



## Effect of Fermented Dough on the Organoleptic Quality and Shelf-Life of Bread

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### Abstract

The study was carried out to determine the effect of fermented dough (sourdough) containing lactic acid bacteria on the organoleptic quality and shelf life of bread. Spontaneous fermentation of three commonly used wheat flour was carried out for 120 hours (5 days) at ambient temperature and at 33° C . The average total lactic acid bacteria counts during dough fermentation were  $2.0 \times 10^7$ ,  $1.8 \times 10^7$  and  $1.7 \times 10^7$  cfu/g for flour A, B and C fermented doughs respectively. A total of seven *Lactobacillus* species were isolated; *Lactobacillus fermentum*, *Lactobacillus sanfranciscensis*, *Lactobacillus casei*, *Lactobacillus pontis*, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus acidophilus*. Percentage overall acceptance of the experimental bread samples was highest (79.00%) in the bread samples with 20% fermented dough concentration. The highest minimum mould-free shelf life was 8 days in both the 15% and 20% fermented dough bread as compared to the control bread baked with yeast and commercially purchased bread. Finally, the use of fermented dough in bread production was found to improve the sensory quality and shelf life of bread.

**Keywords:** Effect, Fermented dough, Organoleptic quality, Shelf life, Spontaneous fermentation

### INTRODUCTION

Bread and bakery products have an important role in human nutrition. Generally, wheat bread is considered to be a good source of energy and irreplaceable nutrients for the human body. This is especially true for the product made from whole grain or high-fibre flour. There is increasing evidence that the intake of whole grain foods and cereal fibre helps to fight against chronic diseases, such as type 2 diabetes and cardiovascular disease (Mellen *et al.*, 2008).

In recent years traditional sourdough bread production has enjoyed renewed success with the ever-increasing demand by the consumer for more natural, tasty and healthy foods. Fermented dough (sourdough) has

proven useful in improving the texture and palatability of cereal products (Meignen *et al.*, 2001, Gül *et al.*, 2005, Katina *et al.*, 2006).

Sourdough is an intermediate product of bread preparation and contains metabolically active microorganisms. It comprises a mixture of lactic acid bacteria (LAB) and yeasts (De Vuyst and Vancanneyt, 2007), where the dominant flora are the lactic acid bacteria (Gobbetti *et al.*, 1994, Ottogalli, 1996, Gobbetti 1998, Corsetti and Settanni 2007). Lactic acid bacteria have a long history of safe use commonly referred to as GRAS (Generally Recognised as Safe) status which has led to their wide industrial applications (Dike and Sanni, 2010).

Several species belonging to the genera *Leuconostoc*, *Weisella*, *Pediococcus*, *Lactococcus*, *Enterococcus* and *Streptococcus* have been isolated from wheat sourdough, but *Lactobacillus* strains are the most abundant (Rollán *et al.*, 2010). Yeasts such as *Saccharomyces exiguous*, *Torulopsis holmii*, *Candida krusei*, *Pichia norvegensis* and *Hansenula anomala*, are also present in wheat sourdoughs but *S. cerevisiae* is frequently present or added (Saeed, 2009).

Lactic acid bacteria produce several natural antimicrobials, including organic acids (such as lactic acid, acetic acid, formic acid, phenyllactic acid, caproic acid), carbon dioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins, reuterin, and reutericyclin (Messens and De Vuyst, 2002). The LAB of the naturally fermented wheat sourdoughs may be used in the production of novel fermented foods such as sourdough bread, which is likely to have superior quality and longer shelf life (Saeed, 2009).

Sourdough is a dough containing a lactobacillus culture, usually in symbiotic combination with yeasts. It is one of the two principal means of leavening in bread baking, along with the use of cultivated forms of yeast (*Saccharomyces*). It is of particular importance in baking rye-based breads, where yeast does not produce comparable results. In comparison with yeast-based breads, it produces a distinctively tangy or sour taste, mainly because of the lactic acid produced by the lactobacilli (Vogel *et al.*, 2009).

The sourdough fermentation is central to acceptability in flavour, as chemically acidified breads prepared with pure commercial starter cultures are not well scored in sensory preference assessments (Lund *et al.*, 1989; Rehman *et al.*, 2006). The synergistic metabolic activities of microorganisms produce acidification or souring, influencing the final characteristics of bread, notably the texture and generate typical flavor compounds yielding typical sourdough sensory attributes (Gobbetti, 1998; Katina *et al.*, 2006). Sourdough bread

may vary in flavouring compounds as a result of ingredients selection and lactic acid bacteria fermentation (Saeed, 2009). Moreover, lactic acid bacteria contribute to the production of safer foods by inhibiting the growth of pathogenic microbes or by removing chemicals or toxic contaminants. Certain *Lactobacillus* spp, in the process of souring of dough, produce proteinases; an enzyme that breaks down gluten in wheat flour which is known to be toxic to people with celiac disease (Gobbetti *et al.*, 1998; Di Cagno *et al.*, 2009).

