

## Microbiological and physicochemical properties of sourdough bread from sorghum flour

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### Abstract

Sorghum is the world's fifth most important cereal grain after wheat, maize, rice and barley. Sourdoughs have been successfully used to improve the quality of gluten-free bread. The production of a novel food product with improved health benefit for gluten intolerance individuals necessitated this study. Microbiological and physicochemical qualities of the bread samples were determined. Sorghum grains were germinated and processed into flour for the production of sourdough. Thirty-six lactic acid bacteria (LAB) and thirteen yeast isolates were obtained. Among the LAB and yeasts, *Pediococcus pentosaceus* and *Saccharomyces cerevisiae* were employed as starter cultures, singly and in combination for the production of sorghum bread samples. The moisture content of the sorghum flour was determined to be 7.52%; the crude protein was 10.62% while the crude fat, crude fibre, ash and carbohydrate contents were 1.99%, 2.0%, 1.29% and 78.58% respectively. There was a significant decrease in the pH (6.77 to 4.31), increase in the acid equivalent (0.47ml to 1.69ml) and leavening (0.00cm to 1.40cm) at the end of the fermentation period. The proximate composition of the sorghum bread samples showed that moisture content ranged from 34.68 to 50.40%; crude protein, 11.27 to 16.60%; crude fat, 2.98 to 10.97%; ash, 1.50 to 2.13% while carbohydrate, 65.58 to 77.39%. The mineral compositions in (mg/100g) were Calcium (6.6-13.15), Magnesium (68.65-120.75), and Potassium (126.2-233.25). The nutritional properties and physical parameters of the sorghum bread samples were improved relative to the sorghum flour and met the expectation for sourdough bread.

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### Introduction

Sorghum (*Sorghum bicolor* L. Moench) is one of the oldest known grains (cereals) originating in Africa and India where it is commonly used in a variety of foods. Sorghum has unique adaptation to Africa's climate and it is about the largest cultivated crop in the Northern Guinea Savanna areas of Nigeria, a tropical region of Africa (Taylor, 2004; Kolawole *et al.*, 2007). Quantitatively, it is the world's fifth most important cereal grain after wheat, maize, rice and barley. Nigeria and the United States are the two largest producers of sorghum in the world (FAO, 2003). World annual production is over 60 million tonnes in which Africa produces about 20 million tonnes. This makes sorghum quantitatively the second most important cereal in Africa after maize with Nigeria ranking first among Africa sorghum producing countries and the consumption rate among other cereals all over Nigeria is about 75% (ICRISAT/FAO, 1996; Taylor, 2004; Lead Capital Limited, 2008; USDA, 2011).

Sorghum has achieved the highest growth rate among the food crops in West Africa and believed

to possess the highest capability among available food crops for attaining technological breakthrough to boost production of food in any region (Manyong *et al.*, 1996). Production of sorghum is expected to increase in 2014/15 due to attractive producer prices in the year 2011 and greater availability of inputs (USDA, 2011).

The use of sourdough bread as a form of leavening is one of the oldest biotechnological processes in food production and it is an ancient craft, which is currently undergoing a revival of interest in developed and developing countries (Rocken and Voysen, 1995; Wood, 2004). Sourdough is an acidic or sharp tasting mixture of flour and water for making bread from cereal flours (Stauffer, 1991). Sourdoughs have been successfully used to improve the quality of gluten-free bread (Rakkar, 2007; Arendt *et al.*, 2011). Traditional breads made from these sourdoughs have a palatable taste, soft and elastic texture, and long shelf-life. The microorganisms involved in the sourdoughs may play an important role in quality of these breads (Golshan Tafti *et al.*, 2013). The indigenous microorganisms present in naturally fermented sourdough generally contain a complex mixture of natural yeast and LAB

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(Jespersen, 2003; De Vuyst and Neysens, 2005). The association of yeasts and LAB in sourdough generally is at a ratio of 1:100 (Gobbetti *et al.*, 1994; Ottogalli *et al.*, 1996). The most relevant bacteria isolate from sourdough is *Lactobacillus* species. Other genera are *Leuconostocs*, *Pediococcus*, and *Weissella* (De Vuyst and Neysens, 2005). Sanni *et al.* (1998) isolated *Lactobacillus* and *Pediococcus* genera from sour maize meal. The general aim of this study was to produce sourdough bread from sorghum flour using starter culture(s) and to determine its microbiological and physicochemical qualities.

