POTENTIALS OF CO-CULTURE OF SACCHAROMYCES CEREVISIAE AND AMYLOLYTIC LACTIC ACID BACTERIA ON CASSAVA AND WHEAT DOUGH FERMENTATION

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DEPARTMENT OF MICROBIOLOGY,
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MAY, 2016.

DECLARATION

I declare that the work in this Thesis entitled "POTENTIALS OF CO-CULTURE OF SACCHAROMYCES CEREVISIAE AND AMYLOLYTIC LACTIC ACID BACTERIA ON CASSAVA AND WHEAT DOUGH FERMENTATION" had been carried out by me in the Department of Microbiology, Faculty of Science, Ahmadu Bello University Zaria, Nigeria under the supervision of Prof. J.B. Ameh, Prof. S.A. Ado and Prof. I.O. Abdullahi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another degree or diploma at this or any other institution

Tarfena Yoila AMAPU _____ _____
Signature Date

CERTIFICATION

This Thesis entitled "POTENTIALS OF CO-CULTURE OF SACCHAROMYCES CEREVISIAE AND AMYLOLYTIC LACTIC ACID BACTERIA ON CASSAVA AND WHEAT DOUGH FERMENTATION" by Tarfena Yoila AMAPU meets the regulations governing the award of the degree of Doctor of Philosophy in Microbiology of Ahmadu Bello University, Zaria and is approved for its contributions to knowledge and literary presentation.

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ABSTRACT

Lactic acid bacteria and yeasts were isolated from fruits, wet milled cereals and cassava using De Man Rogosa Sharpe (MRS) media and Potato Dextrose agar respectively. The Lactic acid bacteria colonies were screened for amylase production on MRS starch agar. Colonies with clear zones of inhibition were identified as Amylolytic Lactic acid bacteria (ALAB) and were selected for further studies. Yeasts and ALAB isolates were morphologically and physiologically characterised and thereafter identified to species level using API 20 C AUX and API50 CHL kits Biomerieux respectively. Results obtained showed that all the isolates grew at 30, 37, 40 and few at 45°C.They all tolerated NaCl at 3, 6 and a few at 10%, they utilised glucose and some produced gas. They also utilised common sugars found in cereals and fruits such as sucrose, maltose, fructose, galactose and some ALAB strains utilised raffinose. The predominat yeasts and ALAB characterised and identified were *Saccharomyces cerevisiae* (12) 60% and *Lactobacillus plantarum* 8(57.14%). Fermentation conditions (pH, time and temperature) were optimized for reducing sugars and enzyme activity. The optimum time was found to be 24 h ; pH 6.0 was found to have a strong influence on reducing sugar and on enzyme activity at 0.69mg/ml and 3.11U/ml respectively. Enzyme activity was stable at 70°C. *Lactobacillus plantarum* (AMz5) from maize *and Saccharomyces cerevisiae* YSg2 from sorghum showed considerable potentials for leavening ability in wheat flour(94cm^3).The leavening potentials of *Lactobacillus plantarum and Saccharomyces cerevisiae* in co culture were investigated in wheat/cassava composite flour of different ratios. Results showed that there was no significant difference ($P < 0.05$) among three of the formulations (10, 20%, and Wf (dough started with isolates) in dough rising after four hours of fermentation time. There

was a significant difference from 30% cassava inclusion. The dough rising capacity of control (commercial yeast) was faster, though dough started with *Lactobacillus plantarum* and *Saccharomyces cerevisiae* increased steadily up to four hours. The proximate analysis of the flours and baked products was conducted. Results showed that all the flours met standard specifications for moisture content of less than 14%, carbohydrate and protein 80-90% and 1-3% respectively, while ash content for market cassava was at variance with standard specifications of 1.64%. Generally, the proximate (fat, protein and carbohydrate) values of wheat/cassava blended bread decreased with increasing levels of cassava flour substitutions, while moisture and ash contents increased with increase in cassava flour ratios. However, there was no significant difference between the products made from industrial and market cassava flours.The crude protein content of products baked with industrial cassava had higher protein values than the market cassava products. The sensory evaluation which was used to assess the quality of baked products using the five point Hedonic scale, showed a general acceptance of the products. The average scores however decreased as the level of cassava flour substitution increased. There was no significant difference ($P > 0.05$) in taste, aroma, appearance, texture ratings of the bread samples of 100% wheat bread, 10% and 20%. Wheat bread started with isolates was highly rated by the panelists. A strong correlation existed between dough pH and viable cell growth of the inoculants, where decrease in pH led to increase in viable counts. Bread produced from 10% and 20% wheat cassava rated higher in all the attributes tasted for than those produced with commercial yeast and routine bread.

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ABBREVIATIONS

Acronyms	Meanings
ALAB	Amylolytic Lactic acid bacteria
API	Analytical profile index
CFP	Composite flour programme
DNS	Dinitro salicylic acid
EPS	Exopolysaccharides
FAO	Food and Agriculture organisation
FOSHU	Food for specified Health use
GRAS	Generally regarded as safe
HQCF	High quality cassava flour
LAB	Lactic acid bacteria
MRS	de Man Rogosa –Sharpe
PSD	Particle size distribution
WHO	World health organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Background of the Study

Lactic acid bacteria (LAB) and yeasts are known to be involved in African indigenous fermented foods (Al-Askari *et al*., 2002; Cheirship *et al*., 2003; Ogunbanwo *et al*.,2008).They occur naturally in a wide variety of nutrient-rich environments which include plants, cereals, vegetables, milk and dairy products. These organisms ferment carbohydrates directly into organic acids and other metabolites as end products (Reddy *et al*., 2008)

The acidic environment created by LAB, tend to favour the proliferation of yeast in food, while the growth of bacteria is simulated by the presence of yeast, which may provide growth factors such as vitamins and soluble nitrogen compounds. Metabolites produced during LAB and yeast associations are found to impact taste and flavor to foods (Kimaryo *et al*., 2000; Varnam and Awamaria, 2007; Ali and Mustafa, 2009; Byrne, 2009). The presence of LAB can also enable the utilization of substances such as starch that can ordinarily not be made to be fermentable thereby increasing the microbial adaptability to the complex food ecosystem (Hansen *et al*., 2002; Holzapfel, 2002).

It has been reported that about fifty (50) different species of LAB had been isolated from sourdough (Corsetti and Settanni, 2007). *Lactobacillus* strains are the most frequently observed bacteria in this matrix, but the species belonging to the genera *Leuconostoc*, *Weissella*, *Pediococcus*, *Lactococcus* or *Enterococcus* had been isolated as well (Hansen *et*

al., 2002; Ehrmann and vogel, 2005; Hervé *et al*., 2006; Gerekova *et al*., 2011). Lactic acid bacteria are found to be useful in modifying the structure and properties of starches for diverse applications. This is made possible by the presence of amylases which have the ability to partially hydrolyze raw starch. Amylolytic lactic acid bacteria (ALAB) had been reported from different tropical amylaceous fermented foods, prepared mainly from cassava and cereals (e.g., maize and sorghum) (Agati *et al*., 1998). *Lactobacillus Plantarum* was found to be predominating in 'ogi'. It was also isolated from fermented cassava roots, fermented fish and rice (Agati *et al*., 1998; Reddy *et al*., 2008).