Bakery products are known to have a very short shelf-life. Important losses in the bakery industry due to microbial spoilage have been reported all over the world (Hamdi *et al.*, 2002). Fungal contamination which is known to be the most common source of microbial spoilage is a costly problem in the baking industries and the major factor in many cases, governing the shelf-life. Fungal growth may also be responsible for off flavours and may produce mycotoxins and allergenic compounds (Gobbetti *et al.*, 2009). In the EU regulations, the level of additives have been reduced allowing the concentrations of propionate (0.3% weight by weight) which is the most commonly used for packaged sliced breads. Studies have shown that mould growth still occurs in these conditions indicating that food preservation is not guaranteed (Rollan *et al.*, 2010). Ethanol has also been said to inhibit fungi in baked goods, but in some cases insufficient in preventing contamination (Gobbetti *et al.*, 2008). This study was therefore aimed at determining the effect of sourdough containing lactic acid bacteria on the organoleptic quality and shelflife of bread.

## Materials and Methods

### Sample Collection

Three popular wheat flour brands were purchased from terminus market in Jos metropolis, Plateau state.

### Preparation of Dough

The dough preparation was carried-out by mixing 1000g of the flour with 1000ml of

sterile tap water manually under aseptic conditions (Teiking, 2005; Saeed, 2009). Each flour brand dough was prepared in triplicates.

### **Fermentation of Dough to Yield Sourdough**

The dough samples were allowed to ferment spontaneously for 120 hours (5days) at room temperature.

### **Analysis of dough during fermentation**

Microbial counts, pH and titratable acidity (TTA) were determined at 0hrs, 24hrs, 48hrs, 72hrs, 96hrs and 120hrs intervals of fermentation.

### **Determination of pH and TTA**

Ten grams (10g) of the fermented dough sample was homogenized in 90ml of sterile distilled water and filtered and pH taken using a pH meter (HANNA HI 9025). Ten millilitres of the homogenate from each sample was titrated against 0.1N sodium hydroxide (NaOH) for the determination of TTA (Saeed, 2009; Bolourin and Khodaparast, 2010)..

Percentage titratable acidity calculated as lactic acid:

$$\text{TTA} = \frac{\text{Titre} \times \text{Normality of base} \times 0.009018}{\text{Weight of sample in gram}} \times 100$$

(Kim *et al.*, 2008; Wakil *et al.*, 2008).

### **Microbial counts**

A serial dilution of each sample was made and the last two dilutions ( $10^{-5}$  and  $10^{-6}$ ) were plated out in duplicates on appropriate agar for microbial counts as described below:

### **Lactic acid bacteria count (LABC)**

The last two dilutions ( $10^{-5}$  and  $10^{-6}$ ) were inoculated on De Man Rogosa Sharpe's (MRS) agar plates. The plates were incubated under anaerobic condition at  $30^{\circ}\text{C}$  for 48hours. Colonies were counted and results expressed as CFU/g.

### **Aerobic plate count (APC)**

The last two dilutions ( $10^{-5}$  and  $10^{-6}$ ) were inoculated on plate count agar (PCA) plates. The plates were incubated at  $37^{\circ}\text{C}$  for 24hours. Colonies were counted and results expressed as CFU/g.

### **Fungal Count**

The last two dilutions were inoculated on potato dextrose agar (PDA) (containing 0.5ml/l streptomycin sulphate) plates. The plates were incubated at room temperature. Colonies were counted and results expressed as CFU/g.

### **Identification of LAB isolates**

Colonies on the MRS plates were further purified by successive streaking on fresh MRS agar plates. The LAB isolates were identified based on their Gram reaction, catalase test and sugar fermentation test as described by Mehmood *et al.* (2009), Rubayyi *et al.*, 2010, Sameen *et al.* (2010).

### **Production of Experimental Bread**

The bread samples were produced using Supreme flour sourdough which has the highest lactic acid bacteria count and the highest number of heterofermentative LAB species. The experimental bread samples were produced at 5% (w/w), 10% (w/w), 15% (w/w) and 20% (w/w) sourdough concentrations. Other bread dough formulation include 950g, 900g, 850g and 800g flour, 640ml tap water, 44g salt and 40g sugar. The dough samples were divided into 100g dough balls, molded and placed in pans. Final proofing was done for 45 minutes and  $35^{\circ}\text{C}$  and bread was baked at  $232^{\circ}\text{C}$  for 25 minutes (Saeed, 2009; Rehman *et al.*, 2007). Control bread samples were also prepared using baker's yeast only.

