samples, the pH remained relatively stable. Following the Analysis of Variance (ANOVA), the Tukey test illustrated that several factors, including the type of fermentation, the fermentation duration, and the proportion of sourdough added, had a significant impact ($P < 0.05$) on the pH value. The pH variation observed in the FFL could be due to the presence of a highly acidifying microbial consortium, as is often the case in mature sourdough starters. Certain lactic acid bacteria (e.g., *Lactobacillus plantarum*, *L. fermentum*, *L. brevis*, *Pediococcus spp*.) rapidly metabolize carbohydrates and produce a significant amount of lactic acid, thus causing a sharp drop in pH at the beginning of fermentation. Yeasts commonly found in sourdough starters (e.g., *Saccharomyces cerevisiae*, *Candida milleri*) could amplify this effect by stimulating bacterial growth. Although the microbial composition of the sourdough starter has not been analyzed, these mechanisms likely explain the acidification profile observed in the natural sourdough. These observations are supported by the work of Gobbetti et al. [20], who reported that the addition of natural starter cultures, often rich in lactic acid bacteria, increases the production of organic acids, resulting in a rapid decrease in pH. Furthermore, Adesulu-Dahunsi et al. [21] and Fang et al. [22] pointed out that natural starter culture helps maintain better pH stability, thus creating conditions conducive to homogeneous lactic acid fermentation while inhibiting the growth of pathogenic microorganisms. The observed difference between FFL and FFS samples can be attributed to the specificity and stability of the microbial consortium present in natural starter culture, as mentioned by De Vuyst et al. [23] and De Vuyst and Neysens [13], who reported that natural starter cultures harbor stabilized microbial communities, which are particularly efficient in producing significant amounts of lactic acid and

acetic acid. This microbial dynamic is responsible for the rapid decrease in pH, thus reinforcing the importance of the choice of sourdough in fermentation processes.

3.3 *Determination of titratable acidity*

Titratable acidity, measured in grams per liter of lactic acid, showed a variation over time, both in samples fermented with natural sourdough (FFL) and in those fermented spontaneously (FFS). This trend was influenced by a double factor: high hydration level associated with a high proportion of sourdough added (Figure 4). After 48 h, the acidity in the FFL sample with 10% sourdough with the hydration level of 1 : 3 showed a significant increase, from 4.68 ± 0.00707 to 13.63 ± 0.035 g/L.

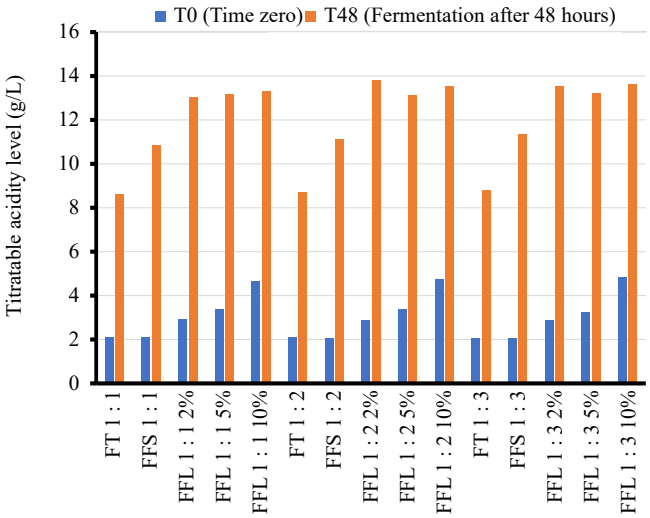


Figure 4. Titratable acidity level obtained on samples during fermentation

For FFL samples with 10% sourdough at 1 : 2 and 1 : 1 hydration levels, acidity ranged from 4.78 ± 0.021 to 13.53 ± 0.045 g/L and from 4.87 ± 0.025 to 13.31 ± 0.057 g/L over the same period, respectively. It was also observed that the variation in acidity depended on the proportion of sourdough added. For example, in the FFL sample with 2% sourdough and a hydration ratio of 1 : 2, the acidity ranged from 2.91 ± 0.00707 to 11.19 ± 0.014 g/L, while in the sample containing 5% sourdough at an identical hydration ratio, it was measured between 3.37 ± 0.032 and 16.04 ± 0.707 g/L during the first 24 hours. Regarding the FFS samples, the hydration level also had an impact on the acidity. After 48 h, the acidity ranged from 10.87 ± 0.078 g/L for the FFS sample at a hydration ratio of 1 : 1, reaching 11.15 ± 0.22 g/L and 11.37 ± 0.014 g/L for the samples with hydration ratios of 1 : 2 and 1 : 3, respectively. In comparison, the control sample (FT) remained relatively stable, with an acidity of approximately 8.81 ± 0.018 g/L, thus demonstrating the effect of the applied treatments.

