MOLECULAR CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM SOURDOUGH AND THEIR BAKING POTENTIALS

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DECLARATION

I declare that the work in this thesis entitled **‘’ Molecular Characterization of Lactic acid Bacteria Isolated from Sourdough and their Baking Potentials’’** has been performed by me in the Department of Microbiology under the supervision of Prof. S.A. Ado, Prof. J.B. Ameh and Prof. C.M.Z. Whong. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled ‘MOLECULAR CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM SOURDOUGH AND THEIR BAKING POTENTIALS’ by Michael Macvren DASHEN meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) in Microbiology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

In memory of my late dad, Mr. Emmanuel Dagwel Dashen.

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ABSTRACT

This study was aimed at determining the lactic acid bacteria composition of Nigeria's type II sourdough, their baking potentials and the type of exopolysaccharides genes they carry. Doughs made from three different brands of Nigeria's widely used wheat flour were fermented spontaneously to produce sourdough. Microbial counts, pH and titratable acidity of the sourdoughs were determined. Lactic acid bacteria were isolated from the sourdoughs and identified. Selected lactic acid bacteria from the sourdoughs and selected sourdough were used to bake bread and doughnuts. The lactic acid bacteria were also screened for exopolysaccharides (EPS) production ability. Molecular characterization and sequencing of the exopolysaccharide genes were also carried out. The results obtained revealed that the lactic acid bacteria were the dominant population in all the sourdough samples. The highest total mean counts were log 6.826±0.68, 6.551±0.57 and 6.414±0.59 for lactic acid bacteria, fungal count and aerobic plate count respectively at room temperature. At 40^0C, the highest total mean counts were log 7.051±1.04, 4.925±0.64 and 4.968±0.88 for lactic acid bacteria count, fungal count and aerobic plate count respectively. Significant difference exists between these counts at $P \leq 0.05$. The pH values decreased with increase in fermentation time; it ranged between 6.73 ±0.05 and 4.20 ±0.29 while the TTA values increased with increase in fermentation time; it ranged between 0.168±0.00 and 0.676±0.01. A total of seventy-nine (79) isolates of lactic acid bacteria were obtained. The lactic acid bacteria isolated from the sourdough samples included *Lactobacillus plantarum* (34.17%), *Lactobacillus brevis* (29.11%), *Lactobacillus pentosus* (17.72%), *Pediococcus pentocaseus* (8.86%), *Lactobacillus buchneri* (2.53%), *Lactobacillus collinoides* (2.53%), *Lactobacillus fermentum* (2.53%) and *Pediococcus acidilactici* (2.53%). Nineteen (24.05%) out of the total of seventy-nine (79) lactic acid bacteria isolates produced exopolysaccharides. Sixteen (84.21%) of the nineteen EPS positive isolates phenotypically expressed their EPS in form of mucoid and ropy colonies while three (15.79%) produced only mucoid colonies. Fourteen (73.68%) of the nineteen EPS positive isolates were heterofermenters. The best gas producers were *Lactobacillus plantarum* SP2 and *Lactobacillus brevis* SP5. The highest dough leavening was obtained from the combination of *Lactobacillus plantarum* and baker's yeast which was 108.00±2.00cm^3 compared to 92.00±1.00cm^3 for baker's yeast alone both at 180 minutes of dough leavening. Significant difference exists between the dough leavening at $P \leq 0.05$. All the lactic acid bacteria based bread and doughnuts also compared favourably with the baker's yeast bread and doughnuts and commercial bread and doughnuts in terms of organoleptic quality. All the lactic acid bacteria based bread and doughnuts had longer minimum mould free shelflife than the baker's yeast and commercial bread and doughnuts. All the bread and doughnuts were within standard with respect to moisture content and protein contents. Seven (36.84%) of the nineteen (19) exopolysaccharides producing lactic acid bacteria were levansucrase (LevV) gene positive while none was glucansucrase gene positive. Molecular Phylogenetic analysis indicated close relationship between the isolates. Therefore the lactic acid bacteria isolated from the type II sourdough have the potentials for use as starter culture in baking for the improvement of the organoleptic quality and shelflife of bakery products in Nigeria.

TABLE OF CONTENTS

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

LIST OF TABLES

Table **Title** **Page**

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDECES

Appendix **Title** **Page**

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

For several thousand years, bread has been one of the major constituents of the human diet, making the baking of yeast – based sourdough breads one of the oldest biotechnological processes. Generally, wheat bread and other bakery products are considered to be good sources of energy and nutrients for the human body. This is especially true for the bakery products made from whole grain or high – fibre flour (Rollan *et al*., 2010).

The spontaneous fermentation of dough leads to the development of sourdough. Sourdough fermentations improve dough properties, enhance both bread texture and flavour and delay bread spoilage (Wehrle and Arendt, 1998; De vuyst *et al*., 2007; Zugic – Petrovic *et al*., 2009). Sourdough fermentation is a traditional process used in baking industry to improve the quality and flavour of baked products. The addition of sourdough in bread baking affects in several ways the final properties of the bread. During the fermentation process several metabolic activities take place and each is responsible for the changes that occur in the characteristics of wheat flour as well as in aroma, taste, nutritive value and shelf-life of bakery products (Zotta *et al*., 2006; Corsetti *et al*., 2007).

Lactic acid bacteria (LAB) and yeasts play a key role in sourdough fermentation processes (Scheirlink *et al*., 2007; Scheirlink *et al*., 2008; Sadeghi *et al*., 2008; Bolourin and Khodaparest, 2010). Sourdough LAB had been extensively studied with respect to their carbohydrate metabolism, proteolysis and amino acid metabolism, lipid metabolism and production of volatile compounds (De Vuyst *et al*., 2007). Lactic acid bacteria have shown a major potential for use in biopreservation because of their safety for human consumption (generally regarded as safe (GRAS) status) and prevalent microflora during storage in many foods. Lactic acid bacteria can produce a wide range of antimicrobial metabolites such as organic acids, hydrogen peroxide and bacteriocins which can contribute to the microbiological safety of foods by controlling the growth of pathogenic microorganisms (El-Gaali *et al*., 2009).

A common trend of sourdough fermentations is the unique symbiosis of certain heterofermentative and homofermentative lactic acid bacteria with yeasts. The organoleptic and nutritional properties of sourdough breads depend on their metabolism (Sadeghi *et al*., 2008).

Sourdough has traditionally been used as a leavening agent in bread production (Sadeghi, 2008). Sourdough fermentations, as well as baking agents based on sourdoughs, have retained their importance in contemporary baking technology because of the improved aroma, texture and shelf life of sourdough breads (Ganzle and Vogel, 2002). The production of a wide variety of traditionally prepared baked goods continues to rely exclusively on the use of sourdough as a leavening agent. In most industrial applications, sourdough or dried sourdough preparations are added to bread doughs which also contains baker’s yeast as a leavening agent (Teiking, 2005).

It has been reported that certain LAB produce exopolysaccharides (Welman *et al*., 2003; Maneerat *et al*., 2010). Lactic acid bacteria that produce exopolysaccharides (EPS) are already known to play an important role in dairy industries because of their contribution to the consistency and rheology of fermented milk products (Katina, 2005). Exopolysaccharides can replace hydrocolloids used as texturizing, antistalling, or prebiotic additives in bread production, improving the texture and nutritional properties of the product (Zotta *et al*., 2006).

Sourdough represents a natural food ecosystem in which the fermentative activities of LAB and yeasts largely determine the typical characteristics of the resulting baked products. Based on production technology, sourdough fermentations can be divided into three types: type I, or traditional, sourdoughs characterized by continous propagation of the dough at ambient temperature (20^0C – 30^0C); type II, or industrial sourdoughs are incubated at high temperatures (> 30^0C), with longer fermentation times and a high water content; and type III sourdoughs which are dried preparations of industrial doughs (Teiking 2005; Scheirlinck *et al*., 2007; Saeed, 2009).

1.2 Statement of the Research Problem

A variety of high – molecular weight polysaccharides produced by plants (cellulose, pectin and starch) seaweeds (alginate and carrageenan), and bacteria (alginate, gellen and xanthan), found applications as viscosifying, stabilizing, emulsifying, gelling or water binding agents in food and non-food industries (Adebayo-tayo and Onilude, 2008). All of these polysaccharides are additives, however, and therefore they are considered less desirable in food industries (Dijkhuizen *et al*., 1999).

Bakery products have a very short shelf-life. All over the world microbial attacks cause very important losses in bakery industries (Hamdi *et al*., 2007). Contamination by fungi is the most common source of microbial spoilage and is a costly problem in bakery industries. In many cases it is the major factor governing shelf-life. Besides the repellent side of visible growth, fungi, may be responsible for off-flavours and may produce mycotoxins and allergenic compounds (Gobbetti *et al*., 2008). Currently, the protection of baked products from fungal spoilage is mainly achieved through the use of organic acids such as propionic, sorbic, acetic and benzoic acids and some of their salts as inhibitors. Present trends in the bakery industry have included the desire for high – quality foods, which are minimally processed and do not contain chemical preservatives (Rollan *et al*., 2010).

The appearance of cancer-like tumours in rats fed propionic acid at concentrations of up to 4% has led to prohibition of the use of calcium propionate in some European countries and/or to the limited use of organic acids in foods (Rollan *et al*., 2010). Propionic acid has also been reported to cause irritability, restlessness, inattention and sleep disturbance in some children (Dengate and Ruben, 2002). The level of additives has been reduced in the new EU regulations, allowing the concentrations of propionate the most commonly used, up to 0.3% (wt/wt) for packaged sliced breads. However, fungal growth still occurs in these conditions, meaning that food preservation is not guaranteed (Rollan *et al*., 2010). Ethanol is also inhibitory to fungi in baked goods, but in some cases it is not sufficient to prevent contamination (Gobbetti *et al*., 2008).

International trend towards elimination of chemical preservatives from bread and other foodstuffs is expected to increase the risk of bread spoilage by rope-producing *Bacillus* strains (Rollan *et al*., 2010).

Several proteins of wheat flour and products of their hydrolysis have biological activities that can affect human health when wheat or it's by products are consumed as foods. In 1953, it was first recognised that ingestion of wheat gluten causes celiac disease in sensitive individuals (Gobbetti *et al*., 2002; Zotta *et al*., 2006). Celiac disease (CD) is one of the most common food intolerance problems that occur in one out of every 130 – 300 persons of the European and United States populations and one out of every 100 persons in Argentina (Rollan *et al*., 2010). However, gluten exclusion results in very serious problems for bakers, and at present many gluten - free products in the market are of low quality, exhibiting poor mouth feel and flavour. It has been reported that gluten - free breads have a tendency for rapid stalling and a weak aroma (Rollan *et al*., 2010).

1.3 Justification of the Study

Lactic acid bacteria are food-grade organisms that possess Generally Regarded As Safe (GRAS) status and are known to contribute positively to the taste, smell or preservation of the final products (Dijkhuizen *et al*., 1999; Saeed *et al*., 2009). One important attribute of many LAB is their ability to produce antimicrobial compounds called bacteriocins. In recent years interest in these compounds had grown substantially due their potential usefulness as natural substitute for chemical food preservatives in the production of foods with enhanced shelf life and/or safety (Anwaar *et al*., 2002).

Consumer demands for more natural foods have stimulated the research on biological (i.e vegetal and microbial) preservation systems. In this aspect, LAB are organisms of interest for preservation since they have been used for centuries in various fermented foods, either by their natural presence in raw materials (spontaneous fermentation) or their addition as pure starter cultures (Anwaar *et al*., 2002). Lactic acid bacteria had received scientific attention because of their antifungal potential since LAB from cereals with antifungal activity have been reported (Anwaar *et al*., 2002). However, the application of these antifungal LAB cultures in baked food is still limited despite the advances on the characterization of antifungal metabolites from lactic acid bacteria (Anwaar *et al*., 2002; Rollan *et al*., 2010).

In wheat fermentation, sourdough LAB fermentation creates an optimum pH for the activity of endogenous enzymes which improve dough properties and texture, contributes directly to bread aroma and flavour, increases phytate breakdown, loaf volume and digestability (Sadeghi *et al*., 2007; Moroni *et al*., 2009). The application of LAB in the form of sourdough has been reported to have positive effects on bread stalling. One such effect is an improvement in loaf specific volume, which is associated with a reduction in the rate of stalling. The use of sourdough in wheat bread has gained popularity due to its ability to improve the quality and flavour of wheat bread (Arendt *et al*., 2002).

During sourdough fermentation, LAB produce a number of metabolites such as organic acids and exopolysaccharides (EPS). Lactic acid bacteria EPS have been recognised to have antitumor, antiulcer and blood cholesterol lowering activities, as well as the ability to enhance the immune system. The food industry is interested in EPS producing food grade organisms such as lactic acid bacteria (Moroni *et al*., 2009).

Sourdough has traditionally been used as a leavening agent in bread production. Sourdough fermentations as well as baking agents based on sourdough have retained their importance in contemporary baking technology because of the improved aroma, texture and shelf-life of sourdough breads (Tieking *et al*., 2003; Corsetti and Settanni, 2007). Different approaches are under investigation to produce breads that can be tolerated by celiac disease patients. One such approach is the use of sourdough to improve the quality of gluten-free bread (Arendt *et al*., 2002; Rollan *et al*., 2010).

Knowledge of the metabolic activities and corresponding genes of sourdough lactic acid bacteria that are responsible for their positive influence on bread quality is a prerequisite for the deliberate choice of starter cultures for specific applications (Tieking *et al*., 2003; Teiking, 2005).

1.4 Aim

The aim of this research was to isolate, characterize, determine the baking potentials and screen for some important genes of lactic acid bacteria from type II sourdough produced in Nigeria.

1.5 Specific Objectives of the Study

The objectives of this study were to:

1. Carryout total microbial counts, isolate and characterize LAB isolates from sourdoughs made from different brands of wheat flour.
2. Screen the LAB isolates for exopolysaccharides production.
3. Screen the LAB isolates for dough leavening ability.
4. Produce bread and doughnuts using selected lactic acid bacteria isolates.
5. Determine the physico-chemical properties and proximate composition of flour, fermented dough (sourdough), bread and doughnuts produced using the selected lactic acid bacteria isolates.
6. Determine the organoleptic quality and shelf-life of the bread and doughnuts produced using the selected lactic acid bacteria isolates.
7. Screen the exopolysaccharides producing isolates for the presence of levansucrase gene (Lev V) and glucansucrase gene (gtf).
8. Sequence the levansucrase gene and glucansucrase gene of the EPS positive isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An Overview of the Lactic Acid Bacteria

Lactic acid bacteria (LAB) constitute a group of Gram-positive bacteria united by a constellation of morphological, metabolic, and physiological characteristics (Axelsson, 2004). The general description of the bacteria included in the group is gram-positive, nonsporing, nonrespiring cocci or rods, which produce lactic acid as the major end product of carbohydrates fermentation (Axelsson, 2004; Makarova and Koonin, 2007; Auld, 2010) . They are nutritionally fastidious, requiring carbohydrates, amino acids, peptides, nucleic acids and vitamins (Rattanachaikunsopon and Phumkhachorn, 2010). The term lactic acid bacteria was gradually accepted in the beginning of the 20th century. Other terms as ‘’milk souring’’ and ‘’lactic acid producing’’ bacteria had previously been used for the bacteria causing a slight confusion. This ended with the publication of a monograph about lactic acid bacteria written by Orla-Jensen in 1919, a work that had great impact on the systematic of lactic acid bacteria (Khalid, 2011).

Lactic acid bacteria are widespread in nature; they were first isolated from milk and have since been found in meat, raw fish and prawns, milk products, vegetables, beverages and bakery products. They have also been isolated from soil, water, manure and sewage. They are commonly found in the mucosal membranes and gastrointestinal tracts of humans and animals (Nanasombat *et al*., 2012).

The boundaries of the group have been subject to some controversy, but historically the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* form the core of the group. Taxonomic revisions of these genera and the description of new genera mean that

LAB could, in their broad physiological definition, comprise around 20 genera. However, from a practical, food-technology point of view, the following genera are considered the principal LAB: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, Weissella, Lactosphaera* and *Paralactobacillus* (Axelsson, 2004; Auld, 2010; Khalid, 2011).

The genus *Bifidobacterium* is historically also considered to belong to the LAB group. In the 7th edition of Bergey's Manual of 1957, the bifidobacteria were designated *Lb. bifidum*. Although *Bifidobacterium* species do fit the general description above, they are phylogenetically more related to the Actinomycetaceae group of gram-positive bacteria. In addition, they have a special pathway for sugar fermentation, unique to the genus, which clearly separates them from the LAB group (Axelsson, 2004). Bifidobacteria will, therefore, not be considered in this general overview of LAB.

The genera of LAB belong to five families which include carnobacteriaceae comprising of the genera *Carnobacterium* and *Lactosphaera*, enterococcaceae comprising of the genera *Enterococcus*, *Tetragenococcus* and *Vagococcus*, lactobacillaceae comprising of the genera *Lactobacillus*, *Paralactobacillus* and *Pediococcus*, leuconostocaceae comprising of the genera *Leuconostoc*, *Oenococcus* and *Weissella*, streptococcaceae comprising of the genera *Streptococcus*, Lactococcus (Auld, 2010; Zhang *et al.*, 2011).

2.1.1 The Genus *Lactobacillus*

Lactobacilli are gram positive bacteria and vary in morphology from long slender rods to short coccobacilli, which frequently form chains. They are fermentative, catalase-negative, microaerophilic and chemoorganotrophic, producing lactic acid as the major product of carbohydrates fermentation. *Lactobacillus* belongs to the phylum: Firmicutes, order:

Lactobacillales and family: Lactobacillaceae. They have complex growth requirements and thrive well at low pH (between 4.5 and 6.2). The genus is one of the largest, comprising over 100 species and subspecies (Canchaya *et al*., 2006).

Lactobacilli are widespread in nature, and many species have found applications in the food industry. They are generally the most acid-tolerant of the LAB and will, therefore, terminate many spontaneous lactic fermentations such as silage and vegetable fermentations (Axelsson, 2004). Lactobacilli are also associated with the oral cavity, gastrointestinal tract, and vagina of humans and animals. Some species, e.g., *L. brevis*, *L. casei* and *L. plantarum* can be found in many habitats. Others are more specialized and are found only in certain niches, e.g., the sourdough organism *L. sanfransiscensis* and the yogurt-associated *L. delbrueckii* subsp. *bulgaricus* (previously *L. bulgaricus*) (Axelsson, 2004).

2.1.2 The Genus *Leuconostoc*

The genus *Leuconostoc* was first described by Van Teighein in 1878. They are heterofermentative cocci that are sometimes oval or even short rods and occur in pairs or short chains. They are Gram positive, non-sporeforming, facultative anaerobes. They form grey, flat colonies on agar and are commonly found on the surface of and inside of fruits and vegetables and in dairy products (Mavhungu, 2005). Leuconostocs may form significant amounts of diacetyl from citrate in milk, and some species, mainly *Ln. mesenteroides* subsp. *cremoris*, have been used in the dairy industry for this purpose (Edema, 2010). Leuconostocs are also important in spontaneous vegetable fermentations, e.g., sauerkraut and pickles, where they often initiate the lactic fermentation (Axelsson, 2004; Mavhungu, 2005). Leuconostocs are catalase negative, lack cytochromes, do not hydrolyse arginine and are non-proteolytic. Their growth is dependent on the presence of fermentable carbohydrates (Mavhungu, 2005). In spontaneous fermentation processes, the other lactic acid bacteria tend to dominate the growth of *Leuconostoc* species because of their slow growth rates and weak acidifying properties. However, in a well-balanced starter culture, Leuconostocs play an important role in the rheological and flavouring quality of the fermented product (Edema, 2010). In carrot and cabbage fermentations, *Leuconostoc mesenteroides* and *Lactobacillus pentosus* are used as starter cultures; *Leuconostoc mesenteroides* produces acetic acid that inhibits the growth of *Listeria monocytogenes* and most other spoilage bacteria and increases the organoleptic quality of the products. The presence of *Leuconostoc* species associated with lactobacilli is considered very important in the fermentation of sourdough (Edema, 2010).

2.1.3 The Genus *Pediococcus*

This genus was first decribed by Wochnschre F. Balcke in 1884 (Mavhungu, 2005). *Pediococcus* can be described as “the only acidophilic, homofermentative, lactic acid bacteria that divide alternately in two perpendicular directions to form tetrads” (Engesser *et al.,* 1994). Pediococci are important in food technology in both a negative and positive sense. *Pediococcus damnosus* is a major spoilage organism in beer manufacture, since growth may lead to diacetyl/acetoin formation, resulting in a buttery taste. *Pediococcus acidilactici* and *P. pentosaceus* are used as starter cultures for sausage making and as silage inoculants. Pediococci may also be important constituents of the complex known as the nonstarter lactic acid bacteria (NSLAB), which is involved in the ripening of cheese (Fox *et al.,* 1990). The main characteristics for distinguishing between the species are the range of sugars fermented, hydrolysis of arginine, growth at different pH levels (7.0 and 4.5), and the configuration of lactic acid produced. *Pediococcus pentosaceus* and *P. acidilactici* are difficult to distinguish using these characteristics but have been shown to be distinct species with DNA-DNA homology studies. These species are also similar in that they may produce a nonheme "pseudocatalase."Genetic fingerprinting methods are also available for distinguishing pediococci (Satokari *et al.*, 2000).

2.1.4 The Genera *Streptococcus*, *Enterococcus*, *Lactococcus* and *Vagococcus*

The genera *Enterococcus*, *Lactococcus*, *Streptococcus* and *Vagococcus* were earlier included in one genus, *Streptococcus* (Axelsson, 2004). They are all homofermentative, gram-positive cocci and also occur as chains (coccobacilli). They are widely distributed in raw milk and dairy products, on plant material and in the mouths and intestines of humans and animals (Mavhungu, 2005).

Members of the genus *Streptococcus* are gram – positive cocci, catalase negative, that form pairs and chains of cells when cultured on liquid media. The streptococci are a large group consisting of thirty-seven species. The streptococci that are important in foods are divided into four groups, the pyogenic, viridans, lactic and enterococcus group (Mavhungu, 2005).
Enterococci are typically found in the intestinal tract and faeces of man and other animals. Some species have also been isolated from soil, food, water and plants, and their ability to grow and survive under a wide range of environmental conditions, probably accounts for the almost ubiquitous distribution of the genus (Hardie and Whiley, 1997). The most common humans infections associated with enterococci are those involving the urinary tract, in which enterococci are implicated in about 10% of cases and up to 16% of nosocomial urinary tract infections. They are also known to cause neonatal, central nervous system and respiratory tract infections on rare occasions (Hardie and Whiley, 1997).

Lactococci are intimately associated with dairy products, but out of the five species currently recognized, only one, *Lc. lactis,* is actually used in dairy technology. *Lactococcus garviae* strains have been associated with bovine mastitis and fish lactococcosis. Three subspecies of

Lc. lactis can be distinguished: *Lc. lactis* subsp. *lactis, Lc. Lactis* subsp. *cremoris*, and *Lc. lactis* subsp. *hordniae*. Only the first two are important in dairy manufacture. *Lactococcus lactis* subsp. *lactis* includes species formerly designated *Streptococcus lactis* subsp. *lactis*, *Strep. lactis* subsp. *diacetylactis* and *Lactobacillus xylosus* (Teuber and Geis, 2000).

Species of the genus *Vagococcus* are easily confused with lactococci, but the genera are clearly distinguished by fatty acid composition. Some strains of vagococci are motile. Genus- and species-specific oligonucleotide probes are available for vagococci, which make a reliable identification of these bacteria feasible (Axelsson, 2004).

2.1.5 The Genera *Aerococcus* and *Tetragenococcus*

The genus *Aerococcus* is a monospecific genus which is related to *Pediococcus* and *Tetragenococcus*. The genus consists of gram positive cocci, catalase activity is weak or absent and when present is a non-hemepseudocatalase. This genus is different from other genera of gram-positive cocci primarily by its wide range of negative carbohydrates characteristics that are positive for other genera. They grow sparsely on de Mans Rogosa Sharpes (MRS) agar and cause greening of blood agar (Mavhungu, 2005).

The genus *Tetragenococcus* contains strains previously regarded as *Pediococcus halophilus*. Only two species, *Tetragenococcus halophilus* and *Tetragenococcus muriaticus*, are currently recognized, but one enterococcal species, *Enterococcus solitarius*, has been shown to be related to *Tetragenococcus* phylogenetically. In addition to extreme salt tolerance (18% NaCl), which distinguishes them from other LAB, tetragenococci generally require salt in the range of 5% NaCl for growth. *Tetragenococcus* species are important in lactic fermentation of high-salt-containing food, e.g., soy sauce (Axelsson, 2004).

2.1.6 The Genus *Oenococcus* and *Weissella*

The genus *Weissella* comprises of eight species; *W. Confuse, W. halotolerans, W. hellinica, W. kandleri, W. minor*, *W. paramesenteroides*, *W. thailandensis* and *W. viridescens*. *Weissella* strains have been isolated from a variety of sources. *Weissella paramesenteroides* is one of the predominant strains in fresh vegetables and it also plays an important role in the first phase of silage fermentation. *Weissella hellinica* and *W. viridescens* have been commonly associated with meat or meat products (Bjorkroth *et al*., 2002).

The genus *Oenococcus* is a heterofermentative, coccoid lactic acid bacteria producing only D-lactic acid from glucose and not producing ammonia from arginine. Oenococci are easily distinguished from other lactic acid bacteria by their extreme acid and ethanol tolerance. (Yang and Woese, 1989). The best known member of this genus, *Oenococcus oeni* is best adapted to the wine environment and concomitantly the majority of LAB present in wine belong to this species. *Oenococcus oeni* strains are also the selected bacteria used for commercial starter cultures (Lerm, 2010).

2.1.7 The Genus *Carnobacterium*

Species of the genus *Carnobacterium* were originally classified as group III lactobacilli under the designations *Lb. divergens*, *Lb. carnis*, and *Lb. piscicola*. Later studies showed that these bacteria were separate from lactobacilli and warranted a separate genus and that the metabolism of glucose was predominantly homofermentative. Generally, carnobacteria grow at relatively high pH (e.g., pH 9), while lactobacilli do not. Furthermore, the fatty acid composition of carnobacteria differs from that of lactobacilli. Carnobacteria are characteristically found in meat and meat products, where they are able to proliferate even at low temperatures (Schillinger and Holzapfal, 1995).

A summary of the differential characteristics of the LAB is given in Table 2.1 below:

Table 2.1: Differential characteristics of lactic acid bacteria genera

Character	***Carnobacterium***	***Lactobacillus***	***Aerococcus***	***Enterococcus***	***Lactococcus*** ***Vagococcus***	***Leuconostoc*** ***Oenococcus***	***Pediococcus***	***Streptococcus***	***Tetragenococcus***	***Weisella***
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Cocci	Cocci	Cocci	Cocci^a
Tetrad formation	-	-	+	-	-	-	+	-	+	-
CO_2 from glucose	-^{c}	±	-	-	-	+	-	-	-	+
Growth at 10^0C	+	±	+	+	+	+	±	-	+	+
Growth at 45^0C	-	±	-	+	-	-	±	±	-	-
Growth in 6.5% NaCl	ND	±	+	+	-	±	±	-	+	±
Growth in 18% NaCl	-	-	-	-	-	-	-	-	+	-
Growth at pH 4.4	ND	±	-	+	±	±	+	-	-	±
Growth at pH 9.6	-	-	+	+	-	-	-	-	+	-
Lactic acid	L	D, L, DL^f	L	L	L	D	L, DL^f	L	L	D, DL^f

+ positive; - negative; ± response varies between species; ND not determined.^{a}*Weissella* strains may also be rod-shaped; ^{c}Small amounts of CO_2 can be produced depending on media; ^{e}Configuration of lactic acid produced from glucose; ^{f}Production of D-, L-, or DL- lactic acid varies between species; (Axelsson, 2004).

2.2 Metabolism of Lactic Acid Bacteria

Lactic acid bacteria possess two main pathways for the metabolism of glucose and a single pathway for the metabolism of pentose sugars. The two pathways for the metabolism of glucose include the glycolysis (Embden-Meyerhof-Parnas (EMP) pathway and the 6 phosphogluconate/phosphoketolase (6-PG/PK) pathway (Lerm, 2010). Lactic acid bacteria can be classified into two groups on the basis of their metabolism, homofermentative and heterofermentative. While the homofermentative LAB convert glucose almost exclusively into lactic acid, the heterofermentative LAB catabolise glucose into ethanol and CO_2 as well as lactic acid and acetic acid. The homofermentative LAB usually metabolize glucose *via* the Embden-Meyerhof pathway (*i.e.* glycolysis). Since glycolysis results only in lactic acid as a major end-product of glucose metabolism, two lactic acid molecules are produced from each molecule of glucose with a yield of more than 0.90 g/g (Ryu *et al*., 2006).

Glycolysis, used by all LAB except *Leuconostoc*, oenococci, and *Weissella*, is characterized by the formation of fructose-1,6-diphosphate (FDP), which is split by a FDP aldolase into dihydroxyacetonephosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). Glyceraldehyde -3-phosphate (and DHAP via GAP) is then converted to pyruvate in a metabolic sequence including substrate-level phosphorylation at two sites. Under normal conditions, that is, excess sugar and limited access to oxygen, pyruvate is reduced to lactic acid by a NADP-dependent lactate dehydrogenase (nLDH), thereby reoxidizing the NADH formed during the earlier glycolytic steps. A redox balance is thus obtained, lactic acid is virtually the only end product, and the metabolism is referred to as homolactic fermentation (Corsetti and Settanni, 2007).

The other main fermentation pathway is the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway, which also involves phosphoketolase but does not have 6 - phosphogluconate as an intermediate. It is characterized by initial dehydrogenation steps with the formation of 6-phosphogluconate, followed by decarboxylation. The remaining pentose-5-phosphate is split by phosphoketolase into GAP and acetyl phosphate. Glyceraldehyde -3- phosphate is metabolized in the same way as in the glycolytic pathway, resulting in lactic acid formation. When no additional electron acceptor is available, acetyl phosphate is reduced to ethanol via acetyl CoA and acetaldehyde. Since this metabolism leads to significant amounts of other end products (CO_2, ethanol) in addition to lactic and acetic acids, it is referred to as heterolactic fermentation (Axelsson, 2004; Corsetti and Settanni, 2007).

Hexoses other than glucose enter these major pathways at the level of glucose of glucose-6-phosphate or fructose-6-phosphate after isomerisation and/or phosphorylation. Disaccharides are split by specific hydrolases and/or phosphohydrolases to monosaccharides which then enter the major pathways. Pentoses are phosphorylated and converted to riboluse-5-phosphate or xyloluse-5-phosphate and subsequently metabolized through the lower half of the 6 - PG/PK pathway. Fermentation of pentoses results in the production of equimolar amounts of lactic and acetic acids; no CO_2 is formed (Corsetti and Settanni, 2007).

A classification of the LAB based on fermentation pattern is given in Table 2.2 below:

Table 2.2: Classification of lactic acid bacteria based on fermentation pattern

Homofermentative LAB	Facultative Homofermentative LAB	Heterofermentative LAB
Enterococcus faecium	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Enterococcus faecalis	*Lactobacillus casei*	*Lactobacillus buchneri*
Lactobacillus acidophilus	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus lactis	*Lactobacillus curvatus*	*Lactobacillus confuses*
Lactobacillus amylovorus	*Lactobacillus pentosus*	*Lactobacillus frumentii*
Lactobacillus delbrueckii	*Lactobacillus plantarum*	*Lactobacillus coprophilus*
Lactobacillus johnsonii	*Lactobacillus alimentarius*	*Lactobacillus higardii*
Lactobacillus leichmannii	*Lactobacillus sake*	*Lactobacillus fermentum*
Lactobacillus salivarius		*Lactobacillus sanfrancisco*
Streptococcus bovis		*Leuconostoc dextranicum*
Streptococcus thermophilus		*Leuconostoc mesenteroides*
Pediococcus acidilactici		*Leuconostoc paramesenteroides*
Pedicoccus damnosus		*Lactobacillus panis*
Pediococcus pentocaceus		*Lactobacillus reuterin*
Lactobacillus amylolyticus		*Lactobacillus rossiae*
Lactobacillus farciminis		*Lactobacillus zymae*
Lactobacillus mindensis		*Lactobacillus spicheri*
		Lactobacillus collinoides

(Corsetti and Settanni, 2007)

2.3 Probiotics Value of Lactic Acid Bacteria

The increasing consumer awareness of diet and health has stimulated the development of functional foods containing probiotics defined as “living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition’’. Scientific evidence is accumulating to support various health beneficial effects of probiotics including pathogen interference, immunostimulation and immunomodulation, anticarcinogenic and antimutagenic activities, alleviation of symptoms of lactose intolerance, reduction in serum cholesterol, reduction in blood pressure, decrease in incidence and duration of diarrhea, prevention of vaginitis and maintenance of mucosal integrity (Hongpattarakere *et al*., 2008). Many lactic acid bacteria (LAB) are known to function as probiotics, which are beneficial to the host health, when ingested in sufficient quantities. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion and by the production of organic acid and antimicrobial compounds (Hongpattarakere *et al*., 2008).

Promising probiotic strains include the members of the genera *Lactobacillus, Bifidobacterium* and *Enterococcus*. The representative species include *Lactobacillus acidophilus, Lactobacillus johnsonii*, *Lactobacillus casei*, *Lactobacillus gasseri*, *Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium bifidum, Bifidobacterium infantis*, *Enterococcus faecalis* and *Enterococcus faecium* (Kaur *et al*., 2002).

Probiotic microorganisms including lactic acid bacteria (LAB) positively influence the composition of the gut microflora; they stimulate the production of secretory IgA; they affect the targeted transportation of the luminal antigens to Peyer’s patches and they increase the production of IFN-γ. Lactic acid bacteria stimulate the activity of non-specific and specific immune cells. They are able to eliminate damage to the gut microenvironment; they stimulate local and systemic immune responses and they maintain the integrity of the gut wall (Herich and Levkut, 2002).

Probiotic preparations of *L. reuterii* have been found beneficial in the prevention and treatment of infantile viral diarrhea and antibiotic associated diarrhoea. *Lactobacillus casei* when administered orally was found to reduce recurrence of superficial bladder carcinoma in humans. There have been reports of improved production of immune factors such as immunoglobulins, interferons, interleukins, tumour necrosis factors and an increased phagocytic activity by some administered LAB. Examples of most commonly used strains in probiotic compositions include species *L. plantarum, L. rhamnosus, L. acidophilus, L. salivarius, L. reuteri* and *Enterococcus faecum* (Kutima *et al*., 2010).

There are a variety of proposed beneficial health effects of probiotics. Several probiotics including *Streptococcus boulardii*, *Enterococcus faecium* and *Lactobacillus* species have been shown to be clinically effective in preventing antibiotic-associated diarrhoea. The modulation of the intestinal microbiota by probiotics can be a useful tool for both the dietary management and the prevention of some allergic diseases. Perinatal administration of *L. rhamnosus* GG decreased subsequent occurrence of eczema in at-risk infants by one-half (Isolauri *et al*., 2000). Probiotics may also be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein. Consumption of certain strains of lactobacilli has shown an improvement in symptoms of inflammatory bowel disease (IBD), pouchitis and ulcerative colitis. There is evidence that supplementation of the diet with fermented milks (yoghurt) has a beneficial hypocholesterolemic effect. Isolates of *L. acidophilus* from human intestinal material are able to assimilate cholesterol and actively deconjugate bile salts into free acids that are excreted more rapidly from the intestinal tract than are conjugated bile acids (Bamrungna, 2009).

Lactobacilli are safe bacteria and they have a number of properties that render them highly suited as vehicles for the delivery to the mucosa of compounds that are of pharmaceutical interest. The immunomodulating capacity of lactobacilli together with the possibility of targeting antigens to specific sites of the bacterium offers attractive opportunities for the treatment of infectious diseases, auto-immune diseases, or other immune disorders by modulating the immune response in a directed and predetermined way (Havenith *et al*., 2002).

2.4 Antimicrobial Compounds Produced by Lactic Acid Bacteria

Lactic acid bacteria display a wide range of antimicrobial activities. Amongst these activities, the production of lactic acid and acetic acid is obviously the most important. However, certain strains of LAB are further known to produce bioactive molecules such as ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. Many strains also produce bacteriocins and bacteriocin-like molecules that display antibacterial activity (De Vuyst and Leroy, 2007). Besides the production of bacteriocins, some LAB are able to synthesize other antimicrobial peptides that may also contribute to food preservation and safety. For instance, strains of *Lactobacillus plantarum* , isolated from sourdough and grass silage, display antifungal activity, due to the production of organic acids, other low-molecular- mass metabolites, and/or cyclic dipeptides (Lavermicocca *et al*., 2000; Strøm *et al*., 2002; Schnürer and Magnusson, 2005).

2.4.1 Bacteriocins

In the past two decades, there have been many reports on the bacteriocins produced by LAB (De Vuyst and Leroy, 2007). These bacteriocins are of a proteinaceous nature and on a sound scientific basis three defined classes of bacteriocins have been established: Class I, the lantibiotics; class II, the small heat stable non lantibiotics; and class III, large heat labile bacteriocins. A fourth class of bacteriocins is composed of an undefined mixture proteins, lipids and carbohydrates (Aly *et al*., 2006; Phumkhachun and Rattanachaikunsopon, 2010). Nisin is the only bacteriocin with GRAS (Generally Regarded as Safe) status for use in specific foods and this was awarded as a result of a history of 25 years of safe use in many European countries and was further supported by the accumulated data indicating its nontoxic, nonallergenic nature (Phumkhachun and Rattanachaikunsopon, 2010).

Other important bacteriocins include, pediocin A, pediocin AcH, leucocin, helviticin J, carnobacteriocin. Table 2.3 gives a summary of these important bacteriocin, the producing organisms and their properties.

Table 2.3: Properties of some well characterized bacteriocins produced by lactic acid bacteria

Bacteriocin	**Producer organism**	**Properties**
Nisin	*Lactococcus lactis subsp.lactis ATCC 11454*	Lantibiotic, broad spectrum, chromosome/plasmid mediated, bactericidal, produced late in the growth cycle.
Pediocin A	*Pediococcus pentosaceus FBB61 and L-7230*	Broad spectrum, plasmid Mediated.
Pediocin AcH	*Pediococus acidilactici H*	Broad spectrum, plasmid mediated.
Leucocin	*Leuconostoc gelidum UAL 187*	Broad spectrum, plasmid Mediated, bacteriostatic, produced early in the growth cycle.
Helveticin J	*L. helveticus 481*	Narrow spectrum, chromosomally mediated, bactericidal.
Carnobacteriocin	*Carnobacterium piscicola LV17*	Narrow spectrum, plasmid mediated, produced early in the growth cycle.

(Aly *et al*., 2006)

2.4.2 Organic acids (Lactic, acetic and propionic acids)

The antimicrobial effect of organic acids lies in the reduction of pH, as well as the undissociated form of the molecules which are toxic to many bacteria, fungi and yeasts. Acetic and propionic acids produced by LAB strains through heterofermentative pathways, have greater antimicrobial activity than lactic acid due to their higher pKa values (lactic acid 3.08, acetic acid 4.75, and propionic acid 4.87), and higher percentage of undissociated acids than lactic acid at a given pH (Earnshaw, 1992).