## Materials and Methods

### Sample collection and processing

Sorghum grains of the red variety (*Sorghum bicolor*), were purchased from a retail market in Ibadan, South Western Nigeria. Red variety of sorghum was chosen due to its concentrated pigmentation. Sorghum seeds were germinated using the method of (Elkhalifa and Bernhardt, 2010). Briefly, 4000 g of sorghum grains were soaked in tap water for 24 h at room temperature with two changes of water to remove dirt and husk. Then, the grains were spread out thinly on moist jute sacs and covered with another jute sac. The grains were germinated for two days in the dark at room temperature (27°C). During germination, the grains were kept moist by spraying with water in order to control the moisture. The germinated sorghum grains were sun-dried in accordance with the procedure of Kolawole *et al.* (2007). The root portions were manually removed and the seeds were milled using a hammer mill into flour. The flour was sieved to obtain a particle size between 0.2 and 0.6 mm.

### Proximate analysis of the sorghum flour

The sorghum flour was analyzed for ash, crude protein (N x 6.25), lipid (ether extract) or crude fat and fibre according to the methods of Association of Official Analytical Chemists (A.O.A.C. 1990) on dry matter basis. All the measurements were in duplicates. Carbohydrate was calculated by difference (A.O.A.C, 1990).

% Carbohydrate = 100 – (sum of the percentage for ash, fat, protein and crude fibre).

### Natural fermentation of sorghum meal

The sorghum flour and tap water were mixed in ratio 1:1 (weight per volume) and then allowed to ferment naturally at room temperature according to the method of Lonner *et al.* (1986). The period of

fermentation was 48 h, after which the pH became stable.

### Temperature

At 0, 8, 24 and 48 h, the temperature of the fermenting sorghum meal was taken using a laboratory thermometer.

### pH

The pH of the fermenting sorghum meal was determined by the method of Tansey (1973). One gram of the meal was suspended in 9 ml sterile distilled water and homogenized. The electrode of the pH meter was dipped into the homogenate and readings were taken.

### Titratable acidity (TTA)

The amount of lactic acid produced in the fermenting sorghum meal was determined using the standard titration procedure according to A.O.A.C. (1990). A 10 ml of aliquots (triplicates) were pipetted and titrated against 1N NaOH to phenolphthalein end-point. Each ml of 1N NaOH is equivalent to 90.08 mg of lactic acid.

### Leavening

The level of leavening of the fermenting meal was determined by placing a short meter rule along the side of the bottle containing the fermenting meal and the level of the meal was read on the meter rule at 0, 8, 24 and 48h. The difference between the initial reading at 0 h and the final reading at 48 h was recorded as the level of leavening (Edema and Sanni, 2008).

### Microbiological analysis of the fermenting sorghum meal

At the intervals of 0, 8, 24 and 48 h, 1.0 g of the fermenting meal were homogenized in 9.0 ml sterile peptone water for about 30 s. The mixture was serially diluted in sterile peptone water by method of Meynell and Meynell (1970). From appropriate 10 fold dilutions, 0.1 ml of each dilution was plated out using pour plate method. The media used were deMann Rogosa Sharpe medium (MRS) of deMann *et al.* (1960) for lactic acid bacteria which was adjusted to pH 5.4 with HCl before sterilization, Malt extract agar (MEA) (LAB M, Lancashire, U.K) and Yeast Extract Agar supplemented with streptomycin were used for the isolation and enumeration of yeasts.

### Characterization and identification of microbial isolates

After enumeration, distinct colonies of lactic acid

bacteria and yeasts were randomly picked from the sample, purified by streaking on MRS and MEA agar respectively and observed for their morphological properties and colonial characteristics. The identification of LAB isolates was facilitated by the use of Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986) and The Genera of Lactic Acid Bacteria (Wood and Holzappel, 1995). Representative yeast isolates were identified to the level of species according to the procedure of Kurtzman and Fell (1998).