Amylolytic enzymes are widely distributed in bacteria and fungi. They have almost surpassed the synthetic sources in some industries (Horvathova *et al*., 2001; Gupta *et al*., 2003). Microbial amylases are however preferred to other sources because of their plasticity and vast availability, (Pandey *et al*., 2000; Guyot *et al*., 2001; Sohail *et al*., 2005). After potassium bromate was banned in many countries, interest increased in finding an enzyme to replace this chemical oxidant. Numerous studies had reported that these enzymes play key roles in bread making such as increasing loaf volume, producing finer crumb cells, and extending shelf-life (Guy, 2001; Hille and Schooneveld-Bergmans 2004; Ribotta and Le Bail, 2007). Amylolytic Lactic acid bacteria had been isolated from traditional fermented foods and they are found to ferment different types of amylaceous raw materials such as wheat, potato, cassava (*Manihot esculenta* Crantz*)* and some starchy substances (Ben-Omar *et al*., 2000; Reddy *et al*., 2008; Fossi and Tavea, 2013).

Nigeria, being the largest producer of Cassava (*Manihot esculenta* Crantz*)* in the world, is spending about ₦6,356 billion annually on wheat importation. This has negative effects on its reserves (Sanni *et al*., 2009).Cassava (*Manihot esculenta* Crantz*)* which serves as one of

the basic food sources of the Nation and is consumed in forms of *Gari, Akpu, Laffu ,Abacha* and starch. However, the introduction of the cassava policy is to expand production of cassava through the addition of another widely consumed staple food, bread (Aroputin, 2007; Sobowale *et al*., 2007; Putri *et al*., 2011).

The Food and Agriculture Organization (FAO) of United Nations initiated the composite flour programme (CFP) to develop bakery products from locally available raw materials, especially cassava, corn, and sorghum in 1964 to help countries meet their wheat requirements. Many countries are making effort to produce bread from a blend of wheat flour with cassava added. Products or confectionaries made with this composite flour is gaining acceptance in Colombia, Kenya, Nigeria, Senegal, Sri Lanka and Sudan. Bread made from composite flours of 30% sorghum and 70% wheat is prepared in Nigeria and Senegal and is comparable to 100% wheat bread. The popularity of bread made from composite flour is also increasing in many other countries (Ali and Mustafa, 2009; Aboaba and Obakpolar, 2010).

Bread is consumed worldwide on daily basis. It is a dietary staple food for people living in cities and rural communities. The consumption of bread in Nigeria is steady and is increasing especially with the change in their food habit to fast food, which requires minimal packaging and can be kept at room temperature for a long period of time. (Ahlborne *et al*., 2005; Fang , 2008).

1.2 Statement of the Research Problems

Nigeria is the only country that imports 100% wheat it consumes and it is the highest producer of cassava in the World (Sobowale *et al*., 2007; Aroputin, 2007), while countries that produce less are using cassava for various secondary means and contributing 80% of added cassava and cassava products globally. According to the Nigerian Minister of Agriculture and Rural development, Nigeria is at the bottom of the value chain as it continues to supply raw cassava at its most basic price. It also supplies other markets with cassava from which they produce sophisticated products and sell at higher prices, while importing wheat at about ₦6, 356 billion annually (Sanni *et al*., 2009). The industrial utilization of cassava is still limited in the country. This limits the market alternatives for cassava producers. The inclusion of cassava flour in bread making has raised a lot of doubts amongst Nigerians concerning its acceptability and sustainability.

Cassava flour which is supposed to form the main ingredients of bread and similar products lacks the necessary gluten network for holding gas produced during dough fermentation, which is a vital component of bread baking. Bread made from cassava flour will yield a brittle or hard loaf which crumbles easily and can therefore not be easy to slice. (Gallagher *et al*., 2003; Eduardo *et al*., 2013).Similarly, large amount of money is spent by bakers on importation of yeast and magic solutions (enzymes) which form vital components of baking. Studies have shown that these can be sourced locally to help improve these products (Foss andTavea, 2013). Potential amylolytic lactic acid bacteria strains that will give high yield efficiency for utilization of cheaply available renewable agricultural starchy biomass are not yet available (Songré-Ouattara *et al*., 2009; Fossi *et al*., 2011)

1.3 Justifications

Nearly all food fermentations are the results of more than one microorganism either working together or in a sequence (Odunfa and Oyewole, 1998). Bread, a product of fermentation is well accepted worldwide and Nigeria is one of the highest consumers (Ogunbanwo *et al*., 2008). It is one of the ancient foods produced with the assistance of microorganisms. Bread is widely consumed because of its low cost, ease of preparation, versatility, sensory attributes and nutritional properties. In human nutrition, bread is not only a source of energy, but also a supplier of irreplaceable nutrients for the human body. It provides little fat, but high quantities of dietary fibers (Baysal and Over, 1994).The Federal Government of Nigeria has been pushing for a minimum of 20% inclusion of cassava flour derived from a crop widely grown locally with the view to saving significant foreign exchange and enhancing the local market. Having an indigenous cassava bread market will boost the nation's economy by saving the country the billions of naira it spends on wheat importation; it will also reduce our dependence on raw materials from abroad. Besides the use of cassava bread could be an alternative solution for celiac disease syndrome. Nigeria needs to have an aggressive strategy to add value to its cassava, to expand markets for farmers and raise farm incomes. Yeasts and amylolytic Lactic acid bacteria (ALAB) are found to be suitable raw materials for bread making involving the use of cassava. Although, the use of bakers yeast (*Saccharomyces cerevisiae*) shortens the time for bread making, it does not give bread good taste, texture and flavour except in association with ALAB (Paramithiotis *et al*., 2006).Yeasts and Amylolytic lactic acid bacteria (ALAB) are not only cheap, but also generally regarded as safe (GRAS) and readily available (Byrne, 2000; Sohail *et al*., 2005; Foss and Tavea, 2013). Also The

amylases of microorganisms have a broad spectrum of industrial applications as they are more stable than when obtained from plants and animals (Horvathova *et al*., 2001; Gupta *et al*., 2003). The use of enzymes has become a common practice in the baking industry, because enzymes can promote effects similar to those of chemical additives but with the advantage of being considered as natural additives by regulators (Ribotta and Le Bail, 2007; Paula *et al*., 2010).

1.4 Aim of Study

The aim of this study was to determine the bread making potentials of cassava and wheat flour using co - culture of *Saccharomyces cerevisiae* and amylolytic lactic acid bacteria.