2.4.3 Hydrogen peroxide

The antimicrobial effect of hydrogen peroxide (H_2O_2) may result from the oxidation of sulfhydryl groups causing denaturing of a number of enzymes; it may also be due to the production of bactericidal free radicals such as superoxide and hydroxyl radicals which can damage DNA. It has been reported that the production of H_2O_2 by *Lactobacillus* and *Lactococcus* strains inhibited *Staphylococcus aureus*, *Pseudomonas* sp. and various psychotrophic microorganisms in foods (Cords and Dychdala 1993). In raw milk, H_2O_2 activates the lactoperoxidase system, producing hypothiocyanate (OSCN-), higher oxyacids (O2SCN- and O3SCN-) and intermediate oxidation products that are inhibitory to a wide spectrum of Gram-positive and Gram-negative bacteria (Conner, 1993).

2.4.4 Carbon dioxide

Carbon dioxide contributes to food safety by creating anaerobic conditions which inhibit the growth of oxygen - dependent microorganisms such as moulds and gram - negative bacteria. Carbon dioxide also curbs the growth of pathogenic and spoilage bacteria by disrupting enzyme – dependent reactions due to a decrease in pH (Madoroba, 2009). Carbon dioxide is mainly produced by heterofermentative LAB. Carbon dioxide effectively inhibits the growth of many food spoilage microorganisms, especially gram-negative psychrotrophic bacteria (Yang, 2009).

2.4.5 Aroma compounds

Diacetyl is produced by strains within all genera of LAB by citrate fermentation. The antimicrobial effect of diacetyl has been known since the 1930s. It inhibits the growth of Gram-negative bacteria by reacting with the arginine-binding protein, thus affecting the arginine utilization. Diacetyl at 344 μg/mL inhibited strains of *Listeria, Salmonella, Yersinia, Escherichia coli* and *Aeromonas* (Yang, 2009).

Acetaldehyde is produced by *L. delbrueckii* ssp. *bulgaricus* by the action of a threonine aldolase, which cleaves threonine into acetaldehyde and glycine. Acetaldehyde at 10-100 ppm inhibits the growth of *Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli* in dairy products (Yang, 2009).

2.4.6 Fatty acids

Under certain conditions, some lactobacilli and lactococci possessing lipolytic activities may produce significant amounts of fatty acids, e.g. in dry fermented sausage and fermented milk. The antimicrobial activity of fatty acids has been recognized for many years. The unsaturated fatty acids are active against Gram-positive bacteria. The antimicrobial action of fatty acids has been thought to be due to the undissociated molecule, not the anion, since pH had profound effects on their activity, with a more rapid killing effect at lower pH (Kabara, 1993).

2.4.7 Reuterin

Reuterin exhibits a broad spectrum of antimicrobial activity against certain Gram-positive and Gram-negative bacteria, yeast, fungi and protozoa. Spoilage organisms sensitive to reuterin include species of *Salmonella, Shigella, Clostridium, Staphylococcus, Listeria, Candida* and *Trypanosoma* (Yang, 2009; Kutima *et al*., 2010).

2.5 Fermented Dough (Sourdough)

Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB), mainly hetero-fermentative strains, elaborating lactic acid and acetic acid in the mixture, and hence, resulting in a pleasant sour taste of end product (De Vyust and Neysens, 2005). Spontaneous sourdough fermentation begins with aerobic fermentation immediately upon mixing flour and water. Once oxygen is depleted, anaerobic fermentation begins with the growth of LAB and yeasts. The production of acid by LAB enables their rapid growth when the pH of the sourdough has dropped to a level that is too low for other microorganisms to grow. The LAB become the dominant microflora in the sourdough and are therefore responsible for the final stages of sourdough development (Zugic – Petrovic *et al*., 2009).

The sourdough fermentation is a traditional process for improving bread quality and producing different wheat and rye breads (Thiele *et al.*, 2002). Sourdough is employed in the manufacture of breads, cakes and crackers. The typical characteristic of sourdough is mainly due to its microflora, basically represented by lactic acid bacteria (LAB) and yeasts. These microorganisms ensure acid production and leavening upon addition of flour and water (Ghulam, 2009).

In the present era, consumers wish to have a wide range of foods that are nutritious, of good flavour and have long shelf life without added preservatives. Sourdough is an important modern fermentation of cereal flours and water based upon an earlier spontaneous process (Chavan and Chavan, 2011). The sourdough microflora is dominated by lactic acid bacteria and, along with yeast; they play a key role in the fermentation of bread dough. Factors that affect the quality of sourdough are dough yield, temperature, type of starter culture, acidity of the medium and the substrate (Chavan and Chavan, 2011).

Sourdough is classified into three types; Types I, II and III (Saeed, 2009; Chavan and Chavan, 2011). The sourdough fermentation has a number of beneficial effects that include prolonged shelf life, accelerated volume gain, delayed staling, improved bread flavour and good nutritional value. Sourdough also improves sensory characteristics such as loaf volume, evenness of baking, colour, aroma, taste and texture of breads. Sourdough has been reported to contribute to extended shelf life by inhibiting the growth of spoilage bacteria and moulds (Chavan and Chavan, 2011).

2.5.1 Classification of sourdough

Sourdoughs have been classified into three types, based on the kind of technology applied for their production, as used in artisan and industrial process (Scheirlinck *et al*., 2007):

(i) Type I sourdough or traditional sourdough.

(ii) Type II sourdough or accelerated sourdough.

(iii) Type III sourdough or dried sourdough.

(iv) Type 0 dough

Type - I sourdoughs are traditional doughs maintained by continuous propagation at ambient temperature (20 – 30°C). Mostly, traditional three - stage fermentation processes are used. *Lactobacillus (L.) sanfranciscensis* and *L. pontis* are the dominant LAB in these sourdoughs (Saeed, 2009). Some type I sourdoughs have been found to contain *L. fructivorans*, *L. fermentum* and *L. brevis*. The type I sourdough include pure culture, sourdough starter isolated from different origin (type Ia), mixed culture sourdoughs made from wheat and rye and prepared with multiple stage fermentation process (type Ib) and finally the sourdough made in tropical regions fermented at high temperature (Type Ic) (Palomba, 2010).

Type II sourdoughs are used as dough - souring supplements during bread making. These are semi-fluid silo preparations prepared in long fermentation periods (from 2 to 5 days) and at elevated fermentation temperature often >30°C to speed up the microbial activity (Scheirlinck *et al*., 2007; Ghulam, 2009). The dominant strains in industrial processes of type - II doughs are mostly *L. panis*, *L. pontis*, *L. reuteri* (Vogel *et al*., 1999), *L. johnsonii*, *L. sanfranciscensis* (Hammes and Ganzle, 1998), *L. fermentum*, *L. delbrueckii*, *L. acidophilus*, *Lactococcus lactis*, *L. brevis* and *L. amylovorus* (Vogel *et al*., 1999).

Type - III doughs are dried preparations of doughs which are made by traditional sourdough fermentation with subsequent water evaporation by freezedrying, roller spray drying or drying in a fluidized bed reactor. The type - III sourdoughs are the most convenient way to introduce superior bread taste into modern bakery industry (Saeed, 2009, Rollan *et al*., 2010). This type of sourdough is used especially as acidifier supplements and to increase the aroma of bakery products. It contains LAB that are resistant to the drying process such as *L. brevis, P. pentosaceus* or *L. plantarum*. It is convenient, simple in use and result in standardized end products (De Vuyst and Neysens 2005, Palomba, 2010).

Type 0 dough, for which baker’s yeast is the main fermenting agent, is not made with sourdough technology (Ghulam, 2009).

Sourdoughs type I and type II requires the addition of baker’s yeast as leavening agent (Palomba, 2010).

2.5.2 Microorganisms of sourdough

Sourdoughs are considered extremely complex ecosystems where lactic acid bacteria (LAB) represent the prevailing microflora (De Vuyst and Vancanneyt, 2007). Numerous LAB genera and species of LAB have been identified in sourdoughs. The dominant microbes in spontaneously fermented doughs are homofermentative lactobacilli and pediococci, which are found in wheat sourdoughs at the level of 3 x 10^8 – 3 x 10^9CFU/g (Corseti and Settanni, 2007). Typical homofermentative lactic acid bacteria in spontaneous sourdough are *Lactobacillus casei*, *L. delbruekii*, *L. farciminis*, *L. mindenensis*, *L. crospactus*, *L. johnsonii*, *Pediococcus pentosaceus* etc (Katina, 2005; Gerez *et al*., 2006; Corseti and Settanni, 2007). Typical heterofermentative lactic acid bacteria in spontaneous sourdoughs include *L. brevis*, *L. buchneri*, *L. fermentum*, *L. frumenti*, *L. hilgardii*, *L. panis*, *L. pontis*, *L. sanfriciscencis*, *L. spicheri*, *L. zymae*, *L. plantarum* among others (Katina, 2005; Corseti and Settanni, 2007).

Typical sourdough LAB, responsible for the acidification of dough are lactobacilli. They consist of obligately and facultatively heterofermentative and obligately homofermentative species (Corsetti *et al*., 2004). Cereals fermentation, particularly sourdoughs, are dominated by specifically adapted LAB occurring at numbers above 10^8 CFU/g, which may be in coexistence or possibly in symbiosis with typical yeasts (Gobbetti *et al*., 1999; Vogel *et al*., 2002). The majority of species regularly isolated from sourdough or used as sourdough starter belong, with only few exceptions, to one of the four genera *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* (De Vuyst and Vancanneyt, 2007). The highest number of different species (>23 species) is found in the genus *Lactobacillus*. The continuous propagation of sourdough by back - slopping leads to a stable microflora, characterized by a high acid tolerance and a metabolism well adapted to the cereal environment. This microflora is dominated by members of the genus *Lactobacillus* mainly constituting heterofermentative species such as *L. pontis*, *L. sanfranciscensis*, *L. fermentum*, *L. reuteri*, *L. panis, L. amylovorus* and *Weissella confusa* (Theile, 2003). The microflora of type one sourdoughs consists mainly of strains of *L. sanfranciscensis*. Strains of *L. reuteri*, *L. fermentum*, *L. pontis*, *L. amylovorus* and *L. panis* are most frequently isolated from type II sourdoughs (Kazanskaya *et al.*, 1983; Vogel *et al.,* 1999). Type III doughs consists of mainly *L. plantarum*, *L. brevis* and *Pediococcus pentosaceus* (Theile, 2003). The dominant *Lactobacillus* species in Italian wheat sourdoughs have been reported to be *Lactobacillus sanfranciscensis*, *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus fructivorans*, belonging to the obligately heterofermentative group of lactobacilli; *Lactobacillus plantarum* and *Lactobacillus alimentarius*, belonging to the facultatively heterofermentative group; and *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus farciminis*, belonging to the obligately homofermentative lactobacilli (Corsetti *et al.*,2001; Corsetti *et al.*, 2003). Other species such as *Lactobacillus spicheri*, *Lactobacillus mindensis*, *Lactobacillus frumenti* and *Lactobacillus paralimentarius* were also isolated from sourdough (Corsetti *et al*., 2004).

The majority of yeasts found in sourdoughs belong to the species *Candida humilis, C. holmii*, *S. exiguous* and *S. cerevisiae* (Hammes and Ganzle, 1998). Yeasts are often associated with LAB in sourdough and the yeasts/LAB ratio is generally 1:100 (Gobbetti *et al.,* 1994; Ottogalli *et al.,* 1996). The yeasts found in sourdoughs belong to more than 20 species (Gullo *et al.,* 2002). Typical yeasts associated with LAB in sourdoughs are *S. exiguus, C. humilis* (formerly described as *C. milleri*) and *Issatchenkia orientalis* (*C. krusei*) (Gobbetti, 1998; Succi *et al.*, 2003). Other yeast species detected in sourdough ecosystem are *Pichia anomala, P. saitoi*, *P. membranifaciens*, *Hansenula anomala*, *Torulaspora delbrueckii* and *Debaryomyces hansenii* (Gobbetti *et al.*, 1994; Succi *et al.*, 2003; Druvefors *et al*., 2005).

2.5.3 Impact of sourdough on the texture and structure of baked products

Generally, the fermented dough (sourdough) is used to improve flavour but its addition also has an effect on the dough and the final baked product structure. In fact, there is a wide consensus with regard to the positive effects of fermented dough addition for bread production, including improvements in bread volume and crumb structure (Maleki *et al*., 1980; Arendt *et al*., 2007). During the dough fermentation different organic acids are produced. These organic acids improve the flavour of bread, help the swelling of gluten and increase gas retention, which result in products with good texture and massive volume and also function as natural dough conditioners (Park *et al.,* 2007).

The exopolysaccharides produced by lactic acid bacteria during fermentation is one of the aspects of fermented dough technology with the potential for the replacement of hydrocolloids. These compounds, commonly named as gums, are used as texturizing, antistaling, or prebiotic additives in bread production (Tieking *et al.,* 2003).

In comparison to bread prepared with baker's yeast, the fermented dough breads are characterized by moist, dense grains and rather chewy texture. The application of fermented dough to wheat breads has a positive impact on bread volume which is a primary quality characteristic of bread (Ghullam, 2009).

2.5.4 Proteolysis during sourdough fermentation

Lactic acid bacteria contribute to overall proteolysis during the fermentation of sourdough by creating optimum conditions for activity of cereal proteinases. The lactic acid bacteria having high proteolytic activity contribute to the hydrolysis of wheat proteins in a strain-specific manner (Saeed, 2009). The proteolysis process leads to the production of precursor compounds needed for the formation of aroma volatiles during baking as well as substrates for microbial conversion of amino acids to flavour precursor compounds (Palomba, 2010). Generally, sourdough fermentation with lactic acid bacteria results in an increase of amino acid concentrations during fermentation, whereas the concentration of free amino acids reduced when the dough was fermented with yeast. The level of individual amino acids in wheat dough's depends on the pH level of the dough, fermentation time and the consumption of amino acids by the fermentative microorganisms (Thiele *et al*., 2002). Bacterial proteolysis during sourdough fermentation resulted in the production of typical sourdough flavours of baked breads as compared to the chemically acidified or yeasted breads (Hansen *et al*., 1989; Loponen *et al*., 2004). The level of amino acids in dough's may be affected by the proteolytic strains of LAB, but cereal proteases play a major role in degradation of proteins in sourdoughs (Thiele *et al.*, 2002, Thiele *et al.*, 2004).

In wheat sourdoughs, *L. brevis* subsp. *lindneri*, *L. sanfranciscencis*, *L. brevis* and *L. plantarum* increased the levels of aliphatic, dicarboxylic and hydroxyl amino acids. The yeasts; *S. cerevisiae* and *S. exiguus* decrease the total level of amino acids in a similar way (Katina, 2005).

Substantial hydrolysis of gliadinin and glutenin proteins occurs during sourdough fermentation due to pH-mediated activation of cereal enzymes (Thiele *et al.*, 2003, Loponen *et al*., 2004). Cereal proteinases have been shown to be active at pH 3.7, but show no activity at pH 5.5. Thus, proteolysis during sourdough fermentation is highly dependent on formation of acids (Katina, 2005).

2.5.5 Impact of sourdough on shelf life of baked products

Lactic acid bacteria play an important role in increasing the shelf life of fermented foods (Caplice and Fitzgerald, 1999; Rollan *et al*., 2010). Bread is generally viewed as a perishable commodity, while in its many forms is one of the most staple foods consumed by humans, but shelf life is limited by two main factors, including staling and microbial (fungal spoilage and ropiness) attack (Kirschner and Von Holy, 1989; Katina, 2005; Arendt *et al.,* 2007). Bakery products have a very short shelf-life; in fact, during their storage the freshness decreases and in parallel, the crumb will become hard. All of these aspects contribute to a loss of consumer acceptance. It was demonstrated that the use of LAB in dough fermentation have a positive effects on staling process. One effect is an improvement in a loaf specific volume, which is associated with a reduction in the rate of staling (Maleki *et al*., 1980; Zhang, 2011) and a reduction in crumb softness during the storage (Corsetti *et al.,* 2000; Crowely *et al*., 2002). Even under the stringent conditions of production, bread may be contaminated with moulds or bacteria such as *Bacillus subtilis* and *Clostridia* that grow and subsequently spoil the product. The bread is spoiled by rope formation due to *Bacillus spp*., and can be observed from 12–24 h after bread is out from the oven. The spoilage can be observed as an unpleasant odour, discoloration, sticky soft bread crumb and by the extracellular slimy polysaccharides (Rosenquist and Hansen, 1995; Saeed, 2009). When the cell numbers of *B. subtilis* and *B. licheniformis* are upto 10^5 CFU/g, they are a threat of food borne illness (Kirschner and Von Holy, 1989). The microbial contamination can be controlled by the addition of organic acids or fermented dough (15%) to common dough in bread production. The use of lactic acid bacteria as a means of biopreservation, that is, control of one organism by another has shown very good results (Magnusson *et al.,* 2003). Certain fermented dough lactic acid bacteria and their components have an antifungal effect against various fungal species due to the production of organic acids, particularly acetic acid. Mould growth is the most common cause of microbial spoilage and deterioration in the quality of bread during storage (Saeed, 2009). The fermented dough inhibits the growth of the pathogens by synthesizing the antimicrobial compounds, like lactic acid, acetic acid, benzoic acid and hydrogen peroxide (Park *et al.,* 2007). Positive effects of the use of fermented dough on the mould free shelf life of wheat bread have also been reported by Barber *et al,* (1992). Although, antimould activities may vary greatly among the strains and are mainly detected within obligately heterofermentative *Lactobacillus* species, rate of inhibition of a number of fungal species is highly strain dependent (Corsetti *et al.,* 1998). The fermented dough addition with its additive - free image has been reported to be the best preservation method of bread and other bakery products (Rosenquist and Hansen, 1998; Coloretti *et al*., 2007).

2.6 Antimycotoxigenic Activity of Lactic Acid Bacteria

Lactic acid bacteria, and in particular the species belonging to the genera *Lactobacillus*, have long been known to possess antimycotoxigenic activity against the most harmful mycotoxins like zearalenone, fumonisin, ochratoxin, aflatoxin and deoxynivaleon. Strains of LAB, such as *L. acidophilus* VM20 (Fuchs *et al*., 2008), *L. acidophilus* CH-5, *L. plantarum* BS, *L. brevis* and *L. sanfrancisciensis* (Piotrowski and Zakowski, 2005) have been reported to bind ochratoxin A (OTA) in a strain specific manner causing its decrease by up to 95%. This allows reduction in the absorption of these toxins from the intestine and hence reducing their estrogenic effects in humans (Arendt *et al*., 2011).

2.7 Exopolysaccharides (EPS) from Lactic Acid Bacteria

Exopolysaccharides (EPS) are long-chain polysaccharides produced extracellularly mainly by bacteria and microalgae. Exopolysaccharides consist of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose, mannose, N-acetylglucosamine, N-acetyl galactosamine and rhamnose, in variable ratios. Exopolysaccharides are not permanently attached to the surface of the microbial cell and are secreted into their surroundings during growth as loose slime. This distinguishes them from the structurally similar capsular polysaccharides, which remain permanently attached to the microbial cell surface (Patel *et al*., 2012). Exopolysaccharides play vital role in protection of the microbes from adverse conditions as desiccation, nutrient shortage, toxic compounds, bacteriophages, osmotic stress and antagonists. Exopolysaccharides also play key role in initial adhesion and firm anchorage of the bacteria to solid surfaces, cation sequestration, biofilm formation, cellular recognition and pathogenecity (Maina, 2012, Patel *et al*., 2012).

Exopolysaccharides from lactic acid bacteria sources can be classified into two groups based on their monosaccharide composition and biosynthetic pathway, the homopolysaccharides (HoPS) which include dextran, mutan, alternan, reuteran, pullulan, levan, glucan, curdlan etc. and heteropolysaccharides (HePS) comprising of gellan, xanthan, kefiran (Patel *et al*., 2012). Homopolysaccharides consist of identical monosaccharides, D-glucose or D-fructose and can be divided into two major groups: glucans and fructans. By contrast, heteropolysaccharides from LAB have repeating units showing very little structural similarity to one another (Monsan *et al*., 2001; Pool-Zobel, 2005).

2.7.1 Homopolysaccharides

Glucansucrase produces glucans as dextran (α-1,6 osidic bond), mutan (α-1,3 osidic bond), alternan (α-1,6 and α-1,3 osidic bond) and reuteran (α-1,6 and α-1,4 osidic bond). Similarly, fructansucrase produces levan (β-2, 6 osidic bond) and inulin- type (β-2, 1 osidic bond) of fructans (Maina, 2012, Patel *et al*., 2012).

(i) Dextrans

Dextran is a generic name for several α-glucans produced by LAB that belong to the *Leuconostoc*, *Lactobacillus, Streptococcus*, *Pediococcus* or *Weissella* genera (Naessens *et al*., 2005; Bounaix *et al*., 2009). According to Rehm (2010), dextrans were among the first microbial polysaccharides to be discovered. Studies on dextrans date back to the work of Louis Pasteur on viscosity development in wine in 1861. In 1874, Scheibler showed that viscosity in beet sugar juices was due to a carbohydrate that had a positive optical rotation and he thus called it ‘’dextran‘’ (Naessens *et al*., 2005).

Dextrans are composed of α-1, 6 glycosidic linkages in the main chains and α-1, 2, α-1, 3 and α-1, 4 branched glycosidic linkages. The degree of branching involving α-1, 2, α-1, 3 and α-1, 4 linkages in dextrans vary according to the origin of dextransucrase. Native dextrans, the partially degraded dextrans and their derivatives have immense commercial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer (Goulas *et al*., 2004). Dextrans are used for the matrix preparation of chromatography columns such as sephadex. Clinical dextrans of molecular size 40–100 kDa are used as therapeutic agents to restore blood volume in case of casualties (Naessens *et al*., 2005). They are also used for synthesizing dextran sulphate for blood coagulation prevention and blood flow facilitation. The larger molecular weight dextrans can act as osmotic agents; they are used to treat hypovolemia. Iron dextran is used to treat iron deficiency anaemia. They are used as lubricant in eye drops and to increase blood sugar levels. Use of dextrans have ramified into paper, metal-plating processes and enhanced oil recovery (Padmanabhan and Kim, 2002). They are used as food syrup stabilizers and dough improvers (Vuyst *et al*., 2001).

(ii) Alternan

The exopolysaccharide alternan, produced by alternansucrase contains alternating α-1, 6 and α-1, 3 glucosidic linkages, with some degree of α-1, 3 branchings. The strains producing alternansucrase are *Leuconostoc mesenteroides* NRRL B-1355, NRRL B-1501 and NRRL B-

1498. Due to its unique structure, alternan has high solubility, low viscosity and remarkable resistance to enzymatic hydrolysis. Alternan is commercially exploited as low viscosity bulking agent and extender in foods and cosmetics. Extracellular alternanase depolymerises alternan to oligosaccharides. These alternan oligosaccharides are used as low-glycemic sweetener in confectionaries and as prebiotics (Patel *et al*., 2012).

(iii) Reuteran

Reuteran is a water soluble glucan produced by reuteransucrase. It has70% α -1, 4 linkage, α -1, 6 glycosidic bonds and 16% 4, 6-disubstituted α -glucosyl units at the branching points and a molecular weight of 40 MDa. It is elaborated by *Lactobacillus reuteri* strain LB 121, *Lactobacillus reuteri* strain ATCC 55730 and *Lactobacillus reuteri* strain 35–5 have been reported to produce reuteran. Because of its water solubility, it is used in bakery (Arendt *et al*., 2007).

(iv) Levan

Levan is a fructan having b-2, 6 osidic bonds with b-2, 1-linked side chains. Levansucrase catalyzes the transfer of D-fructosyl residues from fructose to yield levan. Levan producing LAB include *Steptococcus salivarius, Steptococcus mutans, Leuconostoc mesenteroides* NRRL B-512F, *Lactobacillus sanfranciscensis* LTH 2590 and *Lactobacillus reuteri* LB 121. Levan from *L. sanfranciscensis* LTH 2590 exhibits prebiotic effects (Korakli *et al.*, 2003). Levan has attracted attention for its antitumor properties (Yoo *et al.*, 2004), cholesterol-lowering properties and application as an eco-friendly adhesive. Levan also holds promise as biothickener in food industry (De Vuyst *et al*., 2001).

(v) Inulin

Inulin-type EPS are fructans or fructooligosaccharides containing β-1, 2 osidic bonds. *Lactobacillus johnsonii* NCC 533 produces high molecular mass inulin from sucrose by using an inulosucrase enzyme. *Streptococcus mutans* strain JC2, *Leuconostoc citreum* CW28 and *Lactobacillus reuteri* 121 are some other LAB which produce inulins. These are nondigestible and function as prebiotics in humans and animals. Inulins type fructooligosaccharides synthesize butyrate, which prevent pathogenic adherence and decrease pH of lumen (Sartor, 2004). Inulin-type fructans can be employed as vehicles for targeted drug delivery in treating colon cancer (Pool-Zobel, 2005).

2.7.2 Heteropolysaccharides

Heteropolysaccharides (HePS) are composed of regular repeating units consisting of three to eight carbohydrate units. In addition to glucose and galactose, HePS often contain rhamnose, N-acetyl-glucosamine (GlcNAc), N-acetyl-galactosamine (GalNAc) and phosphates (Tieking, 2005).

(i) Kefiran

It is a water soluble heteropolysaccharide produced by *Lactobacillus kefiranofaciens, L. kefirgranum, L. parakefir, L. kefir* and *L. delbrueckii subsp. bulgaricus*. Kefiran consists of approximately equal proportions of glucose and galactose (Micheli *et al*., 1999; Duboc and Mollet, 2001). Microscopic observation of kefir grains reveal that kefiran encapsulates LAB, acetic acid bacteria and yeasts involved in the fermentation. Kefiran improves visco-elastic properties of acid milk gels. Ability of kefiran to form edible transparent films is being explored (Piermaria *et al.*, 2009). Kefiran is reported to have antimicrobial and wound healing properties, ability to lower blood pressure and cholesterol in serum, and also capacity to retard tumour growth (Vinderola *et al*., 2006). Kefiran also serves as an oral antigen, inducing gut mucosal response (Medrano *et al*., 2008). It is reported to confer protective immunity, maintain intestinal homeostasis, enhance IgA level at both the small and large intestine level and influence the systemic immunity through the release of cytokines into the blood (Piermaria *et al.*, 2009).

Table 2.4 gives a summary of important exopolysaccharides produced by lactic acid bacteria and their functional applications.

Table 2.4: Applications of functional exopolysaccharides from lactic acid bacteria

Exopolysaccharides	**LAB producing EPS**	**Uses**
Dextran	*Leuconostoc mesenteriodes* *Streptococcus mutans*	As adjuvant, emulsifier, carrier and stabilizer in food and pharmaceutical industries, plasma substitute, matrix of chromatography column, anticoagulant, paper industry, metal-plating processing, for enhanced oil recovery.
Alternan	*Leuconostoc mesenteriodes*	Prebiotics, sweetener in confectionaries, low viscosity bulking agent and extender in foods.
Reuteran	*Lactobacillus reuterin*	Used in bakery.
Levan	*Steptococcus salivarius* *Steptococcus mutans* *Leuconostoc mesenteroides* *Lactobacillus sanfranciscensis* *Lactobacillus reuterin*	Prebiotic, antitumor property, hypocholesterolaemic, agent, eco-friendly adhesive, Bio-thickener in food industry.
Glucan	*Lactobacillus johnsonii* *Streptococcus mutans* *Leuconostoc citreum* *Lactobacillus reuterin*	Prebiotics, nourishes gut mucosal cells and inhibits pathogens, for targeted drug delivery against colon cancer, substitute of fat in food products.
Kefiran	*Lactobacillus kefiranofaciens* *Lactobacillus kefirgranum* *Lactobacillus parakefir* *Lactobacillus kefir* *Lactobacillusdelbrueckii subsp. bulgaricus*	Improves visco-elastic properties of acid milk gels, antimicrobial and wound healing properties, ability to lower blood pressure and cholesterol in serum, capacity to retard tumour growth, enhance immunity of gut.

(Patel *et al*., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Collection

Three (3) popular brands of wheat flour; Golden penny flour (flour A), Dangote flour (flour B) and Supreme flour (flour C) were purchased from Samaru market, transported to the Microbiology Department, A.B.U Zaria in clean polythene bags and used in this study.

3.2 Preparation of Dough

Dough samples were prepared from the three (3) popular brands of flour by mixing 1000g of the flour with 1000ml of sterile tap water manually under aseptic conditions (Teiking, 2005; Saeed, 2009). Six dough samples were prepared from each flour type; giving a total of 18 dough samples.

3.3 Fermentation of Dough to Yield Sourdough

The dough samples prepared in 3.2 above were fermented in triplicates at room temperature and at 40^{o}C for 120 h (five days).

3.3.1 Analysis of dough during fermentation

Lactic acid bacteria count (LABC), aerobic plate count (APC) and fungal counts were carried-out at 0 h, 24 h, 48 h, 96 h and 120 h of incubation.

3.3.2 Preparation of serial dilutions

Ten grammes (10g) of each dough sample was homogenized in 90ml of sterile peptone water. This was serially diluted up to 10^{-6} and the last two dilutions ($10^{-}5$ and 10^{-6}) were inoculated on appropriate media for LABC, APC and fungal count as follows:

3.3.3 Lactic acid bacteria count (LABC)

The last two dilutions ($10^{-}5$ and 10^{-6}) were inoculated on de Man Rogosa Sharpes (MRS) agar plates in duplicates using the pour plate method. The MRS plates were incubated anaerobically at 30°C for 48 h.

3.3.4 Aerobic plate count (APC)

The last two dilutions ($10^{-}5$ and 10^{-6}) were inoculated on plate count agar (PCA) plates in duplicates using the pour plate method. The plates were at incubated aerobically at 37°C for 24 h.

3.3.5 Fungal count

The last two dilutions ($10^{-}5$ and 10^{-6}) were inoculated on potato dextrose agar (containing 0.5mg/l streptomycin sulphate) plates in duplicates using the pour plate method. The plates were incubated aerobically at room temperature for 3 – 5 days.

In all cases colonies were counted after the incubation periods and results expressed as CFU/g (Wakil *et al*., 2008; Zugic – Petrovic, 2009).

3.3.6 Identification of the lactic acid bacteria isolates

After the LABC, the lactic acid bacteria isolates were identified based on their Gram reaction, catalase test and carbohydrate fermentation profiles using API 50 CH system (Biomerieux, Marcy l'Etoile France) (El Gaali *et al*., 2009; Vural and Ozgun, 2011; Baratto *et al*., 2012).

3.3.6.1 Catalase test

Pure isolates were obtained after repeated subculturing in MRS broth and on MRS agar. The pure isolates were subjected to catalase test as follows:

A drop of 3% hydrogen peroxide was placed on clean grease - free slide. A bit of a discrete colony of each isolate was picked with a sterile wireloop and placed inside the drop of hydrogen peroxide. Absence of gas bubbles indicated a negative catalase test, while presence of gas bubbles indicated a positive test. Lactic acid bacteria are catalase negative.

All isolates that were catalase negative were subcultured on MRS plates for Gram staining.

3.3.6.2 Gram staining of the isolates

All isolates that were catalase negative were subjected to Gram staining as follows:

A drop of normal saline placed on clean grease-free slides. A bit of a colony of each isolate was placed in the drop of the normal saline and smeared. The smears were heat-fixed over burnsen burner flame followed by flooding with crystal violet for one minute and washed with gentle flowing tap water. The smears were flooded with Gram's iodine for one minute and washed with gentle flowing tap water. The smears were decolourize with acetone for ten seconds and washed with gentle flowing tap water. The smears were flooded with safranin for 30 seconds and washed with gentle flowing tap water. Finally, stained smears were blot – dried and a drop of oil-immersion was placed on each stained smear and observed under the 100x objective lens.

3.3.6.3 Identification of Lactic acid bacteria isolates using the API 50 CH system

All isolates that were catalase negative and Gram positive rods or cocci were further identified based on their carbohydrate fermentation profiles using API 50 CH system. The procedure is as follows:

The isolates were harvested from MRS plates using sterile swab sticks and emulsified in two millilitres of sterile distil water until a tick suspension was obtained. Two millilitres of the isolates suspensions were drawn-out with sterile pipettes and introduced dropwise into testtubes containing five millilitres of sterile distilled water. The drops of the isolates suspension introduced into the five millilitres of the sterile distilled water were counted and the turbidity of the suspensions checked until it equals that of McFarland standard 2 (Biomerieux, Marcy l'Etoile France). Twice the number of drops of the isolates suspension that was equivalent to McFarland standard 2 were pipetted into ampoules of ten millilitres API 50 CHL medium. The tiny holes on the incubation trays of the API 50 CH system were filled with sterile distilled water and the API test strips were chronologically arranged in the trays. Sterile syringes were used to fill the 50 wells of the API test strips with the inoculum of the isolates contained in the API 50 CHL medium. All the wells were sealed with mineral oil.

The trays were covered and incubated aerobically at 35^{o}C for 48 hours. The results were read after 24 h and 48 h and recorded in the result sheets provided. A change in colour to yellow indicated a positive test while the colour remain purple in a negative test. The results obtained were fed into the apiweb^{TM} software which gave the identity of the isolates.

All the identified isolates were preserved on MRS agar slants at 4^{o}C and in MRS broth supplemented with 10% glycerol at -20°C (Baratto *et al*., 2012).

3.4 Preliminary Screening of Lactic Acid Bacteria Isolates for Exopolysaccharides Production

The LAB isolates were screened for exopolysaccharides production using modified MRS agar with sucrose rather than glucose as carbon source. The medium composition is as follows: peptone protease (10g/l), meat extract (8g/l), yeast (4g/l), sucrose (20g/l), sodium acetate. $3H_20$ (5g/l), triammonium citrate (2g/l), magnessium sulphate. $7H_20$ (0.2g/l), manganese sulphate.$4H_20$ (0.05g/l), Dipotassium hydrogen phosphate (2.0g/l), polysorbate 80 (sorbitan monoleate) 1ml, agar (14g/l) (Bridson, 1995; ; Savic *et al*., 2006; Valls and Nacente, 2006).

Formation of slimy (mucoid) colonies or mucoid colonies that form long strands when extended with an inoculation loop were regarded as positive EPS producing isolates (Welman *et al*., 2003; Raul-Madiedo and de los Reyes-Gavilan, 2005; Savic *et al*., 2006).

3.5 Heterofermentative Test of the LAB Isolates

The nineteen EPS positive isolates were subjected to heterofermentative tests as described by (Mehmood *et al*., 2009; Rubayyi *et al.*, 2010; Sameen *et al*., 2010). The isolates were tested for their ability to ferment and produce carbon IV oxide (CO_2) from glucose, fructose, galactose, sucrose, lactose and maltose. The procedure for the heterofermentative test is as follows:

One percent of each sugar was prepared in testtubes with Durham tubes containing 10ml of distilled water. The sugars were tyndalised. One hundred mililitres (100ml) of MRS broth was prepared for each sugar and autoclaved at 121^oC for 15 minutes. The autoclaved broth was allowed to cool. Five millilitres of the MRS broth and 100 microlitres of sugars were pipetted into sterilized testtube; the testtubes were properly labelled, covered and incubated at room temperature for 24 h to check for contamination. After 24 h the purified colonies were inoculated into the sugar solutions, the sugar solutions were sealed with mineral oil and incubated aerobically at 37^oC for 48 h. Gas production in the Durham tubes indicated a positive test.

3.5.1 Dough leavening ability of the LAB isolates

The EPS positive lactic acid bacteria isolates that produce considerable amount of gas in the Durham tubes were selected for the dough leavening test. These include *L. collinoides* GP1, *L. fermentum* SP4$_{40}$*,* *L. plantarum* SP2*,* *L. pentosus* SP3$_{40}$ and *L. brevis* SP5. The dough samples were prepared by mixing 20g flour and 15ml water. The isolates were added at floral concentration of 5ml of 9.0 x 10^8 CFU/ml (Mac Farland standard 3) in 200ml volumetric cylinders. The leavening activity was carried-out at 30^{o}C for 120 minutes (Saeed, 2009).

The leavening activity was compared to that of commercial Baker's yeast. Control dough was prepared without inoculum (Thiele *et al*., 2002; Hertel *et* al., 2005).

3.5.2 Dough leavening ability of the (fermented dough) sourdough

The sourdough sample from supreme flour which had the largest number of heterofermenters and EPS producing LAB isolates was tested for dough leavening capacity at 5%, 10%, 15%, 20%, 25% and 30% concentration. The leavening test was carried-out at 30^{o}C for 120 minutes (Saeed, 2009).

3.6 Production of Experimental Bread and Doughnuts

Lactobacillus plantarum SP2 and *Lactobacillus brevis* SP5 were choosen for bread and doughnuts baking based on the results of the dough leavening tests. Bread and doughnuts were also baked using sourdough at 5%, 10% and 15% concentrations. Control bread and doughnuts were also baked using Baker's yeast only.

3.6.1 Production of experimental bread using selected LAB

Bread dough formulation was 1000g of flour, 640ml tap water, 44g salt and 40g sugar. The selected (base on the result of heterofermentative test) lactic acid bacteria isolates

Lactobacillus plantarum SP2 and *Lactobacillus brevis* SP5 were added at concentration of 50ml of 9.0 x 10^8 CFU/ml (Mac Farland standard 3) while 2g of compressed Baker's yeast was used. The ingredients were mixed manually to form dough and the dough was allowed to ferment at 30°C for 5 hours. After the leavening, the dough was divided into 100g dough balls, moulded and placed in pans. Final proofing was done for 45 minutes at 35°C and bread was baked at 232°C for 25 minutes (Rehman *et al*., 2007; Saeed, 2009).

3.6.2 Production of experimental bread using selected sourdough

Bread dough formulations include 950g, 900g and 850g flour mixed with 50g, 100g and 150g selected sourdough respectively. The dough composition, mixing and leavening is as described in 3.10.1.

3.6.3 Production of experimental doughnuts using selected LAB

Doughnuts dough formulation was 1000g of flour, 640ml tap water, 44g salt, 40g sugar, 10g baking powder, four nutmegs and one sachet of Simas butter. Lactic acid bacteria isolates were added at concentration of 50ml of 9.0 x 10^8 CFU/ml (Mac Farland standard 3) while 2g of compressed baker's yeast was used. The ingredients were mixed manually to form dough and the dough was allowed to ferment at 30°C for 5 hours. After the leavening, the dough was divided into doughnuts rings. Final proofing was done for 45 minutes at 35°C and doughnut was fried (Rehman *et al*., 2007; Saeed, 2009).

3.6.4 Production of experimental doughnuts using selected sourdough

Doughnuts dough formulations include 950g, 900g and 850g flour mixed with 50g, 100g and 150g selected sourdough respectively. The dough composition, mixing and leavening is described in 3.10.3.

3.7 pH Determination

Ten grammes (10g) of the sourdough samples were homogenised in 90ml of sterile distilled water and the pH values were taken using pH meter (HANNA HI 9025).

3.7.1 Determination of pH of experimental bread and doughnuts

Ten grammes (10g) of bread and doughnuts crumb were placed in a container with 100ml of distilled water, covered and stirred until the crumbs dispersed into a semi-liquid mixture. The pH was read using pH meter (HANNA HI 9025) (Saeed, 2009; Bolourin and Khodaparast, 2010).