#### *Selection of starter cultures from lactic acid bacteria and yeast isolates*

Thirty-six LAB and thirteen yeast isolates were identified from the spontaneously fermenting sorghum meal. The criteria for the selection of the starter cultures were frequency of occurrence during fermentation and presence at the end of the fermentation, signifying the dominant microflora (Sanni *et al.*, 1998; Edema and Sanni, 2008).

#### *Preparation of starter inoculated fermented sorghum meals*

Sorghum flour and water were mixed and inoculated with the starter organisms, which inoculum sizes were  $2 \times 10^{10}$  cfu/ml of LAB and  $3 \times 10^9$  cfu/ml of yeast according to the method of Edema and Sanni (2008). The selected test cultures were used singly and as mixed cultures in the fermentation of the sorghum meal. 2.5 % baker's yeast was used as the control (Edema and Sanni, 2008).

#### *Bread making*

The ingredients used per 100 g of sorghum flour were: water (100-120 ml), baking fat (10 g), sugar (30 g), salt (1g) and egg (1 whole egg). Batter method was used for the bread baking. To make bread, all the ingredients were thoroughly mixed with electric mixer (Binatone electric mixer. Model: HM-350S, United Kingdom) for 10 min at high speed and allowed to stand for 30-40 min. Then, the mixture was gently mixed and the batter scaled (batter weight: 200 g) into greased baking pans (9 cm x12 cm x 6 cm) and allowed to ferment for 24 h at ambient temperature  $28 \pm 2^\circ\text{C}$  (Omoaka and Bokanga, 1994; Sanni *et al.*, 1998; Edema and Sanni, 2008). The pans were carefully transferred into the oven (Sonoko oven Model SK-716, Japan) to prevent collapse and baked at  $180\text{-}200^\circ\text{C}$  for 45-60 min.

#### *Cooling*

In order to prevent deformities in loaf and also to avoid undesirable condensation of moisture within

the packaging material, the breads were cooled to internal crumb temperature of about  $40^\circ\text{C}$  within a short period to minimize moisture loss. Then, the breads were packaged in clean transparent cellophane bags until required for further analysis.

#### *Physicochemical analyses of bread samples*

Proximate analysis of bread samples was carried out on dry matter basis. Ash, crude protein, ether extract (fat) and fibre contents of the bread samples were determined as described earlier for flour samples by the methods of A.O.A.C (1990). Determination of calcium, potassium and magnesium was by atomic absorption spectrophotometry (A.O.A.C., 1990). Bread heights were measured with a meter rule in centimeters (cm). Three different places were measured on the loaves and the mean values of the measurements were recorded as the heights of the loaves (Lonner and Preve-Akesson, 1988). The bread's weights were determined using a weighing balance.

#### *Organoleptic assessment*

Sensory evaluation of the bread was carried out within 24 h of baking. The samples were evaluated by 12-man untrained panel at the University of Ibadan on a 9-point hedonic scale of 9 (like extremely) to 1 (dislike extremely) for appearance, taste, texture, aroma, crumb and overall acceptability.

#### *Determination of shelf life of bread samples*

The bread loaves were wrapped in sterile plastic bags and stored at room temperature ( $28 \pm 2^\circ\text{C}$ ) on the shelf to determine the storage time (in days) until mould growth became visible (Lonner and Preve-Akesson, 1989).

#### *Analysis of data*

The statistical analyses were conducted using either one-way or two-way Analysis of variance (ANOVA) procedures depending on the experimental design. Statistical differences in samples were tested for at  $p \leq 0.05$ . Duncan's multiple-range test (DMRT) was used to differentiate between the mean values. All the analyses were done with SPSS (16.0) software for windows.

## **Results**

#### *Microbial population and frequency of occurrence of lactic acid bacteria and yeasts in the fermenting sorghum meal*

Increase in the microbial population, for both lactic acid bacteria and yeast were observed, the

growth of lactic acid bacteria increased from 3.46 log cfu/ml at 0 h, to 7.83 log cfu/ml at 48 h, while the yeast population increased from 3.41 log cfu/ml to 4.51 log cfu/ml within the same period of observation (Result not shown). The selection of the starter culture from both lactic acid bacteria and yeasts were based on the species variation and dominance at the end of the fermentation period. *Pediococcus pentosaceus* and *Saccharomyces cerevisiae* were selected from predominating LAB and yeasts isolates respectively and were used singly and in combination as starters for sourdough leavening (Result not shown).