The results indicate that an inoculated medium, such as that containing natural sourdough, promotes a faster and more significant accumulation of organic acids. This increase in acidity is correlated with the decrease in pH, highlighting the link between these two parameters during fermentation. Following the Analysis of Variance (ANOVA), the Tukey test established significant effects ($P < 0.05$) of fermentation duration, the proportion of sourdough added, and the hydration level on acidity. In addition, the interaction between the hydration level and the proportion of sourdough also showed a significant influence on acidity ($P < 0.05$), highlighting the importance of these two factors in the fermentation process. The variations in acidity observed during fermentation reflect the formation of organic acids, due to the consumption of fermentable substrates by microorganisms. According to the research of Jideani et al. [24] and Nsabimana et al. [4], this variation is a key indicator of the efficient progression of the fermentation process and reflects the dynamic activity of the microflora involved. In our study, fermentation

with natural starter (FFL) produced significantly higher acidity levels compared to spontaneous fermentation (FFS) and control samples, at all hydration levels. This phenomenon can be attributed to the presence of an active and pre-adapted microflora in natural starter, mainly composed of lactic acid bacteria and yeasts, which are particularly efficient in synthesizing organic acids. These results corroborate those of Dev et al. [25], who noted that early fermentation leads to more marked acidification. Interestingly, in the FFL sample with 5% sourdough and a hydration ratio of 1 : 2, the acidity initially increased from 3.37 ± 0.032 and 16.04 ± 0.707 g/L in 24 h, before stabilizing at about 13.13 ± 0.021 g/L after 48 h. This dynamic is supported by the work of Adebo et al. [26] 48, 72, 96, and 120 h, who observed that acidity increases rapidly during the first 24 h, followed by stabilization or a slight decrease around 48 h, which could be due to the depletion of fermentable substrates or the accumulation of metabolites. Furthermore, Samtiya et al. [27] trace minerals (iron, zinc, magnesium, manganese, etc. pointed out that lactic acid bacteria present in cereal flours play a crucial role in lactic acid production, which improves the stability and microbiological safety of the fermentation process.

3.4 *Determination of phytic acid content*

The analyses on the determination of phytic acid in the samples according to the hydration level and fermentation duration led to the results mentioned in Figures 5, 6, and 7. Thus, the raw flour initially analyzed contained 0.369 ± 0.043 g of phytic acid per 100 g. During the fermentation process, a significant decrease ($p < 0.05$) of this content was observed in all FFL and FFS samples, with more marked results in the FFL samples compared to those of the FFS. After 48 h of fermentation, the measured phytic acid levels were 0.249 ± 0.057 g/100 g (a reduction of 32.43%), 0.230 ± 0.071 g/100 g (a reduction of 37.73%), and 0.196 ± 0.106 g/100 g (a reduction of 46.92%) for the FFS samples with the flour/water ratios of 1 : 1, 1 : 2, and 1 : 3, respectively (Figure 5).

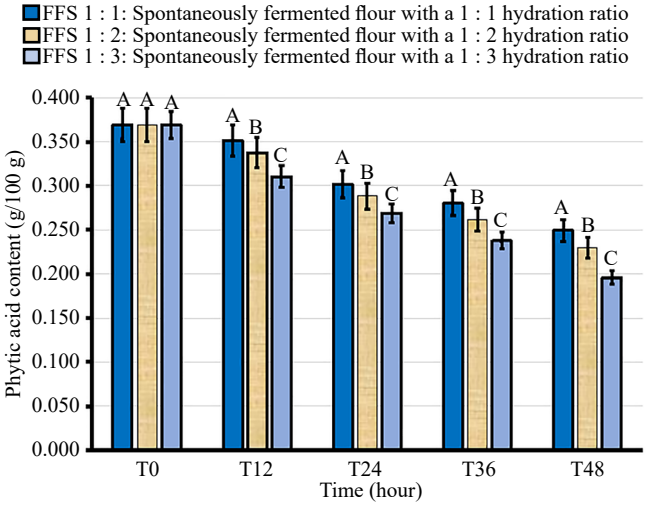


Figure 5. Phytic acid content of FFS samples according to fermentation time and hydration level