3.7.2 Preparation of standard (0.1N) sodium hydroxide solution

Aliquots of 83.33g of 96% NaOH pellets were dissolved in a 200ml beaker using distilled water. The solution was quantitatively transferred to a one litre volumetric flask and made-up to the one litre mark with distilled water. It was then allowed to stay for six hours and standardized using potassium hydrogen phthalate, a primary standard salt. The procedure for the standardization of the NaOH solution is as follows:

Five grammes of potassium hydrogen phthalate was dried in an oven at a temperature of $100^{o}C – 106^{o}C$ for one hour. Two grammes of the salt was weighed out of the five grammes above into a 250ml conical flask. One hundred millilitres of distilled water was added to dissolve the salt. The solution was heated on a burnsen flame to completely dissolve the salt followed by addition of three drops of phenolphthalein indicator. The potassium hydrogen phthalate solution was titrated against the NaOH solution until the colour changed to faint pink. The normality of the NaOH solution was calculated using the following relationship:

$$\text{Normality of NaOH} = \frac{\text{Weight of phthalate x 1000}}{\text{Volume of NaOH consumed x molar mass of phthalate (204.20g)}}$$

The concentration of the NaOH solution was found to be 2.03N and from this solution 0.1N NaOH was prepared using the formula $M_1V_1 = M_2V_2$. Where,

$M_1 =$ Initial normality of NaOH = 2.03N.

$V_1 =$ Initial volume of NaOH (required volume) = ?

$M_2 =$ Final or required normality of NaOH = 0.1N.

$V_2 =$ Final volume of NaOH = 1000ml.

Thus,

$$2.03 \times V_1 = 0.1 \times 1000$$

$$V_1 = \frac{0.1 \times 1000}{2.03}$$

$$= 49.26\text{ml}$$

49.26ml of NaOH was transferred to 1000ml volumetric flask and made-up to the one litre mark with distilled water to obtain 0.1N NaOH solution (Mendham *et al*., 2000).

3.7.3 Titratable acidity (TTA) determination

Ten millilitres (10ml) of the homogenate from each sourdough sample were titrated against 0.1N sodium hydroxide (NaOH) for the determination of TTA. The TTA was calculated using the formular below:

Percentage titratable acidity calculated as lactic acid:

$$\text{TTA} = \frac{\text{Titre x Normality of base x chemical factor (0.009018) x 100}}{\text{Weight of sample}}$$

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

3.7.4 Determination of titratable acidity of experimental bread and doughnuts

Aliquots of 0.1N NaOH was added to 25 mililitre of the semi-liquid above until the pH reached 6.6. Titratable acidity was determined based on the quantity of NaOH consumed using the formula used in 3.7.3 (Saeed, 2009; Bolourin and Khodaparast, 2010).

3.7.5 Proximate Analysis of the Flours, Fermented Dough and Experimental Bread and Doughnuts Samples

The flours, sourdough, baked bread and doughnuts samples were analysed for moisture content, ash content, crude protein, carbohydrate and fat content as described in AOAC (2010).

3.7.5.1 Moisture content determination

Five grammes (5g) of each sample were weighed into a silica dish which had been previously dried and weighed. The silica dishes containing the samples were placed in a hot air oven for 24 h at 70^{o}C until it was dry to constant weight. The moisture content was calculated using the relationship below:

$$\text{\% moisture} = \frac{\text{wt of biological material – wt of biological material taken after drying x 100}}{\text{Wt of biological material}}$$

3.7.5.2 Total ash content determination

The residue from the moisture determination was charred in a muffle furnace between 600^{0}C for twelve hours until the ash was grey or nearly white. The charred ash was cooled and weighed to determine total ash.

3.7.5.3 Crude protein determination

The crude protein was determined by first determining the nitrogen content of the protein using the micro Kjeldahl method. A known weight of the sample was placed in Kjeldahl flask and about 200mg of catalyst mixture added. Ten millilitres of concentrated tetraoxosulphate VI acid (H_2SO_4) was added to the content and heated gently for a few minutes until frothing ceased, the heat was increased to digest for three hours. It was allowed to cool and made-up to a known volume with 100ml of distilled water. Ten millilitres of the dilute solution of the digest was distilled by pippetting the volume into distillation chamber of macro kjeldhal distillation apparatus. Ten milliliters of 40% sodium hydroxide (NaOH) solution and steam from the distillation chamber was added into 10ml of 2% boric acid containing mixed indicator until colour change from red to green was noted. The mixture was titrated agaisnt 0.01N hydrochloric acid (Hcl) to grey end point. The nitrogen content was calculated using the relationship below:

$$\%N = \frac{(a-b) \times 0.01 \times 14.0057 \times c \times 100}{d \times e}$$

a = Titre value for the sample.

b = Titre value for the blank.

C = Volume to which digest is made up with distilled water.

d = Aliquot taken for distillation.

c = Weight of dried sample (mg).

Crude protein content was calculated after determining the nitrogen content using the following relationship:

% crude protein = % N x 5.70.

3.7.5.4 Fat content determination

Two grams of each sample was transferred to a fat-free extraction thimble, plugged lightly with cotton wool and $150cm^3$ of petroleum ether added until it siphoned once. More ether was added until barrel of extractor was half full and then boiled for eight hours. The content of the barrel was siphoned into the ether stock bottle and dried in an oven to constant weight. The fat content was calculated using the following relationship:

$$\text{Ether extract} = \frac{\text{wt of oil x100}}{\text{Wt of biological material}}$$

3.7.5.5 Crude carbohydrate content determination

Five hundred mililitres (500ml) of glacial acetic acid was mixed with 450ml of water and 50ml concentrated nitric acid (HNO_3) followed by the addition of 20g of trichloroacetic acid (TCA) to the mixture. One gram of defatted sample was weighed into a 250ml conical flask and 100ml TCA mixture was added. It was refluxed for exactly 40 minutes. A condenser was used to prevent loss of liquid. The flask was disconnected, the content of the flask was cooled and filtered through No.14 wathman filter paper previously dried and weighed. The residue on the filter paper was washed 10 times with hot distilled water and once with absolute alcohol. The washed residue was dried in an oven at 105^0C, cooled and weight. The crude carbohydrate content was determined by subtracting the weight of the filter paper from the weight of both the filter paper and the residue.

3.8 Sensory Evaluation of Experimental Bread and Doughnuts Samples

The bread and doughnuts samples were subjected to sensory evaluation using a panel of 12 enlightened judges who evaluated the following physical parameters: taste, texture, appearance and aroma. Nine points hedonic scale was used for the analysis, this include: 9 is Like Extremely, 8 is Like Very Much, 7 is Like Moderately, 6 is Like Slightly, 5 is Neither

Like nor Dislike, 4 is Dislike Slightly, 3 is Dislike Moderately, 2 is Dislike Very Much and 1 is Dislike Extremely (Aboaba and Obakpolor, 2010; Breashears and Crowe, 2013).

3.9 Evaluation of Shelf-life of Bread and Doughnuts Samples

The bread and doughnuts 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).

3.10 Screening of Exopolysaccharides Producing LAB for the Presence of Levansucrase (Lev V) Gene and Glucansucrase (Gtf) Gene

3.10.1 Isolation of DNA

Deoxyribonucleic acid (DNA) was isolated from the 19 EPS positive isolates using the QIA$_{amp}$ DNA extraction kit (Qiagen, S.P.A., Milan, Italy) based on the manufacturer’s instructions as described below.

Twenty microlitres (20µl) of proteinases K was pipetted into the bottom of 1.5ml microcentrifuge tubes labelled 1 -21. Two hundred microlitres (200µl) of the broth containing the isolates were added to each tube, followed by the addition of two hundred microlitres (200µl) of Buffer AL. All the tubes were incubated at 56^{o}C for 10 minutes. The tubes were centrifuged at 8000 rpm for 15 seconds to remove drops from the inside of the lid. Two hundred microlitres (200µl) of absolute ethanol was added to each tube and mixed again by pulse-vortexing for 15 seconds. After which the tubes were centrifuged at 8000 rpm for 15 seconds to remove drops from the inside of the lid. The contents of the tubes were carefully placed in QIA$_{amp}$ Mini spin columns (in a 2 ml collection tube), care was taking not to wet the rim. The caps were closed and the tubes were centrifuged at 8000 rpm for one minute. The QIA$_{amp}$ Mini spin columns were placed in clean 2ml collection tubes, while the tubes containing the filtrate were discarded. The QIA_{amp} Mini spin columns were carefully opened and 500µl Buffer AW1 was added to each column. Care was taking not to wet the rim. The caps were closed and centrifuged at 8000 rpm for one minute. The Mini spin columns were placed in clean 2 ml collection tubes, while the tubes containing the filtrate were discarded. The QIA_{amp} Mini spin columns were carefully opened and 500µl Buffer AW2 was added to each column. Care was taking not to wet the rim. The caps were closed and centrifuged at full speed (14,000 rpm) for 3 minutes. The QIA_{amp} Mini spin columns were placed in new 2ml collection tubes, the old collection tubes were discarded with the filtrate. The columns were centrifuged at full speed (14,000 rpm) for 1 minute. The QIA_{amp} Mini spin columns were placed in clean 1.5 ml microcentrifuge tubes while the collection tubes containing the filtrates were discarded. The spin columns were carefully opened and 200µl of Buffer AE was added to each tube. All the tubes were incubated at room temperature for five minutes and centrifuged at 8000 rpm for one minute. The yield and quality of the extracted DNA was checked by loading 20µl of the extracted DNA in one percent (1%) agarose gels followed by staining with ethidium bromide and the DNA fragments were visualised at 312nm by UV transillumination (Querre, *et al.*, 1997; Teiking *et al.*, 2003).

3.11 Polymerase Chain Reaction Amplification of DNA

The glucansucrase (gtf) and Levansucrase (Lev) genes were screened using primers that targeted the two genes as described by Blaiotta *et al.* (2008). The PCR master mix for each sample consists of 16µl of nuclease free water, 25µl Dream Taq polymerase mix, 1µl each of the two primer pairs $\text{Gtf}_{\text{forward,}}$ $\text{Gtf}_{\text{reverse,}}$ $\text{LevV}_{\text{forward}}$ and $\text{LevV}_{\text{reverse.}}$ Five microlitres of the extracted DNA was added to the master mix to give a total volume 50 µl.

The PCR conditions consisted of 35 cycles (95^{o}C for 5minutes, 95^{o}C for 30seconds, 42^{o}C for 45 seconds, 72^{o}C for 1minute, 72^{o}C for 7minutes and 4^{o}C for 1minute).

3.11.1 Agarose gel electrophoresis of PCR products

Gel electrophoresis was carried-out by applying 25µm of sample to submerged horizontal 1% agarose gels slab gels (Querre, *et al.*, 1997; Tieking *et al.*, 2003). Gels were run for 30 minutes at 50V in TEB electrophoresis buffer (89mmol^{-1} boric acid, 89 mmol^{-1}Tris, 2 mmol^{-1}EDTA) without cooling. A 100 bp DNA molecular weight marker (superladder mid-1, Eurogentee) was used as standard (Querre, *et al.*, 1997; Ulrich and Hughes, 2001). Gels were stained with ethidium bromide and DNA fragments were visualised at 312nm by UV transillumination (Querre, *et al.*, 1997; Tieking *et al.*, 2003).

3.11.2 Optimization of PCR

After visualization of the DNA bands none of the isolates showed the expected band size of glucansucrase genes (gtf). The PCR was therefore optimized to increase the chances of amplifying the glucansucrase genes (gtf) if present as shown in table below

The PCR conditions are as detailed below:

Table 3.1: Primer pairs and PCR conditions for screening of levansucrase (Lev V) and glucansucrase (Gtf) genes

Primer	**Sequence (5' – 3')**	**Gene target**	**Expected fragment size (bp)**	**PCR conditions**	**References**
Gtf fw Gtf rev	GACAACACIAACCCTACIGTIC ADATCICCATAATAIAAICGIG	Glucansucrase	660	35 cycles of 95^{o}C (30s), 42^{o}C (45s), 72^{o}C (1min)	Hijum *et al.*, 2004.
LevVfw LevVrev	GACGTATGGGACACATGGC TCATCCTCATCACAAAACAT	Levansucrase	500	35 cycles of 95^{o}C (30s), 42^{o}C (45s), 72^{o}C (1min)	Hijum *et al.*, 2004.

Table 3.2: **Optimization of PCR**

Tube	**dNTP**	**$MgCl_2$**	**Primers**	**Taq polymerase**	**10xPCR buffer**	**Water**	**DNA**
1	0.25	2.0	0.20	0.25	5.0	37.30	5.0
2	0.25	2.5	0.25	0.50	5.0	36.50	5.0
3	0.25	3.0	0.50	1.00	5.0	35.25	5.0
4	0.50	2.0	0.25	1.00	5.0	36.25	5.0
5	0.50	2.5	0.50	0.25	5.0	36.25	5.0
6	0.50	3.0	0.20	0.50	5.0	35.80	5.0
7	1.00	2.0	0.50	0.50	5.0	36.00	5.0
8	1.00	2.5	0.20	1.00	5.0	35.30	5.0
9	1.00	3.0	0.25	0.25	5.0	35.50	5.0

The annealing temperature was also varied as part of the optimization process, annealing temperatures of 43°C, 44°C, 45°C, 46°C and 47°C were used.

3.12 Sequencing of the Levansucrase Genes

The levansucrase genes of the seven isolates were sequenced at the Iqaba Laboratory, South Africa. The data received from the sequencing facility was aligned with the National Centre for Biotechnology Information (NCBI) database and was accomplished by using the Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov./BLAST/; (Tamura *et al*., 2011).

3.13 Molecular Phylogenetic Analysis of the Isolates

The sequence data from the NCBI BLAST search was aligned with the ClustalW multiple alignment function in the BioEdit Sequence Alignment Editor program (Hall, 1999). The alignment was used to draw a phylogeny reconstruction tree with the neighbor-joining statistical method and tested with the bootstrap method in Molecular Evolutionary Genetics Analysis (MEGA) version 5 (Tamura *et al*., 2011).

3.14 Statistical Analysis

The statistical analyses of the measured parameters were made using analysis of variance (ANOVA). Significant differences among samples were evaluated by Duncan multiple – range test ($P \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Microbial Counts during Dough Fermentation at Room Temperature

The results of the microbial counts for the dough fermented at room temperature is as shown on Table 4.1. The lactic acid bacteria were the most dominant population in all the sourdoughs. There was a progressive increase in the lactic acid bacteria counts while there was a progressive decrease in the aerobic plate counts and fungal counts with increase in the length of fermentation of all the sourdoughs. At room temperature fermentation; flour C sourdough had the highest total mean lactic acid bacteria count (LABC) of log 6.826 ± 0.68CFU/g followed by flour B sourdough with a total mean LABC of log 6.471 ± 0.62 CFU/g and flour A sourdough with a total mean LABC of log 6.462 ± 0.62 CFU/g. There was a significant difference in the total mean lactic acid bacteria count of flour C sourdough and that of both flour B and flour A ($P \leq 0.05$) but there is no significant difference in the mean lactic acid bacteria counts between flour B and flour A sourdoughs ($P \geq 0.05$). The total mean fungal counts were log 6.551 ± 0.57CFU/g, log 6.451 ± 0.59 and log 6.305 CFU/g ± 0.49 for flour C, flour B and A sourdoughs respectively. There was significant difference in the mean fungal counts of the three different sourdoughs ($P \leq 0.05$). The total mean aerobic plate counts were log 6.460 ± 0.64 CFU/g, log 6.414 ± 0.59 CFU/g and log 6.186 ± 0.64CFU/g for flour B, flour C and flour A sourdoughs respectively. There was no significant difference in the total mean aerobic plate counts of flour B and flour C sourdoughs ($P \geq 0.05$), however, the total mean aerobic plate counts of the two sourdoughs were significantly different from that of flour A sourdough ($P \leq 0.05$).

Table 4.1: Microbial counts during spontaneous fermentation of the dough at room temperature

Length of fermentation (hrs)	**Flour A**			**Flour B**			**Flour C**		
	LABC (Log CFU/g)	**FC (Log CFU/g)**	**APC (Log CFU/g)**	**LABC (Log CFU/g)**	**FC (Log CFU/g)**	**APC (Log CFU/g)**	**LABC (Log CFU/g)**	**FC (Log CFU/g)**	**APC (Log CFU/g)**
0	5.524 ± 0.08^{a}	6.701 ± 0.05^{a}	7.061 ± 0.10^{a}	4.930 ± 0.56^{a}	6.557 ± 0.39^{a}	7.020 ± 0.08^{a}	5.860 ± 0.07^{a}	6.876 ± 0.06^{a}	6.977 ± 0.06^{a}
24	5.729 ± 0.02^{a}	7.095 ± 0.06^{a}	7.071 ± 0.04^{a}	5.760 ± 0.05^{b}	6.851 ± 0.17^{b}	6.881 ± 0.11^{a}	5.877 ± 0.13^{a}	6.908 ± 0.09^{a}	6.890 ± 0.02^{a}
48	5.950 ± 0.16^{a}	6.032 ± 0.04^{b}	6.001 ± 0.04^{b}	6.391 ± 0.24^{c}	6.951 ± 0.03^{b}	6.957 ± 0.07^{a}	7.188 ± 0.03^{b}	6.977 ± 0.02^{a}	6.802 ± 0.36^{a}
72	7.095 ± 0.07^{b}	6.438 ± 0.434^{c}	5.895 ± 0.76^{b}	7.143 ± 0.05^{d}	7.346 ± 0.42^{c}	6.805 ± 0.09^{a}	7.291 ± 0.03^{b}	7.037 ± 0.08^{a}	6.945 ± 0.05^{a}
96	7.168 ± 0.06^{b}	5.924 ± 0.10^{b}	5.590 ± 0.12^{c}	7.250 ± 0.03^{d}	5.957 ± 0.24^{d}	5.611 ± 0.11^{b}	7.287 ± 0.08^{b}	5.827 ± 0.12^{b}	5.787 ± 0.04^{b}
120	7.303 ± 0.05^{b}	5.637 ± 0.15^{d}	5.508 ± 0.21^{c}	7.354 ± 0.07^{d}	5.645 ± 0.14^{e}	5.485 ± 0.03^{c}	7.453 ± 0.02^{c}	5.683 ± 0.06^{c}	5.483 ± 0.05^{c}
Total Mean	$6.462 \pm 0.74_{h}^{c}$	$6.305 \pm 0.49_{i}^{c}$	$6.186 \pm 0.64_{j}^{b}$	$6.471 \pm 0.62_{h}^{c}$	$6.451 \pm 0.59_{h}^{a}$	$6.460 \pm 0.65_{h}^{f}$	$6.826 \pm 0.68_{l}^{e}$	$6.551 \pm 0.57_{k}^{g}$	$6.414 \pm 0.59_{h}^{d}$
P-Value	< 0.003	< 0.001	< 0.021	< 0.033	< 0.032	< 0.003	0.042	< 0.003	< 0.003

All values are mean of triplicate readings. Mean values with different superscripts in the same column are significantly different ($P \leq 0.05$). Mean values with different subscripts in the same row are significantly different ($P \leq 0.05$). Mean values were separated using Duncan Multiple Range test.

LABC = Lactic acid bacteria count; FC = Fungal count; APC = Aerobic plate count

4.2 Microbial Counts during Dough Fermentation at 40^{o}C

For sourdoughs produced at 40^{0}C, flour C sourdough also had the highest total mean lactic acid bacteria count (LABC) of log 7.051 ± 1.04CFU/g followed by flour A sourdough with a total mean LABC of log 6.878 ± 0.99 CFU/g and flour B sourdough with a total mean LABC of log 6.728 ± 0.95 CFU/g. There was a significant difference in the total mean lactic acid bacteria count of flour C sourdough and that of both flour B and flour A ($P \leq 0.05$) but there was no significant difference in the mean lactic acid bacteria counts between flour B and flour A sourdoughs ($P \geq 0.05$). The total mean fungal counts were log 4.925 ± 0.64 CFU/g, log 4.922 ± 0.69 CFU/g and log 4.900 ± 0.64 CFU/g for flour C, flour B and flour A sourdoughs respectively. There was no significant difference between the total mean fungal counts of the three different sourdoughs ($P \geq 0.05$). The total mean aerobic plate counts were log 4.968 ± 0.88 CFU/g, log 4.937 ± 0.84 CFU/g and log 4.794 ± 0.59 CFU/g for flour A, flour B and flour C sourdoughs respectively. There was no significant difference ($P \geq 0.05$) in the total mean aerobic plate counts of flour A and flour B sourdoughs (Table 4.2).

4.3 Microbial Counts and Lactic Acid Bacteria Isolates of Selected Sourdough before Leavening Test

The result for the microbial counts and LAB isolated from the selected sourdough prior to leavening test and baking showed that the lactic acid bacteria were still the most dominant organisms with a mean count of 8.357 ± 0.06, followed by a fungal count of 4.907 ± 0.51 and bacterial load of 4.818 ± 0.42. There is a significant difference ($P \leq 0.05$) between the lactic acid bacteria count and both the fungal and aerobic plate counts. With the exception of *L. buchneri* and *Pediococcus acidilactici* all the other lactic acid bacteria isolates obtained during the spontaneous fermentation of the doughs were again recovered (Table 4.3)

Table 4.2 Microbial Counts during Dough Fermentation at 40^{o}C

Length of fermentation (hrs)	Flour A			Flour B			Flour C		
	LABC (Log CFU/g)	FC (Log CFU/g)	APC (Log CFU/g)	LABC (Log CFU/g)	FC (Log CFU/g)	APC (Log CFU/g)	LABC (Log CFU/g)	FC (Log CFU/g)	APC (Log CFU/g)
0	5.770 ± 0.02^{a}	5.489 ± 0.04^{d}	5.778 ± 0.07^{c}	5.775 ± 0.08^{b}	5.615 ± 0.19^{e}	5.755 ± 0.05^{b}	5.954 ± 0.02^{a}	5.725 ± 0.10^{b}	5.774 ± 0.03^{e}
24	6.002 ± 0.06^{a}	5.601 ± 0.12^{d}	5.811 ± 0.04^{c}	5.841 ± 0.07^{b}	5.654 ± 0.11^{e}	5.804 ± 0.12^{b}	5.956 ± 0.05^{a}	5.514 ± 0.06^{d}	5.512 ± 0.16^{c}
48	6.071 ± 0.02^{a}	5.528 ± 0.05^{d}	5.687 ± 0.05^{c}	5.886 ± 0.03^{b}	5.548 ± 0.08^{e}	5.625 ± 0.11^{b}	6.339 ± 0.05^{e}	5.432 ± 0.07^{d}	5.451 ± 0.03^{c}
72	7.177 ± 0.11^{b}	4.300 ± 0.16^{e}	4.551 ± 0.18^{d}	7.059 ± 0.09^{d}	4.143 ± 0.46^{f}	4.548 ± 0.06^{c}	7.331 ± 0.06^{b}	4.461 ± 0.03^{e}	4.525 ± 0.13^{f}
96	8.024 ± 0.07^{d}	4.279 ± 0.12^{e}	4.577 ± 0.12^{d}	7.665 ± 0.44^{e}	4.324 ± 0.07^{f}	4.367 ± 0.08^{c}	8.290 ± 0.07^{d}	4.254 ± 0.23^{f}	4.217 ± 0.08^{g}
120	8.225 ± 0.01^{d}	4.201 ± 0.14^{e}	3.405 ± 0.16^{e}	8.144 ± 0.05^{f}	4.246 ± 0.09^{f}	3.525 ± 0.04^{d}	8.433 ± 0.02^{d}	4.165 ± 0.10^{f}	3.286 ± 0.08^{h}
Total Mean	$6.878 \pm 0.99_{a}^{c}$	$4.900 \pm 0.64_{c}^{f}$	$4.968 \pm 0.88_{c}^{f}$	$6.728 \pm 0.95_{a}^{g}$	$4.922 \pm 0.69_{c}^{g}$	$4.937 \pm 0.85_{c}^{e}$	$7.051 \pm 1.04_{j}^{b}$	$4.925 \pm 0.64_{c}^{h}$	$4.794 \pm 0.58_{k}^{i}$
P-Value	< 0.023	< 0.014	<0.0043	<0.003	<0.024	<0.043	<0.012	<0.003	<0.004

All values are mean of triplicate readings. Mean values with different superscripts in the same column are significantly different ($P \leq 0.05$). Mean values with different subscripts in the same row are significantly different ($P \leq 0.05$). Mean values were separated using Duncan Multiple Range test.

LABC = Lactic acid bacteria count; FC = Fungal count; APC = Aerobic plate count

Table 4.3 Microbial Counts and Lactic Acid Bacteria Isolates of Selected Sourdough Before Leavening Test

Sample	LABC Log CFU/g	FC Log CFU/g	APC Log CFU/g	LAB isolates
1	8.336	5.539	5.462	*L. plantarum, L. pentosus, P. Pentocaseus*
2	8.301	5.633	5.462	*L. plantarum, L. pentosus,L. brevis, L. collinoides*
3	8.294	4.556	4.602	*L. plantarum, L. brevis, L. collinoides*
4	8.434	4.462	4.477	*L. brevis, L. plantarum, L. fermentum, P. pentocaseus*
5	8.324	4.792	4.491	*L. brevis, L. fermentum*
6	8.455	4.462	4.415	*L. plantarum, L. pentosus, L. brevis*
Mean	8.357 ± 0.06^a	4.907 ± 0.51^b	4.818 ± 0.42^b	

Mean values with different superscripts in the same row are significantly different ($P \leq 0.05$).

TTA = Titratable acidity; LABC = Lactic acid bacteria count; FC = Fungal count; APC = Aerobic plate count

4.4 Morphological and Biochemical Characterization of the Lactic Acid Bacteria Isolates

The morphological and biochemical characterization of the lactic acid bacteria isolates is as shown in Table 4.4. All the isolates were gram positive (Appendix XV) and catalase negative. A summary of the sugar fermentation tests shows slight differences in the sugar fermentation pattern of the isolates. A positive sugar fermentation test was indicated by a change in colour from purple to yellow (appendices XX and XXI). The detail sugar fermentation pattern of the isolates is as shown in Appendix I.

4.5 Lactic Acid Bacteria Isolated from the Fermented Dough

A total of seventy – nine (79) isolates of lactic acid bacteria comprising of six (6) species of the genera *Lactobacillus* and two species of the genera *Pediococcus* were obtained. Forty-two (42) isolates were obtained from dough fermented at 40^0C while thirty-seven (37) isolates were obtained from dough fermented at room temperature. *Lactobacillus plantarum* was the most predominant isolate in both fermented doughs (sourdough) with an overall frequency of occurrence of 27 representing 34.17%, followed by *Lactobacillus brevis* with an overall frequency of occurrence of 23 representing 29.11%. *Lactobacillus pentosus, Lactobacillus buchneri, Lactobacillus collinoides* and *Lactobacillus fermentum* all had overall frequency of occurrence of 2 (2.53%). *Pediococcus pentosaceus* and *Pediococcus acidilactici* had overall frequency of occurrence of 7 (8.86%) and 2 (2.53%) respectively. The highest number of lactic acid bacteria isolates were obtained from flour C dough, a total of thirty-two (32) representing 40.51% of the isolates, followed by flour B dough with a total of 25 isolates (36.65%) and flour A dough with a total of 22 isolates representing 27.85% of the total number of isolates obtained (Table 4.5).

4.4 Morphological and biochemical characterization of the lactic acid bacteria isolates

Test	**SP2**	**SP5**	**DG3**	**SP3$_{40}$**	**GP1**	**SP4**	**SP4**	**SP5$_{40}$**
Gram reaction	+ve rods	+ve rods	+ve rods	+ve rods	+ve rods	+ve rods	+ve cocci	+ve cocci
Catalase test	-	-	-	-	-	-	-	-
Sugar fermentation tests								
D-glucose	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+
D-mannose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
L-sorbose	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	+	+	+	+	+
Mannitol	+	-	-	+	+	+	-	-
B-methyl-Xloside	+	+	+	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	-
Lactose	+	-	-	+	-	-	+	-
5-Keto-gluconate	-	-	-	+	+	+	-	-
D-raffinose	+	-	+	+	+	+	+	-
Sorbitol	+	-	-	+	-	+	-	-
Inference	*L. plantarum*	*L. brevis*	*L. buchneri*	*L. pentosus*	*L. collinoides*	*L. fermentum*	*P. pentosaceus*	*P. acidilactici*

Key: +ve = Positive; -ve = Negative

Table 4.5: Lactic Acid Bacteria Isolated from the Fermented Dough

Room temperature (%)										
Flour type	**No. of samples analysed**	***Lactobacillus plantarum***	***Lactobacillus brevis***	***Lactobacillus pentosus***	***Lactobacillus buchneri***	***Lactobacillus collinoides***	***Lactobacillus fermentum***	***Pediococcus pentocaseus***	***Pediococcus acidilactici***	**Total**
Flour A	36	3 (8.33)	3 (8.33)	2 (5.56)	2 (5.56)	-	-	-	-	10 (12.66)
Flour B	36	5 (13.88)	4 (11.11)	3 (8.33)	-	-	-	1 (2.78)	-	13 (16.46)
Flour C	36	4 (11.11)	4 (11.11)	2 (5.56)	-	2 (5.56)	-	2 (5.56)	-	14 (17.72)
Total	108	12 (32.43)	11(29.73)	7 (18.91)	2 (5.40)	2 (5.40)	0 (0.00)	3 (8.10)	0 (0.00)	37 (46.84)
40^0C										
Flour A	36	5 (13.88)	4 (11.11)	1 (2.78)	-	-	-	1 (2.78)	1 (2.78)	12 (15.19)
Flour B	36	4 (11.11)	3 (8.33)	3 (8.33)	-	-	-	2 (5.56)	-	12 (15.19)
Flour C	36	6 (16.67)	5 (13.88)	3 (8.33)	-	-	2 (5.56)	1 (2.78)	1 (2.78)	18 (22.78)
Total	108	12 (32.43)	11(29.73)	7 (18.91)	2 (5.40)	2 (5.40)	0 (0.00)	3 (8.10)	0 (0.00)	42 (53.16)
Overall Total	216	27 (34.17)	23 (29.11)	14 (17.72)	2 (2.53)	2 (2.53)	2 (2.53)	7 (8.86)	2 (2.53)	79

4.6 Screening of the Lactic Acid Bacteria Isolates for Exopolysaccharides Production

The result obtained from the screening of the isolates for exopolysaccharides production ability showed that nineteen (19) isolates representing 24.05% of the seventy – nine (79) isolates screened have exopolysaccharides production ability. Thirteen (68.42%) of the exopolysaccharides positive isolates phenotypically expressed their exopolysaccharides in form of mucoid and ropy colonies, while six (31.58%) of the exopolysaccharides positive isolates phenotypically expressed their exopolysaccharides in form of mucoid colonies only (Appendices XVI - IX). The exopolysaccharides positive isolates where found among five of the eight species screened; *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus pentosus, Lactobacillus collinoides* and *Lactobacillus fermentum*, while the remaining three species; *Lactobacillus buchneri*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* were found to be exopolysaccharides production negative (Table 4.6).

4.7 Heterofermentative Tests of the Exopolysaccharides Positive LAB Isolates

The result of the heterofermentative test showed that all the nineteen isolates were capable of fermenting glucose, fructose, galactose and maltose. The *Lactobacillus brevis* isolates could not ferment both lactose and sucrose while *Lactobacillus fermentum* and *Lactobacillus collinoides* could not ferment lactose. All the isolates with the exception of *Lactobacillus plantarum* SP4, *Lactobacillus plantarum* SP5, *Lactobacillus plantarum* SP3, *Lactobacillus plantarum* SP4$_{40}$, *Lactobacillus brevis* DG5$_{40}$ and *Lactobacillus pentosus* GP3$_{40}$ produced gas from glucose fermentation. The amount of gas produced in the Durham tubes however differ. *Lactobacillus plantarum* SP2 and *Lactobacillus brevis* SP5 produced a considerable amount of gas in the Durham tubes. Five of the isolates *Lactobacillus plantarum* SP2, *Lactobacillus plantarum* SP2$_{40}$, *Lactobacillus plantarum* DG5, *Lactobacillus plantarum* GP3 and *Lactobacillus plantarum* DG5$_{40}$ produced gas from sucrose fermentation (Table 4.7).

Table 4.6: Screening of the lactic acid bacteria isolates for exopolysaccharides production

Organism	No. of isolates screened	Number positive (%)		
		Mucoid colonies only	Mucoid and ropy colonies	Total positive
Lactobacillus plantarum	27	5 (45.45)	6 (54.55)	11 (40.74)
Lactobacillus brevis	23	1 (33.33)	2 (66.66)	3 (13.04)
Lactobacillus pentosus	14	0. (0.00)	3 (100)	3 (42.88)
Lactobacillus buchneri	2	0. (0.00)	0 (0.00)	0 (0.00)
Lactobacillus collinoides	2	0 (0.00)	1 (100)	1 (50.00)
Lactobacillus fermentum	2	0 (0.00)	1 (100)	1 (50.00)
Pediococcus pentosaceus	7	0. (0.00)	0 (0.00)	0 (0.00)
Pediococcus acidilactici	2	0. (0.00)	0 (0.00)	0 (0.00)
Total	79	6 (31.58)	13 (68.42)	19 (24.05)

Table 4.7 Heterofermentative test for the exopolysaccharides positive LAB isolates

	Sugars					
Isolates	**Glucose**	**Fructose**	**Galactose**	**Lactose**	**Sucrose**	**Maltose**
L. plantarum SP2	+ (G^{+++})	+	+	+	+ (G^{+})	+
L. plantarum SP2$_{40}$	+ (G^{++})	+	+	+	+ (G^{+})	+
L. plantarum SP5	+	+	+	+	+	+
L. plantarum SP3	+	+	+	+	+	+
L. plantarum DG5	+ (G^{++})	+	+	+	+ (G^{+})	+
L. plantarum GP3	+ (G^{++})	+	+	+	+ (G^{+})	+
L. plantarum DG5$_{40}$	+ (G^{++})	+	+	+	+ (G^{+})	+
L. plantarum SP3$_{40}$	+ (G^{+})	+	+	+	+	+
L. plantarum SP4$_{40}$	+	+	+	+	+	+
L. plantarum SP4	+	+	+	+	+	+
L. plantarum GP5	+ (G^{++})	+	+	+	+	+
L. brevis SP5	+ (G^{+++})	+	+	-	-	+
L. brevis DG5$_{40}$	+ (G^{+})	+	+	-	-	+
L. brevis GP4	+ (G^{+})	+	+	-	-	+
L. pentosus SP3$_{40}$	+ (G^{+})	+	+	+	+	+
L. pentosus GP3$_{40}$	+	+	+	+	+	+
L. pentosus GP1	+ (G^{++})	+	+	+	+	+
L. fermentum SP4	+ (G^{++})	+	+	-	+	+
L. collinoides GP1	+ (G^{+})	+	+	-	+	+

+ = Turbidity (growth), - = Absence of turbidity (growth), + (G^{+}) = Growth & bubble of gas, + (G^{++}) = Growth & about quarter of Durham tube gas production, + (G^{+++}) = Growth & about half of Durham tube gas production.

4.8 Comparison of Dough Leavening Ability of Baker's Yeast and the LAB Isolates

The Baker's yeast gave the highest leavening ability at 150 minutes of leavening followed by *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus fermentum*, *Lactobacillus brevis* and the least being *Lactobacillus collinoides* and the control dough. *Lactobacillus plantarum* gave the highest level of dough leavening among the lactic acid bacteria (LAB) isolates followed by *Lactobacillus fermentum*, *Lactobacillus pentosus*, *Lactobacillus brevis* and *Lactobacillus collinoides* all at 210 minutes of leavening (Fig. 4.1).

After 150 minutes of leavening, there was significant difference ($p \leq 0.001$) in the mean dough leavening between Baker's yeast (87.50 ± 6.50 cm^3) and all the lactic acid bacteria isolates as well as the control dough. Among the lactic acid bacteria isolates there was no significant difference ($p \geq 0.01$) in the mean dough leavening between *Lactobacillus fermentum* (37.50 ± 0.50 cm^3), *Lactobacillus pentosus* (37.50 ± 1.00 cm^3) and *Lactobacillus brevis* (33.00 ± 1.00 cm^3). There was also no significant difference ($p \geq 0.01$) in the mean dough leavening between *Lactobacillus collinoides* (30.00 ± 0.00 cm^3) and the control dough (30.00 ± 0.00 cm^3). There was however, a significant difference ($p \leq 0.01$) in the mean dough leavening between *Lactobacillus plantarum* (39.50 ± 0.50 cm^3) and the other lactic acid bacteria isolates. At 210 minutes of dough leavening *Lactobacillus plantarum* gave the highest dough leavening (46.00 ± 2.00 cm^3) in comparison with the other LAB isolates. However, there was no significant difference ($p \geq 0.01$) in mean dough leavening between *Lactobacillus plantarum* (46.00 ± 2.00 cm^3) and *Lactobacillus fermentum* (45.00 ± 1.00 cm^3). There was also no significant difference ($p \geq 0.01$) in mean dough leavening between *Lactobacillus collinoides* (30.00 ± 0.00 cm^3), *Lactobacillus brevis* (32.50 ± 0.50 cm^3) and the control dough (30.00 ± 0.00 cm^3). Significant difference ($p \leq 0.01$) however, exist in the mean dough leavening between *Lactobacillus pentosus* (40.00 ± 1.00 cm^3), *Lactobacillus collinoides* (30.00 ± 0.00 cm^3) and *Lactobacillus brevis* (32.50 ± 0.50 cm^3) (Appendix II).

4.9 Comparison of Dough Leavening Ability of Baker's Yeast and Combination of LAB Isolates/Baker's Yeast

The results obtained for the synergistic dough leavening by a combination of the LAB isolates and the Baker's yeast in comparison with the Baker's yeast alone and the control dough showed that in all cases the combination of the LAB isolates and the Baker's yeast were able to leavened dough more than the Baker's yeast alone. The highest dough leavening was obtained from the combination of *Lactobacillus plantarum* and Baker's yeast, followed by the combination of *Lactobacillus collinoides* and Baker's yeast, *Lactobacillus fermentum* and Baker's yeast, *Lactobacillus brevis* and Baker's yeast, *Lactobacillus pentosus* and Baker's yeast, Baker's yeast alone and the control dough being the least at 180 minutes of leavening (Figure 4.2).

There was no significant difference ($p \geq 0.01$) in the mean dough leavening between the combination of *Lactobacillus plantarum* and Baker's yeast (108.00 ± 2.00cm^3) and the combination of *Lactobacillus collinoides* and Baker's yeast (107.00 ± 2.00cm^3) but significant difference ($p \leq 0.01$) exists between these two combinations and the remaining combinations; Baker's yeast alone (92.00 ± 1.00 cm^3) and the control dough (30.00 ± 0.00 cm^3). There was also no significant difference ($p \geq 0.01$) in mean dough leavening between the combination of *Lactobacillus fermentum* and Baker's yeast (105.00 ± 3.50cm^3) and *Lactobacillus brevis* and Baker's yeast (103.00 ± 2.00cm^3). In all cases significant difference ($p \leq 0.01$) exists in mean dough leavening between the combinations of LAB isolates and Baker's yeast and Baker's yeast alone (Appendix 4.3).