### **Analysis of experimental bread samples**

The pH and TTA of the experimental bread samples were determined as earlier described.

### **Sensory evaluation of bread samples**

The bread samples were subjected to sensory evaluation using a panel of 25 enlightened judges to evaluate the following physical parameters; taste, texture, appearance, and aroma. Five point grade was used for the analysis; Excellent-5, Very good-4, Good-3, Satisfactory-2, and Poor-1 (Boboye *et al.*, 2008). The judges were instructed to rinse their mouth with water before and after taking each sample.

### Evaluation of Shelf-life of Sourdough Bread

The experimental sourdough bread samples, the control bread (without sourdough) and three different types of commercial bread samples were stored at room temperature and evaluated for their shelf life by determining the minimum mould-free shelf life (MMFSL) as described by Pattison *et al.* (2004).

#### Statistical Analysis

The data obtained were subjected to statistical analysis using analysis of variance (ANOVA) and Duncan Multiple Range Test.

#### Results

Table 1 shows the result of the microbial counts, the mean lactic acid bacteria count was  $4.18 \times 10^6$ cfu/g,  $3.88 \times 10^6$ cfu/g and  $1.91 \times 10^6$ cfu/g for flour C, flour A and B respectively. There is no significant difference in the mean LABC between C and A ( $P \geq 0.05$ ) but there is a significant difference in the mean LABC count between the two flours and flour B ( $P \leq 0.05$ ). In all cases the LABC counts increased with increase in fermentation time while both the yeast count and the APC decreased. There is also a significant difference in the mean LABC and both the yeast count and the APC ( $P \leq 0.05$ ).

The lactic acid bacteria composition of the fermented dough (sourdough) is as shown in Table 2. Seven different species of lactic acid bacteria all belonging to the genera *Lactobacillus* were isolated and identified. These include *Lactobacillus sanfransicensis*, *Lactobacillus plantarum*, *Lactobacillus*

*fermentum*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus acidophilus* and *Lactobacillus pontis*. All the seven species were isolated from flour C while five species were isolated from flour B and four species from flour A.

Table 3 shows the pH and TTA values for the sourdough bread samples and the control bread. The pH of the sourdough bread samples are all lower than that of the control bread while the TTA of the sourdough bread samples were higher that of the control bread.

Table 4 shows the sensory evaluation scores for the different bread samples. The twenty percent (20%) sourdough bread sample scored the highest in terms of taste (87%) and appearance (78%) while 10% sourdough bread sample scored the highest in terms of texture (81.54%) and aroma (83.85%). In terms of overall acceptance, the 20% sourdough bread sample was the most preferred with an overall score of 79% followed by the 15% sourdough bread (73%), the 10% sourdough bread sample 63.76%, 5% sourdough bread 56.66% and the least was the control bread sample 52%.

The minimum mould – free shelflife of the bread samples is as shown in Table 5. The 15% and 20% sourdough bread samples had the longest shelflife of eight (8) days followed by the 5% and 10% bread samples with a shelflife of 7 days. Two of the three commercial bread samples have a shelflife of 6 days while the least shelflife of 5 days was obtained for the control bread samples and the third commercial bread sample.

