



THE EFFECT OF PROCESSING VARIABLES ON THE NUTRITIONAL QUALITY OF PITO - A LOCALLY BREWED ALCOHOLIC BEVERAGE IN NIGERIA

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Abstract: *This study investigated the effect of different processing variables, such as varieties of grains, supplementation with pineapple juice, use of starter cultures (*L. fermentum* and *Saccharomyces cerevisiae*), and varied steeping time, on the nutritional quality of pito. The vitamin A and C content, proximate analysis, anti-nutritional values, and mineral content of pito produced under various variables were determined. In all the variables, the highest vitamin A content was recorded in pito produced using the combination of starter cultures (6.99 mg/100 mL), while the highest vitamin C content was recorded in pito produced from grains steeped for 48 hours (5.27 mg/100 mL). The highest moisture content (98.49 %) and alcohol content (2.35 %) were observed in pito produced from maize. The pito produced from grains steeped for 48 hours had the lowest oxalate (5.25 mg/100 mL) and phytate (1.03 mg/100 mL) content, and the highest magnesium (32.50 mg/100 mL) and calcium (82.50 mg/100 mL) content. Pito produced from the combination of both starter cultures (*L. fermentum* and *Saccharomyces cerevisiae*) had the highest iron content (6.65 mg/100 mL). All the pito produced using the different variables showed that the beverage is highly nutritious; therefore, Pito produced from either maize or red sorghum as base grain, addition of pineapple juice, combination of *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae* as starter cultures, and steeping the grains for 48 hours will ensure consistent quality and maximize health benefits of the beverage.*

Keywords: *Fermentation, starter culture, processing variables, varieties of grains, steeping time, pito*

1. Introduction

Fermentation is a widely utilized method across Africa for food processing and preservation [1, 2]. Its popularity stems from diverse applications, including daily consumption, social occasions, and traditional customs. Indigenous fermented foods play a significant role in combating hunger and malnutrition in many households [3, 4]. This process involves the transformation of carbohydrates into alcohol under anaerobic conditions, typically driven by the action of bacteria or fungi [5]. African traditional beverages, rich in live microorganisms like bacteria

and yeast, offer multiple health benefits. These microbes and the bioactive compounds they produce during fermentation interact with the gut microbiota, supporting immune function [5, 2]. Fermentation techniques vary, with key types including alcoholic, lactic acid, acetic acid, and alkaline fermentation. Pito is a traditional alcoholic drink commonly prepared from sorghum, millet, or maize, and is popular in regions such as Ghana, Togo, and northern Nigeria. Pito is a traditional golden to dark brown alcoholic beverage, known for its sour or sweet flavor profile and rich composition of sugars,

lactic acid, amino acids, alcohol, and essential vitamins. [6]. Recent studies confirm that pito contains notable levels of nutrients, including calcium, magnesium, potassium, and iron, along with B-group vitamins and organic acids that contribute to its taste and nutritional value. Its composition is shaped by fermentation dynamics and the type of grains used, typically sorghum or maize, which influence both its sensory attributes and health-promoting properties [6]. The beverage is made through spontaneous fermentation involving yeast and lactic acid bacteria [5]. Species of *Saccharomyces* and other yeast genera have been associated with the alcoholic fermentation of Konkomba and Nadom pito varieties in northern Ghana [7]. Notably, *Saccharomyces cerevisiae* is the dominant yeast found in the fermentation of pito and its Burkinabe counterpart, dolo [8]. During fermentation, bacteria such as *Limosilactobacillus* and *Leuconostoc* species play a key role in lowering pito's pH, contributing to its sour profile [9]. The diversity of yeast strains present in these beverages is influenced by factors including the use of back-slopping techniques, the nature of raw materials, and the fermentation substrate [8]. Currently, there is a limited standardized procedure for pito production, resulting in varied product quality. Because information on optimizing pito for large-scale, nutritionally enhanced production remains limited, this study seeks to address that gap. The core aim is to identify optimal fermentation conditions and evaluate the impact of various processing factors on the beverage's quality and consistency.