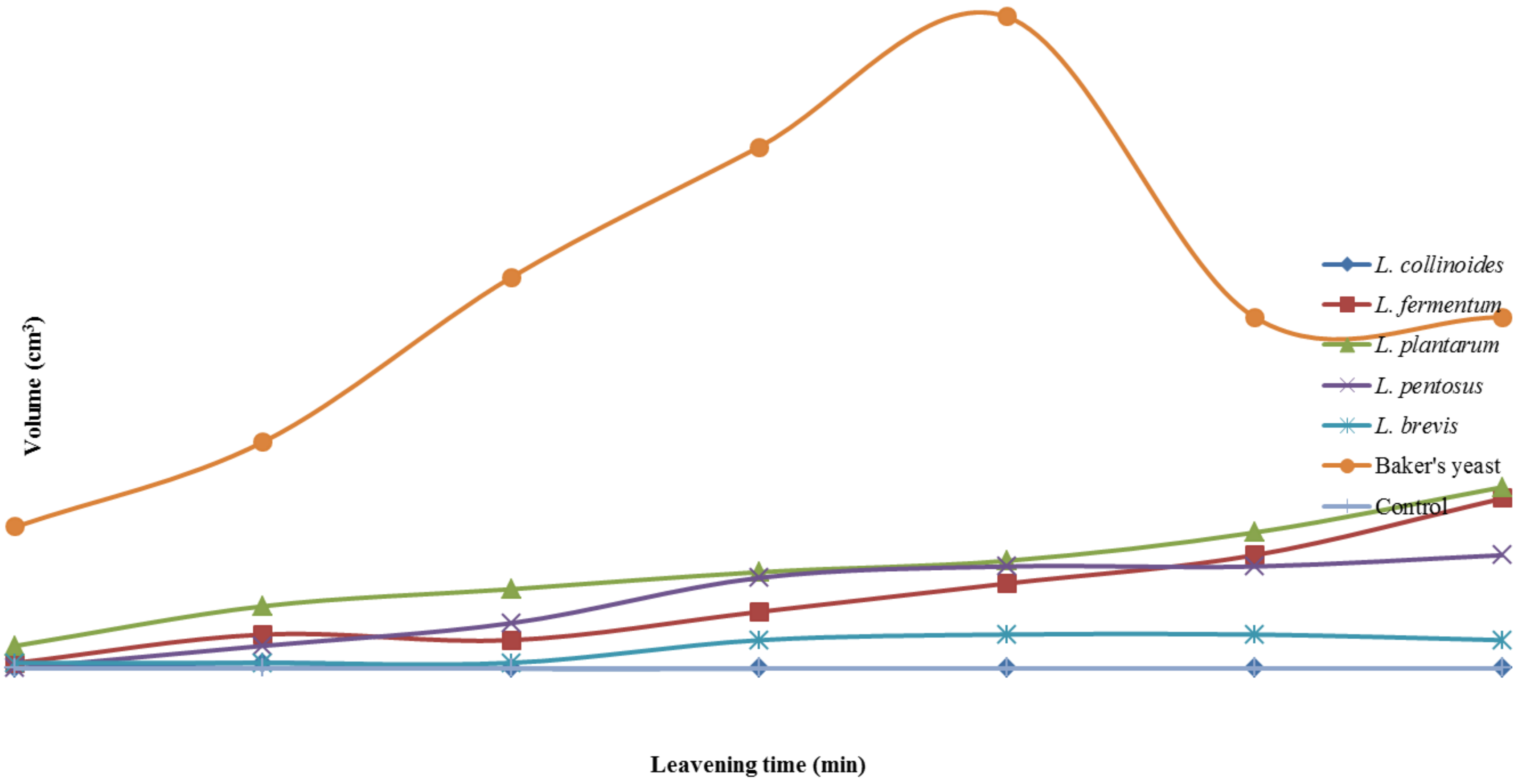

Figure 4.1: Leavening capacity of the lactic acid bacteria isolates in comparison with baker's yeast

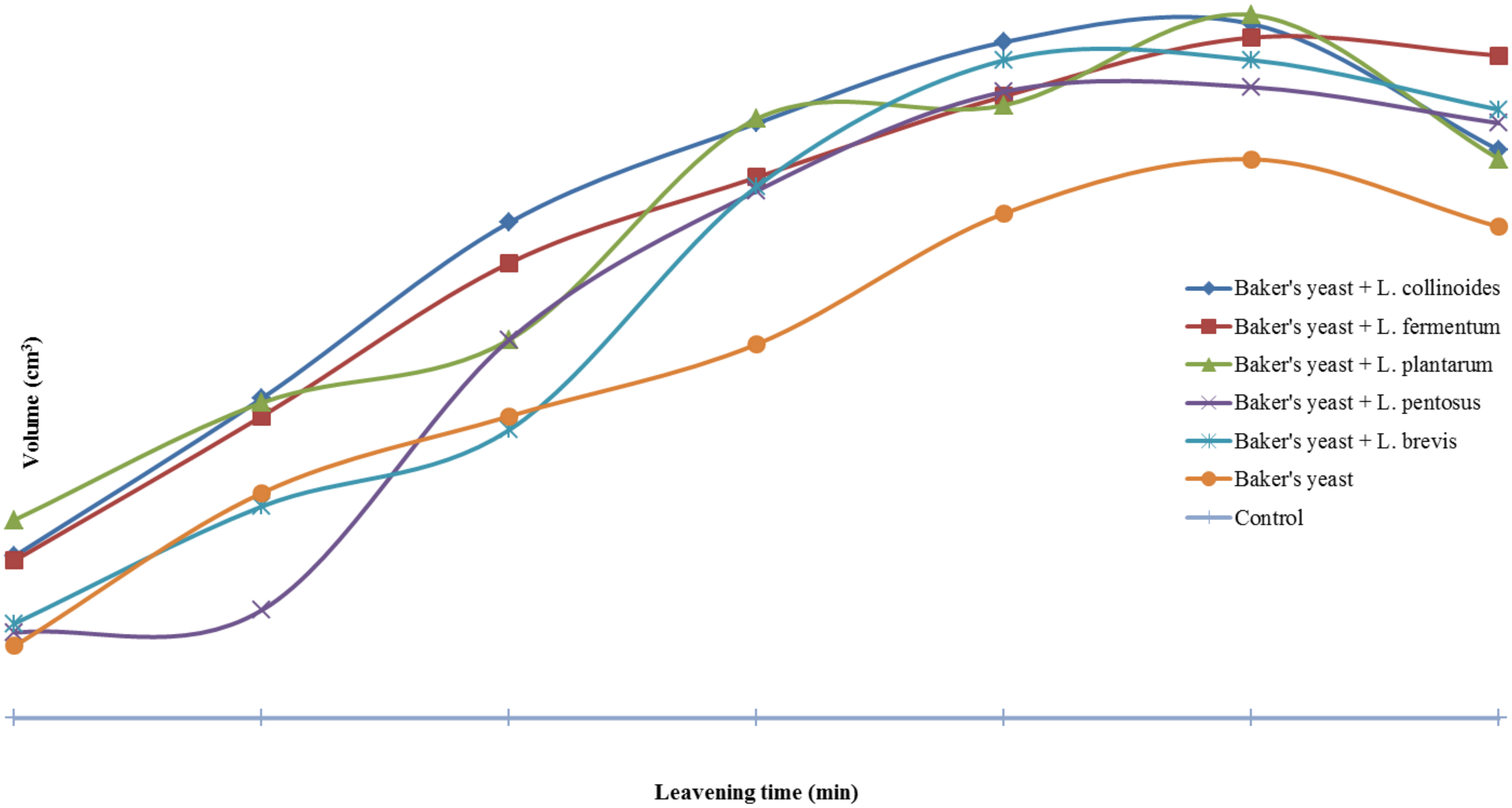

Figure 4.2: Leavening capacity of the lactic acid bacteria isolates and baker’s yeast in comparison with baker's yeast alone

4.10 Comparison of Dough Leavening Ability of Baker's Yeast and Different Percentages of Sourdough

The dough leavening ability of different percentages of the sourdough samples in comparison with Baker's yeast and control dough is as shown in Figure 4.3. Baker's yeast gave the highest level of dough leavening at 180 minutes of leavening, followed by 25% and 30% sourdough, 20% sourdough, 10% and 15% sourdough and the least being 5% sourdough and control dough. There was a slight improvement in the level of dough leavening at 210 minutes of leavening by 15% sourdough, 25% sourdough and 30% sourdough.

There is a significant difference ($p \leq 0.001$) in mean dough leavening between the baker's yeast ($84.00 \pm 1.00cm^3$) and the sourdough samples at 180 minutes of dough leavening. There is also a significant difference ($p \leq 0.001$) between the mean dough leavening of 30% sourdough ($36.50 \pm 1.50cm^3$) and 25% sourdough (36.00 ± 2.00). The mean dough leavening by the 25% and 30% sourdough samples were also significantly different ($p \leq 0.001$) from the 5% sourdough ($31.00 \pm 1.00cm^3$), 10% sourdough ($32.00 \pm 0.00cm^3$), 15% sourdough ($32.00 \pm 2.00cm^3$), 20% sourdough and control dough. There was no significant difference ($p \geq 0.001$) in the mean dough leavening between 10% sourdough, 15% sourdough and 20% sourdough, significant difference ($p \leq 0.001$) however, exist between these three dough samples and the 5% sourdough. The highest level of dough leavening by the sourdough samples was obtained from 25% sourdough ($37.50 \pm 1.50cm^3$) and 30% sourdough ($37.50 \pm 1.50cm^3$) at 210 minutes of leavening and there was no significant difference ($p \geq 0.001$) in mean dough leavening between the two (Appendix IV).

4.11 Comparison of Dough Leavening Ability of Baker’s Yeast and Combinations of Sourdough/Baker’s Yeast

The dough leavening by the combinations of different percentages of sourdough and Baker’s yeast, Baker’s yeast alone and control dough showed that the combination of 30% sourdough and Baker’s yeast gave the highest dough leavening followed by the combination of 25% sourdough/Baker’s yeast and Baker’s yeast alone, combination of 15% sourdough/Baker’s yeast and combination of 20% sourdough/Baker’s yeast, combination of 10% sourdough and Baker’s yeast and the least being control dough. In all cases the highest dough leavening were obtained at 180 minutes (Figure 4.4).

The mean dough leavening by the combination of 30% sourdough and Baker’s yeast at 180 minutes of leavening which was 104.50 ± 0.50cm^3 was significantly different ($p \leq 0.001$) from that of Baker’s yeast (101.50 ± 3.00cm^3) alone and all the other combinations (Appendix V).

4.12 Bread and Doughnuts Produced using the Selected LAB Isolates and Baker’s Yeast

Appendices XXIII to XXVI shows the bread and doughnuts produced using the selected LAB isolates and baker’s yeast. All the baked products look similar interms of appearance.

4.13 pH Values during Dough Fermentation

The pH values obtained during the dough fermentations are as shown on Table 4.8. The values ranged from 3.33 ± 0.21 (flour C dough at 40^{0}C and at 120 hours of fermentation) to 6.73 ± 0.05 (flour C dough at 0 hours of fermentation). A pH of range of 4.60 ± 0.17 to 6.37 ± 0.26 and 3.70 ± 0.13 to 6.37 ± 0.26 was obtained for flour A fermented at room temperature and at 40^{0}C respectively, a pH of range of 4.00 ± 0.06 to 6.63 ± 0.05 and 3.80 ±

0.12 to 6.63 ± 0.05 was obtained for flour B fermented at room temperature and at 40^0C respectively. Generally, there was a progressive decrease in pH with increase in length of fermentation. In all cases, there was no significant difference in the mean pH values obtained at 0 hour and at 24 hours fermentation ($P \geq 0.05$), but there was significant difference between mean pH values at 24 hours and at 48 hours of fermentation ($P \leq 0.05$) with the exception of flour B fermented at room temperature where the difference in pH values between the two doughs was not significant ($P \geq 0.05$). There was also significant difference in mean pH values at 72 hours and 96 hours of fermentation ($P \leq 0.05$) with the exception of flour C dough fermented at 40^0C. In all cases, there was also significant difference in mean pH at 120 hours of fermentation and at 0 hour to 96 hours of fermentation ($P \leq 0.05$).

4.14 Titratable Acidity Values during Dough Fermentation

With respect to titratable acidity (TTA), the TTA range from 0.168 ± 0.00 to 0.490 ± 0.03, 0.168 ± 0.00 to 0.536 ± 0.02 and 0.17 ± 0.001 to 0.68 ± 0.01 for flour A dough, flour B dough and flour C dough respectively. An inverse relationship between pH and TTA was observed. There was a significant difference in mean TTA between 0 hour and 24 hours fermented doughs ($P \leq 0.05$) with the exception of flour A and flour B doughs fermented at room temperature (33^0C). There was no significant difference in mean TTA between 24 hours and 48 hours fermented doughs ($P \geq 0.05$) with the exception of flour A and flour B doughs fermented at room temperature where there was significant difference in TTA between the 24 hours and the 48 hours fermented doughs ($P \leq 0.05$). There was also a significant difference in TTA between doughs fermented at 48 hours and 72 hours ($P \leq 0.05$) with the exception of flour A and flour B doughs whose TTA values at 48 hours and at 72 hours were not significantly different ($P \geq 0.05$). In all cases, there is a significant difference in TTA of doughs fermented at 72 hours, 96 hours and 120 hours ($P \leq 0.05$) (Table 4.9).

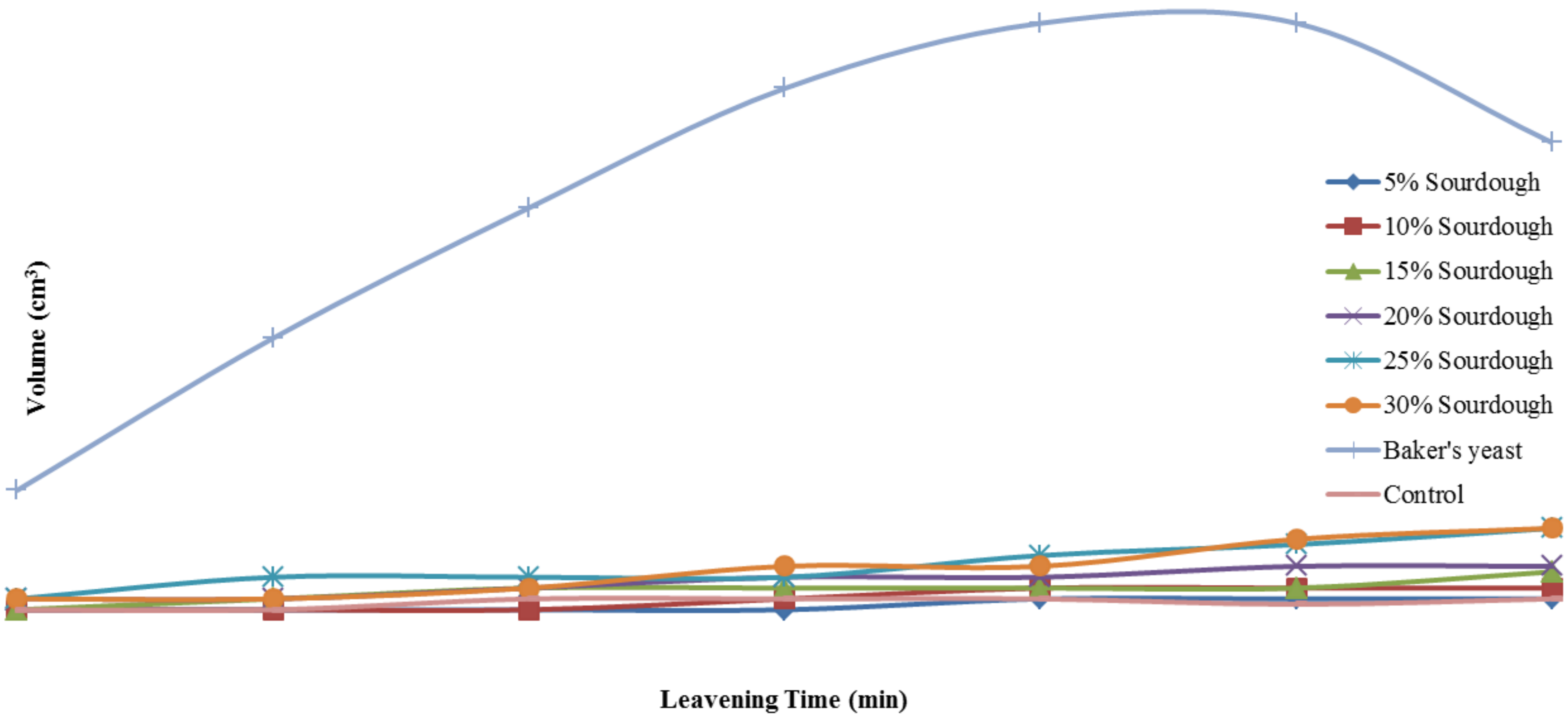

Figure 4.3: Leavening capacity of sourdough in comparison with baker's yeast alone

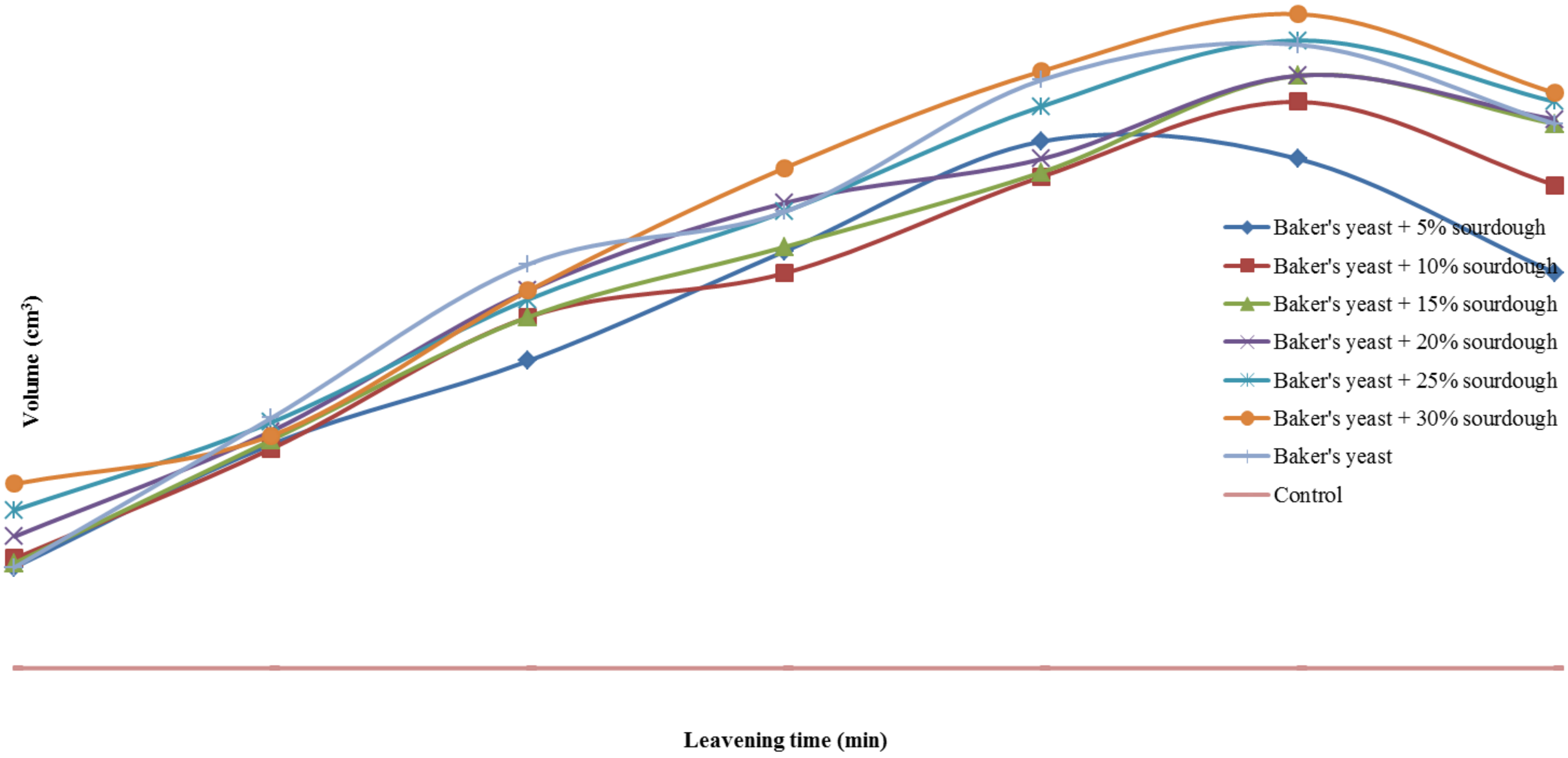

Figure 4.4: Leavening capacity of sourdough and baker’s yeast in comparison with baker's yeast alone

Table 4.8: pH values during spontaneous fermentation of the dough

Length of fermentation (hrs)	Flour A		Flour B		Flour C	
	Room temperature	40^{0}C	Room temperature	40^{0}C	Room temperature	40^{0}C
0	6.37 ± 0.26^{a}	6.37 ± 0.26^{a}	6.63 ± 0.05^{a}	6.63 ± 0.05^{a}	6.73 ± 0.05^{a}	6.73 ± 0.05^{a}
24	6.23 ± 0.26^{a}	6.27 ± 0.17^{a}	6.43 ± 0.05^{a}	6.50 ± 0.08^{a}	6.23 ± 0.13^{a}	6.07 ± 0.25^{a}
48	5.83 ± 0.12^{b}	5.67 ± 0.29^{b}	6.10 ± 0.08^{a}	5.87 ± 0.21^{b}	5.70 ± 0.25^{b}	5.30 ± 0.33^{c}
72	5.43 ± 0.21^{b}	5.20 ± 0.14^{b}	5.43 ± 0.34^{c}	5.23 ± 0.34^{c}	5.23 ± 0.50^{b}	4.83 ± 0.17^{d}
96	5.07 ± 0.25^{c}	4.83 ± 0.25^{c}	4.83 ± 0.13^{d}	4.60 ± 0.16^{d}	4.27 ± 0.31^{c}	4.20 ± 0.29^{d}
120	4.60 ±0.17	3.70 ±0.13	4.00 ±0.06	3.80 ±0.12	3.53 ±0.17	3.33 ±0.21
P-Value	<0.030	<0.043	<0.010	<0.045	<0.030	<0.013

All values are mean of triplicate readings. Mean values with different superscripts in the same column are significantly different ($P \leq 0.05$).

Table 4.9: Titratable acidity values during spontaneous fermentation of the dough

Length of fermentation (hrs)	**Flour A**		**Flour B**		**Flour C**	
	Room temperature	**40^{0}C**	**Room temperature**	**40^{0}C**	**Room temperature**	**40^{0}C**
0	0.168±0.00^a	0.168±0.00^a	0.168±0.00^a	0.168±0.00^a	0.169±0.00^a	0.169±0.00^a
24	0.187±0.00^a	0.210±0.01^b	0.186±0.01^a	0.205±0.01^b	0.227±0.01^b	0.243±0.02^b
48	0.213±0.01^b	0.222±0.01^b	0.220±0.00^b	0.243±0.01^b	0.264±0.03^b	0.260±0.02^b
72	0.276±0.03^b	0.309±0.01^c	0.270±0.01^b	0.315±0.00^c	0.312±0.02^c	0.342±0.02^c
96	0.373±0.02^c	0.407±0.01^d	0.383±0.02^c	0.415±0.00^d	0.404±0.01^d	0.410±0.04^d
120	0.476±0.02^d	0.490±0.03^e	0.469±0.02^d	0.536±0.02^e	0.607±0.02^e	0.676±0.01^e
P-Value	<0.033	<0.043	<0.013	<0.040	<0.033	<0.013

All values are mean of triplicate readings. Mean values with different superscripts in the same column are significantly different ($P \leq 0.05$).

4.15: pH and TTA Analysis of the Selected Sourdough before Leavening Test and Baking

The result obtained for the physico-chemical analysis of the selected sourdough prior to leavening test and baking showed that the sourdough had a mean pH and TTA values of 3.97 ± 0.21 and 0.719 ± 0.06 respectively (Table 4.10).

4.16 pH of Bread and Doughnuts

The pH of the bread samples range between 4.67 ± 0.12 to 6.67 ± 0.09. The lowest pH value is that of the 15% sourdough bread (4.67 ± 0.12). There was no significant difference ($p \geq 0.05$) between the pH of the 15% sourdough bread and 10% sourdough bread (5.00 ± 0.33). There was also no significant difference ($p \geq 0.05$) between the pH of 10% sourdough bread and 5% sourdough bread (5.87 ± 0.21). However, the pH of the 15% sourdough bread significantly differed ($p \leq 0.05$) from that of the 5% sourdough bread, *L. brevis* bread (6.03 ± 0.58), *L. plantarum* bread (5.37 ± 0.00), Baker's yeast bread (6.50 ± 0.30) and commercial bread (6.67 ± 0.09). There was no significant difference ($p \geq 0.05$) between the pH of Baker's yeast bread (6.50 ± 0.30) and commercial bread (6.67 ± 0.09) (Table 4.11).

Table 4.12 shows the pH of the doughnuts samples which ranged between 4.93 ± 0.33 to 6.60 ± 0.16. The lowest pH value was that of the 15% sourdough doughnut (4.93 ± 0.33). There was no significant difference ($p \geq 0.05$) between the pH of the 15% sourdough doughnut, 10% sourdough doughnut (5.00 ± 0.08) and 5% sourdough doughnut (5.23 ± 0.26). There was also no significant difference ($p \geq 0.05$) between the pH of *L. brevis* doughnut (6.27 ± 0.29), *L. plantarum* doughnut (5.73 ± 0.25), Baker's yeast doughnut (6.43 ± 0.25) and commercial doughnut (6.60 ± 0.16).

Table 4.10: pH and TTA of the selected sourdough

Sample	pH	TTA
1	4.1	0.703
2	4.1	0.631
3	3.8	0.758
4	3.6	0.649
5	4.0	0.776
6	4.2	0.794
Mean	3.97 ± 0.21	0.719 ± 0.06

Table 4.11: pH of bread and doughnuts

Type	Bread	Doughnuts
5% sourdough	5.87 ± 0.21^{ac}	5.23 ± 0.26^{a}
10% sourdough	5.00 ± 0.33^{ab}	5.00 ± 0.08^{a}
15% sourdough	4.67 ± 0.12^{b}	4.93 ± 0.33^{a}
Lactobacillus brevis	6.03 ± 0.58^{cd}	6.27 ± 0.29^{b}
Lactobacillus plantarum	5.37 ± 0.00^{ac}	5.73 ± 0.25^{b}
Baker's yeast	6.50 ± 0.30^{d}	6.43 ± 0.43^{b}
Commercial	6.67 ± 0.09^{d}	6.60 ± 0.16^{b}
ANOVA	0.501	7.501
P-value	0.003	<0.001

Significant difference exists at $p \leq 0.05$. Mean values with different superscripts in the same column are significantly different. Means were separated using Duncan Multiple Range Test. All values are mean of triplicate readings.

4.17 Titratable Acidity of the Bread and Doughnuts

The highest TTA was that of 15% sourdough bread (0.685 ± 0.02) which significantly differed ($p \leq 0.05$) from that of all the other bread samples. There was no significant difference ($p \geq 0.05$) in the TTA of 5% sourdough bread (0.568 ± 0.02), 10% sourdough bread (0.585 ± 0.02), *L. brevis* bread (0.544 ± 0.01), *L. plantarum* bread (0.553 ± 0.03), and Baker's yeast bread (0.223 ± 0.03). The least TTA was that of commercial bread (0.227 ± 0.03) which significantly differed ($p \leq 0.05$) from the other bread samples.

The TTA of the 15% sourdough doughnut (0.697 ± 0.02) was the highest but does not differ significantly ($p \geq 0.05$) from that of the 10% sourdough doughnut (0.628 ± 0.03). There was no significant difference ($p \geq 0.05$) in the TTA of 10% sourdough doughnut (0.628 ± 0.03) and *L. plantarum* doughnut (0.607 ± 0.03). There was also no significant difference ($p \geq 0.05$) in the TTA of 5% sourdough doughnut (0.544 ± 0.02) and *L. brevis* doughnut (0.542 ± 0.02). The least TTA are those of baker's yeast (0.269 ± 0.08) and commercial doughnut (0.260 ± 0.03) which did not differ significantly ($p \geq 0.05$) from each other (Table 4.12).

4.18 Proximate Analysis of Flour and Selected Sourdough

The results of the proximate analysis of the three brands of flour and selected sourdough showed that there was no significant difference ($p \geq 0.001$) in the moisture and fat contents of the three flour types, the moisture and fat contents however differed from that of the sourdough ($p \leq 0.001$). The highest protein content was that of the sourdough (12.23 ± 0.13%) which differs significantly ($p \leq 0.001$) from that of the other flour types. There was no significant difference ($p \geq 0.001$) in the carbohydrate content of flour C (77.37 ± 0.78%) and flour B (76.22 ± 0.43%). The least carbohydrate content was that of sourdough (21.42 ± 0.38%) and it differs significantly ($p \leq 0.001$) from that of the three brands of flour (Table 4.13).

Table 4.12: Titratable Acidity of bread and doughnuts

Type	Bread	Doughnuts
5% sourdough	0.568 ± 0.02^{a}	0.544 ± 0.02^{a}
10% sourdough	0.585 ± 0.02^{a}	0.628 ± 0.03^{bc}
15% sourdough	0.685 ± 0.02^{b}	0.697 ± 0.02^{c}
Lactobacillus brevis	0.544 ± 0.01^{a}	0.547 ± 0.02^{a}
Lactobacillus plantarum	0.553 ± 0.03^{a}	0.607 ± 0.03^{b}
Baker's yeast	0.223 ± 0.03^{c}	0.209 ± 0.08^{d}
Commercial doughnut	0.227 ± 0.03^{c}	0.260 ± 0.03^{d}
ANOVA	0.601	0.601
P-value	0.260	<0.001

Significant difference exists at $p \leq 0.001$. Mean values with different superscripts in the same column are significantly different. Means were separated using Duncan Multiple Range Test. All values are mean of triplicate readings.

Table 4.13: Proximate analysis of flour and selected sourdough

Sample	**% Moisture**	**% Ash**	**% Fat**	**% Protein**	**% Carbohydrate**
SPF	7.18 ± 0.03^{b}	0.05 ± 0.00^{a}	10.39 ± 0.19^{b}	5.01 ± 0.16^{c}	77.37 ± 0.78^{a}
DGF	7.31 ± 0.19^{b}	0.73 ± 0.02^{b}	10.39 ± 0.21^{b}	5.36 ± 0.19^{c}	76.22 ± 0.43^{ab}
GPF	7.34 ± 0.18^{b}	0.35 ± 0.03^{c}	10.39 ± 0.22^{b}	6.84 ± 0.13^{b}	75.08 ± 0.34^{b}
SD	44.54 ± 0.20^{a}	0.73 ± 0.02^{b}	21.09 ± 0.10^{a}	12.23 ± 0.13^{a}	21.42 ± 0.38^{c}
ANOVA	12592.149	331.424	799.344	475.164	5347.726
p-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001*

All values are mean of triplicate readings. Significant difference exists at $p \leq 0.01$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

SPF = Supreme flour, DGF = Dangote flour, GPF = Golden penny flour, SD = Sourdough

4.19 Proximate Analysis of Bread

The result of the proximate analysis of the bread samples showed that the 5% sourdough bread and the *L. brevis* bread have the highest mean moisture content of 34.89 ± 0.69% and 33.67 ± 0.35% respectively. There was no significant difference ($p \geq 0.001$) in the mean moisture content of the two but the mean moisture content of the two differ significantly ($p \leq 0.001$) from the mean moisture content of the other bread samples. The least mean moisture content were those of Baker's yeast alone bread (24.12 ± 0.47%) and commercial bread (25.05 ± 0.46%), no significant difference exist between the two ($p \geq 0.001$). The highest ash content were obtained from 10% sourdough bread (0.67 ± 0.03%), *L. plantarum* bread (0.67 ± 0.04%) and 15% sourdough bread (0.64 ± 0.03%) all of which differ significantly ($p \leq 0.001$) from the rest but do not significantly differed ($p \geq 0.001$) from each other. The least ash content is those of 5% sourdough bread and commercial bread with ash content of 0.52 ± 0.02% and 0.42 ± 0.02% respectively. The highest fat content is that of 15% sourdough bread (10.14 ± 0.24%) followed by *L. plantarum* bread (9.90 ± 0.06%), there was no significant difference ($p \geq 0.001$) in the mean fat content of the two but the two significantly differ ($p \leq 0.001$) from the other bread samples. There was no significant difference ($p \geq 0.001$) in the mean protein content of Baker's yeast alone (6.34 ± 0.13%), commercial bread (6.23 ± 0.19%), 15% sourdough bread (6.18 ± 0.56%), *L. plantarum* bread (6.15 ± 0.12%) and 10% sourdough bread (5.99 ± 0.10%). The least protein content were those of 5% sourdough bread (4.47 ± 0.26%) and *L. brevis* bread (5.10 ± 0.18%), the protein content of these two did not significantly differ ($p \geq 0.001$). The highest carbohydrate content was that of Baker's yeast alone bread (65.76 ± 0.28) which did not differ significantly ($p \geq 0.001$) from that of commercial bread (60.35 ± 0.58) (Table 4.14).

4.20 Proximate Analysis of Doughnut

Table 4.15 shows the result of the proximate analysis of the doughnuts. *Lactobacillus brevis* doughnut had the highest moisture content of 31.19 ± 0.35% which did not differ significantly ($p \geq 0.001$) from that of commercial doughnut (30.06 ± 0.27%). There was no significant difference ($p \geq 0.001$) in the mean ash content of 15% sourdough doughnut (0.55 ± 0.03%), 5% sourdough doughnut (0.41 ± 0.03%), *L. plantarum* doughnut (0.40 ± 0.01%) and 10% sourdough doughnut (0.39 ± 0.01%). Commercial doughnut had the highest mean ash content of 1.13 ± 0.12% which differed significantly ($p \leq 0.001$) from that of Baker's yeast alone doughnut 0.86 ± 0.03%. The least mean ash content was that of *L. brevis* doughnut (0.20 ± 0.01%). The 10% sourdough doughnut had the highest fat content of 25.66 ± 0.18%, followed by 5% sourdough doughnut (24.99 ± 0.24%), *L. plantarum* doughnut (22.45 ± 0.28%), *L. brevis* doughnut (20.07 ± 0.13%), commercial doughnut (18.57 ± 0.39%), Baker's yeast alone doughnut (18.15 ± 0.06%) and 15% sourdough doughnut (15.47 ± 6.63%). The 10% sourdough doughnut also had the highest protein content of 11.08 ± 0.12%, followed by *L. plantarum* doughnut (8.10 ± 0.16%), 15% sourdough doughnut (7.92 ± 0.06%), *L. brevis* doughnut (6.59 ± 0.06%), commercial doughnut (6.34 ± 0.16%), 5% sourdough doughnut (5.61 ± 1.06%) and Baker's yeast alone doughnut (5.21 ± 0.11%). There was significant difference ($p \leq 0.001$) in the protein content of all the doughnuts except for commercial doughnut (6.34 ± 0.16%) and Baker's yeast alone doughnut (5.21 ± 0.11%) which do not differ significantly ($p \geq 0.001$) from each other in protein content. There was significant difference ($p \leq 0.001$) in the carbohydrate content of Baker's yeast alone doughnut (50.55 ± 0.26%), commercial doughnut (43.90 ± 0.37%), *L. brevis* doughnut (41.94 ± 0.46%), 10% sourdough doughnut (41.46 ± 0.20) and 5% sourdough doughnut (40.36 ± 0.28%).

Table 4.14: Proximate analysis of bread samples

Bread	**% Moisture**	**% Ash**	**% Fat**	**% Protein**	**% Carbohydrate**
5% sourdough bread	34.89 ± 0.69^{a}	0.52 ± 0.02^{b}	7.19 ± 0.26^{d}	4.47 ± 0.26^{b}	52.85 ± 0.78^{c}
10% sourdough bread	27.89 ± 0.46^{c}	0.67 ± 0.03^{a}	8.94 ± 0.10^{b}	5.99 ± 0.10^{a}	56.51 ±0.56^{c}
15% sourdough bread	30.23 ± 0.41^{b}	0.64 ± 0.03^{a}	10.14 ± 0.24^{a}	6.18 ± 0.56^{a}	52.81 ± 0.60^{c}
Lactobacillus brevis bread	30.73 ± 0.32^{b}	0.67 ±0.04^{a}	9.90 ± 0.06^{a}	6.15 ± 0.12^{a}	52.94 ± 0.51^{c}
Lactobacillus plantarum bread	33.67 ± 0.35^{a}	0.50 ± 0.02^{bc}	4.68 ± 0.42^{e}	5.10 ± 0.18^{b}	56.05 ± 0.35^{c}
Baker's yeast bread	24.12 ± 0.47^{d}	0.50 ± 0.02^{bc}	3.29 ± 0.15^{f}	6.34 ± 0.13^{a}	65.76 ± 0.28^{b}
Commercial bread	25.05 ± 0.46^{d}	0.42 ± 0.02^{c}	8.01 ± 0.04^{c}	6.23 ± 0.19^{a}	60.35 ± 0.58
ANOVA	77.219	14.113	139.658	7.346	78.746
p-value	< 0.001**	< 0.001**	< 0.001**	0.001**	< 0.001*

All values are mean of triplicate readings. Significant difference exists at $p \leq 0.001$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

Table 4.15: Proximate analysis of doughnut samples

Doughnut	% Moisture	% Ash	% Fat	% Protein	% Carbohydrate
5% sourdough doughnuts	28.29 ± 0.69^{b}	0.41 ± 0.03^{c}	24.99 ± 0.24	5.61 ± 1.06^{cd}	40.36 ± 0.28^{e}
10% sourdough doughnuts	21.41 ± 0.34^{d}	0.39 ± 0.01^{c}	25.66 ± 0.18	11.08 ± 0.12^{a}	41.46 ± 0.20^{d}
15% sourdough doughnuts	26.64 ± 0.57^{c}	0.55 ± 0.03^{c}	15.47 ± 6.63	7.92 ± 0.06^{b}	42.76 ± 0.49^{c}
Lactobacillus brevis doughnuts	31.19 ± 0.35^{a}	0.20 ±0.01^{d}	20.07 ± 0.13	6.59 ± 0.06^{c}	41.94 ± 0.46^{cd}
Lactobacillus plantarum doughnuts	26.25 ± 0.57^{c}	0.40 ± 0.01^{c}	22.45 ± 0.28	8.10 ± 0.16^{b}	42.80 ± 0.16^{c}
Baker's yeast doughnuts	25.23 ± 0.22^{c}	0.86 ± 0.03^{b}	18.15 ± 0.06	5.21 ± 0.11^{d}	50.55 ± 0.26^{a}
Commercial doughnuts	30.06 ± 0.27^{a}	1.13 ± 0.12^{a}	18.57 ± 0.39	6.34 ± 0.16^{d}	43.90 ± 0.37^{b}
ANOVA	50.380	43.362	2.235	23.124	98.201
p-value	< 0.001**	< 0.001**	0.101	< 0.001**	< 0.001**

All values are mean of triplicate readings. Significant difference exists at $p \leq 0.01$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

4.21 Sensory Evaluation of Bread

The results of the sensory evaluation of the bread samples showed that in all cases there was no significant difference ($p \geq 0.05$) between all the bread samples. However, on a scale of 9, the 5% sourdough bread with a score of 7.77 ± 0.36 was the most preferred in terms of appearance, followed by the *Lactobacillus plantarum* (*L. plantarum*) bread with a score of 7.62 ± 0.37. The 10% and 15% sourdough bread with scores of 7.54 ± 0.51 and 7.54 ± 0.35 respectively and the *Lactobacillus brevis* (*L. brevis*) bread with a score of 7.15 ± 0.25 were more preferred than the commercial bread with a score of 6.92 ± 0.27. In terms of aroma, the 5% sourdough bread (with a score of 7.62 ± 0.35), the 15% sourdough bread (7.00 ± 0.52), *L. brevis* bread (7.23 ± 0.28), the *L. plantarum* bread (7.54 ± 0.27) and the baker’s yeast alone bread (7.31 ± 0.33) were all more preferred than the commercial bread (6.38 ± 0.31). However, only the 5% sourdough bread and the *L. plantarum* bread are more preferred than the Baker’s yeast alone bread.

