Effect of Bacterial and Fungal Isolates on the whole Grain Millet Sourdough Breads

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Abstract: The sourdough fermentation technique has the potential to improve the nutritional profile and safety of cereal fermented foods which forms the bulk of the diet of rural dwellers in sub-Saharan Africa. This study enumerates the bacterial and fungal isolates responsible for the fermentation of millet sourdoughs, evaluates the antimicrobial safety of the sourdough starters and the quality of bread produced thereafter. The individual flours were spontaneously fermented in the ratio 1:1 (w/v) for 48 h. At the end of the fermentation, the microbial consortium of the sourdough meals obtained were determined and also screened for antagonistic activity against selected pathogens. Chemical analysis analyses were carried out on the breads following well established procedures. Semi trained panellists were employed to access the organoleptic attributes of the product. All the sourdough starters revealed strong clearance zones against the selected food borne pathogens. The proximate compositions of the breads were improved by the sourdough fermentation process compared with the raw flour. The moisture content of flour determined ranged from 7.48 to 11.20% while the protein, fat, ash, crude fibre and carbohydrate were 9.53 to 12.4%; 2.13 to 2.78%; 0.82 to 2.87%; 2.10 to 4.67% and 71.83 to 76.33% respectively. The carbohydrate content of the breads reduced with fermentation and ranged from 63.10% to 73.33% while the protein, ash, crude fibre and fat content ranged from 11.57 to 15.31%; 2.39 to 3.08%; 1.76 to 3.62% and 7.15 to 12.32% respectively. The highest value for calcium 48.86 mg/100 g was exhibited by pearl millet bread. Sensory evaluation showed significant difference in the bread samples. The findings have suggested that indigenous wholegrain cereals could be employed for bread production, thus reduce importation and over dependence of wheat flour and white pan bread consumption.

Key words: Fermentation, probiotics, pathogen, sub-Saharan, indigenous.

1. Introduction

Consumers’ interest in health-promoting, nutritious, functional and wholegrain rich foods is significantly increasing, particularly against several western chronic diseases such as diabetes, coeliac disease and other cardiovascular diseases [1, 2] White pan bread and other bakery products produced from the endosperm flour represents 83% of the kernel, characterized by a high soft elastic crumb structure and high volume owing to the development of gluten network which entraps the gas produced from the fermentation by yeast and adds to the unique cellular structure. Nutritionaly, white pan bread is inferior to the wholegrain which comprise germ 3% and bran 12% [3, 4]. Present in the bran are healthy metabolites such as oligosaccharides, phytochemicals and dietary fibre [5]. Consumption of high rich fibre foods have long been proven to provide health benefits [6]. Utilizing the sourdough technique in the development of fibre rich functional foods such as wholegrain sourdough bread, cake and other baked goods is an emerging technology in sub Saharan Africa and could be suitable in enhancing the technological and nutritional properties of indigenous under-utilized cereals. Sourdough fermentation is spontaneous involving the mixture of flour and water fermented with LAB and yeast [7]. Sourdough technique had been studied and known to distinctively improve dough properties of bread, flavour and taste, offer microbial safety, conveniences, enhanced nutritional value through increased mineral bio-availability,
provide some exopolysaccharides with prebiotic behaviour, lowers the post prandial glucose level, and enhance shelf life of the bread due to the lengthy fermentation period of sourdough [8, 9]. The positive effect of sourdough on the quality of bread dwells on the specific metabolic activities of LAB and yeast which possess the potential to produce different organic acids, enzymes and exopolysaccharides thereby prolonging the shelf stability of the sourdough bread (by prolonging the period of mould attack, anti-staling and prevent ropiness in the bread) [10]. Microbial safety of foods is of immense concern to public health and of interest are Gram negative bacteria such as E.coli, Salmonellasp, Klebsiellasp, along with some Gram positive organisms such as Staphylococcus aureus, Streptococcus faecalis which have been implicated in foodborne illnesses. Inactivation of α-amylase activity, production of bread with suitable physical property such as extensibility, and development of acid flavour as reported by Hansen et al. (2002) [11] are some of the functions of sourdough in the production of rye bread.