1.5 Specific Objectives

The specific objectives of this work were to:

1 Determine the physico-chemical and proximate composition of cassava and wheat flour samples as well as their products.

2 Isolate and chatacterise the selected fermenting organisms from fruits, cassava and wet milled cereals

3 Determine the amylolytic activity of the ALAB isolates and optimise fermentation conditions

4 Assess the leavening ability of the two organisms in co-culture on varying proportions of the two flours as composite.

5 Compare the proximate composition as well as the organoleptic properties of the cassava and wheat bread with those of commercial bread.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and uses of Dough

Dough refers to a wet mass developed after mixing of wheat flour, water and other ingredients. Physico-chemical properties of dough play important role in the bakery, pasta and ready-to-eat cereals processing industry. The process of making and shaping dough is a precursor for a wide variety of dough made foodstuffs. A wide variety of flours can be used for dough. The most commonly used flour is wheat flour. Flours from maize, rice, other cereals and crops have been used. Dough vary widely, depending on ingredients, kind of product to be made and leavening agent type (Cauvain and Young, 2006)

The Greeks, who learned baking from the Egyptians and the Israelites, began making dough similar to those of the East. After keeping their "cooked wheat" system a secret for many years, they finally taught the Greeks to make bread in 600 B.C. With the addition of brewer's yeast, which was known to both the Greeks and Romans since very ancient times, a softer and more flavourful bread was obtained (Cauvain 2003; Angioloni *et al*., 2008) .It took a long time for any new development to appear in the making of dough. At last, in the 19th century, the yeast which fed on wheat was discovered and came into use. During the second half of the 20th century, modern techniques began to appear and dough making became automated. In the United States in particular, automation continually developed and other countries followed suit. At the same time, more attention was paid to the details of the bread's flavour, and with this, many different varieties began to be offered for sale and many different kinds of bread came into production. In Nigeria today, there is a steady

increase in automated enterprises using modern techniques to produce dough for bread **(**Cauvain and Young, 2006; Oyeku *et al*., 2008)

2.2 Dough Fermentation

Dough is developed due to complex interactions among flour constituents during mixing operation. The process of dough development begins with addition of water and commencement of mixing operation. Initially all ingredients are hydrated and appear like a sticky paste. On further mixing, the viscosity increases, sticky characteristics of dough disappear and a non-sticky mass is developed at peak consistency of dough. At this stage the dough behaves like a viscoelastic mass with both elastic and extensible characteristics (Nakamura and Kurata 1997; Fermin *et al*., 2005).The role of energy during mixing is crucial in the development of dough. In the process of developing dough particularly bread dough; the aim is to bring about changes in the physical properties of the dough to improve its ability to retain the carbon dioxide gas produced during yeast fermentation (Charalambides *et al*., 2006) This improvement in gas retention ability is particularly important when the dough pieces reach the baking oven. In the early stages of baking the yeast activity is at its greatest and large volume of carbon dioxide gas is generated and released from solution in the aqueous phase of the dough. The dough pieces expand enormously at this time and well-developed dough or gluten structure is essential to withstand the internal pressure of the expanding dough. The physical properties of dough of great concern in bread making process include resistance to deformation, extensibility, elasticity and stickiness. An example of a rubber band may be used to understand the first three of these properties. When the rubber band is stretched between our fingers a degree

of force is required to change its shape as it resists deformation. If a modest force is applied and one end of the band is released, then it returns to its original position. This reflects the elastic properties. If the rubber band is stretched beyond a limit it will eventually reach a point of extension and ultimately breaks, which is taken as a measure of its extensibility. The fourth physical property, stickiness, is self-explanatory that is, anything that stick to a surface has stickiness. Therefore, dough development is viewed as the modification of some very important physical properties of bread dough, which make major contributions to the character of the final product. This modification of gluten structure can be achieved by a number of different physical and chemical processes, and various combinations of these form the basis of the different groups of bread-making processes that are in common use (Uthayakumaran *et al*., 2003; Mis, 2003; Rhazi *et al*., 2003) Most of the desirable changes resulting from 'optimum' dough development are related to the ability of the dough to retain gas bubbles (air) and permit the uniform expansion of the dough piece under the influence of carbon dioxide gas from yeast fermentation during proof and baking. The dough with a more extensible character is especially important for improved gas retention, while reductions in dough resistance and elasticity play a major role in the modification of bubble structures during processing. It is important to distinguish between gas production and gas retention in fermented dough. Gas production refers to the generation of carbon dioxide gas as a result of yeast fermentation. If the yeast cells in the dough remain viable (alive) and sufficient food for the yeast is available, then gas production will continue, but expansion of the dough can only occur if that carbon dioxide gas is retained in the dough. Not all of the gas generated during the processing, proof and baking will be retained within the dough before it finally sets in the

oven. The proportion that will be retained depends on the development of a suitable gluten matrix within which the expanding gas can be held. Gas retention in dough is therefore closely linked with the degree of dough development that occurs, and as such will be affected by a large, number of ingredients and processing parameters (Havet *et al*., 2000; Schwarzlaff *et al*., 2001; Giannou *et al*., 2003; Khatkar, 2012; Angioloni *et al*., 2008).

2.3 Different Cereals used in Dough Making

Wheat is extensively used for the production of breads (Enriquez *et al*., 2003). The unique bread making properties of wheat flour are mainly due to its gluten proteins to form a viscoelastic network when mixed with water (Ajani *et al*., 2012)

The suitability of wheat cultivars for application is influenced by genetic, seasonal and environmental factors. Various wheat varieties have been found suitable for the preparation of breads. Wheat is grown throughout the world and is adaptable to the wide range of environmental conditions. Wheat based foods are major source of nutrients in many regions of the world (Abdelghafor *et al*., 2011). Although, wheat is generally used as a source of carbohydrates; but its products are also a substantive source of protein, vitamins, and minerals, when consumed as a major component of a diet. It is used extensively in many parts of the world for the preparation of different types of bread and many other bakery products. The flour is analyzed for chemical composition to evaluate its quality, processing and storage life. However, moisture has significant effect on its keeping quality. The flour produced from dry and sound grains can be kept for longer periods if properly stored, but that from wet grains deteriorates dramatically within a few days. 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 refining of flour greatly affects the protein content. It is the response to the removal of germ and aleurone layer as bran; both are relatively rich in protein (Anjum and Walker, 2000; Ziaulhaq *et al.* 2004) reported protein contents in six most prevalent bread wheat cultivars that range from 11.99 - 13.80%. Dewettinck *et al.*, (2008) found 12.13% crude protein in whole wheat flour. Rehman *et al*., (2001) analyzed a few wheat varieties of Sindh province for composition and found protein that ranges from 11.86 - 11.95%. Butt *et al*. (2001) estimated protein content in 30 spring wheat cultivars and reported 10.06 - 11.89% in white flour. Farooq *et al.* (2001) noted significant ($p< 0.01$) effect of varieties and lines on chemical composition of wheat, especially on protein fractions. Most of the flours are mixture of soft and hard wheat. Flour from two manufacturers may consist of different blend, which could produce varying results in the kitchen. Various types of wheat (soft and semi hard) are also used for the preparation of flat bread**. S**oft wheat's are suitable for Egyptian, Iranian and Indian flat breads. Flour containing 8.0% moisture, 10.6% protein and 1.45% ash has been used for the preparation of chapattis. Indrani and Venkkateswara, (2007) revealed that better flat breads could be produced from soft and medium hard wheat, which contain low to medium protein content with weak gluten and baking strength. Ash content is important to determine flour grade and extraction rate, since the ash content of the bran is about twenty times that of endosperm. It is liable indicator of the efficiency with which the bran and germ are separated from the endosperm (Banu *et al*., 2012). The ash predicts the level of bran in the flour. The ratio of bran to endosperm is higher in small kernels. It varies from one variety to another and in many cases within the same variety grown at different locations assuming all other variables relevant to kernel structure and milling technology are constant. Wheat with higher

endosperm ash is inherently bound to give higher flour ash values (Posner, 2000; Dewettinck *et al.*, 2008; Banu *et al*., 2012; Agiriga, 2014)