#### Physico-chemical analysis during spontaneous fermentation of the sorghum meal

During the spontaneous fermentation of the sorghum meal, there was gradual decrease in the pH from 6.77 at mixing (0 h) to 4.31 at the end of fermentation (48 h) while the acid equivalent increased from 0.47 ml to 1.69 ml. Lactic acid produced and leavening of the fermenting meal increased from 41.88 mg/l to 152.98 mg/l; 0cm to 1.4cm respectively over the same period of fermentation (Table 1).

Table 1. Physico-chemical changes during spontaneous fermentation of sorghum meal

Time (h)	0	8	24	48
pH	6.77 <sup>d</sup>	6.27 <sup>c</sup>	4.49 <sup>b</sup>	4.31 <sup>a</sup>
Acid equiv. (ml)	0.47 <sup>a</sup>	0.70 <sup>ab</sup>	1.24 <sup>ab</sup>	1.69 <sup>b</sup>
Lactic acid (mg/l)	41.88 <sup>a</sup>	63.06 <sup>ab</sup>	111.24 <sup>ab</sup>	152.98 <sup>b</sup>
Leavening (cm)	0.00 <sup>a</sup>	0.20 <sup>b</sup>	0.90 <sup>c</sup>	1.40 <sup>d</sup>

Means in each column with different superscripts represent significant difference ( $p \leq 0.05$ ) by Duncan Multiple Range Test (DMRT)

#### Proximate composition of sorghum flour and sorghum bread samples

The proximate composition of the sorghum flour are as follows: Moisture content was 7.52%; the crude protein was 10.62% while the crude fat, crude fibre, ash and carbohydrate contents were 1.99%, 2.0%, 1.29% and 78.58% respectively. The breads after baking were evaluated for their proximate composition. The control sample (BYS) leavened with commercial baker's yeast had the highest moisture, crude protein and ash content of 50.40%, 16.60% and 2.13% respectively. The sample leavened with a combination of *S. cerevisiae* and *P.*

Table 2. Proximate composition of sorghum flour and sorghum bread samples

Sample	Moisture	Crude Protein	Crude Fat	Crude Fibre	Ash	Carbohydrate
YSS	34.68 <sup>b</sup>	11.27 <sup>a</sup>	6.80 <sup>b</sup>	3.23 <sup>b</sup>	1.56 <sup>a</sup>	72.96 <sup>c</sup>
LSS	25.92 <sup>a</sup>	11.46 <sup>a</sup>	2.98 <sup>a</sup>	1.73 <sup>a</sup>	1.50 <sup>a</sup>	77.39 <sup>c</sup>
LYS	40.03 <sup>c</sup>	11.49 <sup>a</sup>	10.97 <sup>d</sup>	5.03 <sup>d</sup>	1.71 <sup>b</sup>	68.84 <sup>b</sup>
BYS	50.40 <sup>d</sup>	16.60 <sup>b</sup>	9.28 <sup>c</sup>	4.40 <sup>c</sup>	2.13 <sup>c</sup>	65.58 <sup>a</sup>
SF	7.52	10.62	1.99	2.00	1.29	78.58

Means in each column with different superscripts represent significant difference ( $p \leq 0.05$ ) by Duncan Multiple Range Test (DMRT).

Key: YSS= sample inoculated with *Saccharomyces cerevisiae*; LSS= sample inoculated with *Pediococcus pentosaceus*; LYS= sample inoculated with *Saccharomyces cerevisiae* and *Pediococcus pentosaceus*; BYSS= sample leavened with baker's yeast; SF= Sorghum flour

*pentosaceus* (LYS) exhibited the highest crude fat and fibre content of 10.97% and 5.03% respectively while sample leavened with only *P. pentosaceus* (LSS) had the least of 2.98% for the crude fat. The carbohydrate content of the sample leavened with only *S. cerevisiae* (YSS) had the highest value of 72.96% while BYSS had the least of 65.58%. There were significant differences in the values obtained for the proximate composition of the sorghum flour in comparison to the analysis of the bread samples (Table 2).