The choice of fonio for this study is based upon the backdrop that fonio is still being under-utilized and is quite rich in methionine and cysteine two important amino acids lacking in other cereals. In this part of the world, the majority of the populace in the rural areas depend on the local grains for survival, creating a novel product at low cost with their indigenous grains could serve for variation in their diet and at the same time provide some health significance as most lack adequate and good medical facilities. The objective of this work is therefore to investigate the dominating effect of wholegrain millet sourdough starters on foodborne enteric organisms and wholegrain bread quality.

2. Materials and Methods

2.1 Source of Materials

The raw materials used are pearl millet (P. glaucoma), acha (D. exilis), iburu (D. iburua) and finger millet (E.coracana). They were all purchased from Owena market, Ilesa, Nigeria. Pearl and finger millet were sorted differently to remove stones and debris, while acha and iburu were winnowed separately to remove extraneous materials. All materials were ground into fine flour using the attrition disc mill, packaged in air tight plastic containers and stored in the refrigerator (4°C) until required. All chemicals used were of analytical grade and purchased from Sigma or BDH Company, UK.

2.2 Spontaneous Fermentation of Individual Flours

The millet sourdoughs were prepared by mixing individual flour with tap water in the ratio 1:1 (w/v) as previously described by Adisa and Ifesan (2016) [7].

2.3 Identification of Isolates

Growth of Lactic acid bacteria (LAB) was estimated from each fermenting dough meal using de Mann Rogosa Sharpe (MRS) agar as described by Adisa and Ifesan (2016) [7]. Pure isolates obtained were subjected to gram and spore staining. Biochemical characterization was based on sugar fermentation (glucose, fructose, lactose, sucrose and maltose utilization), motility, catalase production and growth in 10% NaCl were done as described by Sneath et al. (1986) [12].

Isolation and identification of fungal isolates were done on Malt Extract (MEA) agar and by making reference to Efiofurewene and Uwanogho (1990) [13]. Pure isolates were obtained by successive streaking on MEA agar after which they were stained as described by FAO (1979) [14] and then subjected to carbohydrate fermentation and Cycloheximide resistance test.

For urea hydrolysis test, Urea broth medium was dispensed into tubes aseptically, in aliquots of 5 ml then autoclaved at 121°C for 20 min. A loop full of cells from freshly prepared culture was suspended in the sterile broth and incubated at 37°C. The tubes were examined half hourly for a change of colour to red which is an indication of urease activity [15].
2.4 Proximate Analyses of Flour and Bread

Moisture, crude fibre, total ash and crude fat contents were determined using the standard methods of Association of Official Chemists [16]. The crude protein content was determined using the micro Kjedahl nitrogen method. The nitrogen content was converted to protein using the conversion factor 6.25. Carbohydrate content was determined by difference. The experiment was done in triplicate.

2.5 Screening of Sourdoughs for Antagonistic Activity Against Selected Pathogens

This was done using the agar well diffusion method. The test organisms were grown in nutrient broth at 37°C for 24 h and spread evenly by swabbing on Mueller-Hinton agar plates and 3 wells each of 6 mm diameter were bored into the plates with the aid of a sterile cork borer. The wells were then filled with 100 µl aliquot of freshly prepared sourdough in a laminar airflow cabinet in order to maintain sterile condition. The inoculated plates were incubated at 37°C for 24 h and the diameter of inhibition zone measured in millimetres. A negative control was set by using sterile distilled water in the wells against the pathogens [17].

2.6 Determination of Mineral Content of Bread Samples

The mineral elements of bread samples were determined according to AOAC (2005) using Atomic absorption spectrophotometer (GBC Scientific Equipment PTY Ltd). Phosphorus was determined using spectrophotometer at 430 nm.

2.7 Production of Sourdough Breads

This was done according to the method described by Edema et al. (2005) [18]. The production of the wholegrain millet sourdough breads was based on the spontaneous fermentation process. The ingredients are millet sourdough preferment 200 g, millet flour 100 g, water 100 ml, 2 teaspoon salt. The addition of water depended on each flour, the ingredients were mixed together in a mixing bowl and the resulting dough was kneaded manually for 10 min at 30±2°C. The dough was cut into shapes and placed in greased pans. It was allowed to ferment for 24 h in order to allow sufficient leavening by yeast. The oven was preheated for 1 h and the breads baked at 230°C for 30 min. It was cooled at room temperature for further sensory and proximate analysis.