With respect to texture, the 5% sourdough bread (with a score of 7.15 ± 0.36), the 10% sourdough bread (7.08 ± 0.55), *L. brevis* bread (6.69 ± 0.55) and the *L. plantarum* bread (7.23 ± 0.23) were more preferred than the Baker’s yeast alone bread (6.77 ± 0.41) and the commercial bread (6.31 ± 0.41).

In terms of taste, the 5% sourdough bread (with a score of 7.38 ± 0.27), the 10% sourdough bread (7.46 ± 0.29), the *L. plantarum* bread (7.23 ± 0.28) were more preferred than the baker’s yeast alone bread (7.00 ± 0.28). However, the commercial bread (7.69 ± 0.31).was more preferred than all the other bread samples.

The scores for the overall acceptability showed that the 5% sourdough bread was the most preferred with score of 7.48 ± 0.14, followed by the *L. plantarum* bread with a score of 7.41 ± 0.10 and the least being the commercial bread with a score of 6.83 ± 0.32 (Table 4.16).

4.22 Sensory Evaluation of Doughnuts

The result of the sensory evaluation of the doughnuts is shown in Table 4.17. There was no significant difference ($p \geq 0.05$) between all the doughnuts in terms of appearance, aroma, texture, taste and overall acceptability. However, on a scale of 9, the *L. brevis* doughnut with a score of 8.23 ± 0.12, was the most preferred in terms of appearance. In terms of aroma, the 5% sourdough doughnut with a score of 7.85 ± 0.19, the 10% sourdough doughnut (8.23 ± 0.26), the 15% sourdough doughnut (7.23 ± 0.59), *L. brevis* doughnut (7.46 ± 0.24) and the *L. plantarum* doughnut (7.54 ± 0.18) were all more preferred than the Baker's yeast alone doughnut (7.23 ± 0.17) and the commercial doughnut (6.92 ± 0.21). The most preferred was the 10% sourdough doughnut (8.23 ± 0.26).

With respect to texture, the 5% sourdough doughnut with a score of 7.77 ± 0.20, the 10% sourdough doughnut (8.08 ± 0.21), the 15% sourdough doughnut (7.46 ± 0.42), *L. brevis* doughnut (7.85 ± 0.19) and the *L. plantarum* doughnut (7.08 ± 0.38) were all more preferred than the Baker's yeast alone doughnut (7.23 ± 0.23) and the commercial doughnut (7.31 ± 0.18). The most preferred was the 10% sourdough doughnut (8.08 ± 0.21). In terms of taste, the commercial doughnut with a score of 8.00 ± 0.23 was the most preferred.

The scores for the overall acceptability showed that the 10% sourdough doughnut was the most preferred with score of 7.96 ± 0.15, followed by the *L. brevis* doughnut with a score of 7.77 ± 0.17, the Baker's yeast alone doughnut with score of 7.48 ± 0.18 and the least preferred was *L. plantarum* doughnut with a score of 7.41 ± 0.13.

Table 4.16: Sensory evaluation of bread samples

Bread type	Appearance	Aroma	Texture	Taste	Overall acceptability
5% sourdough bread	7.77 ± 0.36	7.62 ± 0.35	7.15 ± 0.36	7.38 ± 0.27	7.48 ± 0.14
10% sourdough bread	7.54 ± 0.51	6.31 ± 0.56	7.08 ± 0.55	7.46 ± 0.29	7.10 ± 0.28
15% sourdough bread	7.54 ± 0.35	7.00 ± 0.52	6.69 ± 0.55	6.46 ± 0.45	6.92 ± 0.23
Lactobacillus brevis bread	7.15 ± 0.25	7.23 ± 0.28	6.92 ± 0.35	6.85 ± 0.22	7.04 ± 0.09
Lactobacillus plantarum bread	7.62 ± 0.37	7.54 ± 0.27	7.23 ± 0.23	7.23 ± 0.28	7.41 ± 0.10
Baker's yeast bread	7.31 ± 0.33	7.31 ± 0.33	6.77 ± 0.33	7.00 ± 0.28	7.10 ± 0.13
Commercial bread	6.92 ± 0.27	6.38 ± 0.31	6.31 ± 0.41	7.69 ± 0.31	6.83 ± 0.32
ANOVA	0.676	1.821	0.577	1.853	1.387
P-value	0.669	0.105	0.748	0.099	0.266

Significant difference exists at $p \leq 0.01$. All values are mean of triplicate readings.

Table 4.17: Sensory evaluation of doughnuts samples

Bread type	Appearance	Aroma	Texture	Taste	Overall acceptability
5% sourdough doughnut	7.85 ± 0.25	7.85 ± 0.19	7.77 ± 0.20	7.23 ± 0.20	7.68 ± 0.15
10% sourdough doughnut	8.00 ± 0.20	8.23 ± 0.26	8.08 ± 0.21	7.54 ± 0.22	7.96 ± 0.15
15% sourdough doughnut	8.00 ± 0.23	7.23 ± 0.59	7.46 ± 0.42	7.23 ± 0.28	7.48 ± 0.18
Lactobacillus brevis doughnut	8.23 ± 0.12	7.46 ± 0.24	7.85 ± 0.19	7.54 ± 0.22	7.77 ± 0.17
Lactobacillus plantarum doughnut	7.69 ± 0.29	7.54 ± 0.18	7.08 ± 0.38	7.31 ± 0.26	7.41 ± 0.13
Baker's yeast doughnut	8.00 ± 0.20	7.23 ± 0.17	7.23 ± 0.23	7.46 ± 0.27	7.48 ± 0.18
Commercial doughnut	7.92 ± 0.18	6.92 ± 0.21	7.31 ± 0.18	8.00 ± 0.23	7.54 ± 0.26
ANOVA	0.601	2.151	1.783	1.244	1.218
P-value	0.729	0.056	0.112	0.292	0.336

Significant difference exists at $p \leq 0.01$. All values are mean of triplicate readings.

4.23 Minimum Mould – Free Shelflife (MMFSL) of Bread

The results obtained for the Minimum Mould – Free Shelflife (MMFSL) of the bread samples showed that there was a progressive increase in fungal count for all the bread samples with increase in number of days. However, the increase in fungal counts for the lactic acid bacteria based bread samples (5% sourdough bread, 10% sourdough bread, 15% sourdough bread, *L. brevis* bread and *L. plantarum* bread) is lower than those of the Baker's yeast alone bread and the commercial bread. The difference in the fungal counts between the two groups was significant ($p \leq 0.001$). In all cases 15% sourdough bread samples had the lowest fungal counts, followed by the 10% sourdough bread samples. The fifteen percent (15%) sourdough bread had the highest MMFSL of 7.00 ± 0.58 which differed significantly ($p \leq 0.001$) from that of the other bread samples. The lowest MMFSL were those of Baker's yeast bread (3.00 ± 0.00) and commercial bread (3.67 ± 0.33) which did not differ significantly ($p \geq 0.001$) from each other. There was also no significant difference ($p \geq 0.001$) in the MMFSL of commercial bread, 5% sourdough bread, *L. plantarum* bread and *L. brevis* bread (Table 4.18).

4.24 Minimum Mould – Free Shelflife (MMFSL) of Doughnuts

The highest MMFSL was that of 15% sourdough doughnut (11.67 ± 1.20), followed by that of 10% sourdough doughnut (7.67 ± 0.33), 5% sourdough doughnut and *L. plantarum* doughnut *both* with MMFSL of 7.33 ± 0.33. *Lactobacillus brevis* doughnut had MMFSL of (7.00 ± 0.57). Commercial doughnut and baker's yeast alone doughnut have MMFSL of (6.67 ± 0.33) and (6.00 ± 0.00) respectively. The MMFSL of 15% sourdough doughnut was significantly different ($p \leq 0.001$) from that of the other doughnut samples. There was no significant difference ($p \geq 0.001$) in the mean MMFSL of 5% sourdough doughnut, 10% sourdough doughnut, *Lactobacillus brevis* doughnut, *L. plantarum* doughnut, Baker's yeast alone doughnut and commercial doughnut (Table 4.19).

Table 4.18: Minimum Mould – Free Shelflife (MMFSL) of Bread Samples

				Fungal counts	**Log CFU/g**				
Bread type	**0hr**	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**120hrs**	**144hrs**	**196hrs**	**MMFSL**
5% sourdough bread	2.401± 0.14^{a}	4.519± 0.00^{a}	5.585± 0.21^{a}	6.047± 0.02^{a}	6.297± 0.07^{a}	7.205± 0.06^{a}	7.325± 0.02^{a}	7.360± 0.03^{a}	4.00± 0.00^{bc}
10% sourdough bread	1.634± 0.68^{b}	3.477± 0.00^{b}	5.273± 0.04^{b}	5.778± 0.01^{b}	6.035± 0.05^{b}	6.250± 0.14^{b}	7.079± 0.02^{b}	7.306± 0.05^{a}	5.00± 0.00^{b}
15% sourdough bread	1.634± 0.67^{b}	2.968± 1.80^{d}	4.560± 0.00^{c}	5.266± 0.00^{c}	5.874± 0.08^{c}	6.085± 0.05^{c}	6.286± 0.00^{c}	6.388± 0.06^{c}	7.00± 0.58^{a}
Lactobacillus brevis bread	2.360± 0.08^{a}	4.660± 0.01^{ac}	5.590± 0.02^{a}	5.901± 0.01^{a}	6.140± 0.07^{d}	6.330± 0.06^{d}	7.160± 0.01^{d}	7.370± 0.04^{a}	4.33± 0.33^{bc}
Lactobacillus plantarum bread	1.693± 0.69^{b}	4.492± 0.03^{a}	5.517± 0.03^{a}	5.924± 0.00^{a}	6.214± 0.13^{a}	6.216± 0.13^{b}	7.022± 0.12^{b}	7.306± 0.02^{a}	4.63± 0.33^{bc}
Baker’s yeast bread	2.359± 0.08^{a}	4.992± 0.02^{e}	5.784± 0.01^{d}	6.251± 0.00^{d}	6.330± 0.07^{e}	7.294± 0.01^{a}	7.355± 0.05^{a}	7.491± 0.07^{d}	3.00± 0.00^{d}
Commercial bread	2.460± 0.12^{a}	4.881± 0.02^{ce}	5.664± 0.01^{d}	6.152± 0.01^{d}	6.295± 0.03^{a}	7.195± 0.12^{a}	7.312± 0.02^{a}	7.424± 0.04^{d}	3.67± 0.33^{cd}
ANOVA	0.188	5.718	5.731	10.998	7.906	43.577	41.717	0.417	17.056
P-value	0.975	0.003	0.003	<0.001	<0.001	<0.001	<0.001	0.815	<0.001

Significant difference exists at $p \leq 0.001$. Mean values with different superscripts in the same column are significantly different. Means were separated using Duncan Multiple Range Test. All values are mean of triplicate readings.

Table 4.19: Minimum Mould – Free Shelflife (MMFSL) of doughnuts

				Fungal counts	**Log CFU/g**				
Doughnut type	**0hr**	**24hrs**	**48hrs**	**72hrs**	**96hrs**	**120hrs**	**144hrs**	**196hrs**	**MMFSL**
5% sourdough doughnut	1.534± 0.63^{a}	2.968± 0.13^{a}	4.761± 0.12^{a}	5.237± 0.12^{a}	5.369± 0.07^{a}	5.534± 0.04^{a}	5.845± 0.04^{a}	6.073± 0.05^{a}	7.33± 0.33^{b}
10% sourdough doughnut	1.593± 0.65^{a}	2.985± 0.22^{a}	4.719± 0.17^{a}	5.141± 0.15^{b}	5.252± 0.09^{bc}	5.425± 0.07^{a}	5.804± 0.06^{a}	5.967± 0.04^{a}	7.67± 0.33^{b}
15% sourdough doughnut	1.534± 0.63^{a}	2.867± 0.17^{b}	4.634± 0.05^{b}	4.903± 0.16^{c}	5.198± 0.12^{b}	5.406± 0.08^{b}	5.763± 0.07^{a}	5.852± 0.05^{b}	11.67± 1.20^{a}
Lactobacillus brevis doughnut	1.634± 0.68^{a}	2.968± 0.22^{a}	4.783± 0.13^{a}	5.238± 0.09^{a}	5.337± 0.09^{c}	5.547± 0.03^{c}	5.871± 0.04^{b}	6.345± 0.44^{c}	7.00± 0.57^{bc}
Lactobacillus plantarum doughnut	2.460± 0.74^{b}	4.502± 0.14^{c}	4.820± 0.06^{a}	5.090± 0.10^{a}	5.306± 0.04^{a}	5.526± 0.03^{c}	5.764± 0.03^{a}	6.009± 0.03^{a}	7.33± 0.33^{b}
Baker's yeast doughnut	1.651± 0.08^{a}	4.460± 0.12^{c}	5.066± 0.10^{c}	5.272± 0.11^{d}	5.506± 0.08^{d}	5.766± 0.09^{d}	5.975± 0.06^{c}	6.224± 0.06^{d}	6.00± 0.00^{d}
Commercial doughnut	2.492± 0.16^{b}	4.683± 0.11^{d}	5.277± 0.09^{d}	5.339± 0.12^{d}	5.620± 0.12^{d}	5.796± 0.09^{d}	5.808± 0.49^{a}	6.145± 0.06^{d}	6.67± 0.33^{d}
ANOVA	0.188	5.718	5.731	10.998	7.906	43.577	41.717	0.417	17.056
P-value	0.975	0.003	0.003	<0.001	<0.001	<0.001	<0.001	0.815	<0.001

Significant difference exists at $p \leq 0.001$. Mean values with different superscripts in the same column are significantly different. Means were separated using Duncan Multiple Range Test. All values are mean of triplicate readings.

4.25 Characterization of the Exopolysaccharides Producing Isolates for Levansucrase Gene (LevV) and Glucansucrase Gene (gtf)

The results obtained for the molecular characterization of the nineteen exopolysaccharides positive isolates for levansucrase gene (LevV) and glucansucrase gene (gtf) show that seven isolates representing 36.84% are levansucrase gene (LevV) positive. These isolates; *Lactobacillus plantarum* SP2, *Lactobacillus plantarum* $SP2_{40}$, *Lactobacillus plantarum* $SP3_{40}$, *Lactobacillus plantarum* SP5, *Lactobacillus plantarum* DG5, *Lactobacillus plantarum* GP5, *Lactobacillus pentosus* $SP3_{40}$ all show the expected amplicon size of 500bp which corresponds to that of levansucrase gene (LevV). The other 12 isolates; *Lactobacillus plantarum* GP3, *Lactobacillus plantarum* SP3, *Lactobacillus plantarum* $SP4_{40}$, *Lactobacillus plantarum* SP4, *Lactobacillus plantarum* $DG5_{40}$, *Lactobacillus brevis* SP5, *Lactobacillus brevis* $DG5_{40}$, *Lactobacillus brevis* GP4, *Lactobacillus pentosus* $GP3_{40}$, *Lactobacillus pentosus* GP1, *Lactobacillus fermentum* SP4 and *Lactobacillus collinoides* GP1 do not carry the levansucrase gene (LevV) (Plate I).

None of the nineteen isolates show the expected amplicon size of 660bp corresponding to the glucansucrase gene (gtf).

4.26 Sequencing of the levansucrase genes (LevV)

The Basic Local Alignment Search Tool (BLAST) result for the nucleotide sequence of the seven levansucrase gene positive isolates is as shown on Table 4.20. The BLAST search reveals that the isolate *Lactobacillus plantarum* SP2 has 99% similarity to *Lactobacillus plantarum* JDM1 with accession number CP001617 and e - value of 0.0. There are only three nucleotide base positions of dissimilarity; Y, C and T which are all coloured in blue (Appendix VII). *Lactobacillus plantarum* $SP2_{40}$ has 96% similarity to *Lactobacillus* *plantarum* 16 with accession number CP006033 and e – value of 0.0. There are twelve nucleotide base positions of dissimilarity; W, -, R, A, K, Y, S, Y, K, M, K and T which are all coloured in blue (Appendix VIII). *Lactobacillus plantarum* SP5 has 95% similarity to *Lactobacillus plantarum* ZJ316 with accession number CP004082 and e – value of 7e-168. There are thirteen nucleotide base positions of dissimilarity; M, T, K, -, R, Y, Y, M, A, G, S, A, T and T which are all coloured in blue (Appendix IX). *Lactobacillus pentosus* SP3$_{40}$ has 81% similarity to *Lactobacillus pentosus* IG1 with accession number FR874854 and e-value of 7e-125. There are 71 nucleotide base positions that differ from that of *Lactobacillus pentosus* IG1 nucleotide base sequence which are all coloured in blue (Appendix X). *Lactobacillus plantarum* SP3$_{40}$ has 97% similarity to *Lactobacillus plantarum* subsp. *plantarum* P-8 with accession number CP005942 and e-value of 0.0 in the Genbank. There are only seven (W, R, A, G, T, A and T) nucleotide base positions that differ from that of *Lactobacillus plantarum* subsp. *plantarum* P-8 nucleotide base sequence which are all coloured in blue (Appendix XI).

Lactobacillus plantarum DG5 has 95% similarity to *Lactobacillus plantarum* WCFS1 with accession number AL935263.2 and e – value of 2e-163. There are seventeen nucleotide base positions of dissimilarity; M, T, K, -, R, A, C, Y, Y, S, C, A, G, C, S, G and T which are all coloured in blue (Appendix XII). *Lactobacillus plantarum* GP5 has 94% similarity to *Lactobacillus plantarum* subsp. *plantarum* ST-III with accession number CP002222 and e-value of 0.0. There are twenty-three nucleotide base positions of dissimilarity; Y, K, M, S, M, W, C, K, T, S, T, W, K, M, T, R, Y, S, S, A, S, T and T which are all coloured in blue (Appendix XIII).

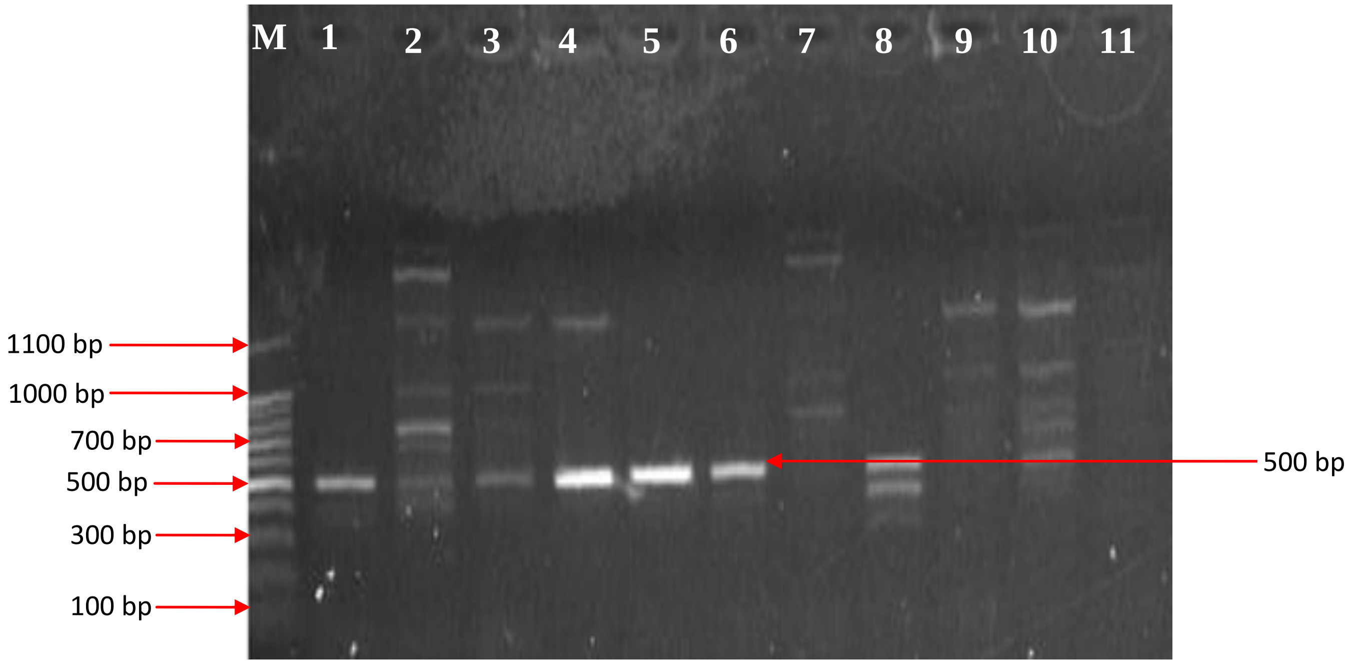

Plate I: Agarose gel photograph of PCR products of exopolysaccharides positive isolates

Lane M is 100bp molecular weight ladder; lanes 1, 3, 4, 5, 6, 8 & 10 are *L. plantarum* SP2, *L. plantarum* SP2$_{40}$, *L. plantarum* SP3$_{40}$, *L. plantarum* SP5, *L. plantarum* DG5, *L. pentosus* SP3$_{40}$ & *L. plantarum* GP5

Table 4.20: BLAST result for the nucleotide sequences of the levansucrase genes

Isolate	GenBank organism	E - value	Identity	Accession number
Lactobacillus plantarum SP2	*Lactobacillus plantarum* JDM1	0.0	99%	CP001617.1
Lactobacillus plantarum $SP2_{40}$	*Lactobacillus plantarum* 16	0.0	96%	CP006033.1
Lactobacillus plantarum SP5	*Lactobacillus plantarum* ZJ316	7e-168	95%	CP004082.1
Lactobacillus pentosus $SP3_{40}$	*Lactobacillus pentosus* IG1	7e-125	81%	FR874854.1
Lactobacillus plantarum $SP3_{40}$	*Lactobacillus plantarum* subsp. *plantarum* P-8	0.0	98%	CP005942.1
Lactobacillus plantarum DG5	*Lactobacillus plantarum* WCFS1	2e-163	95%	AL935263.2
Lactobacillus plantarum GP5	*Lactobacillus plantarum* subsp. *plantarum* ST-III	0.0	94%	CP002222.1

4.27 Molecular Phylogenetic Analysis of the Isolates

The phylogeny tree has two major branches and four sub-branches. The tree shows that isolate 11LEV VREVA0401 representing *L. plantarum* DG5 belong to the same group with *L. plantarum* with accession number AL935263. The tree further shows that *L. plantarum* CP001617.1, 18 LEV VREVB066 (*L. plantarum* GP5), 7 LEV VREVF0416 (*L. plantarum* SP5), 1 LEV VREVB04 (*L. plantarum* SP2), 6 LEV VREVE0413 (*L. plantarum* $SP3_{40}$), 5 LEV VREVD04 (*L. plantarum* $SP2_{40}$), *L. plantarum* CP002222.1, 8 LEV VREVG0419 (*L. pentosus* $SP3_{40}$), *L. plantarum* CP0040821, *L. plantarum* CP006033.1 and *L. plantarum* CP005942.1 are all closely related since the belong to the same branch. *Lactobacillus pentosus* belong to the same group with the isolates listed above (Fig. 4.5).

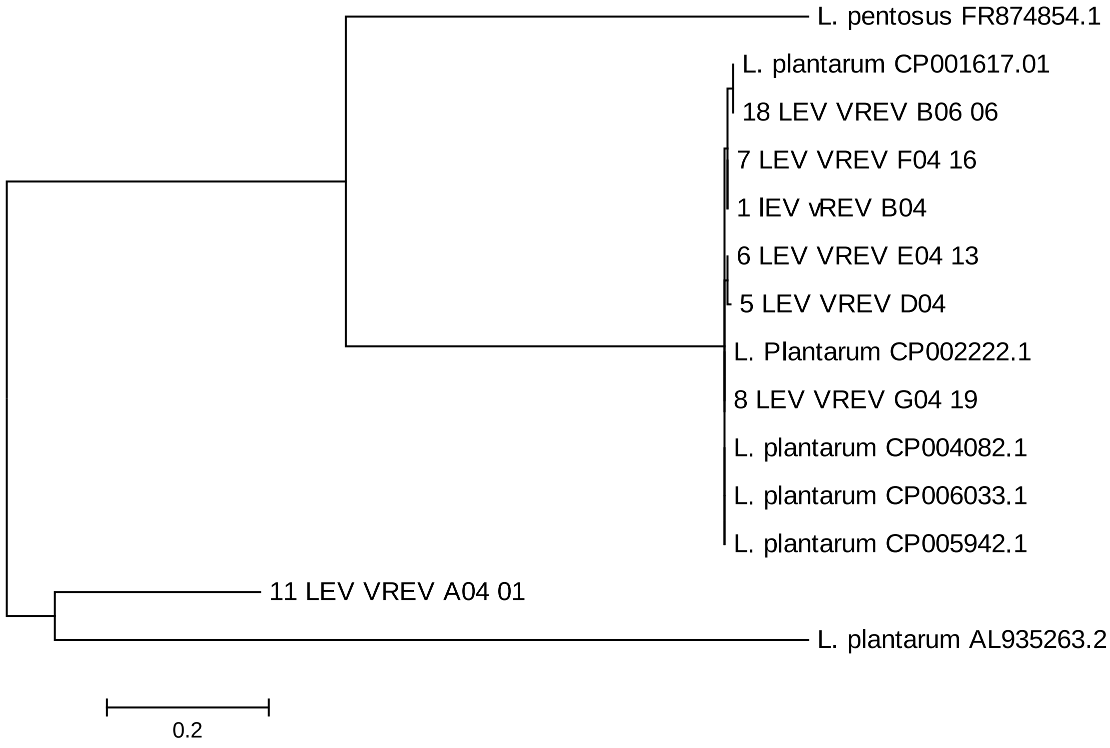

Figure 4.5: Molecular Phylogenetic analysis of the isolates

CHAPTER FIVE

5.0 DISCUSSION

5.1 Microbial Counts of the Sourdough Samples

The higher counts of lactic acid bacteria compared to fungal and aerobic plate counts agreed with the findings of other workers in this area; Hammes *et al*. (2005) reported that in mature sourdoughs LAB counts range from log 9.000 - 9.477 cfu/g and fungi from log 6.000 - 7.699 cfu/g sourdough. According to Katina (2005), the dominant microbes in spontaneously fermented doughs are homofermentative lactobacilli and pediococci, which are found both in wheat and rye sourdoughs at the level of log 8.477 - 9.477 cfu/g. Luangsakul *et al*. (2009) reported lactic acid bacteria counts of log 4.255 - 8.000 cfu/g sample, and low fungal counts of log 1.000 - 2.361 cfu/g. Hamtel *et al*. (2007) reported higher lactic acid bacteria count of log 5.477 - 8.477 cfu/g compared to fungal and aerobic plate counts ranging from log 4.699 - 7.602 cfu/g and log 2.000 - 8.707 cfu/g respectively. Gobbetti *et al*. (2001), Hertel *et al*. (2003), Scheirlink *et al*. (2007), Iacumin *et al*. (2009a), Zhang *et al*. (2011) and Annabelle *et al*. (2011) have all reported higher lactic acid bacteria counts than fungal counts. Matthias and Vogel (2005) reported that cereal fermentations are dominated by specifically adapted lactic acid bacteria occurring at numbers greater than log 8cfu/g, which may be in coexistence or possibly in symbiosis with typical yeasts.

Several factors account for the dominance of lactic acid bacteria during dough fermentation. First, their carbohydrate metabolism is highly adapted to the main energy sources (maltose and fructose) in dough (De Vuyst and Neysens, 2005). Second, sourdough lactic acid bacteria possess several stress response mechanisms to overcome acid, high/low temperatures, high osmolarity/dehydration, oxidation and starvation. Important mechanisms to resist acidic conditions include intracellular proton removal through H^+-ATPase activity, alterations in cell membrane composition, and amino acid conversions (De Angelis *et al*., 2001; Weckx *et al*., 2011). Third, production of lactic and acetic acids by the 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). Fourth, the production of antimicrobial compounds, both organic acids (lactate, acetate) and proteinaceous compounds (for instance, bacteriocins), improves their competitiveness and may contribute to their stable persistence in sourdough fermentations (Gobbetti, 1998; Ga¨nzle & Vogel, 2002; Messens & De Vuyst, 2002). In addition, the competitive advantage of LAB in the sourdough environment is due to their capability to metabolize maltose, originating from the breakdown of starch by cereal amylases, by means of a maltose phosphorylase without ATP expenditure (De Vuyst *et al*., 2011), the use of the arginine diaminase (ADI) pathway to enhance ATP generation and improve acid tolerance (Vrancken *et al*., 2009a; Vrancken *et al*., 2009b), and the reduction of fructose to mannitol by mannitol dehydrogenase to regenerate NAD^+ and to produce ATP (Gobbetti *et al*., 2005; Korakli *et al*., 2003; Vrancken *et al*.,2008).

The findings of this work with respect to lactic acid bacteria count for flour B at room temperature agrees with that of Saeed (2009) who reported lactic acid bacteria as the dominant bacteria in sourdough with counts in the range of log 4.795 cfu/g - 7.840 cfu/g. The findings of this study also tallies with the findings of Faucher *et al*. (1999) who reported lactic acid bacteria count of log 5.477 - 8.477cfu/g. The fungal count obtained in this study agrees with that reported by Saeed (2009) while the aerobic plate count was much lower. The lactic acid bacteria counts reported in this work also tallies with the findings of other researchers in this field (Picozzi *et al*., 2005; Kim *et al*., 2008; Banu *et al*., 2011; Zhang *et al*., 2011; Aplevicz *et al*., 2013). The progressive increase in lactic acid bacteria counts and progressive decrease in fungal and aerobic plate counts seen in this study had also been reported by Hertel *et al*. (2003). The aerobic plate counts obtained in this study also agrees with the findings of Savic *et al*. (2006). The findings of this study with respect to LAB and APC counts however differ from that reported by Gatto and Torriani, (2004) and Korakli *et al*. (2006).

5.2 Lactic Acid Bacteria Isolates Obtained from the Sourdough Samples

The findings of this study in which *Lactobacillus* species was found to be the most dominant genera of LAB in sourdough is in agreement with that of several workers in this field who also reported *Lactobacillus* species as the most dominant genera of LAB in sourdough (Corsetti *et al.,* 2001; Corsetti *et al.,* 2003; Hertel *et al.,* 2003; Gobetti *et al*., 1994; Korakli *et al*., 2004; De Vuyst and Neysens, 2005, Korakli *et al*., 2006; Rehman *et al*., 2006; Herve *et al*., 2009; Luangskul *et al*., 2009; Saeed *et al*., 2009; Rollan *et al*., 2010; De Vuyst *et al*., 2011; Annabelle *et al*., 2011). Typical sourdough prokaryotes are members of the LAB genus *Lactobacillus* with the species *Lactobacillus sanfranciscensis, Lactobacillus plantarum* and *Lactobacillus brevis* being most frequently isolated (Gobbetti, 1998; Rehman *et al.,* 2006; Ghulam, 2009).

The occurrence of *Lactobacillus plantarum* as the dominant isolate in this study agrees with the findings of other workers (Ricciardi *et al*., 2005; Van der Meulen *et al*., 2007; Scheirlinck *et al.,* 2008; Zotta *et al.,* 2008; De Vuyst *et al.,* 2010; Minervini *et al.,* 2010; Moroni *et al.,* 2011; Wan Aida *et al.,* 2011). In a study of four different types of sourdough obtained from bakeries in Northern Italy, *Lactobacillus plantarum* was found to be the most dominant isolate in two of the four sourdoughs (Iacumin *et al*., 2009a). *Lactobacillus plantarum* is a ubiquitous species, found in several food ecosystems, including sourdoughs (Minervini *et al*., 2012b). According to Boekhorst *et al*. (2004) and De Vuyst *et al*. (2010), *Lactobacillus plantarum* is more acid tolerant and often dominate fermentation processes of vegetables and cereals in particular because of its ability to transport and metabolize different carbohydrates. However, the findings of this study differs from that of other workers (Corsetti *et al.,* 2001; Thiele *et al*., 2002; Coppola *et al*., 2005; Rehman *et al.,* 2006; Ferchichi *et al*., 2007; Arendt *et al*., 2009; De Angelis *et al.*, 2009; De Vuyst *et al*., 2011; Minervini *et al*., 2012a) who reported *Lactobacillus sanfranciscensis* as the dominant isolate in wheat flour sourdough.

The other isolates obtained in this work have also been reported by a number of researchers in this field; *Lactobacillus brevis* (Kazanskaya *et al.*, 1983; Corsetti *et al.,* 2001; Thiele, 2003; Gatto and Toriani, 2004; Katina, 2005; Sagdic *et al*., 2005; Gerez *et al.,* 2006; Rehman *et al.*, 2006; Spier *et al*., 2007; De Vuyst *et al*., 2007b; Corsetti and Settanni, 2007; De angelis *et al*., 2009; Iacumin *et al*., 2009a; Wan Aida *et al.*, 2011), *Lactobacillus pentosus* (Thiele, 2003; Catzeddu *et al*., 2005), *Lactobacillus buchneri* (Thiele, 2003; Katina, 2005; Zotta *et al*., 2008; De Vuyst and Neysens, 2005; Rollan *et al*., 2010), *Lactobacillus fermentum* (Kazanskaya *et al*., 1983; Corsetti *et al.*, 2001; Thiele *et al.*, 2002; Katina, 2005; Riggio *et al*., 2005; Rehman *et al.,* 2006; Spier *et al*., 2007; De vuyst *et al*., 2007*;* Arendt *et al*., 2009; Vogelmann *et al*., 2009; Stefan *et al*., 2010), *Pediococcus pentosaceus* (Thiele, 2003; Stolz, 2003; Gerez *et al.,* 2006; Katina, 2005; Sagdic *et al*., 2005; Rehman *et al.,* 2006; Corsetti *et al*., 2007; Iacumin *et al*., 2009a; Sterr *et al*., 2009; De Angelis *et al*., 2009; Arendt *et al*., 2011)), *Pediococcus acidilactici* (Stolz, 2003; Sagdic *et al*., 2005; Rehman *et al.,* 2006; Wakil *et al.,* 2008). There is no published information on the isolation of *Lactobacillus collinoides* from sourdough. *Lactobacillus collinoides* is reported to be commonly found in fermenting apple juice (Sauvageot *et al*., 2000). This may be the first report of the isolation of *Lactobacillus collinoides* from sourdough.

Although the lactic acid bacteria composition of sourdough produced in different parts of the world had been well-documented, there is a dearth of information on the lactic acid bacteria composition of sourdough produced in Nigeria. The lactic acid bacteria of Italian sourdough include *Lactobacillus sanfranciscensis*, *L. alimentarius*, *L. brevis*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* Subsp. *delbrueckii*, *L. fermentum*, *L. brevis* ssp. *lindneri*, *Leuconostoc citreum*, *Lactococcus lactis* Subsp. *lactis*, *Weisella confusa*, *Weisella cibaria*, *Enterococcus faecium*, *Pediococcus pentosaceus*, *Pediococcus pentosus* (Gobbetti *et al*., 2001; Coppola *et al*., 2005; Corsetti and Settanni, 2007; Iacumin *et al*., 2009b). In French sourdough, *Lactobacillus sanfranciscensis*, *L. alimentarius*, *L. brevis*, *L. casei*, *L. acidophilus*, *L. delbrueckii*, *L. panis*, *L. nantensis*, *L. hammesii*, *L. spicheri*, *L. pontis*, *L. frumenti*, *L. paralimenteroides*, *Leuconostoc* (*Lc*) *mesenteroides*, *Lc. mesenteroides* Subsp. *mesenteroides*, *Pediococcus pentosaceus* had been reported (Korakli *et al*., 2004; De Vuyst and Neysens, 2005; Ferchichi *et al*., 2007). *Lactobacillus sanfranciscensis*, *L. brevis*, *L. paralimentarius*, *Pediococcus pentosaceus*, *Weisella cibaria* and *Enterococcus faecium* had been reported as LAB of Greek sourdough (Paramithiotis *et al*., 2005; Paramithiotis *et al*., 2006). In Portuguese sourdough, *L. brevis*, *L. curvatus*, *L. lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *lactis*, *Enterococcus casseliflavus*, *Enterococcus durans*, *Enterococcus faecium*, *Steptococcus constellantus* and *Steptococcus equinus* had been reported (Rouch and Malcata, 1999). The LAB isolates of Belgian sourdough had been reported to include, *Lactobacillus sanfranciscensis*, *L. plantarum*, *L. paralimentarius* and *L. pontis* (Scheirlinck *et al*., 2008). *Lactobacillus platarum*, *L. guizhouensis*, *L. rossiae* and *L. zeae* had been isolated from Chinese sourdough. The LAB isolated from traditional Slovak sourdoughs were to include *L. brevis*, *L. paralimentarius*, *L. helveticus*, *L. plantarum*, *L. crispatus*, *L. delbrueckii* and *L. acidophilus* (Gereková *et al*., 2011). Of all these isolates, only *L. brevis*, *L. plantarum* and *L. fermentum* had been isolated from Nigerian sourdough as reported in this work.

5.3 Exopolysaccharides (EPS) Production by the Lactic Acid Bacteria Isolates

The production of EPS from sucrose is a metabolic activity widespread in sourdough lactic acid bacteria. It is likely that any given sourdough contains EPS producing lactobacilli (De Vuyst *et al.*, 2001; Teiking, 2005). The production EPS by LAB had been studied extensively. The exopolysaccharides producing lactic acid bacteria obtained in this study; *L. plantarum, L. brevis, L. pentosus, L. collinoides* and *L. fermentum* differ from those reported by most other workers in this field. Tieking *et al*. (2003) reported EPS producing LAB species to include *L. frumenti, L. pontis* and *L. reuterin.* Krajl *et al*. (2003) reported EPS positive *Lactobacillus* species to include *L. reuteri*, *L. fermentum*, *L. sakei* and *L. parabuchneri. Leuconostoc* (*Leu.*) *lactis, Leu. gelidum, Leu. mesenteroides, Leu. amelibiosum, Leu. dextraninicum* and *Leu. curvatus* had been reported as EPS producers by Palomba *et al.* (2012). Raul-Madiedo and de los Reyes-Gavilan (2005) reported *Leu. lactis,* subsp *cremoris, Streptococcus thermophiles, L. delbrueckii* spp. *bulgaricus, L. helviticus, L. sakei, L. reuteri, L. rhamnosus, L. rhamnosus* and *Bifidobacterium longum* as EPS producers. Other exopolysaccharides LAB producers that differ from the findings of this work include *L sanfranciscencis, L. panis, Weisella confusa, S. salivarius* and *S. mutans* (Van Hijum *et al*., 2006; Patel *et al.*, 2012). Song *et al.* (2013) reported EPS production in *Pediococcus acidilactici*, none of the two isolates of *Pediococcus acidilactici* screened in this study were found to be EPS positive. However, *L. plantarum, L.* brevis, *L. pentosus* and *L. fermentum* reported in this work as EPS producers agrees with the findings of other workers in this field; *L. plantarum* and *L. fermentum* had been reported by Adebayo-Tayo and Onilude, (2008), Babol *et al*. (2011) and Haroun *et al*. (2013); *L. plantarum* and *L. pentosus* had also been reported by Hamza *et al*. (2009) and Rodriguez-Carvajal *et al.* (2008). Jaiswal *et al*. (2014) also reported *L. brevis* and *L. plantarum* as EPS producers.