#### Mineral composition of the sorghum bread samples

Table 3 shows the mineral composition of the sorghum bread samples. The sample inoculated with only *S. cerevisiae* (YSS) exhibited the highest calcium content of 13.15mg/100g while the sample inoculated with only *P. pentosaceus* (LSS) had the least of 6.6 mg/100g. The control sample (BYS) leavened with commercial Baker's yeast had the highest magnesium and potassium content of 120.75 and 233.25 mg/100g while LSS displayed the least of 68.65 and 126.2 mg/100g respectively.

#### Physical properties of the sorghum bread samples

Table 4 shows the physical properties of the bread samples prepared with the test cultures by batter method. All the bread had similar cracks (medium), crust colour (dark brown), texture (hard) and shape (raised). The highest bread weight of 201.5 g was recorded for LYS while the least was 153.5 g for

Table 3. Mineral composition of bread samples

Sample	Calcium (mg/100g)	Magnesium (mg/100g)	Potassium (mg/100g)
YSS	13.15	98.75	228.05
LSS	6.6	68.65	126.2
LYS	7.75	104.25	195.55
BYS	9.25	120.75	233.25

Key: YSS= sample inoculated with *Saccharomyces cerevisiae*; LSS= sample inoculated with *Pediococcus pentosaceus*; LYS= sample inoculated with *Saccharomyces cerevisiae* and *Pediococcus pentosaceus*; BYS= sample leavened with baker's yeast.

LSS. The highest bread height was 2.0 cm for LYS and BYS while the least was 1.35 cm for LSS. The shelf-life of the bread samples was between 4 to 9 days.

#### Organoleptic properties of the sorghum bread samples

The sensory evaluation of the sorghum bread samples is shown on Table 5. The bread samples inoculated with starters singly and combination (YSS, LSS and LYS) had significant values compared with the control sample (BYS) in preference to taste, aroma and overall acceptability. The values ranged from 6.83 to 3.83 for taste, 6.92 to 4.75 for aroma while overall acceptability was 6.58 to 4.25 for YSS and BYS respectively. There were no significant differences among the bread samples for appearance, texture and crumb.

#### Discussion

The population of indigenous LAB and yeasts increased drastically with the fermentation time in the fermenting sorghum meal as observed in this study. This co-existence between LAB and yeasts confirmed the synergistic relationship between the organisms in a fermenting food matrix (Wood, 2004). According to Wood and Hodge (1985), the ability of LAB and yeast to grow together is very crucial in food fermentation. As the period of fermentation increased, the LAB population was higher than the yeasts. This is in agreement with the report that LAB tend to dominate sourdough fermentations by the production of acid in the fermenting dough (Ottogalli et al., 1996). Wakil and Daodu (2011) reported cell count increase of LAB and yeast in fermenting maize gruel. This may be as a result of the fermentation conditions that tend to favour the growth of LAB than

Table 4. Physical properties of sorghum bread samples

Parameter	Bread samples			
	YSS	LSS	LYS	BYS
Weight (g)	195.3	153.5	201.5	181.4
Height (cm)	1.85	1.35	2	2
Volume (cm <sup>3</sup> )	199.8	145.8	216	216
Cracks	Medium	Medium	Medium	Medium
Crust colour	Dark brown	Dark brown	Dark brown	Dark brown
Texture	Hard	Hard	Hard	Hard
Shape	Raised	Raised	Raised	Raised
Shelf life (Day)	5	6	4	9

Key: YSS= sample inoculated with *Saccharomyces cerevisiae*; LSS= sample inoculated with *Pediococcus pentosaceus*; LYS= sample inoculated with *Saccharomyces cerevisiae* and *Pediococcus pentosaceus*; BYS= sample leavened with baker's yeast.

yeasts. Gobbetti et al. (1994) reported that yeasts are associated with LAB in sourdough, generally at a ratio of 1:100.