**Table 1: Lactic acid bacteria count, Aerobic Plate Count and Yeast count during dough Fermentation (At 25°C)**

Fermentation Time (in hours)	Golden penny flour			Dangote flour			Supreme flour		
	LABC	APC	YC	LABC	APC	YC	LABC	APC	YC
0	1.2x10 <sup>4</sup>	3.6x10 <sup>8</sup>	1.3x10 <sup>2</sup>	2.7x10 <sup>4</sup>	5.8x10 <sup>7</sup>	2.1x10 <sup>2</sup>	1.7x10 <sup>4</sup>	7.4x10 <sup>7</sup>	1.1x10 <sup>2</sup>
24	1.4x10 <sup>5</sup>	4.0x10 <sup>6</sup>	3.5x10 <sup>3</sup>	2.4x10 <sup>5</sup>	5.1x10 <sup>5</sup>	3.8x10 <sup>3</sup>	1.8x10 <sup>5</sup>	5.5x10 <sup>6</sup>	2.8x10 <sup>3</sup>
48	2.5x10 <sup>5</sup>	4.5x10 <sup>5</sup>	5.8x10 <sup>3</sup>	3.7x10 <sup>6</sup>	3.6x10 <sup>5</sup>	5.2x10 <sup>4</sup>	3.2x10 <sup>6</sup>	3.8x10 <sup>5</sup>	4.5x10 <sup>4</sup>
72	2.1x10 <sup>6</sup>	5.5x10 <sup>4</sup>	3.4x10 <sup>4</sup>	5.1x10 <sup>6</sup>	1.5x10 <sup>5</sup>	6.4x10 <sup>4</sup>	8.7x10 <sup>6</sup>	2.7x10 <sup>4</sup>	5.3x10 <sup>4</sup>
96	1.2x10 <sup>7</sup>	2.9x10 <sup>3</sup>	5.5x10 <sup>5</sup>	3.4x10 <sup>7</sup>	3.7x10 <sup>4</sup>	2.6x10 <sup>5</sup>	4.4x10 <sup>7</sup>	5.8x10 <sup>3</sup>	7.1x10 <sup>5</sup>
120	3.1x10 <sup>7</sup>	1.2x10 <sup>3</sup>	8.1x10 <sup>5</sup>	5.8x10 <sup>7</sup>	1.1x10 <sup>3</sup>	4.8x10 <sup>5</sup>	5.3x10 <sup>7</sup>	2.6x10 <sup>3</sup>	8.8x10 <sup>5</sup>
<b>Mean</b>	1.91 x10 <sup>6</sup> <sub>a</sub>	3.62 x10 <sup>5</sup> <sub>bc</sub>	4.60 x10 <sup>4</sup> <sub>ab</sub>	3.85x10 <sup>6</sup> <sub>b</sub>	3.47x10 <sup>5</sup> <sub>bc</sub>	4.15x10 <sup>4</sup> <sub>ab</sub>	4.18x10 <sup>6</sup> <sub>b</sub>	4.63x10 <sup>5</sup> <sub>bc</sub>	4.93x10 <sup>4</sup> <sub>ab</sub>

Values with same subscript are not significantly different ( $P \leq 0.05$ ). LABC= Lactic acid bacteria count, APC= Aerobic plate count, YC= Yeast count.

**Table 2: Lactic acid bacteria isolated during the dough fermentation**

Type of flour	Isolates
Golden penny flour	<i>Lactobacillus sanfransicensis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus pontis</i> .
Dangote flour	<i>Lactobacillus sanfransicensis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus pontis</i> .
Supreme flour	<i>Lactobacillus sanfransicensis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus fermentum</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus pontis</i> .

**Table 3: Physico-chemical properties of the bread samples**

Bread sample	pH	TTA
5%	5.5	0.5
10%	5.5	0.7
15%	5.4	0.6
20%	5.4	0.9
Control	5.9	0.4

**Table 4: Sensory evaluation of the bread samples**

Type of bread	Taste (%)	Texture (%)	Appearance (%)	Aroma (%)	Overall acceptance (%)
5% sourdough bread	77.69	72.31	72.31	72.35	56.66
10% sourdough bread	84.62	81.54	81.54	83.85	63.76
15% sourdough bread	80.00	60.00	78.00	72.00	73.00
20% sourdough bread	87.00	62.00	82.00	79.00	79.00
Control bread	52.00	55.00	72.00	75.00	52.00

**Table 5: Minimum Mould Free Shelflife (MMFSL) of the bread samples**

Bread sample	MMFSL
5% sourdough bread	7 days
10% sourdough bread	7 days
15% sourdough bread	8 days
20% sourdough bread	8 days
Control bread	5 days
Commercial bread I	6 days
Commercial bread II	5 days
Commercial bread III	6 days

### Discussion

The higher counts of lactic acid bacteria compared to yeast and aerobic plate counts agrees with the findings of other workers in this area; Saeed (2009) reported LAB as the dominant bacteria in sourdough with counts in the range of  $6.24 \times 10^4$  CFU/g to  $6.92 \times 10^7$  CFU/g. In mature sourdoughs LAB counts range from  $1 \times 10^9$  to  $3 \times 10^9$  cfu per gram and yeasts from  $1 \times 10^6$  to  $5 \times 10^7$  cfu per gram sourdough (Hammes *et al.*, 2005). Luangsakul *et al.*, 2009 reported LAB counts of  $1.8 \times 10^4$  to  $10^8$  colonies/g sample, and low fungal counts of 10 to  $2.3 \times 10^2$  colonies/g sample. The high LAB counts compared to yeasts and APC counts is due to the production of (lactic and acetic acids) by LAB which enhances their rapid growth when the pH value has dropped too low for other microorganisms to grow (Savic *et al.*, 2006; Gerekova *et al.*, 2011).