2.3.1 Wheat flour as basic bread ingredients

Flour is the chief ingredient of bread and is produced by milling the grains of wheat, various species and varieties of which are known. For flour production, most countries use *Triticum vulgare.* A few countries use *Triticum durum*, but this yellow colored variety is more familiarly used for semolina and macaroni in many countries. The chief constituents of flour are starch (70%), protein (7-15%), sugar (1%), and lipids (1%).In bread-making from *Triticum vulgare* the quality of the flour depends on the quality and quantity of its proteins. Flour proteins are of two types. The first type forming about 15% of the total is soluble in water and dilute salt solutions and is non-dough forming. It consists of albumins, globulins, peptides, amino acids, and enzymes. The remaining 85% are insoluble in aqueous media and are responsible for dough formation. They are collectively known as gluten (Kadam *et al*., 2012). It also contains lipids. Gluten has the unique property of forming an elastic structure when moistened with water. It forms the skeleton which holds the starch, yeasts, gases and other components of dough. Gluten can be easily extracted, by adding enough water to flour and kneading it into dough. After allowing the dough to stand for an hour the starch can be washed off under a running tap water leaving a tough, elastic, sticky and viscous material which is the gluten. Gluten is separable into an alcohol soluble fraction which forms one third of the total and known as gladilins and a fraction (two thirds) that is not alcohol-soluble and known as the glutenins. Gladilins are of lower molecules weight than glutenins; they are more extensible, but less, elastic than glutenins.

Glutelins are soluble in acids and bases whereas glutenins are not. The latter will also complex with lipids, whereas glutelins do not. 'Hard' wheat with a high content of protein (over 12%) are best for making bread because the high content of glutenins enables a firm skeleton for holding the gases released curing fermentation. 'Soft' wheat with low protein contents (9-11%) is best for making cakes. This is especially true for the product made from wholegrain or high-fibre flour. There is increasing evidence that the intake of whole grain foods and cereal fibre against chronic diseases, such as type 2 diabetes and cardiovascular disease (Mellen *et al*., 2008, Kadam *et al*., 2012). Although health benefits can be expected, the addition of whole-meal flours or fiber causes technological changes. In recent years traditional sourdough bread production has enjoyed renewed success with the ever-increasing demand by the consumer for more natural, tasty and healthy foods (Petrulakova *et al*., 2009)

The available carbohydrates in wheat flour are maltose followed by sucrose, glucose and fructose, along with some trisacchrides like maltotriose and reffinose. The glucose increases during fermentation, whereas sucrose decreases in the presence of yeast due the reaction of invertase .The yeasts present in sourdoughs are not able to ferment maltose, a sugar common in flour. However, it can nevertheless develop because of glucose released into the medium by some lactic acid bacteria species, e.g. *Lactobacillus sanfranciscensis* (Gobbetti, 1998)

2.3.2 Cereals used in dough making

Other cereals the can be used in dough making include Maize rye, sorghum, barley, millet and corn. As their proteins have different structures. Millets, like sorghum, are predominantly starchy. The protein content is comparable to that of wheat and maize. The

protein content in millet is very close to that of wheat; both provide about 11% protein by weight, on dry matter basis. Millets contain no gluten, so they are not suitable for raised bread. When combined with wheat (or xanthan gum for those who have celiac disease) they can be used for raised bread. Alone, they are suited for flatbread. *Digitaria exilis* (Acha) is widely grown in Nigeria. Some investigators are currently using starter cultures of lactic acid bacteria and yeast for sour acha, cassava flours, and cassava starch in production of sour dough (compared with sour maize bread) based on conventional technique. Preliminary results show the great potential inherent in acha and iburu for sour dough (Jideani and Jideani, 2011)

In many parts of the world sorghum has traditionally been used in food products and various food items such as unleavened bread, cookies, cakes, couscous. Traditional food preparation of sorghum is quite varied. A popular fermented sorghum product is kisra, aflat bread which constitutes a major part of the staple diet of the people in Sudan. Several authors examined sorghum sourdoughs for kisra production (Elyas *et al*., 2002; Lu *et al*., 20009). In developing countries, maize is mainly used for human consumption .Pozol, made by fermenting and diluting maize dough is produced in small household scales (Sanni *et al*., 2002; Omemu *et al*., 2011)

2.4 Cassava Production in Nigeria

Cassava, *Manihot esculenta* Crantz, is a perennial woody shrub with an edible root, which grows in tropical and subtropical areas of the world. It is also called *yuca*, *manioc*, and *mandioca*. Cassava has the ability to grow on marginal lands where cereals and other crops do not grow well; it can tolerate drought and can grow in low-nutrient soils. Because

cassava roots can be stored in the ground for up to 24 months, and some varieties for up to 36 months, harvest may be delayed until market, processing, or other conditions are favorable (Burrell, 2003; Aerni, 2005; IITA, 2009,)

Cassava (*Manihot esculenta Crantz*) was introduced into central Africa from South America in the sixteenth century by the early Portuguese explorers. It was probably the emancipated slaves who introduced the cassava crop into southern Nigeria, as they returned to the country from South America via the islands of Sao Tome and Fernando Po. At that time there were Portuguese colonies off Nigeria's shores. Cassava, however, did not become important in the country until the end of the nineteenth century when processing techniques were introduced, as many more slaves returned home (Montagnac, et *al*., 2009b; Ukwuru and Egbonu, 2013)

In Africa and Latin America, cassava is mostly used for human consumption; the crop contributes significantly to the diets of over 800 million people, with per capita consumption averaging 102 kilograms per year. In some areas of Africa it constitutes over 50 percent of the daily diets of the people. While in Asia and parts of Latin America it is also used commercially for the production of animal feed and starch-based products. In Africa, cassava provides a basic daily source of dietary energy. Roots are processed into a wide variety of granules, pastes, flours, etc., or consumed freshly boiled or raw (Nweke, 2003; Denton *et al*., 2004; Aerni,2005). In most of the cassava-growing countries in Africa, the leaves are also consumed as a green vegetable, which provides protein and vitamins A and B. In Southeast Asia and Latin America, cassava has taken on an economic role (IITA 2009; Eke-Okoro and Njoku, 2012)

Nigeria is currently the largest producer of cassava in the world with an annual output of over 34 million tonnes of tuberous roots. This increase in production between 2004 and 2006 came about as a result of the interventions of the Nigerian Government and some developmental agencies. The Nigerian Government facilitated the development of new disease-resistant cassava varieties by the joint efforts of IITA, National Root Crops Research Institute (NRCRI), Root and Tuber Expansion Programme (RTEP), and the Federal Ministry of Agriculture, in conjunction with State Agricultural Development Programs and cassava farmers (Sanni *et al*., 2009) Cassava production has been increasing for the past 20 or more years in area cultivated and in yield per hectare. On average, the harvested land area was over 80% higher during 1990–1993 than during 1974–1977 (Akpomie *et al*., 2012)

The growth in cassava production has been primarily due to rapid population growth, large internal market demand, complemented by the availability of high yielding improved varieties of cassava, a relatively well developed market access infrastructure, the existence of improved processing technology and a well-organized internal market structure.