2. Methodology

2.1 Sample collection

A fresh sample of pre-fermented sorghum-based on pito, which was obtained, along with red and white sorghum grains, yellow

maize, and pineapple fruits, all sourced from Bodija Market in Ibadan, Oyo State.

2.2 Processing method for Pito

Sorghum and maize were carefully weighed, sorted, graded, and thoroughly cleaned. The cleaned sorghum grains were steeped in water for 24 to 48 hours, after which the excess water was drained. These soaked grains were then malted over a period of three days by placing them in a basket lined with banana leaves or wrapped in a clean polythene bag. Following the malting stage, the grains were dry-milled and mixed with water at a temperature of approximately 27 °C. This mixture was boiled for roughly eight hours. Once boiled, it was filtered using a fine mesh and then allowed to cool. A second filtration was done using a muslin cloth to obtain a clear extract. Fermentation began by leaving the filtrate overnight, utilizing a natural inoculum derived from an unfermented boiled mixture. After this initial fermentation, the mixture was boiled again for 12 hours, cooled, and then fermented for an additional 12 to 24 hours. In this final stage, the sediment from the first fermentation was used as inoculum to promote microbial activity [10].

2.3 Processing variables: The effects of the following variables were investigated:

Varieties of grains: 250 g of yellow maize, 250 g of red and white sorghum grains each were used to prepare 300 mL of pito.

Supplementation with pineapple juice: 250 g of red sorghum were prepared, and 50 mL of fresh pineapple was added to 300 mL of pito.

Period of steeping: 250 g of red sorghum was cleaned and sorted, and was steeped for 24 and 48 hours.

Use of starter cultures: Pure culture of *Limosilactobacillus fermentum*, *Saccharomyces cerevisiae*, and the combination of *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae*, which was isolated from traditionally

brewed pito, characterized, and used as starter cultures.

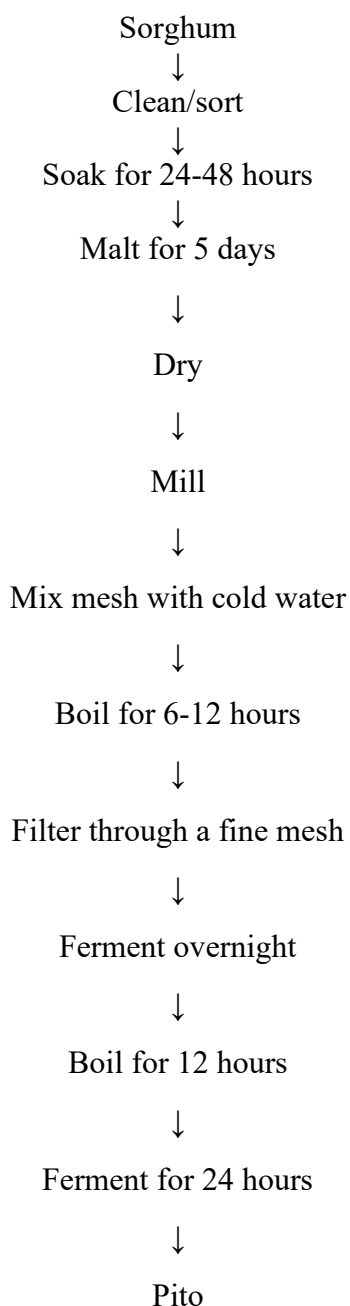


Fig. 1. Flow chart for traditionally brewed Pito

2.4 Proximate properties

2.4.1 Determination of moisture content:

Moisture content was assessed using the standard oven-drying method, as outlined by AOAC [13], and supported by more

recent studies in food analysis protocols [14].

2.4.2 Ash content determination: the ash content was determined using the AOAC official method [13], and supported by more recent protocols in food analysis [14].

2.4.3 Determination of crude fat content: The Soxhlet extraction technique, as described by AOAC [15], was employed to determine the crude fat content.

2.4.4 Crude protein determination: The crude protein content of the sample was analyzed using the micro Kjeldahl technique, following AOAC guidelines.