The LAB isolates obtained in this work tallies with the findings De Vuyst *et al.* (2011) and Scheirlink *et al.* (2007) who reported the isolation of *Lactobacillus paralimentarius*, *L. sanfranciscensis*, *L. Plantarum* and *L. Pontis* from sourdough. Scheirlink *et al.* (2007) reported that species of *Lactobacillus sanfranciscensis*, *Lactobacillus paralimentarius*, *Lactobacillus plantarum*, and *Lactobacillus pontis* dominated the LAB population of Belgian type I sourdoughs. Several workers in this field reported *Lactobacillus* species as the most dominant genera of LAB in sourdough (Gobetti *et al.*, 1994; Korakli *et al.*, 2004; De Vuyst and Neysens, 2005, Luangskul *et al.*, 2009; Saeed *et al.*, 2009; Vuyst *et al.*, 2011).

The increase bread acidity in relation to increase in sourdough concentration agrees with the findings of Bolourin and Khodaparast, (2010) who reported that there was a linear increase in the acidity of bread with increase in sourdough concentration.

The higher score of the 10% and 20% sourdough bread compared to others with respect to texture and aroma; taste and appearance respectively is statistically significant ( $P \leq 0.05$ ). Generally, all the sourdough bread were preferred to the control bread. This is in agreement with the findings of researchers in this field. According to Rehman *et al.* (2006) sourdough fermentation is central to acceptability in flavour, as chemically acidified bread and bread prepared with pure commercial starter cultures are not well scored in sensory preference assessments. Tafti *et al.* (2013) reported that the addition of spray-dried sourdough at the level of 9% can be successfully used for Sangak breadmaking, leading to bread with an improved flavor. Sensory evaluation of sourdough of wheat bread crumb showed that bread made from sourdough containing heterofermentative *Lb. sanfranciscensis* had a pleasant, mild, sour odour and taste (Rehman *et al.*, 2006). According Chavan and Chavan, (2011) sourdough improves sensory characteristics such as loaf volume, evenness of baking, color, aroma, taste, and texture of breads. However, the findings of this work differs from that of Bolourin and Khodaparast, (2010) who reported that 5% sourdough bread is most preferred by the test panellist.

It has been reported that during sourdough fermentation, lactic acid bacteria (LAB) produce a number of metabolites e.g. organic acids, exopolysaccharides (EPS) alcohols, aldehydes, ketones, esters, ether derivatives, furan derivatives, hydrocarbons, ketones, lactones, pyrazines, pyrrol derivatives and sulphur compounds which have been shown to have a positive effect on the texture and/or aroma of bread. Organic acids affect the protein and starch fractions of flour. In addition, the drop in pH associated with acid production causes an increase in the proteases and amylases activity of the flour, thus leading to a reduction in staling. While improving the textural qualities of bread, sourdough fermentation also results in increased mineral bioavailability and reduced phytate content (Thiele *et al.*, 2002; Arendt *et al.*, 2007).

All the sourdough bread have longer minimum mould-free shelflife (MMFSL) than the control and the commercial bread samples. Fungal spoilage is the main cause of economic loss in the baking industry and the ability of sourdough to increase the shelflife of bread has been widely reported. Zannini *et al.* (2013) reported that the use of 20% sourdough fermented with *L. hammesii* in bread making increased the mold-free shelf life by 2 to 3 days or from 2 to more than 6 days. A 10-fold concentrated culture

filtrate of *Lb. plantarum* 21B isolated from sourdough and grown in wheat flour hydrolysate was shown to possess an efficient antifungal activity against *Penicillium corylophilum*, *Penicillium roqueforti*, *Penicillium expansum*, *Aspergillus niger*, *A. flavus*, and *Fusarium graminearum* (Dalie *et al.*, 2010).

Several other researchers have also reported increased shelflife of sourdough bread in comparison with bread baked with only yeast (Gobetti *et al.*, 2000; Gobetti *et al.*, 2005; Dal Bello *et al.*, 2007; Plessas *et al.*, 2008; Saeed, 2009; Chavan and Chavan, 2011; Muhialdin and Hassan, 2011).

The antifungal activity of sourdough is due to a number of metabolites produced by LAB during sourdough development; this include lactic, acetic, caproic, formic butyric, n-valeric, phenyllactic, 4-hydroxy-phenyllactic and mono-hydroxy octadecenoic acids (Gobetti *et al.*, 2000; Gobetti *et al.*, 2005; Zannini *et al.*, 2013).

### Conclusion

Addition of sourdough at a concentration of between five percent and twenty percent (5% and 20%) has the potential of improving both the organoleptic quality and shelflife of bread.

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