The frequency of occurrence of the EPS positive isolates obtained in this work, 19 EPS positive isolates out of 79 isolates (24.05%) tallies with the findings of Dijkhuizen *et al*. (2009) and Savadogo *et al*. (2004) who reported 18 out of 63 LAB isolates representing 28.57% and 13 out of 50 (26%) as EPS positive respectively. Tieking *et al*. (2003), Gee-Schutten *et al*. (2007), Yang (2009) and Palomba *et al*. (2012) reported a frequency of occurrence of EPS positive LAB in sourdough of 19.82%, 32.97%, 5% and 19.20%, respectively which differs from the findings of this work by being either much higher or lower.

The ropy and mucoid phenotypes exhibited by some of the isolates in this study may be due to the ability of such isolates to produce two different types of exopolysaccharides which differ in chemical composition (De Vuyst *et al.* 2001; Raul-Madiedo and de los Reyes-Gavilan, 2005).

The Generally Recognized As Safe (GRAS) status of LAB allows the in-situ production of EPS in different food products such as cheese and yoghurt, which meets consumers demand for products with low levels of additives (De Vuyst *et al*., 2001; Jolly *et al*., 2002; Raul-Madiedo and de los Reyes-Gavilan, 2005). Galle *et al*. (2012) reported that EPS formed from sucrose during sourdough development can improve the technological properties of gluten-free breads and potentially replace hydrocolloids. Korakli *et al.* (2003) also reported that formation of EPS *in situ* from sucrose results in metabolites such as mannitol, glucose and acetate, which may contribute to improved bread quality. Exopolysaccharides produced from lactobacilli thus may be expected to beneficially affect one or more of the following technological properties of dough and bread: (i) water absorption of the dough, (ii) dough rheology and machinability, (iii) dough stability during frozen storage, (iv) loaf volume and (v) bread staling (Tieking and Ganzle, 2005).

Patel *et al*. (2010) further reported that EPS play an important role in improving the appearance, stability and rheological properties of novel food products. The relevance of EPS-producing probiotic LAB as efficient starter cultures for the production of fermented foods has increased due to fascinating technological properties of EPS (Tieking *et al*., 2005). In addition to their technological properties, EPS produced by LAB may also have biological roles, such as immunomodulation, antitumour and cholesterol-lowering activities (Peant *et al*., 2005). The increasing interest of the food industry in these so-called biothickeners results in an increasing demand for these nature-made polysaccharides (De Vuyst *et al*., 2007). Since LAB are able to produce a great variety of both homopolysaccharides (HoPS) and heteropolysaccharides (HePS), the exploration of the biodiversity of wild LAB strains concerning their EPS production may seem to be a very suitable approach (De Vuyst *et al*., 2001, Mozzi *et al*., 2001 and Raul-Madiedo and de los Reyes-Gavilan, 2005).

5.4 Heterofermentative ability of the Lactic Acid Bacteria Isolates

The production of carbon IV oxide (CO_2) from sugars is a characteristic of heterofermentative LAB, while the homofermenters produce only lactic acid from sugar metabolism (Ghulam, 2009; Kihal *et al*., 2013). The inability of *Lactobacillus plantarum* SP5, *Lactobacillus plantarum* SP3, *Lactobacillus plantarum* $SP4_{40}$ and *Lactobacillus plantarum* SP4 to ferment any of the sugars with gas production may be because they used the homofermentative pathway for the catabolism of the sugars; *L. plantarum* is a facultative heterofermentative bacterium (Corsetti and Settanni, 2007; Plumed-Ferre *et al*., 2008). However, seven out of the eleven *L. plantarum* strains showed heterofermentation ability. According to Plumed-Ferre *et al*. (2008) carbohydrate fermentation by *L. plantarum* appeared to be very flexible, allowing the bacterium the simultaneous use of different fermentable sugars. Obinna-Echema *et al*. (2014) reported that *L. plantarum* strains, either as single or mixed starter cultures with yeasts, demonstrated strong fermentation ability. *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus collinoides* are all obligate heterofermenters (Ganzle *et al*., 2007) and this explains why all the isolates of these organisms produce gas from glucose fermentation in this study. The fermentation of maltose by *Lactobacillus brevis* and *Lactobacillus fermentum* is in agreement with findings of Ganzle *et al*. (2007). Sugars used by lactic acid bacteria as energy source vary by species and even by strain. The most common lactic acid bacteria identified in sourdoughs are capable of fermenting pentoses, hexoses, sucrose and maltose, although some species such a *L. sanfransiscensis*, are specific to maltose. Some lactic acid bacteria common in sourdough systems are fructose negative and grow faster in maltose than in glucose, on the other hand, *L. plantarum* prefers maltose and glucose over fructose for rapid growth and weakly ferments sucrose (Katina, 2005; Madoroba, 2009)).

5.5 Dough leavening Ability of the Lactic Acid Bacteria Isolates and selected Sourdough

The higher dough leavening capacity of Baker's yeast in comparison with the lactic acid bacteria isolates and the various percentages of the sourdough used is said to be due to the shorter lag phase of Baker's yeast compared to the lactic acid bacteria isolates (De Vuyst *et al*., 2011). Generally, lactic acid bacteria are slow growers and have a limited metabolic capacity (Weckx *et al*., 2009; De Vuyst *et al*., 2011). The higher dough leavening capacity of the synergistic combinations of Baker's yeast and LAB and Baker's yeast and sourdough is due to a number of reasons. According to Coppola *et al*. (1998) during sourdough fermentation lactic acid bacteria use carbohydrate present in the flour for energy supply, which results in the formation of acids and carbon IV oxide. They further reported that starter cultures characterized by different combinations of lactic acid bacteria can promote the leavening process, achieving the required increase in volume. The proteolytic activity of lactic acid bacteria also leads to increased availability of free amino acids, enhancing the yeast growth leading to higher carbon IV oxide production. Gobbetti *et al*. (1995) reported that yeast fermentation with heterofermentative lactic acid bacteria is faster with corresponding increase in carbon IV oxide production. According to Didar *et al.* (2010), heterofermentative lactic acid bacteria cause increase in yeast's metabolic activity resulting in higher CO_2 production. They further reported that sufficient acidity causes increase in gluten ability to hold gas. *Lactobacillus plantarum* is reported to have high proteolytic activity (Coppola *et al*., 1998), this may account for its higher dough leavening capacity in combination with Baker's yeast compared to the other LAB isolates in combination with Baker's yeast. According to Ghulam, (2009) the used of *L. sanfranciscensis, L. plantarum* and *Saccharomyces cerevisiae* for leavening wheat doughs results in a dough with a more balanced microbiological and biochemical characteristics than dough's started with *Saccharomyces cerevisiae* alone, in which alcoholic fermentation end products largely predominated. By using LAB starters, the greatest lactic acid bacteria cell number and acetic acid production were achieved. The starters resulted in more complete profiles of volatile compounds and greater structural stability (Ghulam, 2009). In their study on the effects of sourdoughs on carbon IV oxide gas generation in dough during fermentation, Kim *et al*. (2005) reported that sourdough samples made from polished flours produced significantly large amounts of carbon IV oxide gas. They attributed this to the good buffering capacity caused by the bran fraction in polished flours which promoted efficient growth of microbial cells, producing a larger amount of carbon IV oxide gas during fermentation. Katina *et al*. (2006) also reported that sourdough addition leads to better gas holding capacity of gluten. It is generally assumed that the use of sourdough improves gas retention and many sourdough microorganisms produce carbon IV oxide gas (Saeed, 2009). Korakli, (2002) further reported that sucrose addition to dough led to substantially more glucose accumulation through the metabolism of sucrose by lactic acid bacteria. This accumulation of glucose may affect yeast metabolism in co-cultures of yeast and lactobacilli. High glucose concentrations support the gas production by yeasts and thus contribute to dough leavening. According to Galle *et al*. (2012) increased release of glucose and fructose from sucrose during lactic acid bacteria fermentation enhanced CO_2 production by yeast thus leading to enhanced dough leavenning. Sourdough has traditionally been used as a leavening agent in bread production (Ghulam, 2009; Galle *et al*., 2012). In most industrial applications sourdough and baker's yeast are used as leavening agents (Hammes *et al.*, 1996; Tieking *et al*., 2003; Vermeulen, 2006). Sourdough fermentations, as well as baking agents based on sourdoughs, have retained their importance in contemporary baking technology because of the improved aroma, texture, and shelf life of sourdough breads (Corsetti *et al*., 2000; Thiele *et al*., 2002). The production of a wide variety of traditionally prepared baked goods continues to rely exclusively on the use of sourdough as a leavening agent. The use of sourdough as leavening agent requires the maintenance of a starter sponge in a metabolically active state by continuous propagation (Ganzle *et al*., 2007).

5.6 pH and TTA Values during Spontaneous Fermentation of the Dough

The fermentation time showed a significant impact on pH exhibiting a decreasing trend with increase in the fermentation time. The decrease in pH is due to increase in acid production in the fermented dough by lactic acid bacteria present in the dough. The lower pH values were obtained in sourdoughs fermented at elevated temperatures of 40^0C, this is due to increase metabolic activities of the lactic acid bacteria. Lactic acid bacteria are thermophilic in nature; high temperature therefore favours their growth and metabolic activities leading to increase in organic acids (lactic and acetic acids) production which are responsible for the lower pH values. Decrease in pH is essential for optimal swelling and baking of bread and also for development of good sensory properties in bread (Arendt *et al.*, 2007; Kam *et al.*, 2012).

The pH range obtained in this work; 3.33 - 6.73 agrees with that reported by Arendt *et al*. (2002), Thiele, (2003), Seed, (2009), Kockova *et al*. (2011) and Zhang *et al*. (2011) which ranged from 4.7 - 5.4, 3.6 - 6.2, 3.53 - 5.74, 3.27 - 6.21 and 2.78 - 5.13 respectively. However, the pH values reported in this study differ from those reported by De Angelis *et al*. (2009), Luangsakul *et al*. (2009), Annabelle *et al*. (2011) and Tafti *et al*. (2013). The pH values of this study (3.3 - 4.6) at day five of fermentation agrees with that of Arendt *et al*. (2002), Hertel *et al*. (2003b), Katina (2005), Kim *et al*. (2005b), Minervini *et al*. (2012a) and Minervini *et al*. (2012b) who reported a final pH of 3.5 - 4.3, 3.4 - 3.6, 3.6 - 3.8, 3.8 - 4.4, 4.5 - 4.7 and 3.8 - 4.1 respectively.

The titratable acidity and pH of the dough are important during sourdough fermentation. Sourdoughs with longer incubation times are said to be characterized by low pH and high titratable acidity values as observed in this study. The decrease in pH and increase in titratable acidity due to microbial activity has been well documented in cereals fermented with endogenous microflora (Wakil *et al*., 2008). The observed increase in titratable acidity could be due to the dominance of the environment by lactic acid bacteria which degrade carbohydrates resulting in acidification. These observations are in agreement with earlier studies by Nout *et al*. (1989), Ariahu *et al*. (1999), Katina (2005), De Vuyst *et al*. (2007). Sadeghi *et al*. (2007) also reported that increasing fermentation time and temperature during sourdough development leads to increase in titratable acidity and lower pH. They further reported that most of the beneficial properties attributed to sourdough are determined by the acidification activity of lactic acid bacteria (Scheirlinck *et al*., 2008).

The titratable acidity obtained in this study at day five of fermentation 0.676 ± 0.01 is similar to the findings of Hertel *et al*. (2003b) and Hertel *et al*. (2005) who reported values of 0.623 to 0.715 and 0.721 respectively while the titratable acidty values obtained at the initial stage of fermentation and after 24 hours which was 0.168 - 0.243 is similar to the findings of Tafti *et al*. (2013) who reported titratable acidity values of 0.137 - 0.242. The level of titratable acidity reported in this study is however, much higher than the value of 0.115 after 24 hours of fermentation reported by Gatto and Torriani (2004). According to Chavan and Chavan (2011), in the initial phase of sourdough development, both acidity and pH remain constant, whereas during the intermediate phase, titratable acidity increases a trend similar to that observed in this study.

5.7 pH and TTA of the Bakery Products

The bread and doughnuts produced using the lactic acid bacteria starter cultures have lower pH values than that produced using Baker's yeast alone and the commercial bread samples; this could be explained by the fact that the lactic acid bacteria are able to convert hexoses to lactic acid, acetic acid/ethanol and carbon IV oxide. These acids are responsible for the drop in pH of the doughs which inturn was responsible for the lower pH of the sourdough and lactic acid bacteria bread and doughnuts. This finding agrees with the findings of Saeed (2009) who reported a pH range of 3.88 - 6.10 for bread. Generally, the pH of the bread and doughnut samples decreases with increase in the concentration of the sourdough; this also tallies with the findings of Saeed (2009).

The results for pH and acidity are in agreement with the findings of Park *et al.* (2007) who reported increased acidity and lower pH values of sourdough breads in contrast to yeast leavened bread which showed less acidity and higher pH values. Similarly Martinez-Anaya *et* *al.* (1990) reported lower pH and higher TTA in breads fermented with LAB than in yeast fermented breads. The reported pH values ranged from 4.92 - 5.33 and TTA values for breads ranged from 0.305 - 0.462.

These findings however, differ from that of Banu *et al.* (2011a) who reported the pH range of 5.1 - 5.3 and TTA of 0.451 - 0.487 for sourdough rye bread compared to pH range of 4.67 - 6.67 and TTA range of 0.568 - 0.585 reported in this work. It also differs from the findings of Bolourin and Khodaperest (2010) who reported a pH of 6.5 and TTA of 0.439 for bread baked with 15% sourdough.

According to Katina *et al.* (2009), the antimicrobial activity of sourdoughs fermented with *L. plantarum, L. brevis* or with *Pediococcus pentosaceus* was evident if the pH of sourdough bread is between 4.8 - 5.1. This pH range tallies with that of both the bread and doughnuts of this work baked using 10% and 15% sourdough which were 4.67 - 5.00 for bread and 4.93 - 5.00 for doughnuts. This also further explained why the 10% and 15% bread and doughnuts have the longest shelflife.

5.8 Proximate Composition of the Baked Products

The Standard Organization of Nigeria (SON, 2004) specifications for wheat bread, among others, are as follows: moisture content 40% maximum, Protein 10% maximum, ash content of 0.6% maximum, fat content of 2.0% maximum and carbohydrate 48% maximum (Eke *et al.*, 2013). Based on this standard, all the bread and doughnuts were within the standard with respect to moisture content. All the bread and doughnuts also fall within standard with respect to protein content; the only exception was the 10% sourdough doughnut which falls slightly outside specifications. With respect to ash content, the 10%, 15% and *L. plantarum* bread did not meet the standard but the remaining bread samples fall within the standard. All the sourdough, *L. plantarum* and *L. brevis* doughnuts met the standard specifications for ash content while both the baker's yeast and commercial doughnuts fall outside the standard. All the bread and doughnuts were far above the standard in terms of fat content with the exception of Baker's yeast bread which was only slightly above the standard of 2.0% maximum. The particularly high fat content of the doughnuts may be due the oil that was used to fry the doughnuts. All the bread samples also had higher carbohydrate content than the maximum of 48% specified by the SON. However, the carbohydrate content of all the lactic acid bacteria based – bread are much lower than that of the baker's yeast and commercial bread. The lower carbohydrate content of the former may be due to co-fermentation of carbohydrate by both the lactic acid bacteria and yeast during the dough proofing. The carbohydrate content of all the doughnuts samples fall within standard specification with the exception of the baker's yeast doughnuts.

The findings of this work differ from that of Ogunbanwo *et al*. (2008) who reported that bread produced using combination of *Lactobacillus brevis* and *Saccharomyces cerevisae* as starter cultures had the highest protein content of 9.43% and the highest carbohydrate content of 56.47%. In this study, highest protein and carbohydrate contents of 6.34% and 65.76% respectively were obtained from baker's yeast bread in both cases.

5.9 Proximate Composition of the Flours and Selected Sourdough

The significantly higher protein content of the fermented dough (selected sourdough) compared to the three brands of flour is an indication that the metabolic activity of the lactic acid bacteria and yeast led to increase in protein content. This increased protein content of the sourdough implies that the application of sourdough during baking may make available more protein for the lactic acid bacteria and yeast to act on producing more amino acids and flavour precursors which may impact on the organoleptic quality of the lactic acid bacteria – based baked products. It also implies that sourdough addition may lead to improvement in nutritional quality of baked goods. Lavermicocca *et al*. (2000), Thiele *et al*. (2002) and Ghulam (2009) reported that sourdough addition improves the nutritional quality of baked products. The nutritional quality of flours produced worldwide is reported to be lower than that of wheat as a result of milling process (Ghulam, 2009). This decreased nutritional quality can be compenseted by addition of sourdough during dough proofing. According to Ghulam (2009), protein content varies significantly in different wheat varieties. It is the best single test that can be applied to determine the quality of flour, because it has a direct correlation with baking quality. The protein content of the flours used in this study differ from that reported by other researchers in this field; Anjum and Walker (2000) reported protein contents, in six most prevalent Pakistani bread wheat flours that range from 11.99 to 13.80%. Ahmed (1993) reported 12.13% crude protein in whole wheat flour. Rehman *et al*. (2001) analyzed a few wheat flour varieties of Sindh province for composition and found protein that ranges from 11.86% to 11.95%. Butt *et al*. (2001) estimated protein content in 30 spring wheat flours and reported 10.06% to11.89% in white flour. The protein content of the three brands of flour used in this work ranged from 5.01% to 6.84%, a value that is much lower than that reported by the researchers mentioned above. However, the protein content of the sourdough used in this work which was 12.23% is similar to the protein content of the flours reported by the researchers mentioned earlier. The proximate values reported in this work also differs from that reported by Rehmen *et al*. (2007), the values reported are moisture 12.20%, crude protein 12.87%, crude fat 0.94% but the ash content of 0.65% is similar to the findings of this work with respect to flour B and sourdough both with ash content of 0.73%.

The ash content of flour has been reported to affect the sensory quality of sourdough bread, the lower the ash content of the flour the better the sensory quality of the bread (Katina, 2005), all the flour types and the selected sourdough used in this work have low ash content of less that one percent.

5.10 Sensory Evaluation of Bread and Doughnuts

The scores for the sensory evaluation for both the bread and doughnut in terms of appearance, aroma, texture and taste reveal that the lactic acid bacteria based bread and doughnuts (that is bread and doughnuts started with lactic acid bacteria and sourdough) score higher than bread and doughnuts started with baker's yeast alone and commercially purchased ones in most cases. In terms of overall acceptability, only the *Lactobacillus brevis* bread scored lower than the baker's yeast alone bread and commercial bread while only the *Lactobacillus plantarum* doughnuts and 15% sourdough doughnut scored lower than the commercial doughnut. Several researchers in this field had reported higher sensory quality score of products baked with lactic acid bacteria and sourdough as starter cultures than those baked with baker's yeast alone.

The sourdough lactobacilli have earned special interest of researchers due to the benefits achieved by their use in breadmaking industry. It has been reported that the lactobacilli contribute to the improvement of the volume, texture and sensory quality of bread (Gerekova *et al*., 2011).

It has been reported that 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). It has also been reported that the synergistic metabolic activities of sourdough LAB and yeasts produce acidification or souring, influencing the final characteristics of bread, notably the generation of typical flavour compounds leading to improved sensory attributes (Gobbetti, 1998; Gobbetti *et al*., 1999; Hertel *et al*., 2005; Katina *et al*., 2006).

It has been reported that during sourdough fermentation, lactic acid bacteria (LAB) produce a number of metabolites such as organic acids, exopolysaccharides (EPS), alcohols, aldehydes, ketones, esters, ether derivates, furan derivates, hydrocarbons, lactones, pyrazines, pyrrol derivates and sulphur compounds which have been shown to have a positive effect on the texture and/or aroma of bread. While improving the textural qualities of bread, sourdough fermentation also resulted in increased mineral bioavailability and reduced phytate content (Thiele *et al.,* 2002**;** Arendt *et al*., 2007, Wang *et al*., 2012a). Furthermore, besides the positive effects of sourdough fermentation by LAB on bread texture, flavour and shelf life, sourdough fermentation by LAB also contributes to the nutritional value of bread, including production of prebiotic dextran and fiber, starch with low glycemic index and vitamin enhancement as reported by Katina (2005); Moroni *et al*. (2009); Poutanen *et al*. (2009) and Zhang, (2011). According to Chavan and Chavan, (2011) sourdough improves sensory characteristics such as loaf volume, evenness of baking, colour, aroma, taste, and texture of breads. Sourdough fermentation had been reported to increase folate and thiamine contents (Kariluoto *et al*., 2004; Liukkonen *et al*., 2003), decrease tocopherol and tocotrienol content (Liukkonen *et al*., 2003). The presence of lactic acid in bread, either added or formed during sourdough fermentation, has also been reported to reduce acute glyceamic and insulinaemic responses. The mineral availability of sourdough baked goods was also improved (Lopez *et al.,* 2003).

The higher score of sourdough and LAB bread and doughnuts in terms of aroma may be due to aroma compounds production by the heterofermentative LAB present in the sourdough used. The concentration of aroma compounds has been reported to increase during sourdough fermentation (Vemeulen, 2006; Arendt *et al.*, 2007; Chavan and Chavan, 2011). *Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus fermentum* all part of the sourdough flora used in baking in this work had been reported to produce aroma compounds such as lactic acid, acetic acid, ethyl acetate, hexanal, heptanal, octanal, nonanal, 1-octanal, octane, heptanes, benzaldehyde, 1-hexanal, 2-methyl-1-pentanal (Rehmen *et al*., 2006), diacetyl, acetaldehyde, 2- and 3-methylbutanol acetaldehyde, 2- and 3-methylbutanol (Gänzle *et al*., 2007; Gerekova *et al*., 2011) during growth in sourdough. It is further reported that diacetyl was produced in high concentration when dough fermentation was carried out with *L. plantarum* and *Pediococcus pentosaceus* (Arendt *et al*., 2011)**.** According to Rehmen *et al*. (2006) loaves made with the addition of 5 to 10% sourdoughs fermented with *L. plantarum* are preferred in odour and taste. Katina *et al*. (2006) and Rehmen *et al*. (2006) reported that sourdough breads fermented with *L. brevis* or *L. plantarum* had the most profound effect on sensory profile, since both of these strains intensified bread flavour. In particular, the ability of these LAB isolates to convert pyruvate and amino acids to various flavour precursors and flavour-active compounds resulted to an improved sensory profile for sourdough and bread (Hansen and Schieberle, 2005; Ganzle *et al*., 2007, De Vuyst *et al*., 2011). In addition, the proteolytic activity of both *L. brevis* and *L. plantarum,* had been reported to provide small peptides and amino acids which serve as precursors for flavour compounds production (Rollan *et al*., 2005; Vermuelen *et al*., 2005; Gerez *et al*., 2006; Rollan *et al*., 2010). Tafti *et al*. (2013) reported that the addition of spray-dried sourdough at the level of 9% improved bread flavour. Kim *et al*. (2005) reported that bread baked with sourdough produces better flavour with improved dough and bread properties than bread baked using commercial Baker's yeast alone. McFeeters (2004) further reported that when sourdough bread is baked, one of the characteristic odour compounds generated in the baking process is 2-acetyl pyrroline, which has a roasted, popcorn-like odour. The addition of *L. paracasi* D5 and *L. fermentum* Te007 in the production of white bread was also reported to result in an improved aroma and a pleasant caramel-like flavour in the baked bread itself (Muhialdin *et al.*, 2011a); *L. fermentum* is one of the LAB in the sourdough used in this work. Several other workers in this field had also reported improved aroma of baked products when lactic acid bacteria are added directly or in form of sourdough (Lavermicocca *et al.*, 2000; Clarke *et al.*, 2002; Thiele *et al.*, 2002; Hertel *et al.*, 2005; Edema *et al.*, 2005; De Vuyst and Vancanneyt, 2007; Rehmen *et al.*, 2007; Scheirlinck *et al.*, 2008; Muhaildin *et al.*, 2011; Ryan *et al.*, 2011; Sharaf *et al.*, 2013). According to Thiele *et al.* (2002), the influence of sourdough on bread flavour is based on three main factors: (i) formation of acidity, (ii) formation of flavour precursors such as amino acids, (iii) formation of volatile compounds.

The higher score of both the sourdough and LAB bread and doughnuts with respect to texture compared to that of Baker's yeast alone bread/doughnuts and commercial bread and doughnuts may be due to insitu production of exopolysaccharides by the LAB in dough which is reported to improve wheat bread texture (Theile, 2003). Acetic and lactic acids produced by LAB during dough leavening are also reported to improve baked goods texture. The most important texture effect by acetic acid is shorter and harder gluten. On the other hand lactic is responsible for the more elastic gluten structure (Arendt *et al.,* 2007; Corsetti and Settanni, 2007; Gerekova *et al.*, 2011). According to Ghulam (2009) sourdough addition resulted in softer bread than control bread without sourdough. Production of organic acids (acetic and lactic acids) during sourdough fermentation helped in the swelling of gluten and increased gas retention which produced products with good texture and increased volume (Park *et al.*, 2007). Plessas *et al.* (2008) reported improvement in texture and palatability of sourdough bread compared to baker's yeast bread. Addition of 20% sourdough is reported to improve crumb softness in wheat bread (Zhang, 2011).

In this study, the *L. plantarum* and *L. brevis* as well as the LAB that are present in the sourdough used in baking the bread and doughnuts are exopolysaccharides (EPS) producers. It has been reported that exopolysaccharides produced by bread associated lactobacilli favourably influenced a host of other technological properties of dough and bread by facilitating water absorption, softening the gluten content of the dough, improving the dough rheology and machinability, increasing specific loaf volume, retarding bread staling and prolonging shelf-life (Ganzle *et al*., 2007, Patel *et al*., 2012, Tamani *et al*., 2013). They are particularly relevant for the textural quality of gluten-free breads that do not contain wheat, rye or barley and thus lack wheat gluten or rye pentosans that are capable of water binding and gas retention at the dough stage (Goulas *et al*., 2004). In situ production of EPS in sourdough was reported to be more effective than external addition (Tieking *et al*., 2003; Arendt *et al*., 2007; Kaditzky and Vogel, 2008; Katina *et al*., 2009). The use of EPS-producing sourdough starters meets the strict requirements of modern baking biotechnology for clean labels and consumer demands for a reduced use of additives (Korakli *et al*., 2002; Di Cagno *et al*., 2006; Rehm, 2010).

The findings of this work agrees with that of Bolourin and Khodaparast, (2010) who reported that 5% sourdough bread is most preferred by the test penalist. It also agrees with the findings of Saeed, (2009) who reported that addition of 5 - 15% sourdoughs containing *L. plantarum* yielded bread with superior odour and taste.

There is no published information on the effect of lactic acid bacteria starter cultures on the organoleptic quality of doughnuts. However, Sharaf *et al*. (2013) reported improved taste and palatability of meatpie when *L. plantarum* and *Leuconostoc diacetylactis* were used as starter cultures compared with control pies baked with only Baker's yeast as starter cultures.

5.11 Shelflife of Bread and Doughnuts

All the lactic acid bacteria and sourdough bread and doughnuts samples had longer minimum mould-free shelflife (MMFSL) than the baker's yeast and the commercial bread and doughnuts samples. Fungal spoilage is the main cause of economic loss in the baking industry and the ability of lactic acid bacteria and sourdough to increase the shelflife of bread is widely reported. Traditionally, chemical preservatives are used to inhibit fungal growth but concerns about environmental pollution and consumer health, along with problems of microbial resistance, favour the demand for alternative methods in controlling the growth of fungi (Druvefors *et al*., 2005; Sadeghi *et al*., 2008). Since LAB are safe for use in foods, they are a significant alternative to chemical preservatives. Several researchers in the area of the bakery industry have successfully added LAB to dough and these strains grew well, producing the desired antifungal compounds in the dough (Rizzello *et al*., 2010).

The findings of this work with respect to the ability *L. plantarum* to improve the shelflife of bread tallies with the findings of many workers in this field. *Lactobacillus plantarum* is reported to have broad antifungal activity (Lavermicocca *et al*., 2000; Dal Bello *et al*., 2007; Ryan *et al*., 2009). A 10-fold concentrated culture filtrate of *L. 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, Aspergilus niger, A. flavus,* and *Fusarium graminearum* (Dalie *et al.,* 2010). Dal Bello *et al*. (2007) reported that addition of *L. plantarum* strains inhibited the outgrowth of *Fusarium* spp. in wheat bread. Gerez *et al*., (2010) reported that combination of *L. plantarum* CRL 778 and calcium propionate (0.4%) increased by 2.6 times the shelf-life of bread compared to bread prepared without LAB. Lavermicocca *et al*. (2000) and Vermeulen *et al*. (2006) reported the ability of *L. plantarum* to produce the antifungal compounds phenyllactic acid and 4-hydroxy compounds. According to Ogunbanwo *et al*. (2008), bread produced using combination of *L. plantarum* and *Saccharomyces cerevisae* as starter culture had shelf life of 12 days while bread produced with chemical preservative (propionic acid at concentration of 3000 ppm) had a shelf life of eight days and bread produced using only baker's yeast had shelflife of four days. *Lactobacillus plantarum* IMAU10014 is also reported to produce antifungal compounds 3-phenyllactic acid and benzeneacetic acid, which have a broad spectrum of antifungal activity, against *Botrytis cinerea*, *Glomerella cingulate*, *Phytophthora drechsleri*, *Penicillium citrinum*, *Penicillium digitatum* and *Fusarium oxysporum* (Wang *et al*., 2012). Bread baked with sourdough fermented with *L. plantarum* showed delayed fungal spoilage for up to one week after inoculation with *A. niger* (Lavermicocca *et al*., 2003). Ryan *et al*. (2008) reduced the use of calcium propionate from 3000 ppm to 1000 ppm when using sourdough fermented with *L. plantarum* FST 1.7 (LP 1.7) and *L. plantarum* FST 1.9 (LP 1.9), in which the growth of *A. niger*, *F. culmorum* and *P. expansum* was delayed for over six days while the growth of *P. roqueforti* appeared after three days of incubation at 30°C.

The ability of *L. brevis* to improve the shelflife of the bakery products in this work is also in agreement with the findings of other workers in this field. Mauch *et al*. (2010) reported that *Lactobacillus brevis* PS1 produces organic acids and proteinaceous compounds which are active against *Fusarium* sp. Four LAB strains (*Lactobacillus brevis* CRL 772, *L. brevis* CRL 796, *L. plantarum* CRL 778, and *L. reuteri* CRL 1100) tested for bread preservation were able to inhibit *Penicillium* sp. growth and lengthen shelf life by twofold in comparison to breads prepared using only *Saccharomyces cerevisiae* (two days shelf life) (Gerez *et al*. 2010).

In this study *L. plantarum* was able to inhibit fungal growth for 4.63 days; this differs from the findings of Lavermicocca *et al*. (2000) who reported a shelflife of seven days but agrees with the findings of this work with respect to 15% sourdough where seven days shelflife was also obtained.

Traditionally sourdough has been used to prevent bread spoilage (Zhang, 2011). The addition of 20% sourdoughs into wheat bread formulations with 0.3 or 0.1% calcium propionate (CP), has been reported to show strong synergistic effect, substantially increasing the shelf life of bread (Rollan *et al*., 2010). According to Zhang, (2011) addition of 10% experimental sourdough, corresponds to 17mM and 74mM propionate and acetate respectively and is able to delay growth of two moulds by one day relative to the control breads.

Gerez *et al*. (2009) reported that the inclusion of antifungal LAB strains (*Lactobacillus plantarum* CRL 778, *Lactobacillus reuteri* CRL 1100, and *Lactobacillus brevis* CRL 772 and CRL 796) allowed for reducing the concentration of calcium propionate by 50% to attain a shelf-life similar to that of traditional bread containing 0.4% calcium propionate. The LAB strains present in this starter had the ability to inhibit *Aspergillus, Fusarium* and *Penicillium*, the major mould genera in bread spoilage.

The ability of *Pediococcus pentosaceus* to contribute to the improvement of the shelflife of bread also tallies with the findings of Dalie *et al*. (2010) who reported that out of a total of 67 isolates of LAB screened for their antifungal activity against *Fusarium proliferatum* and *Fusarium verticillioides*. The most efficient antifungal isolate was identified as *Pediococcus pentosaceus* (L006).

Kim *et al*. (2005) reported that five isolates of lactic acid bacteria identified as *Lactobacillus cruvatus, L. lactis subsp. lactis, L. casei, L. pentosus* and *L. sakei* showed a wide range of antifungal activity against *Aspergillus fumigatus, Aspergillus flavus, Fusarium moniliforme, Penicillium commune* and *Rhizopus oryzae. Lactobacillus pentosus* was among the lactic acid bacteria in the sourdough used in this work.

Several other researchers had also reported increased shelflife of sourdough bread in comparison with bread baked with only yeast (Gobetti *et al.,* 2000; Laitila *et al.,* 2002; Strom *et al.,* 2002; Gobetti *et al.,* 2005; Sagdic *et al*., 2005; Dal Bello *et al.,* 2007; Corsetti *et al.,* 2007; Plessas *et al.,* 2008; Prema *et al.,* 2008; Sadeghi *et al*., 2008a; Saeed, 2009; Wongsuttichote and Nitisinprasert, 2009; Chavan and Chavan, 2011; Kockova *et al*., 2011; Muhialdin and Hassan, 2011; Galle *et al*., 2012; Tamani *et al*., 2013; Zannini *et al.,* 2013).

The antifungal activity of sourdough may be due to a number or metabolites produced by LAB during sourdough development; this include lactic, acetic, propionic, caproic, formic, butyric, n-valeric, phenyllactic, 4-hydroxy-phenyllactic and mono-hydroxy octadecenoic acids (Gobetti *et al.,* 2000; Gobetti *et al*., 2005; Zotta *et al*., 2006; Ghulam, 2009; Zannini *et al.,* 2013), carbon IV oxide, hydrogen peroxide and fungicins reported by Messens and De Vuyst, (2002) and Schn¨urer and Magnusson, (2005), methylhydratoin and mevalonolactone reported by Prema *et al.* (2008), reutericyclin reported by Saeed, (2009), reuterin reported by Zhang (2011), cyclo (L-Phe-traps-4-OH-L-Pro) reported by Wang *et al*. (2012a). The production of reuterin has been reported from *L. brevis, L. buchneri, L. collinoides* and *L.coryniformis* (Claisse and Lonvaud-Funel, 2000; Magnusson *et al*., 2003; Blagojev *et al*., 2012). Among these four lactic acid bacteria isolates, *L. brevis* and *L. collinoides* were part of the LAB flora of the sourdough used in these work and along with *L. platarum* might have contributed to the increased shelflife of the sourdough bread in comparison with the baker's yeast bread.

The results obtained for the counts tallies with that of Saeed (2009) who also reported increase in fungal counts with increase in length of storage and also reported higher fungal counts for the Baker's yeast bread compared to the lactic acid bacteria bread.

There is no published information on the effect of LAB isolates and sourdough application on the shelflife of doughnuts this study shows that sourdough addition improved the shelflife of doughnuts and therefore help bridge this information gap.

5.12 Molecular characterization of the isolates for levansucrase gene (LevV) and glucansucrase gene (gtf)

Seven of the nineteen EPS positive isolates representing (36.8%) were found to be levansucrase gene positive while none of the isolates were glucansucrase gene positive. None of the nineteen isolates was also found to posess both genes. This differs from the findings of Dijkhuizen *et al*. (2009) who reported thirteen out of eighteen (72.2%) isolates to be levansucrase gene positive, five (27.8%) glucansucrase gene positive and nine (50.0%) to posess both levansucrase and glucansucrase genes. It also differs from the findings of De Vuyst *et al*. (2007) who reported 21 out of 174 (12.07%) levansucrase gene positive isolates and 10 out of 174 (5.74%) glucansucrase gene posive isolates. The levansucrase gene was only found in *Lactobacillus* species isolates. This in agreement with the findings of Hijum *et al*. (2004) who reported that *L. reuteri* 121 cultivated on media containing sucrose produced large amounts of both a glucan and a levan. Dijkhuizen *et al*. (1999) further reported that *L. reuteri* 121 is the first *Lactobacillus* species found to possess levansucrase gene. According to Gerekova *et al*. (2011), all relevant EPS in sourdough are homopolysaccharides consisting of identical monosaccharides D-glucose or D-fructose and, on the basis of the constituent saccharide unit they are divided into two major groups: glucans and levans, which significantly influence dough and baked goods properties. The findings of this work in which levan gene is reported in *Lactobacillus plantarum* and *Lactobacillus brevis* difers from that of a number of workers in this field. Levan production has been reported in *Streptococcus* species (Carlson, 1970; Hancock *et al*., 1976; Simms *et al*., 1990), *L. mesenteroides* (Robyt and Walseth, 1979), *L. reuteri* 121 (Van Geel-Schutten *et al.*, 1999, Van Hijum *et al*., 2001) and *Lactobacillus sanfranciscensis* (Korakli *et al*., 2001; Korakli *et al*., 2002). *Lactobacillus frumenti*, *Lactobacillus pontis*, *Lactobacillus panis* and *Weissella confusa* have also been reported to produce levan (Van Hijum *et al*., 2006). Levan production has also been reported in strains of *L. reuteri* LTH5448 and *Weissella cibaria* 10M (Arendt *et al*., 2011). Tieking *et al*. (2005) also reported levansucrase genes in *Lactobacillus mucosae*, *Lactobacillus crispatus* and *Lactobacillus acidophilus*. Korakli *et al*. (2000) reported the production of EPS of the levan type by *Lactobacillus sanfranciscensis* and Korakli *et al*. (2003) have described the ability of several lactobacilli of sourdough and intestinal origin to produce EPS of the levan and glucan types. The frequencies of levan - or glucan - positive strains were highest in the phylogenetically closely related species *L. reuteri*, *L. frumenti*, *L. panis*, and *L. pontis* originating from type II sourdoughs or the intestinal tract (Tieking *et al*., 2003). However, levansucrase gene has been reported in *Lactobacillus plantarum* and *Lactobacillus brevis* in this study; this agrees with the findings of De Vuyst *et al*. (2007b). The findings of this study also agree with that of Wan Aida *et al*. (2012) who reported levan production by lactic acid bacteria isolated from dough. Glucan production has been reported in *Lactobacillus reuteri*, *Lactobacillus fermentum*, *Lactobacillus sakei*, *Lactobacillus parabuchneri*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Leuconostoc lactis*, *Leuconostoc citreum* and *Leuconostoc* *mesenteroides* (Krajl *et al.*, 2003; De Vuyst *et al*., 2007b). However, none of the nineteen isolates screened in this study has the gene for glucan synthesis.