2.4.5 Crude fibre determination: The crude fibre was determined using AOAC method (Weende method) [16].

2.4.6 Alcohol determination: The specific gravity of the sample was measured using the pycnometer method.

2.5 Anti-nutritional components determination

2.5.1 Tannin quantification procedure: Tannin content was determined using a colorimetric method involving ethanol extraction and spectrophotometric analysis [17,18].

2.5.2 Oxalate determination procedure: Oxalate content was analyzed using a titrimetric method involving potassium permanganate [19, 20].

2.5.3 Total carotenoid quantification procedure: Total carotenoids were quantified using a solvent extraction method followed by spectrophotometric analysis [20].

2.5.4 Ascorbic acid determination procedure: Ascorbic acid content was quantified using a spectrophotometric method involving 2,6-dichlorophenolindophenol (DCPIP) as the chromogenic reagent.

Ascorbic acid concentration was calculated using a standard calibration curve, prepared from known concentrations of ascorbic acid. This method remains widely accepted

for its speed, sensitivity, and reliability in food analysis [20].

2.6 Mineral content determination

Potassium content in the samples was determined using flame photometry, a technique well-suited for detecting alkali metals due to its sensitivity and rapid response. Phosphorus was quantified using the phosphovanado-molybdate (yellow) method, following the guidelines outlined by AOAC [15].

For other essential minerals: magnesium (Mg), calcium (Ca), and iron (Fe), analysis was conducted using Atomic Absorption Spectrophotometry (AAS). Prior to measurement, samples underwent wet digestion to break down organic matter and release mineral elements into solution, as recommended by AOAC [15] protocols. All measurements were performed in triplicate to ensure accuracy and reproducibility.

2.7 Statistical analysis: The experimental data were analyzed using Analysis of variance (ANOVA) to determine significant differences between the means, and these were expressed as mean \pm standard deviation (SD). The level of significance was set at $p \leq 0.05$.

3. Results

The following variables: use of different varieties of grains, use of starter cultures, supplementation with pineapple juice, and varied steeping time were used to produce pito, and the results obtained were compared using the following nutritional parameters: proximate analysis, vitamin content, mineral content, and antinutritional content.

3.1 Vitamin contents

As shown in Table 1, pito produced from all the different variables was evaluated for the quantity of vitamins A and C present. Pito produced from maize (M) had the highest value of vitamin A (4.85 ± 0.03 mg/100 mL) and C (3.47 ± 0.08 mg/100 mL), while pito produced from white sorghum (WS) had the

lowest value of vitamin A (1.45 ± 0.04 mg/100 mL). Pito produced by adding pineapple juice (S) had the highest vitamin C (3.95 ± 0.33 mg/100 mL) and the lowest vitamin A value (3.80 ± 0.04 mg/100 mL). The Pito produced using both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae* (B) starter cultures had higher values of both vitamin A (6.99 ± 0.10 mg/100 mL) and C (4.56 ± 0.24 mg/100 mL) when compared with the pito produced from the single starter culture. Pito produced from red sorghum steeped for 48 hours (48 h) had the highest vitamin A (5.27 ± 0.23 mg/100 mL) and C (5.27 ± 0.23 mg/100 mL) values when compared to the one produced using 24 h steeping time. Recent studies confirm that fermentation can preserve or even enhance the vitamin content of cereal-based beverages, especially when supplemented with nutrient-rich ingredients like fruits [21]. The variation in vitamin levels across grain types may be attributed to differences in natural micronutrient content, fermentation efficiency, and bioavailability influenced by grain morphology and processing conditions [22].

3.2 Proximate analysis

The proximate composition of pito produced using different processing variables is presented in Table 2. The results of the analysis of the variance in varieties of grains revealed that the moisture content of pito produced from maize (M) had the highest value (98.49%) and the alcohol content (2.25%), while pito produced from red sorghum (RS) had the highest protein content (2.36 %). The pito produced by adding pineapple juice had the highest moisture (96.92 %), ash (1.86 %), and alcohol content (2.35 %), while the unsupplemented pito (US) had the highest protein content (2.36 %). The pito produced using both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae* (B) as starter culture had the highest value of protein

(2.09 %), ash (1.85 %), and alcohol content (1.35 %). The pito produced from red sorghum steeped for 48 hours (48 h) had the highest value for moisture (89.46 %), fat (0.28 %), ash (1.53 %), fibre (0.28 %), and alcohol content (1.60 %). These findings align with recent studies showing that fermentation can enhance the nutritional profile of cereal-based beverages by modifying moisture, protein, and fat levels through microbial activity [23, 24].