FFL samples showed a more pronounced decrease ($p > 0.05$), reaching 0.102 ± 0.028 g/100 g in the sample containing 10% sourdough with a flour/water ratio of 1 : 3 (Figure 6). The decreases in phytic acid in these samples could be due to the activation of endogenous and microbial phytases, which are particularly active in an acidic environment. For instance, Gupta et al. [28] demonstrated that phytase activity is favored by an acidic pH, as well as by the presence of phytase-producing microorganisms, including certain strains of yeast and lactic acid bacteria. Overall, these results highlight the ability of natural sourdough fermentation to reduce the phytate content of corn flour under local processing conditions. However, a significant limitation of this study is the lack of microbiological characterization of the sourdough starter. Since the functionality of the sourdough starter depends on its microbial community, the lack of information on its lactic acid bacteria and yeast composition restricts the interpretation of phytate degradation

mechanisms. Future studies should include microbial profiling to better correlate the sourdough starter composition with its functional effects.

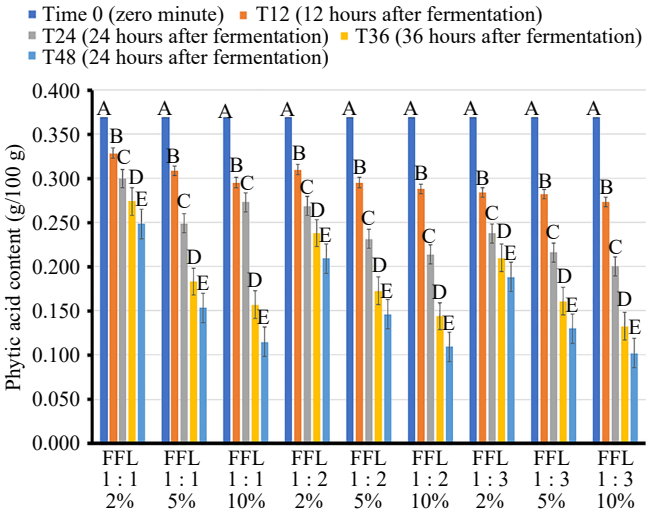


Figure 6. Phytic acid content of FFL samples according to time, proportion of sourdough added, and hydration level

These results indicate that a high water/flour ratio, such as 1 : 3, promotes more efficient phytate hydrolysis. This observation is supported by the work of Comasio et al. [29], who note that increasing the hydration level facilitates the diffusion of substrates and improves the mobility of phytases, making these enzymes more accessible to phytates. Furthermore, as pointed out by De Vuyst et al. [23], the use of natural sourdough intensifies enzymatic activity due to the presence of specific microorganisms such as *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, which play a crucial role in phytic acid hydrolysis. It is important to note that phytic acid hydrolysis took place in acidic environments throughout the fermentation for both FFL and FFS samples. This acidity created optimal conditions for the activity of phytases, whether endogenous (naturally present in cereals) or exogenous (enzymes produced by the sourdough flora or indigenous flora). This observation is corroborated by Castro-Alba et al. [16], who indicated in a study on quinoa that acidic pHs promote the activation of endogenous phytases as well as enzymes produced by the sourdough microflora, thus contributing to the degradation of phytic acid. Furthermore, our results differ from those reported by Nsabimana et al. [4], who found a reduction in phytic acid content of 51.8% in the case of spontaneous fermentation and of 68.7% in fermentation with yogurt containing viable strains of *Lactobacillus casei* after 24 h. The difference between our results and those of Nsabimana et al. [4] likely stems from several factors: variations in native phytase levels among maize varieties, differences in microbial composition and phytase activity between our natural sourdough and their yoghurt starter, and distinct fermentation conditions (temperature, hydration, inoculum size). This discrepancy could be attributed to the quality of the substrate used in our study, as well as the nature of the starter cultures employed. In the FT samples, as shown in Figure 6, the reduction of phytic acid was almost negligible. The slight reduction observed in these FT samples can be attributed to the inactivation of endogenous phytases present in maize and the growth of indigenous flora following sterilization, a phenomenon noted by Atuna et al. [8] in their study on traditional processing methods aimed at reducing phytate content in cereal flours. Indeed, the decrease in phytate content observed during fermentation suggests a potential improvement in the bioavailability of minerals such as iron and zinc, which are strongly complexed by phytates in cereal matrices, as reported by Hurrell et al. [30] and Lopez et al. [31] Phytic Acid (PA). However, this explanation remains hypothetical within the framework of the present study, as the content of free minerals or their availability was not experimentally measured after fermentation. Consequently, it is more appropriate to consider that phytate degradation could reduce interactions by limiting the absorption of iron and zinc, without definitively concluding that there is an improvement in bioavailability.