Majority of LAB isolated in this study were homofermenters. This is in agreement with other workers who reported the predominance of obligately homofermentative LAB in fermenting maize meal for the production of sour bread and homofermentative lactobacilli and *Pediococcus* spp. from the final sourdough for production of Swedish rye bread (Lonner et al., 1986; Sanni et al., 1998; Ricciardi et al., 2005). Gabriel et al. (1999) reported the microbiological survey of nine natural sourdoughs and characterised the isolates into 8 different species with *P. pentosaceus* and *L. mesenteroides* accounting for more than 93% of the total isolates. In this study, *P. pentosaceus* maintained a high population and dominated the fermentation period of 48 h. Sulma et al. (1991), who worked on Sudanese kiswa bread observed similar result. They observed a succession of growth of LAB during the natural fermentation period of 24 h while *L. brevis*, *Lactobacillus* sp., *Pediococcus pentosaceus* and *Enterococcus faecium* were isolated with *Pediococcus pentosaceus* dominating throughout the fermentation period and subsequent fermentations. But this is contrary to the report of Mbata et al. (2009), who observed both homofermentative and heterofermentative lactic acid bacteria at the end of the fermentation period of maize flour fortified with Bambara nuts. The

Table 5. Organoleptic properties of sorghum bread samples

Samples	Appearance	Taste	Texture	Aroma	Crumb	Overall Acceptability
YSS	6.91 <sup>a</sup>	6.83 <sup>b</sup>	6.17 <sup>a</sup>	6.92 <sup>b</sup>	6.67 <sup>a</sup>	6.58 <sup>b</sup>
LSS	6.75 <sup>a</sup>	6.75 <sup>b</sup>	6.08 <sup>a</sup>	6.75 <sup>b</sup>	6.64 <sup>a</sup>	6.33 <sup>b</sup>
LYS	6.58 <sup>a</sup>	6.25 <sup>b</sup>	5.83 <sup>a</sup>	6.75 <sup>b</sup>	6.58 <sup>a</sup>	6.00 <sup>b</sup>
BYS	6.00 <sup>a</sup>	3.83 <sup>a</sup>	5.33 <sup>a</sup>	4.75 <sup>a</sup>	5.50 <sup>a</sup>	4.25 <sup>a</sup>

Means in each column with different superscripts represent significant difference ( $p \leq 0.05$ ) by Duncan Multiple Range Test (DMRT).

Key: YSS= sample inoculated with *Saccharomyces cerevisiae*; LSS= sample inoculated with *Pediococcus pentosaceus*; LYS= sample inoculated with *Saccharomyces cerevisiae* and *Pediococcus pentosaceus*; BYSS= sample leavened with baker's yeast

lactic acid bacteria microflora of sourdough used for the production of Altamura bread, a typical bread of Southern Italy, was dominated by facultative heterofermentative lactobacilli.

The symbiotic association between lactic acid bacteria and yeasts is common in many food fermentations with the lactic acid bacteria providing the acid environment for yeast growth, while the yeasts provide vitamins and other growth factors for the lactic acid bacteria (Odunfa and Adeyele, 1985). In addition, yeasts prevent the accumulation of lactic acid to reach a toxic concentration (Wood, 1981). *Saccharomyces cerevisiae* was among the yeasts with highest percentage occurrence in this study, which is contrary to the report of De Vuyst and Neysens (2005) who reported that *S. cerevisiae* was not found in the raw materials but from the commercial yeast. This is in agreement with the report that *S. cerevisiae* is one of the yeasts most frequently found in sourdoughs and the most dominant yeast species associated with African indigenous fermented foods and beverages (Ottogalli et al., 1996; Jespersen, 2003). The species of yeasts isolated have been encountered in many other native fermented foods (Sulma et al., 1991; Sanni et al., 1998; Yonzan and Tamang, 2010).

A pure single or mixed culture can be used as starters for food fermentation (Frazier and Westhoff, 1986; Reale et al., 2004). In order to have a controlled fermentation, in this study, starter cultures were selected from indigenous yeast and LAB, used singly and in combinations. *Pediococcus pentosaceus* was selected as it had the highest frequency of occurrence among the LAB isolates and present at the end of the

fermentation period which indicated their dominance in the sourdough. *Saccharomyces cerevisiae* was also selected based on its dominance and reported leavening ability (Edema and Sanni, 2008).

There was a significant decrease in the pH of the spontaneously fermenting sorghum meal from 6.77 to 4.31 while the lactic acid and leavening level increased significantly. This is in agreement with the reports of other workers who showed that as fermentation period increases to a certain level, pH decreases while acidity and leavening increases (Arendt et al., 2007; Mbata et al., 2009). Gobbetti et al. (2005) observed that several sourdough LAB contributes to bread aroma and delay of spoilage due to the lactic acid they produce. The acidity level of sourdough is the main factor that determines the inhibitory activity of LAB against rope formation (Oscroft et al., 1990; Corsetti and Settanni, 2007).