3.3 Antinutritional contents

The antinutritional contents (oxalate, phytate, and tannin) of pito present in the different variables were analyzed. Pito produced using white sorghum (WS) had the highest value of oxalate (7.50 mg/100 mL) and tannin (6.20 mg/100 mL), while pito produced from red sorghum (RS) had the lowest value for oxalate (5.63 mg/100 mL) and tannin (3.72 mg/100 mL). When the pito supplemented with pineapple juice (S) is compared with the unsupplemented one (US), it has a high value of phytate (3.37 mg/100 mL) and tannin (4.32 mg/100 mL) with the least oxalate content (5.25 mg/100 mL), though not significantly different from the unsupplemented pito.

The pito produced using *Saccharomyces cerevisiae* (SC) had the highest value of oxalate (7.88 mg/100 mL), phytate (3.24 mg/100 mL), and tannin (5.29 mg/100 mL), while the pito produced using both starter cultures (B) had the least tannin content (4.48 mg/100 mL). Pito produced after steeping for 48 hours had the least oxalate (5.25 mg/100 mL) and phytate content (1.03 mg/100 mL) (Table 3).

Recent studies confirm that fermentation can reduce certain anti-nutritional factors, though grain type and processing conditions still influence their retention and transformation [24, 25].

3.4 Mineral contents

As shown in Table 4, the mineral content (magnesium, calcium, iron, potassium and

phosphorus) of all pito produced using different variables was analyzed. Under varieties of grains, pito produced from maize (M) had the highest calcium (77.50 mg/100 mL) and iron content (3.42 mg/100 mL), red sorghum (RS) had high phosphorus (2.50 mg/100 mL), while the lowest value of all the minerals is in pito produced from white sorghum (WS).

The pito produced from pito supplemented with pineapple juice (S) had the highest value for all the minerals tested when compared with the plain pito (US).

Pito produced using *Limosilactobacillus fermentum* as starter culture had the highest value of magnesium (22.50 mg/100 mL) and phosphorus (2.61 mg/100 mL), while that produced using both starter cultures had the highest mineral value in potassium (1.68 mg/100 mL) and iron (6.65 mg/100 mL). Pito produced from red sorghum steeped for 24 hours (24 h) had the highest mineral content in iron (2.52 mg/100 mL), potassium (2.50 mg/100 mL), and phosphorus (0.69 mg/100 mL), while that steeped for 48 h had high magnesium (32.50 mg/100 mL) and calcium content (82.50 mg/100 mL). Recent studies suggest that fermentation can enhance mineral bioavailability by reducing anti-nutritional factors such as phytates, which often bind minerals and limit absorption [24].

4. Discussion

4.1 Proximate composition of Pito

Proximate analysis serves as a critical method for evaluating the nutritional properties of food items [30]. The notably high moisture content in pito aligns with expectations, given its nature as a liquid-based beverage-suggesting it should ideally be stored sealed or refrigerated to maintain quality. Protein, an essential macronutrient, supports the growth and repair of the body

Table 1.

Vitamin component of Pito produced using different processing variables			
Processing	Vitamins (mg/100 mL)		
Variables	Variables	Vitamin A	Vitamin C
Varieties of grains	M	4.85±0.03 ^e	3.47±0.08 ^d
	WS	1.45±0.04 ^h	3.33±0.29 ^{de}
	RS	3.80±0.04 ^f	3.08±0.05 ^e
Supplementation	S	2.94±0.05 ^g	3.95±0.33 ^e
	US	3.80±0.04 ^f	3.08±0.05 ^e
Starter cultures	LF	6.22±0.05 ^b	4.31±0.19 ^b
	SC	5.51±0.03 ^c	2.15±0.09 ^f
	B	6.99±0.10 ^a	4.56±0.24 ^b
Steeping time	24 h	3.80±0.04 ^f	3.08±0.05 ^e
	48 h	5.27±0.23 ^d	5.27±0.23 ^a

Means along the same column with the different superscripts at each processing variable are significantly different from each other according to Duncan's multiple range test at $p \leq 0.05$.