2.8 Physical Properties of Sourdough Breads

This was determined according to Sanni et al. (1998) [19]. The loaf volume was calculated by using a slide rule to measure the height, length and breadth of the bread and multiplying them all. The loaf specific volume was calculated as the ratio of the loaf volume to the weight of the bread. The crumb and crust colour of the bread were determined by physical observation with the eye. The elasticity of the crumb was determined by the ability of the crumb to return back to its position after it has been pressed down within 5 min.

2.9 Sensory Evaluation of Breads

Sensory evaluation of the wholegrain millet sourdough breads was carried out to determine if there is any significant difference between the breads using a 9-point Hedonic scale (Dislike extremely – 1, dislike very much – 2, dislike moderately – 3, dislike slightly – 4, neither like nor dislike -5, like slightly – 6, like moderately – 7, like very much – 8, like extremely – 9) as reported by Adesokan et al. (2010) [20]. The samples were coded and served within three hours of production to a 20 membered semi-panelist from the final year students of the Department of Food Science and Technology, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun state. They were asked to evaluate the quality of each product for appearance, aroma, texture, taste and overall acceptance of the samples in order to obtain information on their general opinion of the sourdough breads and were given sufficient water to rinse their mouths between each sample.
2.10 Determination of Shelf life of Bread

The bread loaves were stored at room temperature (28±2°C) in the same condition and visually observed every 12 h interval for physical change, mould growth and the change was recorded.

2.11 Statistical Analysis

All analyses were done in duplicate and data was analyzed using analysis of variance and Duncan’s new multiple range test to separate means at 5% significance level (SPSS version 19 computer software).

3. Results and Discussion

3.1 Proximate Composition

Proximate composition is important in determining the quality of raw materials and their products as it often serves as the basis for establishing the nutritional value of food. There was significant difference among the moisture content of the flours (Table 1). The low moisture content of the exhibited by the flour was desirable as it will enhance the keeping quality of the flour and contribute to extension of the shelf life of the product since water available for microbial activity is low.

This phenomenon will in turn reduce microbial growth and hence food spoilage [21]. Fats are relatively minor constituents in cereal grains; however, they are significant in human nutrition as source of energy and fatty acids [21]. The fat content of the flours are significantly different with PEM having the highest value of 4.67%. Serna (2003) [22] also reported high values for pearl millet. There was no significant difference in the crude fibre content of the fonio grains WIF and BAF which were 0.82% and 0.85% respectively. Carbohydrate contributes to the bulk of the energy intake of human diet. High value of carbohydrate content was observed for all the flours and corresponds to the values obtained by other authors [6, 21, 23]. The Ash content indicated a rough estimate of the mineral contents of the flours and the ranges obtained are between 2.13% and 2.78%. Serna (2003) [22] also reported higher values for ash content.

All the parameters analyzed showed that sourdough fermentation enhanced the proximate composition of the breads (Table 2) as there were significant improvements in the values obtained for the sourdough bread when compared with the raw flour.

Sourdough is effective for the production of bread with increased level of flavour compounds and ultimately increasing consumers’ satisfaction. Generally, there was steady decrease in in carbohydrate content of all the bread samples compared with their respective flour with values ranging from 63.1%-73.3% to 71.8-76.3%. Highest crude fat content of 10.8% was observed for PEM bread, while there was no significant difference observed for FIM, WIF and BAF. After baking FIM bread had the highest value for crude protein and crude fibre (13.9% and 3.49%) respectively. This corresponds with the values reported by Rehman et al. (2006) and Cruz et al. (2011) [24, 25].

Table 1  Proximate composition (g 100 g⁻¹ DM) wholegrain millet flours.

| Samples | Moisture content | Crude protein | Total ash | Crude fibre | Fat | Carbohydrate |
|---------|-----------------|---------------|-----------|-------------|-----|--------------|
| PEM     | 9.39±0.78       | 10.8±0.17     | 2.13±0.36 | 2.53±0.12   | 4.67±0.21 | 71.8±1.33    |
| FIM     | 8.31±0.60       | 12.4±0.22     | 2.78±0.36 | 2.87±0.15   | 2.10±0.61 | 74.4±0.13    |
| WIF     | 11.2±0.78       | 10.0±0.19     | 2.33±0.10 | 0.82±0.01   | 2.10±0.20 | 73.4±0.97    |
| BAF     | 7.48±0.15       | 9.53±0.12     | 2.21±0.21 | 0.85±0.01   | 2.78±0.23 | 76.3±0.12    |