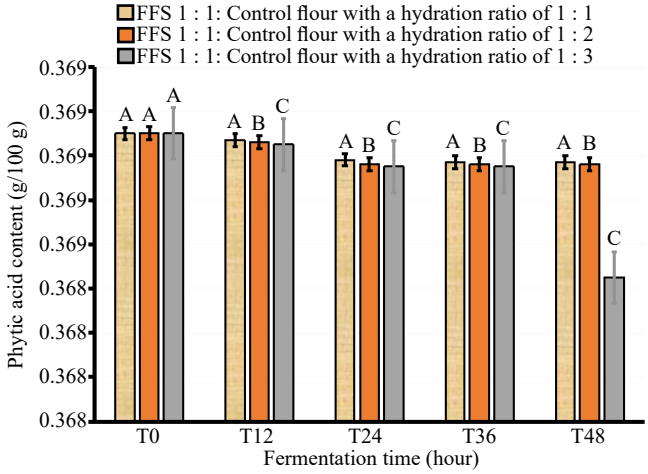
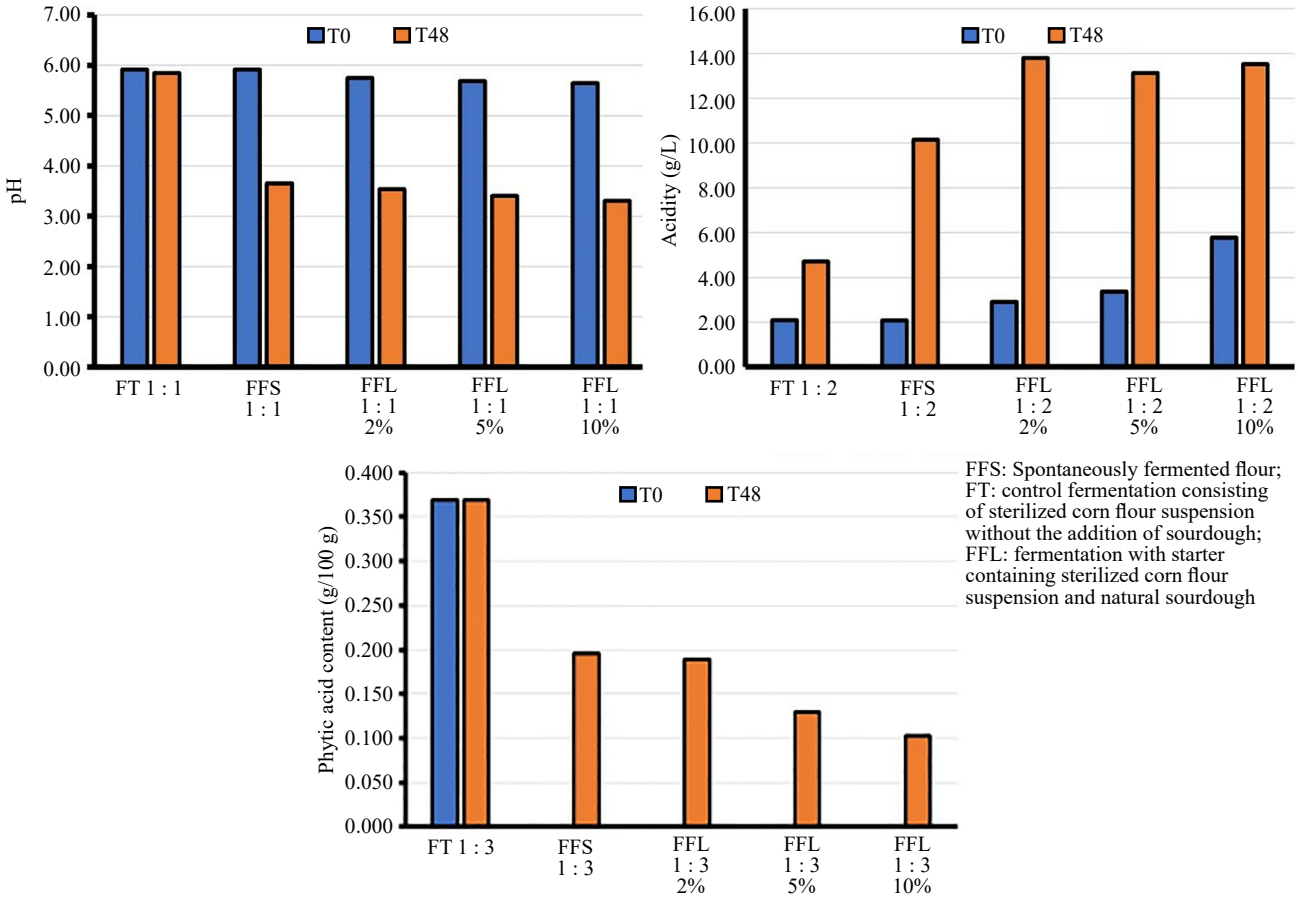