Key: M - Pito produced from Maize, WS - Pito produced from White sorghum, RS - Pito produced from Red sorghum, S - supplemented with pineapple juice, US- Pito produced from red sorghum without pineapple juice, LF - Pito produced from red sorghum, fermented by *Limosilactobacillus fermentum*, SC - Pito produced from red sorghum, fermented by *Saccharomyces cerevisiae*, B - Pito produced from red sorghum, fermented by both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae*, 24 h - Pito produced from red sorghum with steeping period of 24 h, 48 h - Pito produced from red sorghum with steeping period of 48 h

Table 2.

Proximate composition (%)	Proximate composition of Pito produced from different processing variables									
	Varieties of grains			Supplementation		Starter cultures			Steeping time	
	M	WS	RS	S	US	LF	SC	B	24 h	48 h
Moisture content	98.49±0.65 ^a	95.12±3.30 ^a	88.85±1.63 ^b	96.92±2.29 ^a	88.85±1.63 ^b	98.15±1.92 ^a	96.60±1.56 ^a	96.45±1.91 ^a	88.85±1.63 ^b	89.46±2.20 ^b
Ash content	1.54±0.17 ^a	1.82±0.01 ^a	1.35±0.35 ^a	1.86±0.23 ^a	1.35±0.35 ^a	1.38±0.11 ^a	1.35±0.01 ^a	1.85±0.01 ^a	1.35±0.35 ^a	1.53±0.18 ^a
Protein content	2.03±0.20 ^b	1.68±0.03 ^c	2.36±0.06 ^a	1.70±0.07 ^c	2.36±0.06 ^a	1.98±0.02 ^b	1.72±0.01 ^c	2.09±0.01 ^b	2.36±0.06 ^a	2.02±0.01 ^b
Fat content	0.28±0.01 ^{ab}	0.21±0.01 ^b	0.24±0.00 ^{ab}	0.24±0.03 ^{ab}	0.25±0.00 ^{ab}	0.27±0.08 ^{ab}	0.30±0.03 ^a	0.22±0.00 ^b	0.25±0.00 ^{ab}	0.28±0.01 ^{ab}
Fibre content	0.26±0.01 ^b	0.21±0.00 ^d	0.25±0.00 ^b	0.23±0.01 ^c	0.25±0.00 ^b	0.23±0.01 ^{cd}	0.28±0.01 ^a	0.22±0.00 ^{cd}	0.25±0.01 ^b	0.28±0.01 ^a
Alcohol	2.25±0.07 ^a	1.25±0.07 ^c	1.45±0.07 ^{bc}	2.35±0.07 ^a	1.45±0.07 ^{bc}	1.05±0.07 ^d	1.28±0.11 ^c	1.35±0.07 ^c	1.45±0.07 ^{bc}	1.60±0.14 ^b

Means along the same row with the different superscripts at each processing variable are significantly different from each other according to Duncan's multiple range test at $p \leq 0.05$.

Key: M- Pito produced from Maize, WS- Pito produced from White sorghum, RS- Pito produced from Red sorghum, S- supplemented with pineapple juice, US- Pito produced from red sorghum without pineapple juice, LF- Pito produced from red sorghum, fermented by *Limosilactobacillus fermentum*, SC- Pito produced from red sorghum, fermented by *Saccharomyces cerevisiae*, B- Pito produced from red sorghum, fermented by both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae*, 24hr- Pito produced from red sorghum with steeping period of 24hours, 48hr- Pito produced from red sorghum with steeping period of 48 hours

Table 3.