**Means that are significantly different (p < 0.05).
Values are means ± SD of triplicate measurement.
PEM = pearl millet, FIM = Finger millet, WIF = White fonio, BAF = Black fonio, DM = Dry matter
Table 2  Proximate composition (g 100 g⁻¹ DM) sourdough breads.

| Sample  | Moisture content | Crude protein | Total ash | Crude fibre | Fat | Carbohydrate |
|---------|------------------|---------------|-----------|-------------|-----|--------------|
| PEM     | 5.83*±0.01       | 12.9*±0.0     | 2.39*±0.01| 3.44*±0.01  | 12.3*±0.02 | 63.1*±0.01   |
| FIM     | 4.23*±0.01       | 15.3*±0.02    | 3.08*±0.02| 3.62*±0.03  | 7.49*±0.01 | 66.3*±0.01   |
| WIF     | 15.5*±0.03       | 12.2*±0.01    | 2.66*±0.05| 1.76*±0.04  | 7.15*±0.01 | 60.7*±0.02   |
| BAF     | 9.30*±0.03       | 11.6*±0.01    | 2.55*±0.01| 1.82*±0.01  | 7.43*±0.02 | 73.3*±0.01   |

*Means that are significantly different (p < 0.05).
Values are means ± SD of triplicate measurement.
PEM = pearl millet, FIM = Finger millet, WIF = White fonio, BAF= Black fonio, DM = Dry matter

3.2 Microbial Count and Distribution of Isolates during Sourdough Fermentation

Yeast and LAB species dominated the fermentation process and the high population obtained showed that all samples of wholegrain millet sourdoughs met the minimum requirement set by FAO/WHO of 1×10⁶ cfu/g of live organisms (Table 3). The highest number of Lactic acid bacteria was from PEM with count of 4.9×10⁶ cfu/g and the lowest was found in FIM with a count of 2.0×10⁶ cfu/g, going by this initial count, wholegrain millet sourdoughs can be considered as good probiotic sources.

Also the co-interaction between LAB and yeast is common in many food fermentation matrixes, wherein Lactic acid bacteria creates the acidic environment for yeast growth, and yeast in return provides vitamins and other growth factors. In addition, LAB is represented in fermented foods because they are able to survive high acidic conditions and also have the ability to provide a high level of lactic acid [26]. The results of the morphological and biochemical tests used to characterize the isolates obtained were similar to those reported by other authors [27-29] and were also referenced to Bergey’s manual of modern bacteriology.

All the isolates identified on MRS agar were Gram positive, rod shaped, catalase negative and non-spore forming which are characteristic of LAB (Table 4). The result of the biochemical tests showed that the isolates belonged to genus of lactobacillus. The presence of fungi was investigated on malt extract agar and Saccharomyces cerevisiae was observed to be the predominant species in three of the samples, while Candida was found dominating in pearl millet sourdoughs as presented in Table 5. The presence of yeast in these sourdoughs relates well with the identification and characterization of yeast from other African fermented foods. In Ghanaian pito, Demuyakor and Ohta (1991) [30] reported a high predominance of Saccharomyces cerevisiae (33%) and presence of Candida spp. (17%) amongst other genera. While investigating Nigerian sorghum beer, Sanni (1993) [31] observed occurrence of several species, Candida spp., Saccharomyces cerevisiae, Geotrichum candidum, Torulaspora delbrueckii to be dependent on the type of beer.

3.3 Antagonistic Activity Against Selected Pathogens

All the sourdoughs displayed large clear zones of inhibitions to varying degrees against all the tested pathogens, thus confirming the antimicrobial potential of the sourdoughs. The highest inhibition zone was observed by finger millet sourdough (SFM) which gave a clearance diameter of 16.1 mm against Pseudomonas sp, this was closely followed by E. coli with 15.0 mm. the lowest activity was obtained from brown fonio sourdough (SBF) which gave an inhibition of 3mm diameter against Klebsiella sp. Pearl millet sourdough showed lowest activity against all tested pathogens. The result is presented in Fig. 1.