Figure 7. Phytic acid content of control samples according to fermentation time and hydration rate

3.5 *Overall comparison of fermentation methods*

The comparative analysis of the different fermentation methods highlights trends in the evolution of the measured parameters (pH, acidity, and phytic acid values) as shown in Table 1 and Figure 8.



FFS: Spontaneously fermented flour; FT: control fermentation consisting of sterilized corn flour suspension without the addition of sourdough; FFL: fermentation with starter containing sterilized corn flour suspension and natural sourdough

Figure 8. Comparison of fermentation methods after 0 and 48 h

Table 1. Comparison of fermentation methods after 48 h

Parameters	FT 1 : 1	FFS 1 : 1	FFL 1 : 1 2%	FFL 1 : 1 5%	FFL 1 : 1 10%	FT 1 : 2	FFS 1 : 2	FFL 1 : 2 2%	FFL 1 : 2 5%	FFL 1 : 2 10%	FT 1 : 3	FFS 1 : 3	FFL 1 : 3 2%	FFL 1 : 3 5%	FFL 1 : 3 10%
pH T0	5.92	5.92	5.75	5.69	5.65	5.94	5.95	5.78	5.71	5.67	5.96	5.96	5.79	5.75	5.71
pH T48	5.55	3.65	3.54	3.51	3.51	5.51	3.61	3.51	3.49	3.46	5.48	3.53	3.50	3.46	3.41
Acidity (g/L) at T0	2.13	2.12	2.93	3.37	4.68	2.09	2.08	2.91	3.37	4.78	2.05	2.04	2.89	3.25	4.87
Acidity (g/L) T48	8.63	10.87	13.06	13.19	13.31	8.72	11.15	13.80	13.13	13.53	8.81	11.37	15.21	13.24	13.63
Phytic acid (g/100 g) at T0	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.369	0.36	0.36
Phytic acid (g/100 g) at T48	0.36	0.25	0.24	0.15	0.11	0.36	0.23	0.20	0.14	0.11	0.36	0.19	0.18	0.13	0.10

T0: 0 hours; T48: 48 hours; FT: control fermentation consisting of sterilized corn flour suspension without the addition of sourdough; FFS: Spontaneously fermented flour; FFL: fermentation with starter containing sterilized corn flour suspension and natural sourdough. The ratios 1 : 1, 1 : 2 and 1 : 3 are hydration levels (flour: water); the rates 2%, 5% and 10% represent the proportion of leaven added