Cassava is produced largely by small-scale farmers using rudimentary implements. The average land-holding is less than two hectares and for most farmers, land and family labour remain the essential inputs. Land is held on a communal basis, inherited or rented; cases of outright purchase of land are rare. Capital is a major limitation in agriculture; only few farmers have access to rural credit (Eke-Okoro and Njoku, (2012)

Almost all farmers in the main cassava belts of the southeastern, southwestern and central zones grow cassava, which is typically intercropped as a main or minor crop. Rotation and

fallow systems are the traditional systems used by the farmers to maintain soil fertility but population pressure has resulted in reduced fallow, continuous cropping and reduced soil fertility. Farmers are generally aware of the benefit of inorganic fertilizer, but the commodity is scarce and a major constraint in cultivating improved varieties.

Almost all the cassava produced is used for human consumption and less than 5 percent is used in industries. As a food crop, cassava fits well into the farming systems of the smallholder farmers in Nigeria because it is available all year round, thus providing household food security. Compared to grains, cassava is more tolerant to low soil fertility and more resistant to drought, pests and diseases. Furthermore, its roots store well in the ground for months after maturity (Ukwuru and Egbonu, 2013).

Cassava is usually consumed in processed forms. Cassava processing by traditional methods is labour-intensive but the application of improved processing technology has reduced processing time and labour and encouraged further production. Industrial utilization of cassava products is increasing but this accounts for less than five percent of the total production. Its use in the industry and livestock feed, is well known, but is gradually increasing, especially as import substitution becomes prominent in the industrial sector of the economy. As a cash crop, cassava generates cash income for the largest number of households in comparison with other staples. It is produced with relevant purchased inputs as frequently as and in some cases more frequently than other staples. A large proportion of total production, probably larger than that of most staples, is planted annually for sale (Ukwuru and Egbonu, 2013)

Cassava is a staple food consumed in both rural and urban areas of Nigeria, a country with a population of about 120 million people. In recent years, it has also been transformed from being a subsistent crop to industrial cash crop (Nwokoro *et al*., 2002). Cassava is one of the most actively marketed food products and is the most promising in terms of growth and new market opportunities. There is also a regular surplus of cassava in most producing countries. Even so, several governments in Africa have taken positive steps to promote cassava production for industrial processing since many of these countries have large capacities for cassava production.

As a food crop, cassava has some inherent characteristics which makes it attractive, especially to the smallholder farmers in Nigeria. First, it is rich in carbohydrates especially starch and consequently has a multiplicity of end uses. Secondly, it is available all year round, making it preferable to other, more seasonal crops such as grains, peas and beans and other crops for food security. Compared to grains, cassava is more tolerant of low soil fertility and more resistant to drought, pests and diseases. Furthermore, its roots are storable in the ground for months after they mature. These attributes combined with other socioeconomic considerations has been recognized in the crop as lending itself to a commodity-based approach to poverty alleviation (Burell, 2003)

The major beneficiaries of a more diversified world market for cassava will be Sub-Saharan Africa. In Nigeria, the bulk of current cassava production is mostly processed into food products (Eke-okoro and Njoku 2012)**,** indicating an excellent opportunity for raising the production level with the expansion of industrial starch processing. Certainly an

increased cassava production, in order to convert it into an industrial product, is very possible by increasing the area of land farmed without any need for irrigation.

2.4.1 Nutritional value of cassava roots

The composition of cassava depends on the specific tissue (root or leaf) and on several factors, such as geographic location, variety, age of the plant, and environmental conditions. The roots and leaves, which constitute 50 and 6% of the mature cassava plant, respectively, are the nutritionally valuable parts of cassava (Tewe and Lutaladio, 2004). The nutritional value of cassava roots is important because they are the main part of the plant consumed in developing countries. The nutrient composition of raw cassava is compared to other staple crops, such as wheat and corn, and some vegetable and animal foods.

2.4.2 Cassava used in composite flours

In many developing countries bread consumption is continually expanding and there is increasing dependence on imported wheat. Most of these countries, however, grow other staple food than wheat that can be used for bread. Some grow various starchy tubers such as cassava, yam or sweet potatoes and some others grow cereals such as maize, millet or sorghum. It would therefore be economically advantageous for those countries if imports of wheat could be reduced or even eliminated and the demand for bread could be met by the use of domestically grown products instead of wheat (Moreno *et al.*, 2009; Kadam *et al.*, 2012)

The Composite Flour Programme initiated by the Food and Agriculture Organization of the United Nations in 1964 was conceived primarily to develop bakery products from locally available raw materials, particularly in those countries which could not meet their wheat requirements. Although the bakery products obtained were of good quality, similar in some of their main characteristics to wheat-flour bread, the texture and palatability of the composite-flour bakery products were different from those made from wheat flour. Bread made of nonglutenous flour has the crust and crumb structure of cake rather than bread and may not be considered acceptable by people who are accustomed to conventional bread (Ukwuru and Egbonu, 2013 Kadam *et al*., 2012)

The light, evenly structured bread made of wheat flour and the characteristic soft crumbs are due to the swelling properties of wheat-flour gluten in water. If pure starch from another cereal or tuber is used, the product is considerably more rigid and its texture is irregular because gases are insufficiently retained in the dough. Therefore, when starches that do not contain gluten-forming proteins are used, a swelling or binding agent must be added during the preparation of the dough to bind the starch granules (such as, egg white, gums, glyceryl monostearate (Shittu *et al*.,2008; Kadam *et al*., 2012)

Efforts have been made in many countries to produce bread by conventional methods from wheat flour to which other flours such as Sorghum, Plantain, Yam, and Cassava flour were added. It was generally found that the upper limit of such an addition was about 10 percent as the quality of the resultant bread was rapidly impaired beyond this limit of nonwheat flour content. However, recent experiments have shown that it is possible to increase the level of the nonwheat flour considerably without too great a change in the bread

characteristics, provided certain bread improvers such as calcium stearyl lactylate are added or a relatively high percentage of fat and sugar is used. Bread of acceptable quality was obtained by the use of 30 percent of either cassava or corn (maize) starch and 70 percent wheat (Shittu *et al*., 2008). The use of various materials such as maize, sorghum, rice and cassava at varying levels of substitution up to 50% have been reported. Research has shown that substitution of cassava flour up to 20% had no adverse sensory and organoleptic effect on bread (Giami *et al*., 2004; Eddy *et al*., 2008). The combination of 45/30/20/5 cassava flour/wheat/sorgum/soya with a quality score of 72% gave acceptable bread.The inclusion of protein rich materials in composite flour and defatted soy flour and parboiled chickpea flour to supplement wheat flour have been reported. Onabolu *et al*, (1998); Carson *et al*.,(2000); Oyeku, *et al*., (2008) produced 80/20 wheat/cassava flour bread as well as 100% cassava bread in which egg was used as the binding agent. Onwuka (2000) used raw as well as processed mucuna bean flours for making bread in a 50/50 composite with wheat flour. Research work conducted at the International Institute of Industrial Agriculture (IITA) showed that substitution of water yam (*Dioscorea. alata)* flour for wheat at 40% gave acceptable products. Plantain and cocoyam flours are also found to be good substitute for wheat flour in bread making (Olaoye *et al*., 2006; Eddy *et al*., 2007; Oyeku, *et al*., 2008)