Several studies have shown that traditionally fermented foods possess adverse effects against enterotoxigenic pathogens such as E. coli, Salmonella typhimurium and Bacillus cereus [7, 32]. This is as a result of the antimicrobial properties displayed by
Table 3  Microbial counts cfu g$^{-1}$ g of LAB and yeast.

|          | Sourdoughs LAB ($\times 10^6$) | Yeast ($\times 10^5$) |
|----------|-------------------------------|----------------------|
| PEM      | 4.9$^a$                       | 3.0$^b$              |
| FIM      | 2.0$^a$                       | 7.4$^{ab}$           |
| BAF      | 3.9$^a$                       | 9.4$^a$              |
| WIF      | 4.4$^a$                       | 8.5$^a$              |

Means with different superscripts on the same column are significantly different ($p < 0.05$).
Values are means ± SD of triplicate measurement.

PEM = pearl millet, FIM = Finger millet, WIF = White fonio, BAF = Black fonio

Table 4  Morphological and biochemical characteristics of bacterial isolates on MRS agar.

|          | PEM                | FIM                | BAF                | WIF                |
|----------|--------------------|--------------------|--------------------|--------------------|
| Colonial morphology | Pin head, creamy, smooth, moist, circular and convex | Pinpoint, creamy white, smooth, moist, circular and convex | Pinpoint, creamy white, smooth, moist, circular and flat | Pinpoint, creamy white, smooth, moist, circular and flat |
| Cell morphology | G (R)              | G(R)               | G(R)               | G(R)               |
| Catalase test | -                  | -                  | -                  | -                  |
| Motility    | -                  | -                  | -                  | -                  |
| Lactose    | +                  | +                  | +                  | +                  |
| Glucose    | +                  | +                  | +                  | +                  |
| Sucrose    | +                  | V                  | +                  | +                  |
| Raffinose  | -                  | +                  | V                  | +                  |
| Spore forming | -                | -                  | -                  | -                  |
| Co$_2$     | -                  | -                  | -                  | -                  |
| Growth in 10%NaCl | +          | +                  | +                  | +                  |
| Presumptive org | _Lb. fermentum_ | _Lb. plantarum_   | _Lb. plantarum_   | _Lb. plantarum_   |

+positive, -negative, $^a$variable, $^b$G gram positive, $^c$rod shape

PEM = Pearl millet, FIM = Finger millet, BAF = Black fonio, WIF = White fonio

Table 5  Morphological and biochemical characteristics of yeast isolates on MEA agar.

|          | PEM                | FIM                | BAF                | WIF                |
|----------|--------------------|--------------------|--------------------|--------------------|
| Colonial morphology | Large, circular, creamy, mucoid, smooth, with loped margin Budding/presence of pseudo hyphae | Small, smooth white, spherical and flat Budding/ absence of pseudohyphae | Small colonies in chain, dry, rough, round, creamy, mucoid and flat budding/ absence of pseudohyphae | Large, moist, smooth, round, white, raised with grey patches Budding/absence of pseudo hyphae |
| Cell morphology | Budding/presence of pseudo hyphae | Budding/ absence of pseudohyphae | Budding/ absence of pseudohyphae | Budding/absence of pseudo hyphae |
| Glucose    | +                  | +                  | +                  | +                  |
| Galactose  | -                  | +                  | +                  | +                  |
| Lactose    | -                  | -                  | -                  | -                  |
| Melibiose  | -                  | -                  | -                  | -                  |
| Sucrose    | +                  | +                  | +                  | +                  |
| Raffinose  | +                  | -                  | -                  | -                  |
| Spore forming | -                  | -                  | -                  | -                  |
| Urea hydrolysis | -                  | +                  | +                  | +                  |
| Resistance to Cycloheximide | -                  | -                  | -                  | -                  |
| Growth in 10% NaCl | V                  | ND                 | ND                 | V                  |
| Growth at 37°C | +                  | +                  | +                  | +                  |
| Growth at 42°C | +                  | +                  | +                  | +                  |
| Presumptive organisms | Candida sp. | Saccharomyces cerevisiae | Saccharomyces cerevisiae | Saccharomyces cerevisiae |

+positive, -negative, $^a$variable, $^b$not determined

PEM = Pearl millet, FIM = Finger millet, BAF = Black fonio, WIF = White fonio.
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SWF = white fonio sourdough, SBF = black fonio sourdough, SFM = finger millet sourdough, SPM = pearl millet sourdough

Fig. 1  Antimicrobial activity (mm) of sourdoughs against selected pathogens.