Homopolysaccharides (HoPS) of the levan type are known to have prebiotic properties (Teiking *et al*., 2005). The use of these levansucrase positive *Lactobacillus plantarum* and *Lactobacillus pentosus* as starter cultures for baking may improve consumer's health due to the prebiotic value of levan. Levan has attracted attention for its antitumor properties (Yoo *et al*., 2004), cholesterol-lowering properties and application as an eco-friendly adhesive. Levan also holds promise as biothickener in food industry (Vuyst *et al*., 2001; Patel *et al*., 2012).

A preliminary assessment of the performance in baking applications of reuteran, dextran and levan from lactobacilli provided evidence that EPS effectively improve dough rheological parameters and bread quality (Teiking *et al*., 2003). According to Teiking and Ganzle, (2005) levan produced *in situ* was more effective when compared to externally added levan and addition of one percent (flour base) sucrose to wheat doughs sufficed to induce EPS formation by lactobacilli to effective concentrations.

According to Tieking *et al*. (2003) bacterial levansucrases that have been characterized usually have invertase activity in addition to levansucrase activity and because fructose is used as an electron acceptor by heterofermentative lactobacilli from sourdough, resulting in concomitant production of acetate instead of ethanol, sourdough fermentation by levan - forming strains in the presence of sucrose results in higher acetate contents in the dough. The acetate formed affects sensorial qualities and improves the shelf life of the bread. This partly explains why the *Lactobacillus plantarum* selected and used for baking in this work improved the organoleptic quality and shelflife of the bakery products.

5.13 Sequencing of the Levansucrase Genes (LevV)

The degree of similarity of the seven levansucrase genes carrying isolates *Lactobacillus plantarum* SP2, *Lactobacillus plantarum* SP2$_{40}$, *Lactobacillus plantarum* SP5, *Lactobacillus pentosus* SP3$_{40}$, *Lactobacillus plantarum* SP3$_{40}$, *Lactobacillus plantarum* DG5 and *Lactobacillus plantarum* GP5 to levansucrase genes carrying lactic acid bacteria in the Genbank which include *Lactobacillus plantarum* JDM1 (99%), *Lactobacillus plantarum* 16 (96%), *Lactobacillus plantarum* ZJ316 (95%), *Lactobacillus pentosus* IG1 (81%), *Lactobacillus plantarum* subsp. *plantarum* P-8 (97%), *Lactobacillus plantarum* WCFS1 (95%) and *Lactobacillus plantarum* subsp. *plantarum* ST-III (94%) respectively is a confirmation that the isolates are indeed levansucrase gene carrying isolates and also confirms that they are lactic acid bacteria. This is in agreement with the findings of other workers in this field; De Vuyst *et al*. (2007), Guo *et al*. (2009), Malik, (2012), Bivolarski *et al*. (2013) and Crowley *et al*., 2013. The few differences in nucleotide bases between the isolates used in this study and those in the Genbank may be due to substitution or deletion mutations. *Lactobacillus plantarum* JDM1 and *Lactobacillus plantarum* 16 are reported to have several probiotic functions (Guo *et al*., 2009) and broad-spectrum antifungal activity (Crowley *et al*., 2013) respectively.

5.14 Molecular Phylogenetic Analysis of the Isolates

The molecular Phylogenetic analysis indicates close relationship between the isolates. The isolates are clustered into two main groups and four subgroups. The isolates *L. plantarum* CP001617.1, 18 LEV VREVB066 (*L. plantarum* GP5), 7 LEV VREVF0416 (*L. plantarum* SP5), 1 LEV VREVB04 (*L. plantarum* SP2), 6 LEV VREVE0413 (*L. plantarum* SP3$_{40}$), 5 LEV VREVD04 (*L. plantarum* SP2$_{40}$), *L. plantarum* CP002222.1, 8 LEV VREVG0419 (*L. pentosus* SP3$_{40}$), *L. plantarum* CP0040821, *L. plantarum* CP006033.1 and *L. plantarum*

CP005942.1 are in one cluster while *Lactobacillus plantarum* AL935263.2 and isolate 11LEV VREVA0401 (*L. plantarum* DG5) belong to the other cluster. These clusterings are in agreement with the observations of Canchaya *et al*. (2006). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-1586.4243) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 295 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Findings of this work have established the lactic acid bacteria composition of type II sourdough produced in Nigeria to include *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus pentosus, Pediococcus pentocaseus, Lactobacillus buchneri, Lactobacillus collinoides*, *Lactobacillus fermentum* and *Pediococcus acidilactici*, information that was totally lacking.

The exopolysaccharides production potentials of the lactic acid bacteria isolates have also been established.

The sourdough and selected lactic acid bacteria used in this study have baking potentials with respect to improvement in dough leavening compared to baker's yeast alone.

Findings from this study also imply that spontaneously fermented sourdough could be used for the isolation of starter cultures (*L. brevis, L. fermentum, L. plantarum*) which can be used for baking and cereal fermentations.

The bread and doughnuts produced with the addition of the isolated lactobacilli (*L. brevis,* and *L. plantarum*) were found to be superior in terms of consumer's preference than those produced using only control starter culture (*S. cerevisiae*) and the commercially available ones.

Findings of this study study also imply that *L. brevis*, *L. plantarum*, type II sourdough at 5%, 10% and 15% concentration improved the shelflife of bread and doughnuts. Sourdough technology can therefore be applied to reduce or eliminate the level of preservatives often used in baked products.

Molecular studies on the isolates have revealed that some of the lactic acid bacteria isolates from the type II sourdough produced in Nigeria studied in this work possess the gene for homopolysaccharide (levan) production and the nucleotide sequence of levansucrase genes of these isolates have high degree of similarity to those of other lactic acid bacteria in the Genbank. The isolates are also phylogenetically related.

6.2 Recommendations

The utilization of lactic acid bacteria as starter culture and sourdough in bread and doughnuts production should be encouraged to improve the organoleptic quality and shelflife of bread and doughnuts in Nigeria.

The use of indigenously isolated lactic acid bacteria strains should be promoted for the production of value added bakery products.

Further studies are needed to determine the lactic acid bacteria composition of Nigerian type I and III sourdoughs as well their baking potentials and to characterize their EPS genes.

Further studies are also needed to isolate and characterize lactic acid bacteria from sourdough produce from other type of cereals grains grown in Nigeria, determine their baking potentials and the type of EPS genes the carry.

The sourdough technology should be extended to other baked products consumed in Nigeria such as cakes, meat/fish pies, egg rolls and puff- puff.

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APPENDECES

APPENDIX I: Carbohydrate fermentation profile of the sourdough lactic acid bacteria isolates using API 50 CHL

	Isolates							
Carbohydrate	*L. plantarum*	*L. brevis*	*L. buchneri*	*L. pentosus*	*L. collinoides*	*L. fermentum*	*P. pentosaceus*	*P. acidilactici*
Glycerol	-	-	-	+	-	-	-	-
Erythritol	-	-	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+
L-Xylose	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-
B-Methyly-Xyloside	+	+	+	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-
Mannitol	+	-	-	+	+	+	-	-
Sorbitol	+	-	-	+	?	+	-	-
a-Methyly-D-mannoside	+	-	-	-	-	-	-	-
a-Methyly-D-glucoside	-	-	-	+	-	-	-	-
N-acetyl-glucosamine	+	+	+	+	+	+	+	+

Carbohydrate	*L. plantarum*	*L. brevis*	*L. buchneri*	*L. pentosus*	*L. collinoides*	*L. fermentum*	*P. pentosaceus*	*P. acidilactici*
Amygdalin	+	+	-	+	+	+	?	-
Arbutin	+	+	-	+	-	-	+	+
Esculin	+	+	-	+	+	+	+	+
Salicin	+	+	-	+	?	+	+	+
Cellobiose	+	+	-	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	-
Lactose	+	-	-	+	-	-	+	-
Melibiose	+	+	+	+	+	+	+	-
Sucrose	+	-	+	+	+	+	+	-
Trehalose	+	-	-	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	-
Melezitose	+	-	-	-	-	-	-	-
D-Raffinose	+	-	+	+	+	+	+	-
Starch	-	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-
b-Gentobiose	+	+	-	+	+	+	?	+
D-Turanose	+	-	-	-	-	-	-	-
D-Lyxose	-	-	-	-	-	-	-	-
D-Tagatose	-	+	-	+	+	+	+	+
D-Fucose	-	-	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-	-	-
Gluconate	+	+	+	+	+	+	?	-
2-keto gluconate	-	-	-	-	-	-	-	-
5-keto-gluconate	-	-	-	+	+	+	-	-

APPENDIX II: Leavening ability of the LAB isolates in comparison with baker's yeast

	Leavening Time (min)						
Leavening agent	**30**	**60**	**90**	**120**	**150**	**180**	**210**
L. collinoides	30.00 ± 0.00^{b}	30.50 ± 0.50^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{c}	30.00 ± 0.00^{c}	30.00 ± 0.00^{d}
L. fermentum	30.50 ± 0.50^{b}	33.00 ± 1.00^{c}	32.50 ± 0.50^{cd}	35.00 ± 1.00^{bc}	37.50 ± 0.50^{bc}	40.00 ± 0.00^{b}	45.00 ± 1.00^{b}
L. plantarum	32.00 ± 0.00^{b}	35.50 ± 0.50^{b}	37.00 ± 1.00^{b}	38.50 ± 1.50^{b}	39.50 ± 0.50^{b}	42.00 ± 2.00^{b}	46.00 ± 2.00^{b}
L. pentosus	30.00 ± 0.00^{b}	32.00 ± 0.00^{cd}	34.00 ± 0.00^{c}	38.00 ± 2.00^{b}	39.00 ± 1.00^{bc}	39.00 ± 1.00^{b}	40.00 ± 1.00^{c}
L. brevis	30.50 ± 0.50^{b}	30.50 ± 0.50^{d}	30.50 ± 0.50^{d}	32.50 ± 0.50^{cd}	33.00 ± 1.00^{bc}	33.00 ± 1.00^{c}	32.50 ± 0.50^{d}
Baker's yeast	42.50 ± 1.50^{a}	50.00 ± 1.00^{a}	64.50 ± 1.50^{a}	76.00 ± 2.00^{a}	87.50 ± 6.50^{a}	61.00 ± 1.00^{a}	61.00 ± 0.00^{a}
Control	30.00 ± 0.00^{b}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{c}	30.00 ± 0.00^{c}	30.00 ± 0.00^{d}
ANOVA	53.636	128.121	288.000	160.645	64.475	115.238	141.000
p-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**

All values are mean of duplicate readings. All values in cm^3.
* = significant difference exists at $p \leq 0.05$ while ** = significant difference exists at $p \leq 0.01$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

APPENDIX III: Leavening ability of LAB isolates in combination with baker's yeast compared with baker's yeast alone

	Leavening time (min)						
Leavening agent	**30**	**60**	**90**	**120**	**150**	**180**	**210**
Baker's yeast + L. collinoides	48.00 ± 2.00^{ab}	65.50 ± 2.50^{a}	85.00 ± 1.00^{a}	96.00 ± 2.00^{a}	105.00 ± 1.00^{a}	107.00 ± 2.00^{a}	93.00 ± 3.00^{bc}
Baker's yeast + L. fermentum	47.50 ± 1.50^{b}	63.50 ± 2.50^{a}	80.50. ± 1.50^{a}	90.00 ± 2.00^{bc}	99.00 ± 1.00^{b}	105.50 ± 3.50^{ab}	103.50 ± 1.50^{a}
Baker's yeast + L. plantarum	52.00 ± 1.00^{a}	65.00 ± 1.00^{a}	72.00 ± 1.00^{b}	96.50 ± 2.50^{a}	98.00 ± 1.00^{b}	108.00 ± 2.00^{a}	92.00 ± 4.00^{bc}
Baker's yeast + L. pentosus	39.50 ± 1.50^{c}	42.00 ± 1.00^{c}	72.00 ± 1.00^{b}	88.50 ± 2.50^{c}	99.50 ± 0.50^{b}	100.00 ± 1.00^{b}	96.0- ± 7.00^{ab}
Baker's yeast + L. brevis	40.50 ± 0.50^{c}	53.50 ± 0.50^{b}	62.00 ± 2.00^{c}	89.00 ± 1.00^{c}	103.00 ± 3.00^{ab}	103.00 ± 2.00^{ab}	97.50 ± 1.50^{ab}
Baker's yeast	38.00 ± 1.00^{c}	55.00 ± 2.00^{b}	63.50 ± 2.50^{c}	71.50 ± 1.50^{d}	86.00 ± 2.00^{c}	92.00 ± 1.00^{c}	84.50 ± 3.50^{c}
Control	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{e}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}
ANOVA	36.295	66.142	147.543	164.73	303.862	209.749	95.818
p-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**

All values are mean of duplicate readings. All values in cm^3.

* = significant difference exists at $p \leq 0.05$ while ** = significant difference exists at $p \leq 0.01$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

APPENDIX IV: Leavening ability of sourdough in comparison with Baker's yeast

	Leavening Time (min)						
Leavening agent	**30**	**60**	**90**	**120**	**150**	**180**	**210**
5% Sourdough	30.00 ± 0.00^{b}	30.00 ± 0.00^{c}	30.00 ± 0.00^{c}	30.00 ± 0.00^{b}	31.00 ± 0.00^{c}	31.00 ± 1.00^{cd}	31.00 ± 1.00^{c}
10% Sourdough	30.00 ± 0.00^{b}	30.00 ± 0.00^{c}	30.00 ± 0.00^{c}	31.00 ± 0.00^{b}	32.00 ± 1.00^{bc}	32.00 ± 0.00^{c}	32.00 ± 2.00^{bc}
15% Sourdough	30.00 ± 0.00^{b}	31.00 ± 0.00^{bc}	32.00 ± 0.00^{bc}	32.00 ± 1.00^{b}	32.00 ± 1.00^{bc}	32.00 ± 2.00^{c}	33.50 ± 2.50^{bc}
20% Sourdough	31.00 ± 0.00^{b}	31.00 ± 0.00^{bc}	32.00 ± 0.00^{bc}	33.00 ± 0.00^{b}	33.00 ± 1.00^{bc}	34.00 ± 2.00^{c}	34.00 ± 1.00^{bc}
25% Sourdough	31.00 ± 0.00^{b}	33.00 ± 0.00^{b}	33.00 ± 2.00^{b}	33.00 ± 1.00^{b}	35.00 ± 1.00^{b}	36.00 ± 2.00^{bc}	37.50 ± 1.50^{b}
30% Sourdough	31.00 ± 0.00^{b}	31.00 ± 0.00^{bc}	32.00 ± 0.00^{bc}	34.00 ± 3.00^{b}	34.00 ± 1.00^{bc}	36.50 ± 1.50^{b}	37.50 ± 1.50^{b}
Baker's yeast	41.00 ± 1.00^{a}	55.00 ± 0.00^{a}	67.00 ± 1.00^{a}	78.00 ± 1.00^{a}	84.00 ± 2.00^{a}	84.00 ± 1.00^{a}	73.00 ± 3.00^{a}
Control	30.00 ± 0.00^{b}	30.00 ± 0.00^{c}	31.00 ± 0.00^{bc}	31.00 ± 1.00^{b}	31.00 ± 1.00^{c}	30.50 ± 0.50^{d}	31.00 ± 0.00^{c}
ANOVA	113.714	118.143	254.829	163.824	266.057	159.134	61.779
p-value	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**

All values are mean of duplicate readings. All values in cm^3.
* = significant difference exists at $p \leq 0.05$ while ** = significant difference exists at $p \leq 0.01$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

APPENDIX V: Leavening capacity of combination of sourdough and baker's yeast in comparison with baker's yeast alone

	Leavening Time (min)						
Leavening agent	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**
Baker's yeast + 5% sourdough	41.50 ± 1.50^{c}	55.50 ± 0.50^{a}	65.00 ± 1.00^{b}	77.50 ± 1.50^{bc}	90.00 ± 2.00^{ab}	88.00 ± 4.00^{c}	75.00 ± 3.00^{c}
Baker's yeast + 10% sourdough	42.50 ± 0.50^{bc}	55.00 ± 1.00^{a}	70.00 ± 2.00^{ab}	75.00 ± 2.00^{c}	86.00 ± 2.00^{b}	94.50 ± 1.50^{bc}	85.00 ± 3.00^{b}
Baker's yeast + 15% sourdough	42.00 ± 1.00^{c}	56.00 ± 1.00^{a}	70.00 ± 4.00^{ab}	78.00 ± 2.00^{bc}	86.50 ± 1.50^{b}	97.50 ± 1.50^{ab}	92.00 ± 2.00^{ab}
Baker's yeast + 20% sourdough	45.00 ± 1.00^{bc}	57.00 ± 3.00^{a}	73.00 ± 3.00^{a}	83.00 ± 2.00^{ab}	88.00 ± 1.00^{b}	97.50 ± 0.50^{ab}	92.50 ± 3.50^{ab}
Baker's yeast + 25% sourdough	48.00 ± 3.00^{ab}	58.00 ± 2.00^{a}	72.00 ± 1.00^{ab}	82.00 ± 3.00^{ab}	94.00 ± 4.00^{ab}	101.50 ± 2.50^{ab}	94.50 ± 0.50^{a}
Baker's yeast + 30% sourdough	51.00 ± 2.00^{a}	56.50 ± 2.50^{a}	73.00 ± 2.50^{a}	87.00 ± 1.00^{a}	98.00 ± 2.00^{a}	104.50 ± 0.50^{a}	95.50 ± 2.50^{a}
Baker's yeast	41.50 ± 2.50^{c}	58.50 ± 0.50^{a}	76.00 ± 2.00^{a}	82.00 ± 1.00^{ab}	97.00 ± 4.00^{a}	101.00 ± 3.00^{ab}	92.00 ± 1.00^{ab}
Control	30.00 ± 0.00^{d}	30.00 ± 0.00^{b}	30.00 ± 0.00^{c}	30.00 ± 0.00^{d}	30.00 ± 0.00^{c}	30.00 ± 0.00^{d}	30.00 ± 0.00^{d}
ANOVA	12.847	33.154	43.497	106.024	83.265	132.302	93.364
p-value	0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**

All values are mean of duplicate readings. All values in cm^{3}.
* = significant difference exists at $p \leq 0.05$ while ** = significant difference exists at $p \leq 0.01$. Mean values with different superscripts in the same column are significantly different. Means values were separated using Duncan multiple range test.

APPENDIX VI: Nucleotide sequence of the lactic acid bacteria isolates levansucrase genes (LevV)

Nucleotide sequence of *Lactobacillus plantarum* SP2 levansucrase gene (LevV) 477bp

TMCTKGTCTYCTTGGTATCTGTACCATTCTCTACGTGGCTGYATCAGCCATCATG
ACTGGCGTGGTCCCATTTGAAACGTTTGCCAAGTACATTGATCATCCGATCTCGG
CCGTTCTTATTTACTCCGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGC
GATTCTTGGCATGACCACTGTAATGTTAGTCTGTCTCTACGGTCAAACTCGGATTT
CCTTCTCCATGTCGCGGGATGGATTACTCCCCCATGTCTTTAGCGATATTAGCGCT
AAGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGTCGATTGCCGCAATCA
TGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAACTGGTGAATATCGGCACTTT
GACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGCTACGTAAAACACAACCA
GATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCCATACGTC

Nucleotide sequence of *Lactobacillus plantarum* SP2$_{40}$ levansucrase gene (LevV) 471bp

TTTYYTTGGCRWTCTGTACCATWCTCTACGTGGCTGTATCAGYCATCATGACTGG
CGTGGWCCATTARAAAMGKTTGCCAAGTACATTGATCATCCAATCTCGGCCGTT
CTTAKTTACTCYGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGATTC
TTGGTATGACCACTGTAATGTTAGTCTGTCTCTACGGTCAAACTCGGATTTCCTTC
TCCATGTCSCGGGATGGATTACTCCCCCATGTCTTTAGCGATATTAGCGCYAAGA
CCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGTCGATTGCCGCAATCATGGG
TGGCTTKATTCCATTAGCTGACTTAGCTGAACTGGTGAATATCGGTACTTTGACT
GCCTTTACCCTAGTTTCCTTCTCMATCTTACGGKTACGTAAAACACAACCAGATC
TTCGGCGTCCCTTCAAAACGCCATGTGTCCCATACGTC

Nucleotide sequence of *Lactobacillus plantarum* SP3$_{40}$ levansucrase gene (LevV) 475bp

TMKKCYCTCTTGGWATCTGTACCATTCTCTACGTGGCTGTATCAGCCATCATGAC
TGGCGTGGTCCCATTTRAAACGTTTGCCAAGTACATTGATCATCCAATCTCGGCC
GTTCTTAGTTACTCTGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGA
TTCTTGGTATGACCACTGTAATGTTAGTCTGTCTCTACGGTCAAACTCGGATTTCC
TTCTCCATGTCGCGGGATGGATTACTCCCCCATGTCTTTAGCGATATTAGCGCTA
AGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGTCGATTGCCGCAATCAT
GGGTGGCTTTATTCCATTAGCTGACTTAGCTGAACTGGTGAATATCGGTACTTTG
ACTGCCTTTACCCTAGTTTCCTTCTCAATCTTACGGTTACGTAAAACACAACCAG
ATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCCATACGT

Nucleotide sequence of *Lactobacillus plantarum* SP5 levansucrase gene (LevV) 380bp

AWMMKGYCTCTTGGWAWCTGTAMMATTCTCTACGTGGCTGYATCAGCCATCAT
GACTGGCGTGGTCCCATTTGAAACGTTTGCCAAGTACATTGATCATCCGATCTCG
GCCGTTCTTATTTACTCCGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCG
CGATTCTTGGCATGACCACTGTAATGTTAGTCTGTCTCTACGGTCAAACTCGGAT
TTCCTTCTCCATGTCGCGGGATGGATTACTCCCCCATGTCTTTAGCGATATTAGCG
CTAAGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGTCGATTGCCGCAAT
CATGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAACTGGTGAATATCGGCACT
TTGACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGCTACGTAAAACACAACC
AGATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCCATACGTCA

Nucleotide sequence of *Lactobacillus pentosus* SP3$_{40}$ levansucrase gene (LevV) 483bp

TAMWKGTCTCTTGGWATCTGTACCATTCTCTACGTGGCTSCTATCAGCCATCATG
ACTGGCGTGGTCCCATTTRAAACGTTTGCCAAGTACATTGATCATCCGATCTCGG
CCGTTCTTATTTACTCCGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGC
GATTCTTGGTATGACCACTGTAATGTTAGTCTGTCTCTACGGTCAAACTCGGATTT
CCTTCTCCATGTCGCGGGATGGATTACTCCCCCATGTCTTTAGCGATATTAGCGCC
AAGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGTCGATTGCCGCAATCA
TGGGTGGCTTGATTCCATTAGCTGACTTAGCTGAACTGGTGAATATCGGTACTTT
GACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGTTACGTAAAACACAACCAG
ATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCCATACGTCA

Nucleotide sequence of *Lactobacillus plantarum* DG5 levansucrase gene (LevV) 377bp

AWCAWATKGYTCCCMTCCAGTGCATCKACAGGTGATCATCCGTCRACCGCCCGG
CCATTTGTAAATATATCGACTCAAGTGATTGTTGCGGATGTTGCGCTAGTAATTG
YYCAACCSAACCGGTATAAATMAATTGCCCCTGCTTCAAAATTGCCAATCGGTCA
CATAACTGCTGCGCCGTATCCAGATTGTGGGTGGAAAAAATGACCGTCTTACCTT
TGGCGGCATGGGCCTTCATCAAATTTTTCAAATCAAAAGSAGCTTGGGGGTCTAA
CCCTTGCAGCGGCTCGTCTAAAATCCAAATATCGGGGTCGGGAAGCAACGCCCC
AATCAAAATAGCTTTTTGCCGCATCCCGTGTGAATAACTGGCCATGTGTCCCATA
CGTCACTGSC

Nucleotide sequence of *Lactobacillus plantarum* GP5 levansucrase gene (LevV) 477bp

TAMYGCCCTYTTGCSKATCYGTACCATTCTCTACKTGGCTGTATMASCCATCATG
ACTGGMGTGGWCCCATTTGAAACGTTTGCCAAGTACATTGATCATCCGATCTCG
GCCGTTCTTATTTACTCCGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCG
CGATTCTTGGCATGACCACTGTAATGTTAKTCTGTCTCTACGGTCAAACTCGGAT
TTCCTTCTCCATGTCGCGGGATGGATTACTTCCCCATGTCTTTAGCGATATTAKCG
CTAASACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGTTGATTGCCGCAWT
CATGGGKGGMTTTATTCCATTARCTGACTTAGCTGAACTGGYGAATATCSSCACT
TTGACTGCCTTTACCCTAGTTTCCTTCTCAATCTTACGGSTACGTAAAACACAACC
AGATCTTCGGCGTCCTTTCAAAACGCCATGTGTCCCATACGTCARAAWSMGWRC
CCCATACGTCA

APPENDIX VII: BLAST output for *Lactobacillus plantarum* SP2

Lactobacillus plantarum JDM1, complete genome
Sequence ID: gi|254044096|gb|CP001617.1|Length: 3197759Number of Matches: 1
Related Information
Range 1: 2835212 to 2835678GenBankGraphics

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
877 bits(453)	0.0	462/467(99%)	0/467(0%)	Plus/Plus

Features:
amino acid transport protein

```
Query  11        CTTGGTATCTGTACCATTCTCTACGTGGCTGYATCAGCCATCATGACTGGCGTGGTCCCA  70
                 ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  2835212   CTTGGTATCTGTACCATTCTCTACGTGGCTGTATCAGCCATCATGACTGGCGTGGTCCCA
2835271
Query  71        TTTGAAACGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTCCGGT  130
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2835272   TTTGAAACGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTCCGGT
2835331
Query  131       CAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGCATGACCACTGTAATG  190
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2835332   CAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGCATGACCACTGTAATG
2835391
Query  191       TTAGTCTGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTACTC  250
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct  2835392   TTAGTCTGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTACTT
2835451
Query  251       CCCCATGTCTTTAGCGATATTAGCGCTAAGACCGGTGCACCACTTAAATCAACCGTCTTG  310
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2835452   CCCCATGTCTTTAGCGATATTAGCGCTAAGACCGGTGCACCACTTAAATCAACCGTCTTG
2835511
Query  311       TTTGGGTCGATTGCCGCAATCATGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAACTG  370
                 ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2835512   TTTGGGTTGATTGCCGCAATCATGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAACTG
2835571
Query  371       GTGAATATCGGCACTTTGACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGCTACGT  430
                 |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  2835572   GTGAATATCGGCACTTTGACTGCCTTTACCCTAGTTTCCTTCTCAATCTTACGGCTACGT
2835631
Query  431       AAAACACAACCAGATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCC  477
                 ||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  2835632   AAAACACAACCAGATCTTCGGCGTCCCTTCAAAACGCCATGGGTCCC  2835678
```

APPENDIX VIII: BLAST output for *Lactobacillus plantarum* SP2$_{40}$

Lactobacillus plantarum 16, complete genome
Sequence ID: gb|CP006033.1|Length: 3044678Number of Matches: 1
Related Information
Range 1: 2758992 to 2759451GenBankGraphics

Score	**Expect**	**Identities**	**Gaps**	**Strand**
773 bits(856)	0.0	443/460(96%)	1/460(0%)	Plus/Plus

Features:
amino acid transport protein

```
Query  13       TCTGTACCATWCTCTACGTGGCTGTATCAGYCATCATGACTGGCGTGGWCC-ATTARAAA  71
                |||||||||| ||||||||||||||||||| ||||||||||||||||| || |||  |||
Sbjct  2758992  TCTGTACCATTCTCTACGTGGCTGTATCAGCCATCATGACTGGCGTGGTCCCATTTGAAA
2759051
Query  72       MGKTTGCCAAGTACATTGATCATCCAATCTCGGCCGTTCTTAKTTACTCYGGTCAAAATT  131
                 | |||||||||||||||||||||| |||||||||||||||| |||||| ||||||||||
Sbjct  2759052  CGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTCCGGTCAAAATT
2759111
Query  132      GGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGTATGACCACTGTAATGTTAGTCT  191
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2759112  GGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGTATGACCACTGTAATGTTAGTCT
2759171
Query  192      GTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCSCGGGATGGATTACTCCCCCATG  251
                ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  2759172  GTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTACTCCCCCATG
2759231
Query  252      TCTTTAGCGATATTAGCGCYAAGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGT  311
                ||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  2759232  TCTTTAGCGATATTAGCGCCAAGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGGT
2759291
Query  312      CGATTGCCGCAATCATGGGTGGCTTKATTCCATTAGCTGACTTAGCTGAACTGGTGAATA  371
                ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  2759292  CGATTGCCGCAATCATGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAACTGGTGAATA
2759351
Query  372      TCGGTACTTTGACTGCCTTTACCCTAGTTTCCTTCTCMATCTTACGGKTACGTAAAACAC  431
                ||||||||||||||||||||||||||||||||||||| ||||||||| ||||||||||||
Sbjct  2759352  TCGGTACTTTGACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGTTACGTAAAACAC
2759411
Query  432      AACCAGATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCC  471
                |||||||||||||||||||||||||||||||||| |||||
Sbjct  2759412  AACCAGATCTTCGGCGTCCCTTCAAAACGCCATGGGTCCC  2759451
```

APPENDIX IX: BLAST output for *Lactobacillus plantarum* SP5

Lactobacillus plantarum ZJ316, complete genome

Sequence ID: gb|CP004082.1|Length: 3203964Number of Matches: 1

Related Information
Range 1: 1709132 to 1709500GenBankGraphics

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
600 bits(664)	7e-168	352/369(95%)	1/369(0%)	Plus/Minus

Features:
ABC transporter, ATP-binding protein

```
Query  13       CCMTCCAGTGCATCKACA-GGTGATCATCCGTCRACCGCCCGGCCATTTGTAAATATATC  71
                || ||||| ||||| ||| |||||||||||||| ||||||||||||||||||||||||||
Sbjct  1709500  CCCTCCAGCGCATCGACAAGGTGATCATCCGTCAACCGCCCGGCCATTTGTAAATATATC
1709441
Query  72       GACTCAAGTGATTGTTGCGGATGTTGCGCTAGTAATTGYYCAACCSAACCGGTATAAATM  131
                | |||||||||||||||||||||||| |||||||||||  ||||| ||||||||||||| 
Sbjct  1709440  GCCTCAAGTGATTGTTGCGGATGTTGAGCTAGTAATTGTTCAACCGAACCGGTATAAATC
1709381
Query  132      AATTGCCCCTGCTTCAAAATTGCCAATCGGTCACATAACTGCTGCGCCGTATCCAGATTG  191
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1709380  AATTGCCCCTGCTTCAAAATTGCCAATCGGTCACATAACTGCTGCGCCGTATCCAGATTG
1709321
Query  192      TGGGTGGAAAAAAATGACCGTCTTACCTTTGGCGGCATGGGCCTTCATCAAATTTTTCAAA  251
                |||||||||||||||||||||||| |||||||||||||| ||||||||||||||||||||
Sbjct  1709320  TGGGTGGAAAAAAATGACCGTCTTGCCTTTGGCGGCATGAGCCTTCATCAAATTTTTCAAA
1709261
Query  252      TCAAAAGSAGCTTGGGGGTCTAACCCTTGCAGCGGCTCGTCTAAAATCCAAATATCGGGG  311
                ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1709260  TCAAAAGCAGCTTGGGGGTCTAACCCTTGCAGCGGCTCGTCTAAAATCCAAATATCGGGG
1709201
Query  312      TCGGGAAGCAACGCCCCAATCAAAATAGCTTTTTGCCGCATCCCGTGTGAATAACTGGCC  371
                |||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct  1709200  TCGGGAAGCAACGCCCCAATCAAAATGGCTTTTTGCCGCATCCCGTGTGAATAACTGGCC
1709141
Query  372      ATGTGTCCC  380
                ||| || ||
Sbjct  1709140  ATGGGTTCC  1709132
```

APPENDIX X: BLAST output for *Lactobacillus pentosus* SP3$_{40}$

Lactobacillus pentosus IG1, annotated genomic scaffold00001

Sequence ID: emb|FR874854.1|Length: 3687424Number of Matches: 1

Related Information
Range 1: 2307401 to 2307876GenBankGraphics

Score	Expect	Identities	Gaps	Strand
457 bits(506)	7e-125	387/477(81%)	1/477(0%)	Plus/Minus

Features:
amino acid transport protein

```
Query  7         TCTCTTGGWATCTGTACCATTCTCTACGTGGCTSCTATCAGCCATCATGACTGGCGTGGT  66
                 || || || ||||||||| | |||||||| ||   | || || || ||||| ||||||||
Sbjct  2307876   TCACTAGGAATCTGTACCGTGCTCTACGTCGCCGTT-TCTGCGATTATGACCGGCGTGGT
2307818
Query  67        CCCATTTRAAACGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTC  126
                  || ||| |||| || || || ||||| || ||||| ||||| |||||  | || |||||
Sbjct  2307817   GCCTTTTGAAACTTTCGCGAAATACATCGACCATCCAATCTCAGCCGTCTTGATCTACTC
2307758
Query  127       CGGTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGTATGACCACTGT  186
                  ||||||||||||||||| || ||||| ||| | ||||| |||||||| |||||||| ||
Sbjct  2307757   TGGTCAAAATTGGATGGCGGGTATCATCGACTTGGGCGCCATTCTTGGGATGACCACCGT
2307698
Query  187       AATGTTAGTCTGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATT  246
                  ||||| ||||| || || ||||||||||| || || ||||| ||||| ||||| ||  |
Sbjct  2307697   TATGTTGGTCTGCCTGTATGGTCAAACTCGTATCTCGTTCTCGATGTCACGGGACGGCCT
2307638
Query  247       ACTCCCCCATGTCTTTAGCGATATTAGCGCCAAGACCGGTGCACCACTTAAATCAACCGT  306
                 | | || || ||||| ||||| || ||||| |||||||| |||||||| |||||||||||
Sbjct  2307637   ATTACCACACGTCTTCAGCGACATCAGCGCGAAGACCGGAGCACCACTCAAATCAACCGT
2307578
Query  307       CTTGTTTGGGTCGATTGCCGCAATCATGGGTGGCTTGATTCCATTAGCTGACTTAGCTGA  366
                 ||| |||||  |||||||||||||||||||||| || ||||| ||||||||| |||| ||
Sbjct  2307577   CTTATTTGGAACGATTGCCGCAATCATGGGTGGGTTCATTCCGTTAGCTGACCTAGCAGA
2307518
Query  367       ACTGGTGAATATCGGTACTTTGACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGTT  426
                 | |||| || ||||| ||||| || ||||||||||||||||| ||||||||||| ||| |
Sbjct  2307517   ATTGGTCAACATCGGGACTTTAACGGCCTTTACCCTAGTTTCGTTCTCCATCTTGCGGCT
2307458
Query  427       ACGTAAAACACAACCAGATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCCATACGT  483
                 ||||||||| ||||||||| | ||||| || ||||| || || || || || | |||
Sbjct  2307457   ACGTAAAACGCAACCAGATTTGCGGCGCCCATTCAAGACACCCTGGGTTCCGTTCGT  2307401
```

APPENDIX XI: BLAST output for *Lactobacillus plantarum* SP3$_{40}$

Lactobacillus plantarum subsp. *plantarum* P-8, complete genome
Sequence ID: gb|CP005942.1|Length: 3033566Number of Matches: 1
Related Information
Range 1: 2740959 to 2741427

Score	Expect	Identities	Gaps	Strand
814 bits(902)	0.0	461/469(98%)	0/469(0%)	Plus/Plus

Features:
APC family amino acid-polyamine-organocation transporter

```
Query  7        CTCTTGGWATCTGTACCATTCTCTACGTGGCTGTATCAGCCATCATGACTGGCGTGGTCC  66
                ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2740959  CTCTTGGTATCTGTACCATTCTCTACGTGGCTGTATCAGCCATCATGACTGGCGTGGTCC
2741018

Query  67       CATTTRAAACGTTTGCCAAGTACATTGATCATCCAATCTCGGCCGTTCTTAGTTACTCTG  126
                ||||| |||||||||||||||||||||||||||| |||||||||||||||| |||||| |
Sbjct  2741019  CATTTGAAACGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTCCG
2741078

Query  127      GTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGTATGACCACTGTAA  186
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2741079  GTCAAAATTGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGTATGACCACTGTAA
2741138

Query  187      TGTTAGTCTGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTAC  246
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2741139  TGTTAGTCTGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTAC
2741198

Query  247      TCCCCCATGTCTTTAGCGATATTAGCGCTAAGACCGGTGCACCACTTAAATCAACCGTCT  306
                |||||||||||||||||||||||||||| |||||||||||||||||||||||||||||||
Sbjct  2741199  TCCCCCATGTCTTTAGCGATATTAGCGCCAAGACCGGTGCACCACTTAAATCAACCGTCT
2741258

Query  307      TGTTTGGGTCGATTGCCGCAATCATGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAAC  366
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2741259  TGTTTGGGTCGATTGCCGCAATCATGGGTGGCTTTATTCCATTAGCTGACTTAGCTGAAC
2741318

Query  367      TGGTGAATATCGGTACTTTGACTGCCTTTACCCTAGTTTCCTTCTCAATCTTACGGTTAC  426
                |||||||||||||||||||||||||||||||||||||||||||||| |||||||||||||
Sbjct  2741319  TGGTGAATATCGGTACTTTGACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGTTAC
2741378

Query  427      GTAAAACACAACCAGATCTTCGGCGTCCCTTCAAAACGCCATGTGTCCC  475
                ||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  2741379  GTAAAACACAACCAGATCTTCGGCGTCCCTTCAAAACGCCATGGGTCCC  2741427
```

APPENDIX XII: BLAST output for *Lactobacillus plantarum* DG5

Lactobacillus plantarum WCFS1 complete genome

Sequence ID: emb|AL935263.2|Length: 3308273Number of Matches: 1
Related Information
Range 1: 1504338 to 1504703GenBankGraphics

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
585 bits(648)	2e-163	347/366(95%)	1/366(0%)	Plus/Minus