Proximate composition analysis showed that the moisture content, crude protein, crude fat, crude fibre, and ash in the sourdough bread samples generally improved when compared with the raw material (sorghum flour) used for the baking. The bread samples had good mineral composition which indicates that the fermentation had positive influence on the sourdough. Fermentation has numerous beneficial effects on food which include improvements in nutritional qualities like protein and carbohydrate digestibility (Sanni, 1993; Onilude et al., 1999). Sourdough is especially useful in the production of high fibre wheat bread and it had also been used as a tool to improve the quality of barley/oat composite breads (Sahlström et al., 2009). Lactic fermentation of bread dough improves the keeping quality and flavour of the baked products. It also enhances the palatability of bread made from low-grade flours and under-utilized cereals (Lee, 1997).

The bread sample inoculated with *Pediococcus pentosaceus* (LSS) had the least value of weight, height and volume compared with the one leavened with *Saccharomyces cerevisiae* or the combination of both organisms. Apart from the control bread, LSS had the longest shelf life as mould growth was inhibited for up to six days. This is because the *P. pentosaceus* is a homofermentative LAB, producing mainly lactic acid but not carbon dioxide and other compounds. Gas formation by micro-organisms is necessary in order to obtain leavened bread (Hammes and Ganzle, 1998) and here, only the yeast produced gas. Volume had been associated with the type and level of acidification (Clarke et al., 2002) Yeast is important for good batter and leavening while LAB produce acids and other metabolites which inhibit the growth of spoilage organisms (Corsetti et al., 2000).

Although bread is known as a perishable commodity, its shelf-life can be greatly improved by fermentation. Shadi *et al.* (2010), in their study, used lactic acid fermentation to improve the shelf life of baguette and observed that the bread with highest percentage of sourdough had the lowest staling index. Furthermore, in the study of Edema and Sanni (2008), mould growth was inhibited in the sour maize breads for up to seven days. Lactic acid bacteria (LAB) strains are able to improve the shelf life of several food products (Lopez *et al.*, 2001; Di Cagno *et al.*, 2004) since the acids formed during fermentation process, lower the pH thus inhibiting the growth of spoilage organisms (Sanni, 1993).

Preliminary sensory evaluation on the basis of appearance, taste, texture, aroma, crumb and overall acceptability showed no significant difference among the bread samples with the exception of the bread baked with commercial baker's yeast (BYS) which was significantly different in appearance, taste, aroma and overall acceptability. BYS had lowest score for all the parameters tested. This may be due to the high amount of alcohol generated in the sourdough over the fermentation period, as a result of activity of the baker's yeast. This was observed in the taste and aroma of the bread. The bread leavened with *Saccharomyces cerevisiae* had the highest score for appearance, taste, aroma and overall acceptability. It had been reported that yeasts make a useful contribution to the improvement of flavour and acceptability of fermented cereal gruels (Banigo and Muller, 1972; Odunfa and Adeyele, 1985). Many of the taste panel members commented that the sourdough looked like cake because of its dark brown appearance, crumb and crust. The colour resulted from the use of red variety of sorghum for the flour processing. The brownish bread appearance could also be related to the increase in fibre content (Hu *et al.*, 2007). This result is not conclusive because different consumers have different taste preferences and sourdough bread is specialty bread just as we have other bread specialties like whole-meal bread, bagels, fruit breads, etc. (Sanni *et al.*, 1998; Chomiak, 2011).

## Conclusion

The microbiological and physicochemical analysis of sorghum bread showed that there was a co-existence of lactic acid bacteria and yeast in sourdoughs. Furthermore, homolactic fermenters predominated in the fermentation and the use of such organism as starters was able to improve the shelf life of bread. The nutritional properties of the sorghum

flour were improved and the physical parameters met the expectation for sourdough breads. Therefore, sourdough bread using sorghum can become one of Nigerian food varieties. Nevertheless, there will be need to enlighten the public on the health and nutritional benefits of sorghum bread as this will enhance acceptability of such specialty breads.

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