Regarding pH, the fermentation process using natural sourdough (FFL) is distinguished by a significant acidification ($p < 0.05$) compared to spontaneous fermentation (FFS) and the control (FT). The pH of FFL samples with high sourdough concentrations (5% and 10%) recorded the highest decrease, reaching 3.51 ± 0.077 after 48 h in the 1 : 1 hydration level compared to 3.65 ± 0.085 in FFS samples at the same duration and hydration level. This indicates an intense metabolic activity of lactic acid bacteria. This finding is corroborated by the work of De Vuyst et al. [32] and Randazzo et al. [33], who show that a stable microbial consortium favors the rapid production of organic acids, including lactic acid and acetic acid. Furthermore, the control sample (FT) showed little variation in pH, remaining above 5.50 even after 48 h, suggesting low activity of endogenous enzymes and the absence of native microorganisms due to sterilization [34]. Naturally fermented sourdough flour (FFL 1 : 3, 10%) after 48 hours of fermentation has a pH of approximately 3.41, indicating advanced acidification of the medium capable of inhibiting phytase. This is consistent with the findings of Greiner and Konietzny [35], who noted that most plant-derived phytases have an optimum between ~4.5 and 6.0, with their activity dropping sharply at pH levels below 4.0. They indicate that at a pH of 3.41, the activity of endogenous phytases in the flour is very likely significantly reduced. Similarly, Haros et al. [36] report that some lactic acid bacteria exhibit optimal phytase activity around pH 4-7, but that this activity decreases markedly at very acidic pH levels. Thus, even if natural sourdough contains microbially derived phytases, their contribution to phytate degradation at pH 3.41 likely remains limited. Consequently, lowering the pH to 3.41 suggests that the kinetics of phytate degradation are probably slowed, or even halted, despite the presence of microorganisms potentially capable of hydrolyzing this compound. Finally, the lack of analysis of the liquid phase removed by decantation could lead to an underestimation of the total phytate degradation, due to the possibility of the presence of soluble forms of phytate or hydrolysis products.

Regarding titratable acidity, the highest levels (> 13 g/L) were observed in FFL. FFS also reached a significant concentration (approximately 11 g/L) after 48 h, but remained lower than that of sourdough fermentations. This difference is explained by the longer adaptation phase of spontaneous microflora and their variable composition [15]. Furthermore, studies have shown a positive correlation between phytic acid degradation and medium acidification [37]. The most notable reductions (≥ 50%) occurred in FFL, particularly with a sourdough proportion of 10% and high hydration (1 : 3). In contrast, FT showed nearly constant phytic acid levels, suggesting that heat-inactivated endogenous phytases are present in corn. In conclusion, the use of a natural starter at a minimum concentration of 5% and a hydration ratio greater than 1 : 2 allows simultaneous optimization of acidification and phytic acid reduction. It is also plausible that hydration level and inoculum size interacted synergistically, as higher hydration not only improves substrate diffusion but also promotes microbial proliferation and metabolic activity, thereby enhancing acid production and phytase-mediated phytate degradation.

4. Conclusion

The effects of fermentation with natural starter on the phytic acid content of corn flour are essential to improve the nutritional quality of food, promote sustainable food practices, and meet the nutritional needs of communities, particularly in Guinea. Indeed, this study revealed that a greater degradation of phytic acid in corn flour occurs during controlled fermentation with natural starter, compared to spontaneous fermentation. The high levels of acidification observed when using a natural starter made it possible to achieve an optimal pH for the activation of the phytase enzyme, thus promoting increased enzymatic degradation of phytic acid. This advance in nutrition opens promising prospects for artisanal and community applications in Guinea, where improving the nutritional value of food is essential. In our study, the most significant reductions (≥ 50%) were observed in the FFL medium, particularly with a starter culture proportion of 10% and high hydration (1 : 3). Furthermore, the FT medium exhibited nearly constant phytic acid levels, suggesting that heat inactivated the endogenous phytases present in the maize. However, this study revealed some limitations, as microbiological analysis was not performed. Therefore, in future work, we plan to conduct microbiological analysis and explore the characterization and identification of local strains of natural sourdough, particularly lactic acid bacteria and yeasts. We are also considering using HPLC/LC-MS for one of the results obtained from the photochemical analyses.

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Data availability

I don't have any research data outside the submitted manuscript file.

Author contributions

Vamougna Soumaoro: Methodology, writing-review and editing, resource; Moriken Sangaré: Conceptualization, investigation, validation of the methodology, resources, data curation, visualization, writing-original draft, writing -review and editing; Mamady Diawara: Revision, data processing, validation; Sékou Kouyaté: Validation and data processing.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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