Features:
ABC transporter, ATP-binding protein

```
Query  13       CCMTCCAGTGCATCKACA-GGTGATCATCCGTCRACCGCCCGGCCATTTGTAAATATATC  71
                || ||||| ||||| ||| |||||||||||||| ||||||||||||||||||||||||||
Sbjct  1504703  CCCTCCAGCGCATCGACAAGGTGATCATCCGTCAACCGCCCGGCCATTTGTAAATATATC
1504644

Query  72       GACTCAAGTGATTGTTGCGGATGTTGCGCTAGTAATTGYYCAACCSAACCGGTATAAATM  131
                | |||||||||||||||||||||||| |||||||||||  ||||| |||||||||||||
Sbjct  1504643  GCCTCAAGTGATTGTTGCGGATGTTGAGCTAGTAATTGTTCAACCGAACCGGTATAAATC
1504584

Query  132      AATTGCCCCTGCTTCAAAATTGCCAATCGGTCACATAACTGCTGCGCCGTATCCAGATTG  191
                ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  1504583  AATTGCCCCTGCTTCAAAATTGCTAATCGGTCACATAACTGCTGCGCCGTATCCAGATTG
1504524

Query  192      TGGGTGGAAAAAATGACCGTCTTACCTTTGGCGGCATGGGCCTTCATCAAATTTTTCAAA  251
                ||||||||||||||||||||||| |||||||| ||||||||||||||||||||||| |||
Sbjct  1504523  TGGGTGGAAAAAATGACCGTCTTGCCTTTGGCAGCATGGGCCTTCATCAAATTTTTTAAA
1504464

Query  252      TCAAAAGSAGCTTGGGGGTCTAACCCTTGCAGCGGCTCGTCTAAAATCCAAATATCGGGG  311
                ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1504463  TCAAAAGCAGCTTGGGGGTCTAACCCTTGCAGCGGCTCGTCTAAAATCCAAATATCGGGG
1504404

Query  312      TCGGGAAGCAACGCCCCAATCAAAATAGCTTTTTGCCGCATCCCGTGTGAATAACTGGCC  371
                |||||||||||||||||||||||||| ||||||||||||||||||||||||||||| |||
Sbjct  1504403  TCGGGAAGCAACGCCCCAATCAAAATGGCTTTTTGCCGCATCCCGTGTGAATAACTAGCC
1504344

Query  372      ATGTGT  377
                ||| ||
Sbjct  1504343  ATGGGT  1504338
```

APPENDIX XIII: BLAST output for *Lactobacillus plantarum* GP5

Lactobacillus plantarum subsp. *plantarum* ST-III, complete genome
Sequence ID: gb|CP002222.1|Length: 3254376Number of Matches: 1
Related Information
Range 1: 2893055 to 2893515

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
744 bits(824)	0.0	435/461(94%)	0/461(0%)	Plus/Plus

Features:
amino acid permease

```
Query  17       ATCYGTACCATTCTCTACKTGGCTGTATMASCCATCATGACTGGMGTGGWCCCATTTGAA  76
                ||| |||||||||||||| ||||||||| | ||||||||||||| |||| ||||||||||
Sbjct  2893055  ATCTGTACCATTCTCTACGTGGCTGTATCAGCCATCATGACTGGCGTGGTCCCATTTGAA  
2893114

Query  77       ACGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTCCGGTCAAAAT  136
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2893115  ACGTTTGCCAAGTACATTGATCATCCGATCTCGGCCGTTCTTATTTACTCCGGTCAAAAT  
2893174

Query  137      TGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGCATGACCACTGTAATGTTAKTC  196
                |||||||||||||||||||||||||||||||||||||| |||||||||||||||||| ||
Sbjct  2893175  TGGATGGCCGGAATCATTGACCTTGGCGCGATTCTTGGTATGACCACTGTAATGTTAGTC  
2893234

Query  197      TGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTACTTCCCCAT  256
                ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  2893235  TGTCTCTACGGTCAAACTCGGATTTCCTTCTCCATGTCGCGGGATGGATTACTCCCCCAT  
2893294

Query  257      GTCTTTAGCGATATTAKCGCTAASACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGG  316
                |||||||||||||||| ||| || ||||||||||||||||||||||||||||||||||||
Sbjct  2893295  GTCTTTAGCGATATTAGCGCCAAGACCGGTGCACCACTTAAATCAACCGTCTTGTTTGGG  
2893354

Query  317      TTGATTGCCGCAWTCATGGGKGGMTTTATTCCATTARCTGACTTAGCTGAACTGGYGAAT  376
                | |||||||||| ||||||| || || ||||||||| |||||||||||||||||| ||||
Sbjct  2893355  TCGATTGCCGCAATCATGGGTGGCTTGATTCCATTAGCTGACTTAGCTGAACTGGTGAAT  
2893414

Query  377      ATCSSCACTTTGACTGCCTTTACCCTAGTTTCCTTCTCAATCTTACGGSTACGTAAAACA  436
                |||  ||||||||||||||||||||||||||||||||| ||||||||| |||||||||||
Sbjct  2893415  ATCGGTACTTTGACTGCCTTTACCCTAGTTTCCTTCTCCATCTTACGGTTACGTAAAACA  
2893474

Query  437      CAACCAGATCTTCGGCGTCCTTTCAAAACGCCATGTGTCCC  477
                |||||||||||||||||||| |||||||||||||| |||||
Sbjct  2893475  CAACCAGATCTTCGGCGTCCCTTCAAAACGCCATGGGTCCC  2893515
```

APPENDIX XIV: Plates

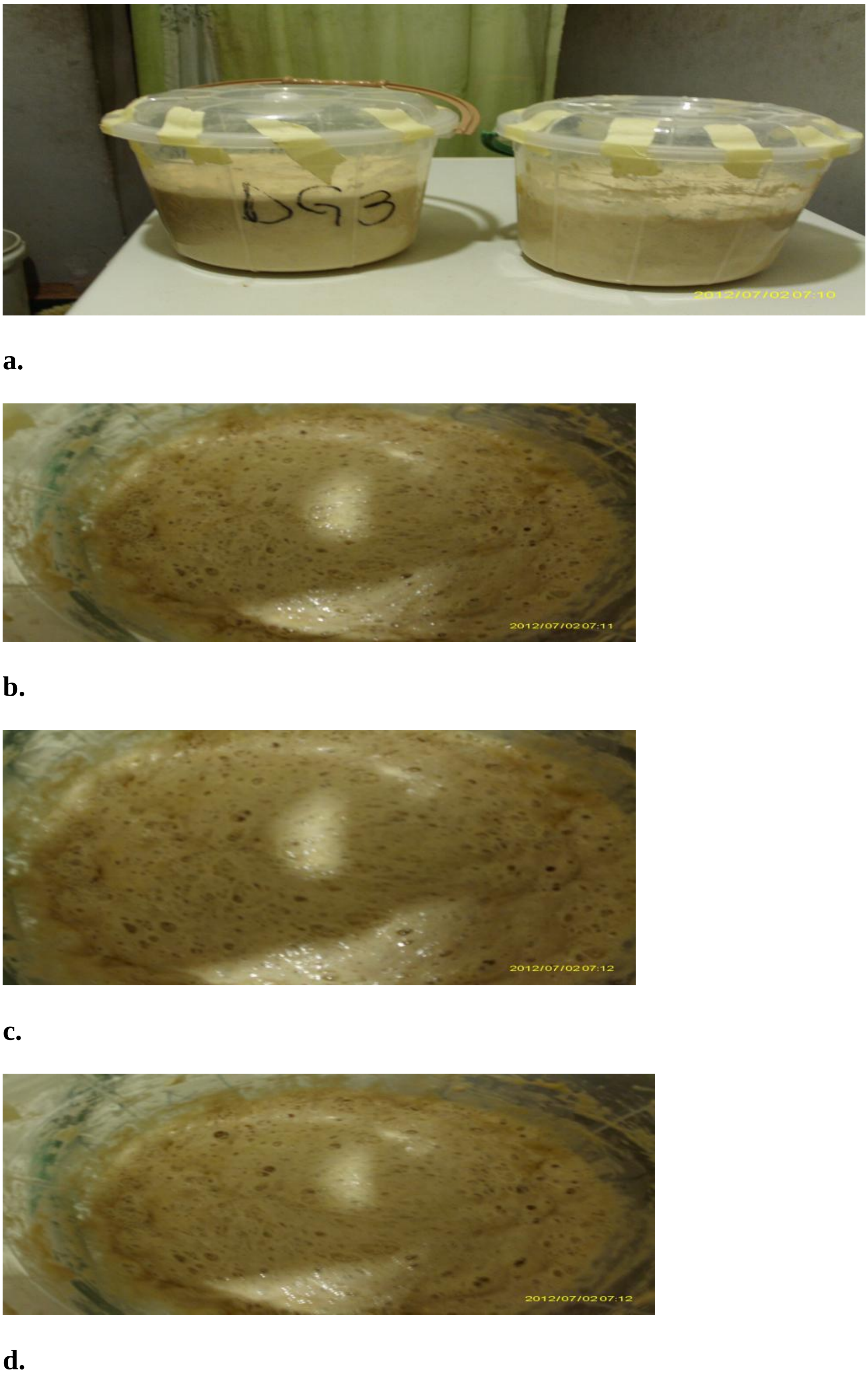


PLATE II: (a) Fermentation of dough (b) Flour A sourdough (c) Flour B sourdough (d) Flour C sourdough

APPENDIX XV

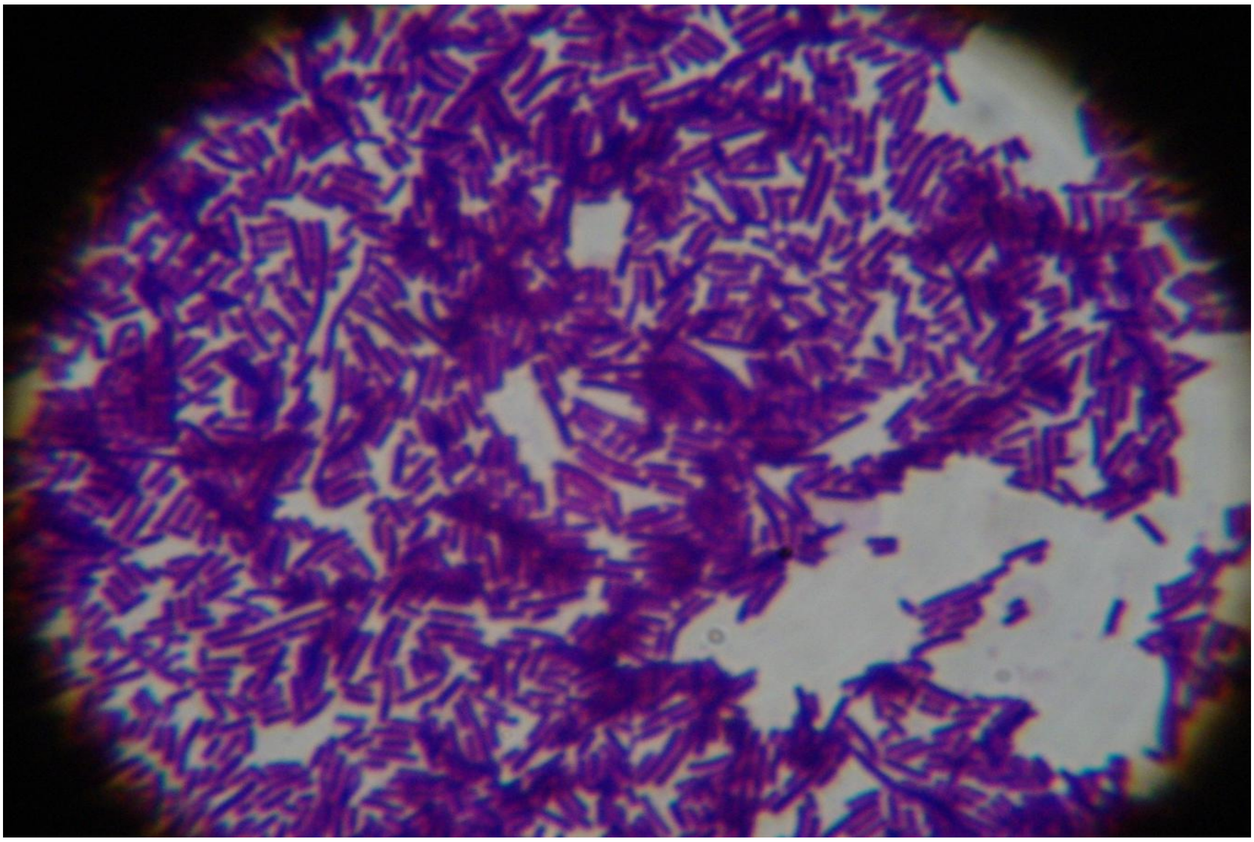


Plate III: Microscopic view of *Lactobacillus plantarum* isolated from the dough

APPENDIX XVI

2011/01/05 06:50

Plate IV: Mucoid colonies of EPS positive *Lactobacillus plantarum*

APPENDIX XVII

Plate V: Mucoid colonies of EPS positive *Lactobacillus pentosus*

APPENDIX XVIII

Plate VI: Mucoid colonies of EPS positive *Lactobacillus brevis*

APPENDIX XIX

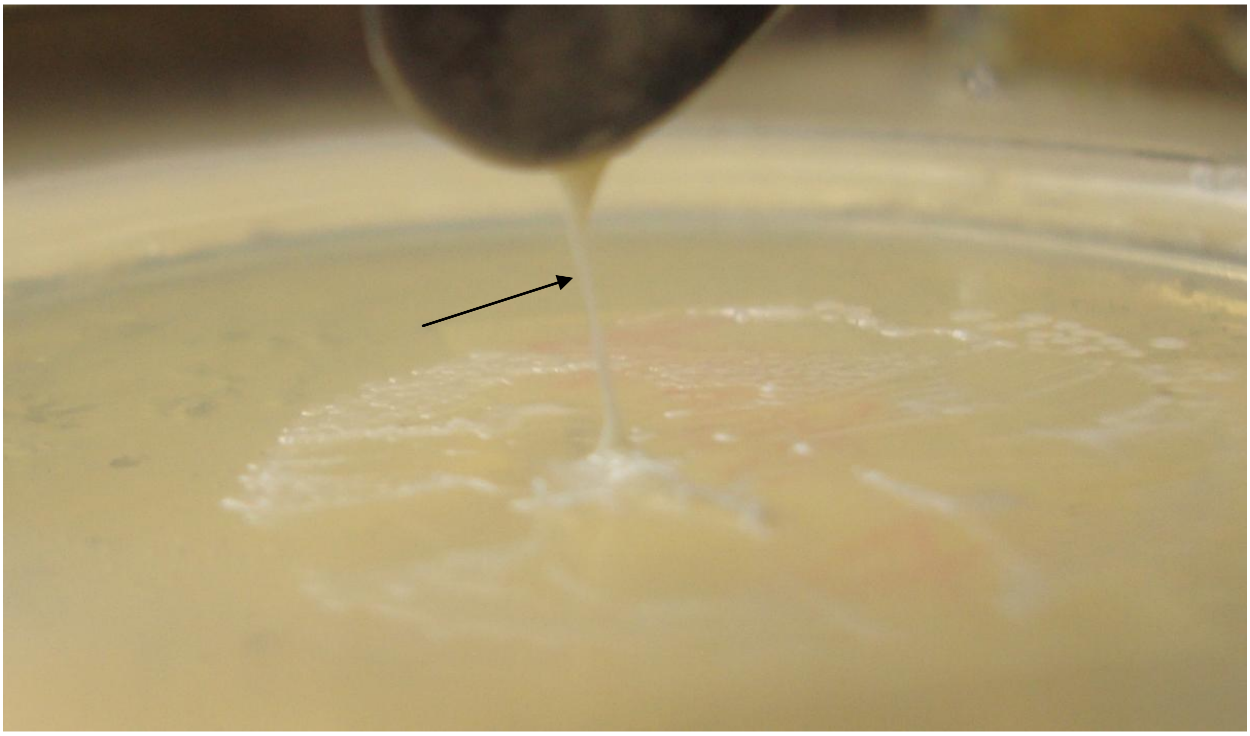


Plate VII: Long strand of EPS positive *Lactobacillus plantarum*

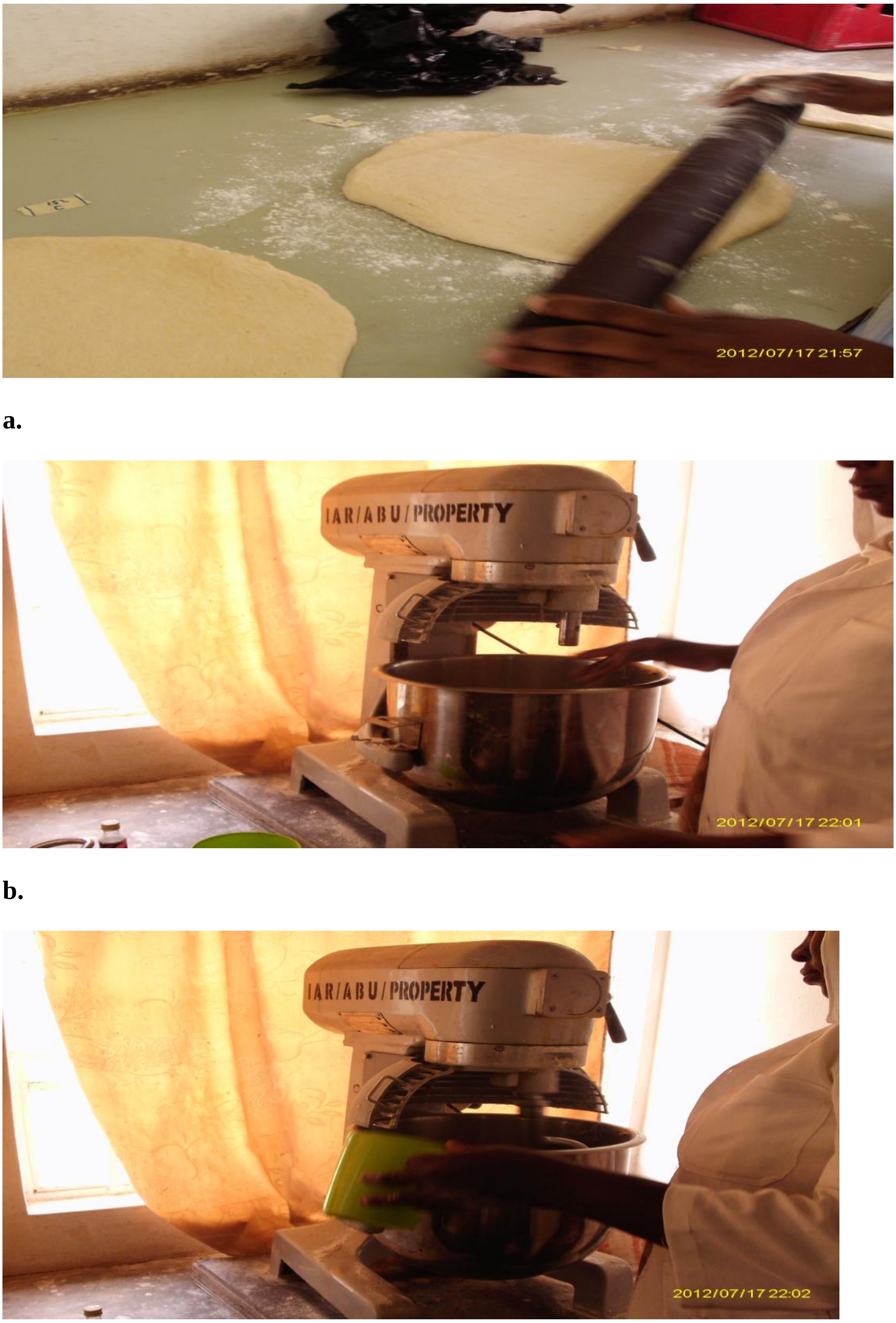
APPENDIX XX

Plate VIII: Inoculated API 50 CH medium before incubation

APPENDIX XXI

Plate IX: API 50 CH medium after incubation

APPENDIX XXII



a.

b.

c.

PLATE X: (a), (b) & (c) Mixing of dough for bread and doughnuts production

APPENDIX XXIII

a.

b.

c.

PLATE XI: (a) 5% Sourdough bread (b) 10% Sourdough bread (c) 15% Sourdough bread

APPENDIX XXIV

a.

b.

c.

PLATE XII: (a) *Lactobacillus brevis* bread (b) *Lactobacillus plantarum* bread (c) Bakers' yeast bread

APPENDIX XXV

a.

b.

c.

PLATE XIII: (a) 5% Sourdough doughnut (b) 10% Sourdough doughnut (c) 15% Sourdough doughnut

APPENDIX XXVI

a.

b.

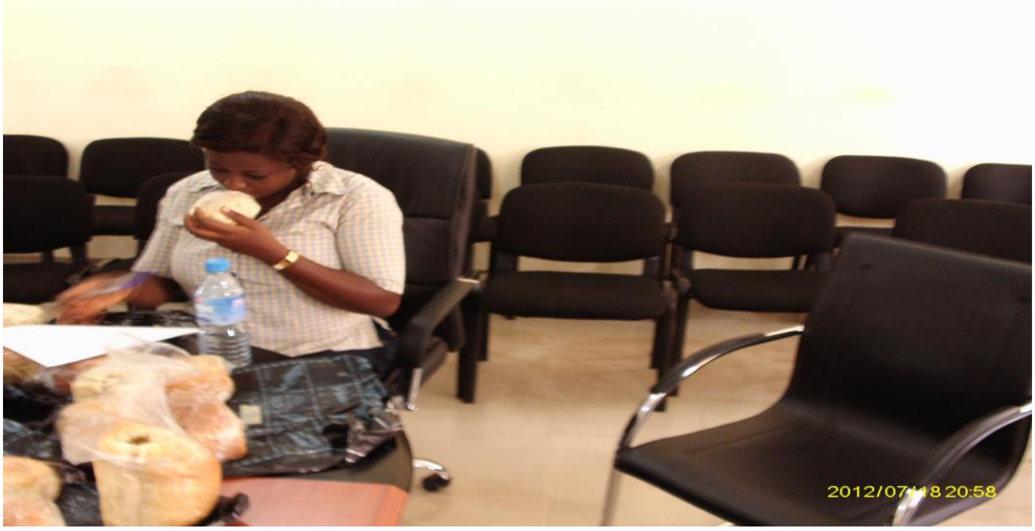
c.

PLATE XIV: (a) *Lactobacillus brevis* doughnut (b) *Lactobacillus plantarum* doughnut
(c) Bakers' yeast doughnut

APPENDIX XXVII

a.

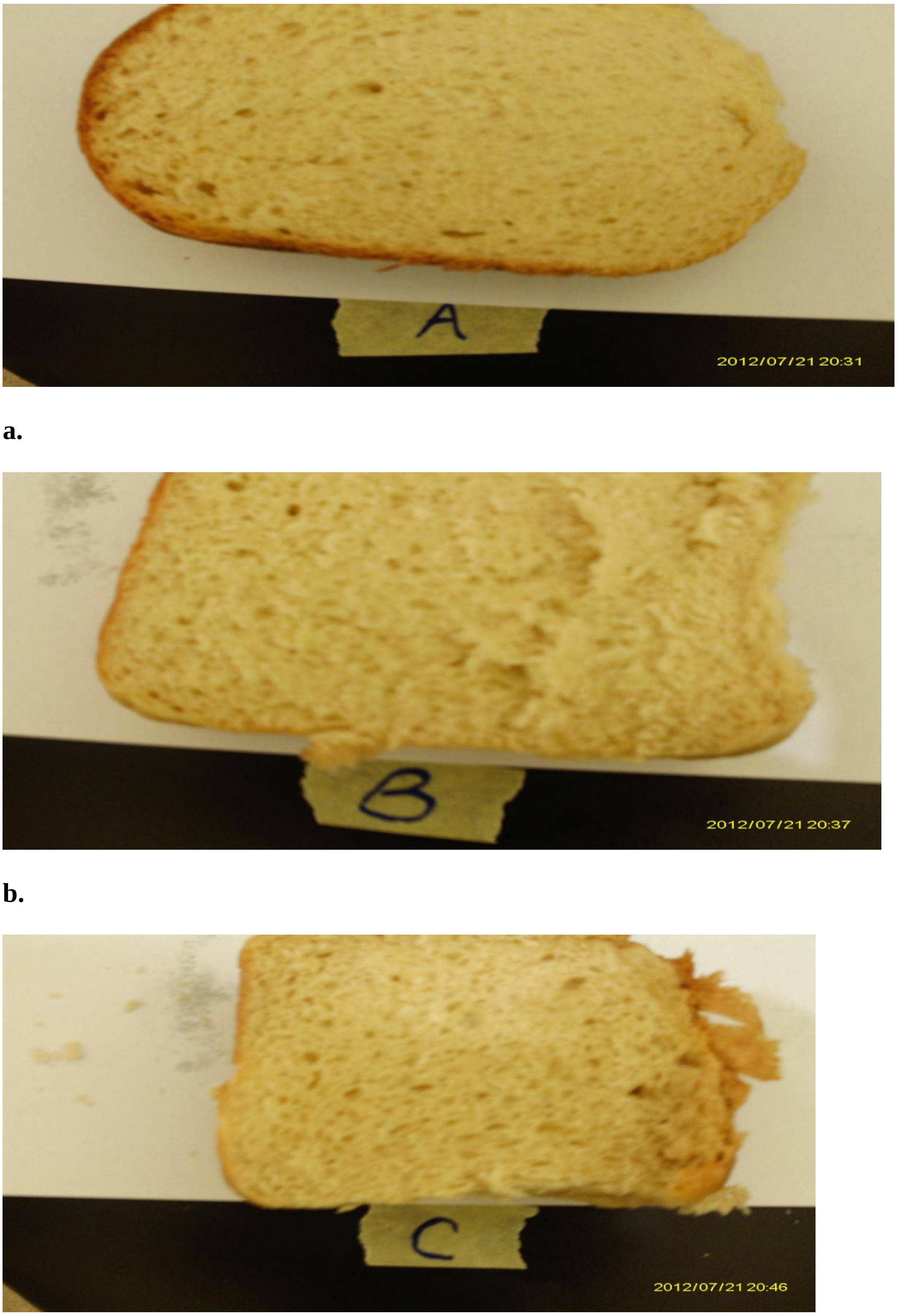
b.



c.

PLATE XV: Test panellists carrying out sensory evaluation of bread and doughnuts

APPENDIX XVIII



c.PLATE XVI: Texture of bread samples at day four shelflife study (a) 5% sourdough bread (b)10% sourdough bread (c) 15% sourdough bread

APPENDIX XXIX

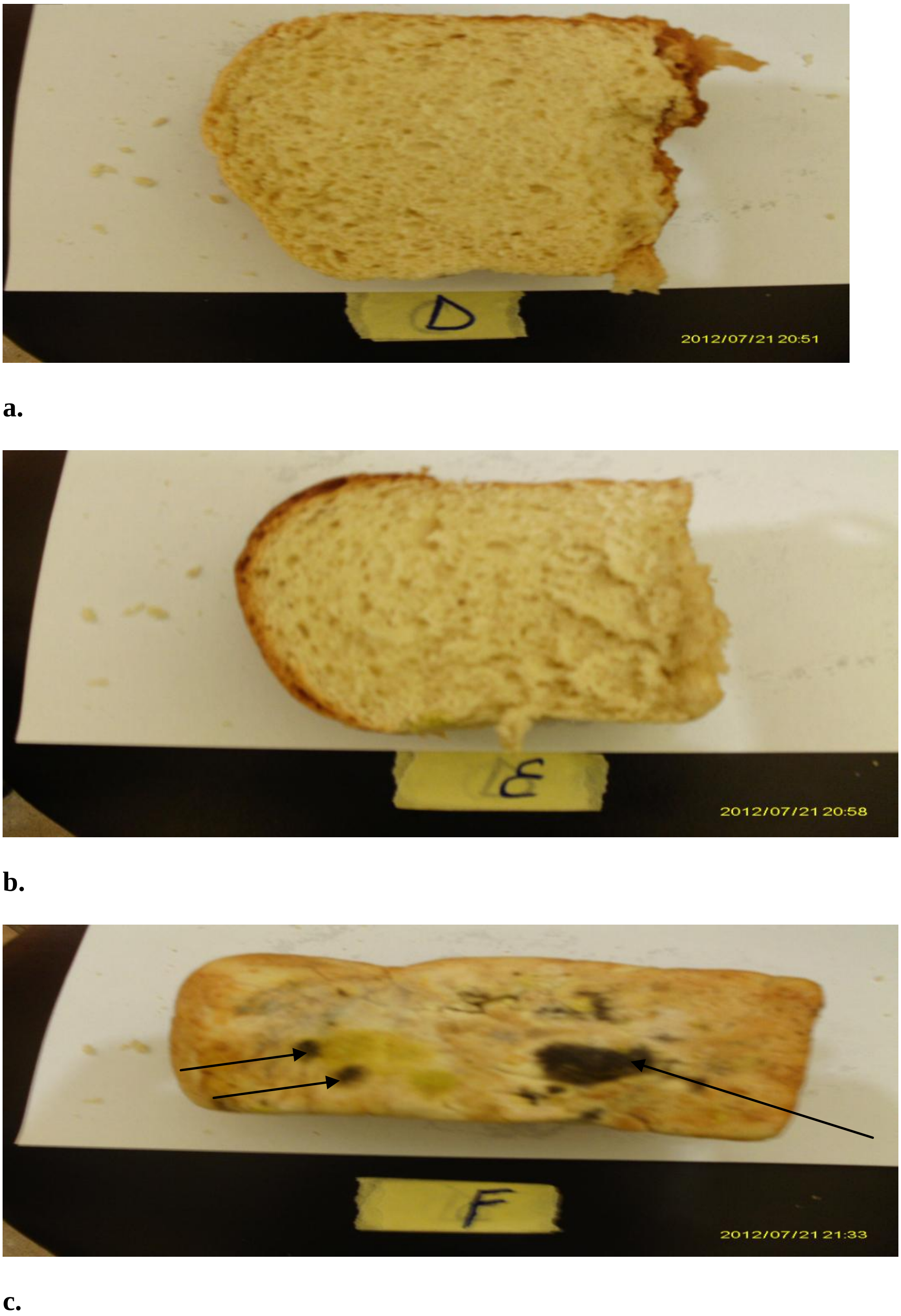
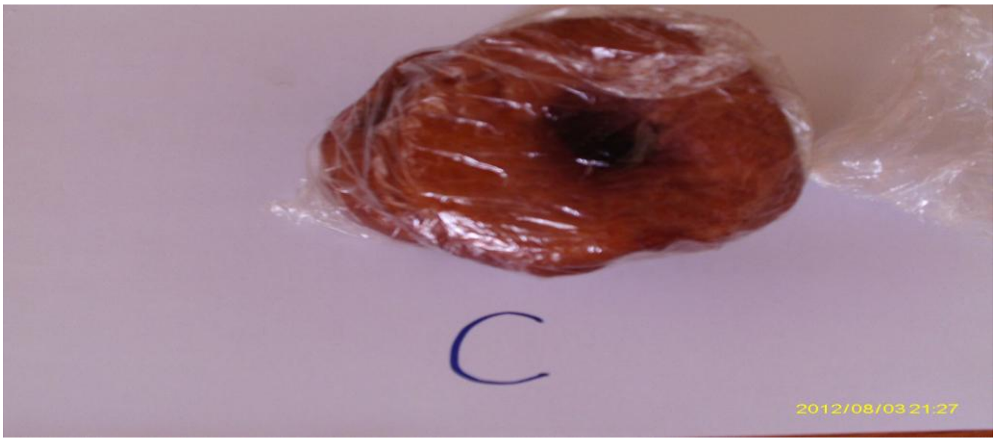


PLATE XVII: Texture of bread samples at day four shelflife (a) *Lactobacillus brevis* bread (b) *Lactobacillus plantarum* bread (c) Baker's yeast bread- notice the visible mould growth

APPENDIX XXX

a.

c.



c.

PLATE XVIII: Sourdough doughnut samples at day seven shelflife study (a) 5% sourdough doughnut (b)10% sourdough doughnut (c) 15% sourdough doughnut

APPENDIX XXXI

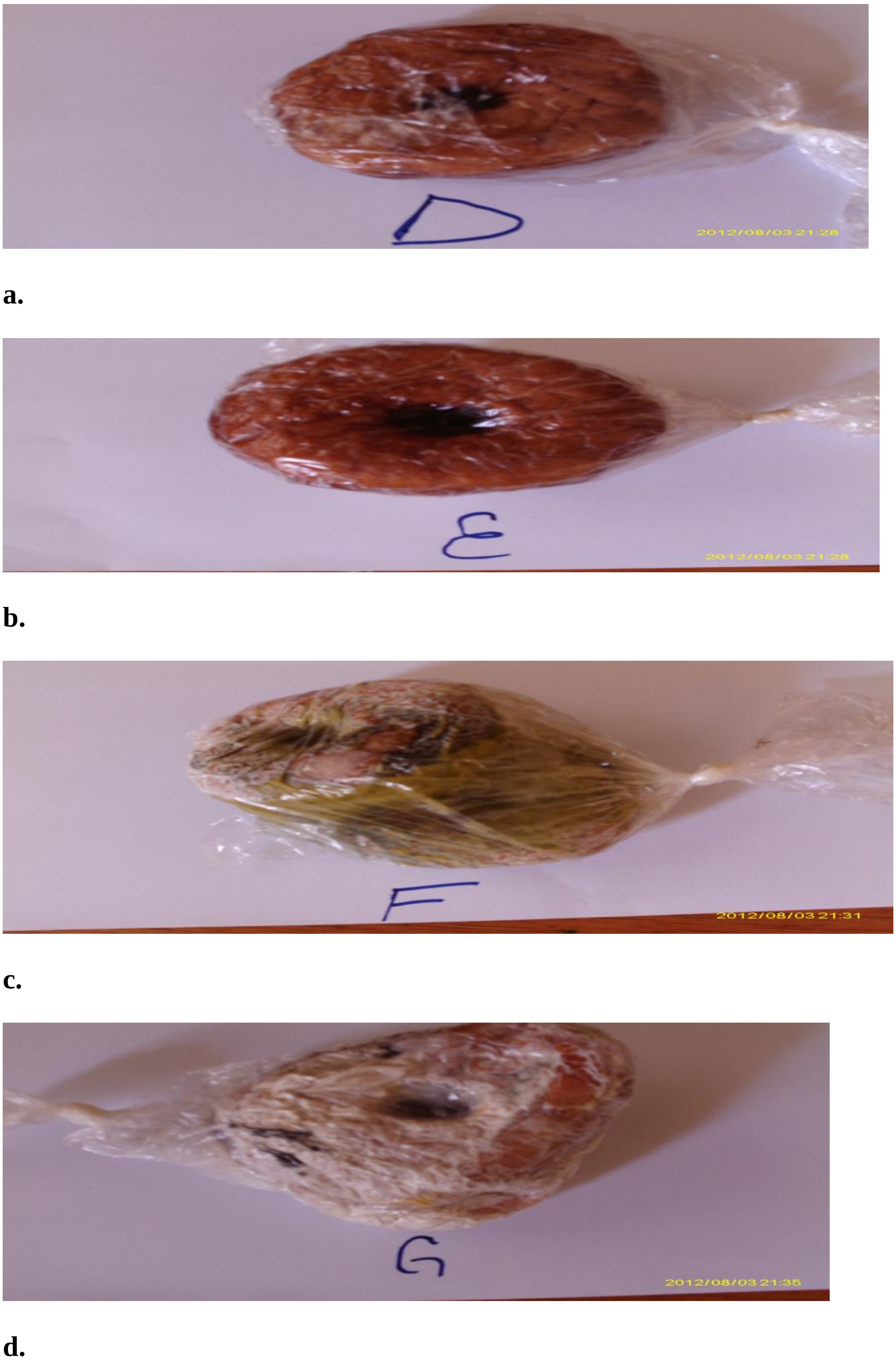


PLATE XIX: Sourdough doughnuts samples at day seven shelflife (a) Mould-free *Lactobacillus brevis* doughnut (b) Mould-free *Lactobacillus plantarum* doughnut (c) Mould-infested Baker's yeast doughnut (d) Mould-infested commercial doughnuts.

APPENDIX XXXII

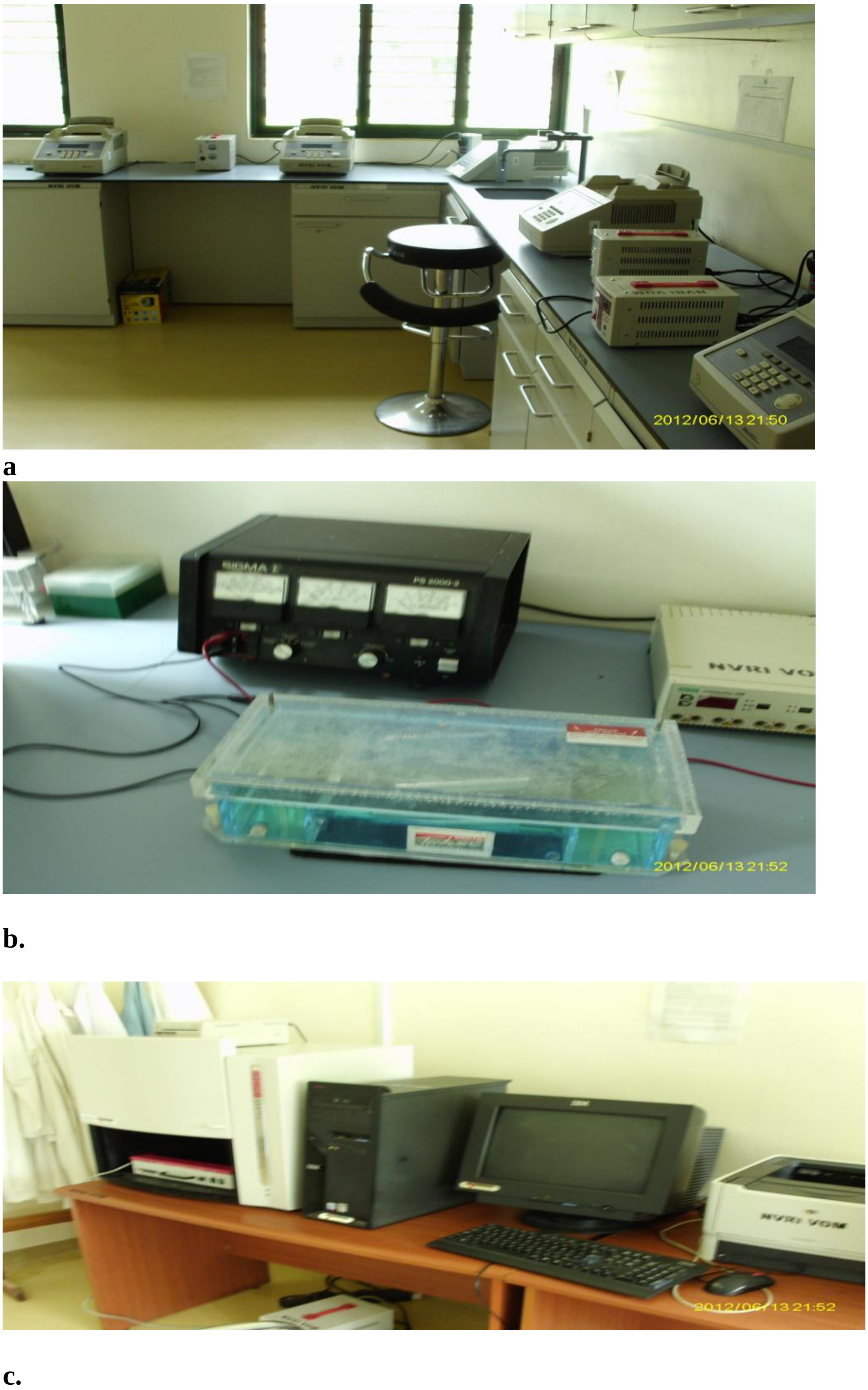


PLATE XX: Some key equipment used during molecular studies (a) PCR machines (b) Gel electrophoresis unit (c) UV transillumination unit