In Nigeria, 20% cassava inclusion has been achieved by Federal institute of industrial Research Oshodi (FIIRO) and Universal trading company (UTC). The International Institute for Tropical Agriculture (IITA) has also achieved a 40% inclusion from high quality cassava flour. The composite bread produced was adjudged by all those who eat it to be of very high quality. The result was according to the Institute's sensory evaluation

reports on cassava bread consumption. In addition to baking bread with the 10 and 20% composite cassava, FIIRO has also successfully baked various acceptable confectionery products with over 20% cassava flour inclusion in wheat flour.

FAO, 2011 reported that experiments made by the Institute of Food Technology in Rio de Janeiro showed that 10 percent flour and 5% cassava or corn (maize) starch can be added to wheat flour of only medium strength (9-11% gluten) and made into a dough containing only one percent shortening which can be baked into loaves of as good quality and appearance as those of the respective wheat-flour samples. Other experiments in some countries have been undertaken to make bread from nonwheat flours alone or mixed with wheat flour. Flours included cassava flour and cassava starch and sources of proteins included full-fat and defatted oilseed flours such as cottonseed, soybean and groundnut, as well as fish meal. In addition, binding agents, water, salt and sugar were used. The proportion of the protein source to starch was varied so as to ensure a protein content of 1820 percent in the composite flour. Results of using nonwheat flours alone suggested that the combination of cassava flour and cassava starch could be used in bread-making and that bread made from cassava flour and defatted soybean flour was of good quality. From the nutritional point of view, the protein quality of both the cassava-soya and the cassava-groundnut breads was higher than that of common wheat bread. In general, as in normal bread-making, the results depended on different factors operating in the bread-making procedure and the quality of the raw materials.

In India, a new product called tapioca macaroni was developed by adding a small percentage of specially prepared groundnut meal and wheat semolina to cassava flour. The

mixture is processed, cooked and consumed in the same way as food grains. The protein content is comparable to that of wheat (about 10%) and the macaroni is nearly twice as nutritious as rice. The Food and Agriculture Organization has lately considered it desirable to investigate the possibility of making bread and similar bakery products of raw materials derived from starchy tubers and defatted oilseeds. An agreement was made between the Organization and some well-known research institutions to study this possibility. The following experiments have been realized: Development of a bread made from nonwheat materials at the Institute of Grain, Flour and Bread (TNO), Wageningen, the Netherlands.Development of abread with partial replacement of wheat flour at the Tropical Products Institute, London (FAO, 2011).

2.5 Use of Composite Flour in Dough Making

The term composite flour refers to the process of mixing wheat flour with flours from root and tubers, cereals, legume or other raw material for bread making. The composite flour programme was initiated by Food and Agriculture Organization (FAO) of the United Nations in 1964 to help Nations meet their wheat requirement(FAO,2004).Efforts has since then been made to produce bread to which other flours were added. Composite flours have the potential to reduce wheat imports by substituting local cereals, starches, or proteins in traditional wheat products. Composite flours are already being used successful in developed nations and are meeting consumer demand for variety and nutrition in the diet. In developing countries, attempted national composite flour programs have not succeeded in reducing wheat imports even though extensive research has indicated that acceptable wheat products can be made with as much as 20–40% substitution with purified starches, 10–30% with rice flour, 5–20% with cereal and root flours, or with 3–15% of

proteinaceous flours (Oyeku *et al*., 2008). According to David and Maura, (2009) Lack of success in developing countries is attributed to, underestimation of the complexity of a national composite flour program and thus the under allocation of adequate management expertise and financial resources, lack of supply of low-cost, high-quality nonwheat flours, starches, or proteins, lack of regulatory methodologies and systems, lack of incentives for wheat millers and bakers to participate in the programme, problems associated with subsidies and commodity pricing and impaired baking quality and/or tolerance that makes the baker's job more difficult or exacting. Wheat imports have been increasing in developing countries, most of which are in the tropics. Contributing factors are its broad availability at low cost, high acceptability of wheat products, urbanization, periodic food shortages, and underdeveloped agricultural systems. The Program has been attempted in Brazil, Senegal, Bolivia, Colombia, and the Sudan, the results are encouraging. While the outlook for national composite flour programs is poor, there may well be opportunities for specialty composite flour products that meet consumer demand for variety, preference, nutrition, and low cost (Oyeku *et al*., 2008)

2.6 Prospects of Cassava Bread in Nigeria

Over the years, researchers have come out with the fact that wheat can be substituted with cassava, rice, maize and sorghum in bread making, however, the government remained undecided until the president came up with a new policy on cassava in 2002 aimed at accelerating the pace of utilisation of cassava by making the country which is the largest producer of cassava in the world, become the largest processor of high value cassava products in the world (Eddy *et al*., 2007; Adetunde *et al*., 2011).

According to reports, Nigeria will save N300 billion annually from substituting wheat with cassava (Shittu *et al*., 2007). In 2010 alone, Nigeria imported wheat worth more than N636 billion, rice worth more than N356 billion and refined sugar worth more than N217 billion. The total amount expended on importing just three items of food in one year was N1.21 trillion which is almost equal to the capital budget of the nation in 2012. The argument has been stretched further that if in the next few years the sum of N1.21 trillion could be returned back into the economy, it could regenerate more income within and form the bedrock for transforming Nigeria: more jobs will be created, agro allied companies will grow and values will be added. This is aside from the export potentials and earnings that could come from being the highest producer of cassava in the world (Boboye and Dayo-Owoyemi, 2009)

The Glycemic Index (GI). The GI measures how much each gram of available carbohydrate raises a person's glucose level following the consumption of the food and it is measured in 100. GI low range falls within 55 and below, medium within 56 and 69, while 70 and above represents the high GI range, However, from available facts, the GI of cassava is moderate, which does not constitute danger to consumers; and cassava flour has a GI of 59.34, which puts it in the medium GI range. On the other hand, white bread, most white rice, corn flakes, cereals, glucose, maltose and white potato are classified as having very high GI, while yam flour was found by a group of researchers from the University of Ibadan to have a GI of 49.81. A research was also conducted by FIIRO to find out how these carbohydrates from the cassava flour is handled by the human body, the Institute has taken up research studies that use the cassava-inclusion loaves of 10 and 20 per cent to generate the result locally. And so far, the preliminary result has been encouraging,

declaring that the cassava is quite safe. The inclusion of cassava flour of lower GI than wheat flour in bread becomes even healthier and more so for even diabetic patients **(**FIIRO, 2008; Scazzina *et al*., 2009).