Anti-nutritional components of Pito produced using different processing variables										
Anti-nutritional components (mg/100ml)	Varieties of grains			Supplementation		Starter cultures			Steeping time	
	M	WS	RS	S	US	LF	SC	B	24 h	48 h
Oxalate	6.75±2.25 ^a	7.50±1.30 ^a	5.63±1.95 ^a	5.25±1.30 ^a	5.63±1.95 ^a	5.63±1.95 ^a	7.88±1.13 ^a	6.00±1.30 ^a	5.63±1.95 ^a	5.25±1.30 ^a
Phytate	1.69±0.02 ^c	2.38±0.09 ^b	3.11±0.08 ^a	3.37±0.45 ^a	3.11±0.08 ^a	3.12±0.11 ^a	3.24±0.02 ^a	3.12±0.10 ^a	3.11±0.08 ^a	1.03±0.0 ^d
Tannin	4.64±0.61 ^{de}	6.20±0.13 ^b	3.72±0.08 ^f	4.32±0.14 ^c	3.72±0.08 ^f	5.05±0.23 ^{cd}	5.29±0.19 ^c	4.48±0.13 ^c	3.72±0.08 ^f	7.63±0.19 ^a

Mean along the same row with the different superscript at each processing variable are significantly different from each other according to Duncan's multiple range test at $p \leq 0.05$.

Key: M - Pito produced from Maize, WS - Pito produced from White sorghum, RS - Pito produced from Red sorghum, S - supplemented with pineapple juice, US - Pito produced from red sorghum without pineapple juice, LF - Pito produced from red sorghum, fermented by *Limosilactobacillus fermentum*, SC - Pito produced from red sorghum, fermented by *Saccharomyces cerevisiae*, B - Pito produced from red sorghum, fermented by both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae*, 24 h - Pito produced from red sorghum with steeping period of 24 h, 48 h - Pito produced from red sorghum with steeping period of 48 h

Table 4.

Mineral Content (mg/ 100 mL)	Mineral composition of Pito produced using different processing variables									
	Varieties of grains			Supplementation		Starter cultures			Steeping time	
	M	WS	RS	S	US	LF	SC	B	24 h	48 h
Magnesium	22.50±3.54 ^b	12.50 ±3.54 ^c	22.50 ± .54 ^b	7.50 ± 3.54 ^c	22.50 ±3.54 ^b	22.50 ±3.54 ^b	5.00 ± 0.00 ^c	10.00 ±0.00 ^c	22.50±3.54 ^b	32.50±3.54 ^a
Calcium	77.50± 3.54 ^{ab}	65.00 ±7.07 ^{cd}	65.00± 0.00 ^{cd}	72.50 ±3.54 ^{bc}	65.00±0.00 ^{cd}	57.50±3.54 ^d	47.50 ± 3.54 ^c	57.50 ±3.54 ^d	65.00 ± 0.00 ^{cd}	82.50 ±3.54 ^a
Iron	3.42±0.03 ^{cd}	1.53±0.11 ^d	2.52 ±0.25 ^{cd}	6.31±3.12 ^a	2.52±0.25 ^{cd}	5.64±0.16 ^{ab}	4.57±0.04 ^{abc}	6.65± 0.35 ^a	2.52±0.25 ^{cd}	2.38±0.11 ^{cd}
Potassium	1.42 ±0.02 ^b	1.46 ±0.02 ^b	0.69±0.02 ^c	1.67±0.01 ^a	0.69 ±0.02 ^c	1.66± 0.06 ^a	1.67± 0.03 ^a	1.68± 0.11 ^a	0.69±0.02 ^c	0.67±0.00 ^c
Phosphorus	1.29± 0.02 ^d	0.56 ±0.10 ^e	2.50±0.07 ^{ab}	2.46±0.03 ^b	2.50±0.07 ^{ab}	2.61± 0.01 ^a	2.44± 0.06 ^b	2.49±0.02 ^{ab}	2.50±0.07 ^{ab}	1.82±0.01 ^c

Mean along the same row with the different superscript at each processing variable are significantly different from each other according to Duncan's multiple range test at $p \leq 0.05$.