These fermented foods such as ogi, kisra, mawe, propelled by the low pH of the foods, production of hydrogen peroxide as well as the metabolic properties of the microorganisms (LAB) associated with the fermentation. In a previous study conducted by Adisa and Ifesan (2016) [7] it was observed that LAB isolates from whole grain millet sourdoughs were able to inhibit the growth of pathogens by both bacteriostatic and bactericidal mode of action.

Generally, *Escherichia coli* an enteric pathogen widely distributed in nature is part of the gut flora of mammals and contaminates food easily through unwashed hands of food handlers especially after using the toilets, changing diapers and other unhygienic practices. *Salmonella typhi* can be transmitted easily through unhygienic handling of contaminated poultry products such as live birds, eggs and other foods. *Staphylococcus aureus* a common agent of food poisoning is an environmental organism occurring almost everywhere. It can be found on the skin and nostrils of some healthy individuals and is responsible for infections such as boils. It grows readily in foods stored at ambient temperature where it multiplies and produces toxins which cause illness [4].

This present study indicates the synergistic effect of the probiotic and prebiotic attributes offered by the fermenting organisms and the substrate in combating undesirable microorganisms of the gut flora. Prebiotic, by definition are non-digestible plant fibres which offer resistance to acid of the stomach and digestive enzymes in the small intestine. The fibre survives passage through the stomach and small intestine and on getting to the large intestine; they act as substrate for the normal gut flora, thereby stimulating growth and activity of the beneficial microorganisms. With increase in the normal gut flora brought about by prebiotics, the ratio of the desirable to undesirable microorganisms is drastically reduced [33].

### 3.4 Mineral Content of Bread Samples

There was no significant difference in the values obtained for iron and zinc content of the sourdough breads. The highest value for calcium 48.86 mg/100 g
was exhibited by PEM while sample FIM had the least value of 46.79 mg/100 g (Table 6). This result shows that the samples may serve as a good source of Calcium and Magnesium to satisfy the nutritional need for consumers because of their relatively high values which may be as a result of the reduction in the anti-nutritional content due to processing.

These anti-nutrients normally bind with some metals like Ca. and Mg. and prevent their bioavailability. Oboh et al. (2003) [34] stated that fermentation and blanching greatly reduced anti-nutrient content such as phytate and oxalate that bind with some metal preventing their bioavailability. These important minerals if present in diet is responsible for the metabolic activities, rigid bone formation, transmission of nerve impulses, regulation of water and salt balance among others [35].

The low values obtained for some mineral such Zinc and iron of the sample may be due to the fact that during fermentation, some microorganisms utilized these mineral elements for their growth. Rainbault (1998) [36] stated that during fungal fermentation, the fermenting fungi utilize mineral salt for metabolic activities. The high mineral composition of the bread is an indication of the positive influence of fermentation on the sourdough. Food fermentation offers numerous benefits which include improvement of nutritional qualities of protein and carbohydrate digestibility [31, 37].

### 3.5 Physical Properties of the Sourdough Breads

The physical properties of the sourdough breads are presented in Table 7. There was significant difference in the breads with regards to the loaf specific volume (LSV) and the values ranged from 1.75 cm$^3$ to 3.66 cm$^3$. Finger millet bread had the highest weight of 141.87 g while brown fonio bread has the lowest weight of 123.0 g.

All the breads had high loaf volume with bread produced from finger millet having the least volume. Barber et al. (1992) [38] reported that breads produced with spontaneous sourdoughs had higher volumes and better crumb grain than those made with conventional baker’s yeast. The crumb colours of the breads were brown with the exception of WFB which was creamy while the elasticity of crumb was weak for all the samples. The crust colour of all the breads was light brown with the exception of finger millet which was dark brown. Crack formation for all the breads showed small and medium cracks.