2.7 Fermentation Processes

The most common yeast *Saccharomyces cerevisiae* is used in baking as a leavening agent, where it converts the food/fermentable sugars present in dough into the gas carbon dioxide. The use of sugar in bread dough accelerates the growth of yeasts (Russell, 2003)

In the bread making processes, mixing plays a major role on forming and developing the gluten structure in the dough and incorporating the necessary gas bubbles for cell structure formation in the baked product. It is the latter which makes bread a light, aerated and palatable food.

In essence mixing is the homogenisation of the ingredients, whereas kneading is the development of the dough (gluten) structure by "work done" after the initial mixing. As fermentation proceeds the gas cells in the dough become larger and larger as more gas is produced. It is necessary to punch or remix the dough. Punching or remixing subdivides the gas cells to produce many smaller cells. To be sure, a large amount of carbon dioxide is lost in the atmosphere, but the important aspect of the process is the creation of the new gas cells (Chavan and Jana, 2008; Cauvain and Cauvain, 2010).

Another important benefit of punching or remixing is the mixing of the dough ingredients. Yeast cells do not have mobility in dough. Therefore, they depend upon the sugar diffusing to them. As fermentation proceeds, the diffusion distances become large, so the

concentration of sugar diminishes, along with the rate of fermentation. Punching or remixing brings the yeast cells and fermentable together again. In addition to its gas-producing capabilities, yeast also affects dough rheology (Wang *et al*., 2011; Toshiyuki and Yasuhide, 2013)

In bread making, yeast growth is carried out under aerobic conditions. This results in increased carbondioxide (CO_2) production and minimum alcohol accumulation. The fermentation of bread involves several steps: alpha- and beta- amylases present in the moistened dough releases maltose and sucrose from starch. Then a baker's strain of the yeast *Saccharomyces cerevisiae*, which produces maltase, Invertase, and zymase enzymes, is added. The carbondioxide (CO_2) produced by the yeast results in the light texture of many bread, and traces of fermentation products contribute to the final flavor. Usually baker's add sufficient yeast to allow the bread to rise within 2- hours-the longer the rising time, the more additional growth by contaminating bacteria and fungi can occur, making the product less desirable (Prescott *et al.,* 2008).

Commercial yeast has optimal reproduction temperature at 28°C or lower. Even though the yeast can ferment well at 32-35°C, its metabolic activity is compromised at high temperatures. Furthermore, the yeast's ability to tolerate high temperature declines dramatically when other stress conditions are introduced, such as high osmotic stress, low pH, limited nutrients and low oxygen levels. Most yeast strains grow well at pH ranging from 3.8-6.0. A yeast culture has an initial pH of 5.2-5.5, and during fermentation, yeast cells keep excreting H^+ ions which lowers the pH of the broth to 4.0-4.5.Growth of lactic acid bacteria in the late fermentation stage can lower the pH further to 3.8 (Russell, 2003;

LeBlanc *et al*., 2004). Since the fermentation rate is much higher using yeast cells in their growth phase, maintaining cells in their growth stage is very important for maximum gas production. *Saccharomyces cerevisiae* is able to utilize sugars in the presence of oxygen (aerobic) and in conditions that are completely devoid of oxygen (anaerobic). Aerobic catabolism produces more energy and is called respiration, while anaerobic catabolism produces less energy and is called fermentation. Compared to most other yeast species, *Saccharomyces cerevisiae* is able to perform fermentation, and can grow under strictly anaerobic conditions (Van Den Brink *et al*., 2008). It is one of the few yeast that is able to grow anaerobically (Visser *et al.*, 1990). When *Saccharomyces cerevisiae* is grown under conditions with abundant oxygen but very limited amount of sugar, it produces very little or no ethanol. However, when the amount of sugar exceeds 0.1%, it produces ethanol, regardless of the presence of oxygen (Piskur *et al.*, 2006). This is called the Crabtree effect. *Schizosaccharomyces pombe* is another example of Crabtree-positive yeast, which ferments in the presence of oxygen. Yeast like *Kluyveromyces lactis* and *Candidia albicans* don't produce ethanol in the presence of oxygen, and thus are Crabtree-negative yeast. Under aerobic conditions, after the depletion of glucose, the metabolism in *Saccharomyces cerevisiae* changes such that the accumulated ethanol is utilized for yeast propagation, which is called the diauxic shift. However, under strict anaerobic conditions, ethanol will not be consumed. Once sugars enter the yeast cell, they are processed through the glycolytic pathway into pyruvate. The fermentation pathway is involved in the conversion of pyruvate to ethanol, producing ATP, CO_2 and NAD^+ (Goddard, 2008). During fermentation (aerobic or anaerobic), yeast recycles NADH in the acetaldehyde-to-ethanol conversion step, which is catalyzed by alcohol dehydrogenase and is readily

reversible. In the presence of oxygen, ethanol can be converted back to acetaldehyde, which allows yeast to grow after depletion of sugars .Yeast other than *Saccharomyces cerevisiae* also requires the supply of oxygen to trigger fermentation. Since it is very difficult to achieve the right oxygen dose in large-scale fermentation, yeast species that are sensitive to oxygen availability are not suitable for large-scale fermentation. Other factors, such as ethanol tolerance, glucose conversion rate, vitality under acidic conditions, and public acceptance, also determine whether a yeast strain can be used for commercial-scale bread production. *Saccharomyces cerevisiae* can tolerate high ethanol concentrations up to 15%, has a glucose conversion rate of about 95% of the theoretical maximum, can grow at low pH conditions, and has the property of GRAS (Vertes *et al.,* 2008). Given its long history of use in industrial fermentation and deep knowledge about its genetics and biochemistry, *Saccharomyces cerevisiae* is still superior to other yeast species for commercial fermentation applications (LeBlanc *et al*., 2004; Vertes *et al.,* 2008).

2.8 Organisms Involved in Dough Fermentation

Saccharomyces cerevisiae, the budding yeast, is an important microorganism not only in various commercial applications such as in the food and beverage industries, but also in biomedical science research. The microorganism is very well known for its superior baking ability, which has been harnessed by humans for brewing and wine production since the very early ages of human civilization. *Saccharomyces cerevisiae* is also widely used for improving bread dough quality (Yabaya and Jatau, 2009).Compared to other closely related yeast species, *Saccharomyces cerevisiae* can be cultivated in large-scale by using a simple growth medium. It has a very broad range of carbon sources that it can utilize. It

has fast growth and fermentation rates, and is highly ethanol-tolerant (Maristela *et al*., 2008). In addition, as a food organism, *Saccharomyces cerevisiae* consists of 48% high-quality proteins and has high public acceptance due to the property of generally regarded as safe (GRAS). Thus, not only does *Saccharomyces cerevisiae* still play vital roles in our daily lives, but it has also been used as a very important tool to advance biomedical science research due to several useful properties. As a eukaryote, *Saccharomyces cerevisiae* has a cellular organization similar to those of plants and animals, but its genetic properties allow it to be manipulated almost as readily as *Escherichia coli*. *Saccharomyces cerevisiae* is a very efficient expression system for heterologous genes and a model system for unraveling molecular, biochemical and genetic details of gene expression and the secretion process (Francisco *et al*., 2002). *Saccharomyces cerevisiae* has been intensively studied to examine how it can be exploited and manipulated to benefit the industry (Yabaya and Jatau, 2009). Romano *et al.,* 2008, reported that *Saccharomyces cerevisiae* is capable of fermenting all sugars present in the dough, for example, glucose, fructose, sucrose and maltose with eight (8) times faster than *Pichia membranificiens* which can only ferment glucose. Bakers and baking companies are in constant pursuit of yeast strains with dough raising properties to meet their daily baking industry requirement (Yabaya and Jatau, 2009; Ozcan, 2001; Ma'aruf *et al*., 2011).