Key: M - Pito produced from Maize, WS - Pito produced from White sorghum, RS - Pito produced from Red sorghum, S - supplemented with pineapple juice, US - Pito produced from red sorghum without pineapple juice, LF - Pito produced from red sorghum, fermented by *Limosilactobacillus fermentum*, SC - Pito produced from red sorghum, fermented by *Saccharomyces cerevisiae*, B - Pito produced from red sorghum, fermented by both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae*, 24 h - Pito produced from red sorghum with steeping period of 24 h, 48 h - Pito produced from red sorghum with steeping period of 48 h.

tissues. The ash content observed across pito samples produced with various processing methods ranged from 1.35% to 1.86%. These values are consistent with findings by Okrah, [31], who reported that germinated sorghum contains ash levels between 0.28% and 1.70%, and noted that germination tends to reduce ash concentration. A similar increase in ash content of sorghum starch was reported after soaking for 12 and 24 h, with values of 1.01 and 1.05 %, respectively [27]. Low fiber levels were evident in all pito variants, a trend that aligns with Alemu's [32] report indicating a reduction in sorghum's crude fiber following fermentation.

4.2 Alcohol content in pineapple-supplemented Pito

Pito enriched with pineapple exhibited the highest alcohol concentration. This aligns with the findings of Adelekan et al. [10], who noted that samples with the highest Brix values, indicating sugar content, were those supplemented with pineapple, resulting in elevated alcohol levels. The underlying reason is that the glucose present in the beverage serves as a substrate for yeast, which converts it into ethanol during fermentation. Consequently, increased sugar levels enhance alcohol production.

4.3 Mineral composition and nutritional importance of Pito

All pito samples prepared using various processing methods contained vital mineral elements. According to Addison et al. [6], minerals such as calcium, magnesium, and iron are commonly found in pito. These nutrients are essential for cellular regulation and development, and may contribute to mood stabilization. Potassium, a key macro-mineral, plays a crucial role in maintaining cellular water balance and regulating the body's acid-base equilibrium. Iron is a fundamental component of hemoglobin, facilitating oxygen transport from the lungs to tissues.

Its deficiency can lead to anaemia. Calcium supports the construction and maintenance of living cells, helps regulate bodily fluid levels, and contributes to overall health. Magnesium aids muscle relaxation and is important for developing strong teeth and bones.

4.4 Antinutritional content

Anti-nutritional factors, such as oxalates, phytates, and tannins, are known to exert both detrimental and beneficial effects on human and animal health. While excessive intake can impair nutrient absorption and contribute to conditions like kidney stones or mineral deficiencies, moderate levels may offer antioxidant and protective benefits.

In this study, pito samples produced under varying processing conditions exhibited relatively low concentrations of anti-nutritional compounds. Notably, a significant reduction in oxalate and phytate levels was observed at a 48-hour steeping duration. This aligns with findings from recent research indicating that soaking and steeping facilitate leaching of anti-nutrients, often evidenced by changes in steep water coloration and composition [27]. The use of *L. fermentum* and *S. cerevisiae* singly has been used successfully to produce *burukutu*, but the combined use of both starter cultures gave improved nutritional quality when comparable with the commercial product [27]. The nutritional profile of grains used in pito production is shaped by both genetic and environmental influences. Soil fertility, moisture availability, and agronomic practices such as fertilization and crop rotation are among the most impactful variables affecting mineral content and overall grain quality. These factors not only determine the baseline nutrient levels but also influence the concentration and activity of anti-nutritional components through plant metabolic responses to stress and soil conditions.

5. Conclusion

Recent investigations into the impact of processing variables on pito production confirm that this traditional beverage is nutritionally rich, offering a broad spectrum of essential nutrients. Pito produced from maize or red sorghum as base grain, addition of pineapple juice, use of both *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae* as starter cultures, and steeping the grain for 48 hours will significantly enhance pito's proximate composition, minerals, and vitamin content. These findings align with current research emphasizing the role of controlled fermentation and ingredient synergy in improving the nutritional and functional properties of fermented beverages. To ensure consistent quality and maximize health benefits, there is a clear need to optimize these processing parameters, particularly steeping time and fruit-based enrichment for scalable and consumer-friendly pito production.

6. References

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