| Table 6 Mineral composition (mg100$^{-1}$ g) of wholegrain millet sourdough breads |
|------------------------------------------------------|
| Samples  | Iron  | Zinc  | Calcium | Phosphorus | Magnesium |
|-----------|-------|-------|---------|------------|-----------|
| WIF       | 2.78±0.00 | 1.95±0.05 | 48.0±0.00 | 7.3±0.50 | 13.75±0.46 |
| BAF       | 2.75±0.00 | 1.99±0.08 | 48.52±0.01 | 8.0±0.00 | 13.25±0.46 |
| PEM       | 2.77±0.14 | 1.93±0.05 | 48.86±0.00 | 9.1±0.00 | 11.85±0.45 |
| FIM       | 2.72±0.37 | 1.90±0.03 | 46.79±0.01 | 8.5±0.01 | 10.5±0.45 |

**Means that are significantly different (p<0.05). Values are means ± SD of triplicate measurement.

PEM=pearl millet, FIM= Finger millet, WIF= White fonio, BAF= Black fonio

| Table 7 Physical properties of wholegrain millet sourdough bread. |
|---------------------------------------------------------------|
| Height (cm)  | Weight (g)  | LV(g/cm$^3$)  | LSV (cm$^3$) |
|---------------|-------------|---------------|--------------|
| FIM           | 5.73±0.15   | 135.27±1.23   | 500.61±16.2  | 3.66±0.15   |
| PEM           | 4.66±0.10   | 141.87±0.58   | 249.17±8.67  | 1.75±0.06   |
| BAF           | 4.66±0.10   | 123.03±0.61   | 447.77±0.70  | 3.59±0.15   |
| WIF           | 5.23±0.58   | 126.57±2.72   | 447.77±0.70  | 3.50±0.08   |

**Means that are significantly different (p < 0.05). Values are means ± SD of triplicate measurement.

FIM = Finger millet bread, PEM = Pearl millet bread, BAF = Black fonio bread, WIF = White fonio bread.

LV = Loaf volume, LSV = Loaf specific volume.
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Table 8  Sensory properties of the sourdough bread.

| Sensory qualities | FIM  | PEM  | BAF  | WIF  |
|-------------------|------|------|------|------|
| Appearance        | 5.63á| 6.41c| 7.15b| 7.36a|
| Taste             | 5.22á| 6.77c| 7.13b| 7.72a|
| Colour            | 5.78á| 6.28c| 7.15b| 7.52a|
| Aroma             | 5.66c| 6.18b| 6.80b| 6.85a|
| Texture           | 5.35c| 6.71b| 7.42a| 7.60a|
| Overall acceptability | 5.22c| 6.37b| 7.13b| 7.56a|

Means with different superscripts across the row are significantly different (p < 0.05). Values are means ± SD of triplicate measurement.

FIM = Finger millet bread, PEM = Pearl millet bread, WIF = White fonio bread, BAF = Black fonio bread.

The panellists were able to detect significant difference (p < 0.05) in the sensory qualities of the wholegrain millet sourdough breads as shown in Table 8. Colour and taste of the sourdough breads are primary factors in determining the overall acceptability of the breads. The breads had a characteristic pleasant aroma and sour taste which is unique to sourdough bread fermented with heterofermentative Lactic acid bacteria [10]. White fonio bread recorded the highest overall acceptability probably due to panellists’ preference for colour and taste. The dark brown colour of finger millet bread, medium cracks and hardness of the bread could be responsible for the low scores in its overall acceptance.

3.6 Shelf Life of Bread

After four days storage at room temperature the breads showed a little growth of mould. This observation can be linked to the use of sourdough in the preparation of bread as it has a positive effect on mould-free shelf life [39] and also due to the exopolysaccharides produced by LAB which enhanced water retention and functioned as hydrocolloids.

4. Conclusion

The synergistic effect of the prebiotic and probiotic activity of the selected wholegrain millet sourdoughs offers a safe and inexpensive alternative to the use of antibiotics of which several microorganisms are becoming resistant to. The potential of the sourdoughs to inhibit the growth of selected pathogens is an affirmation of the probiotic health benefit that could be derived from the consumption of such specialty foods. It is important to establish the fact that sourdough fermentation improved the mineral bioavailability of the bread samples such as calcium, magnesium and phosphorus owing to the acidification provided by the sourdough process which in turn enhanced its nutritional and sensorial qualities. Thus the sourdough technique when applied to local substrates such as millet can provide consumers especially those at the grassroot level with a safe, wholesome, nutritious food with health promoting benefits that could positively better the lives of rural dwellers in sub-Saharan regions.

5. Conflict of Interest

The author(s) did not declare any conflict of interest.

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