Typical yeasts associated with LAB in doughs are *Saccharomyces exiguus, Candida humilis* formerly described as *Candida milleri)* and *Issatchenkia orientalis* (*Candida krusei*); Other yeast species detected in dough ecosystem are *Pichia anomala* as *Hansenula anomala,* Saturnispora saitoi as *Pichia saitoi*, *Torulaspora delbrueckii*, *Debaryomyces hansenii* and *Pichia membranifaciens* (Hammes *et al*., 2005). The presence of *Candida*

humilis in dough was reported by Barnett *et al*. (2000), however the dominance of *Candida* humilis in dough is a new observation**.**

In line with the ability of growing in many ecological niches, *Lactobacillus plantarum* may be used in different food and health applications. Some studies reported that use of *Lactobacillus plantarum* in dough fermentation represent a valid means to improve texture, flavours and shelf-life of bakery products. *Lactobacillus plantarum* 20B showed a good sourdough acidification and acid acetic production when pentosans and L-arabinofuranosidase where also added to the dough (Gobbetti *et al*., 2005). Lactic acid produced by LAB (including *Lactobacillus plantarum*) carbohydrate metabolism, is the main compound responsible for dough acidification and also have a little effect on bread flavour. Acetic acid is also positive because it does improve the sensory properties of the final product. Other studies reported that the time of leavening and the acidification degree detected during dough manufacture were improved by the presence of *Lactobacillus plantarum* strains (Pepe *et al*., 2004). A good dough acidification (pH lower than 5.0) also prevents the growth of undesirable microorganisms, such as, *Enterobacteriaceae* and rope-forming bacteria. Growth inhibition of typical bread spoilage rope-forming (*Bacillus subtilis* and *Bacillus licheniformis*) was observed, when 20–30 % of sourdough fermented with *Lactobacillus plantarum* VTT E-78076, was added to the wheat dough (Katina *et al*., 2002). Furthermore the *Lactobacillus plantarum* antifungal properties in bakery products were also observed. The phenyllactic acid and its 4-hydroxy derivate produced by *Lactobacillus plantarum* 21B possessed antifungal activity against the common spoiling fungi of bakery products belong to genera *Apergillus, Penicillium* and *Fusarium* (Lavermicocca *et al*., 2003; Valerio *et al*., 2009). Recent studies, showed the potential

application of *Lactobacillus plantarum* CRL 778 for improving the nutritional quality and the shelf life of bread made with quinoa dough (Dallagnol *et al*., 2011). A good lactic acid production and protein hydrolysis were observed during fermentation, and an abundant production of phenyllactic and hydroxyphenyllactic acids were also obtained during the study. *Lactobacillus plantarum* is considered one of more important lactic acid bacteria used in fermentation (Sabatini *et al*., 2008.)

Association of yeasts and lactic acid bacteria are often used in the production of beverages and fermented foods. The vast majority of yeasts found in doughs have been allotted to the species *Candida milleri*, *Candida holmii*, *Saccharomyces exiguous* and *Saccharomyces cerevisiae* (Clarke *et al*., 2004). Most of yeast preparations often contain LAB, especially lactobacilli rather than *Pediococcus*, *Lactococcus* and *Leuconostoc spp* (Collado-Fernández, 2003).Which contributes a little to the aroma development acidification and of dough because of the limbed processing time (Clarke *et al*., 2004; Corsetti and Settanni 2007)

2.9 The use of Co-culture in Dough Fermentation

Microorganisms co-exist in the natural environment.This including animal and plant products being either beneficial or detrimental to the process. It is also generally known that the growth of a microorganism as a pure culture can be substantially different from its growth in a mixed culture, due to microbial interactions and can be used as starter culture. Starter cultures are defined as "the preparation containing live or inactive microorganisms with ability to create desirable metabolic activity" (Gerekova *et al*., 2011). The application of starter cultures has become the state of art. Suitable strains combination allows process

standardization, mitigation of hygienic risks and product diversification (Ammor *et al*., 2005; Gul *et al*., 2005). Starter culture development in baking industry was initially based on isolation of native microflora from sourdough, biochemical and taxonomic characterisation of the microorganisms (predominantly Lactobacilli) and the selection of strains appropriate as starter cultures through time-consuming application trials. The superiority of commercial starters is in the improvement of the dough production control while ensuring reliable quality in bread production and allows the production of full dough in a one-stage process. Starter cultures performance within fermentation process has been referred to as pH value decrease, total titratable acidity increase and organic acids production (Robert *et al*., 2006; Gereková *et al*., 2011).The genus *Lactobacillus* are regarded as GRAS (generally recognised as safe) bacteria which means that their addition in food causes no risk for health (Badel *et al*., 2012). The safe character of lactobacilli attracts baking industry by simplifying the regulation and application in dough preparation. The most common lactobacilli species used as single-strain or mixed strain starter cultures in bakery industry are: *Lactobacillus sanfranciscensis, Lactobacillus plantarum, Lactobacillus paralimentarius, Lactobacillus brevis, Lactobacillus pontis*, *Lactobacillus amylovorus, Lactobacillus alimentarius, Lactobacillus delbrueckii, Lactobacillus crispatus, Lactobacillus reuteri, Lactobacillus casei,* and *Lactobacillus fermentum* (Paramithiotis *et al*., 2006; Ganzle, 2009; Weckx *et al*., 2010; Gerekova *et al*., 2011) .

Some additional desirable traits for these microorganisms are their ability to produce flavour components that favour consumption of these foods in traditional and new markets; the capability to break down antinutritional factors (That is phytic acid) present in some substrates; the production of enzymes to utilize recalcitrant wastes as substrates; the

inability to synthesize toxins and other undesirable secondary products; and thermotolerance and osmotolerance, which are important characteristics in solid substrate fermentation processes (Ganzle,2009; Gerekova *et al.*, 2011)

According to Kimaryo *et al*. 2000, the use of the starter cultures would be an appropriate approach for the control and optimization of the fermentation process in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. The prospect of applying starter cultures will become attractive to the small-scale processor only if benefits, such as reduction of costs (e.g. energy), reduced fermentation times, reduced risk of spoilage (increased shelf-life), improved process control, improved sensory quality (taste, aroma, visual appearance, texture, consistency), improved safety attributes (e.g. lower risk of diarrhoea, detoxification of cassava) and reduced preparation procedures for the final product, are perceived. Incorporation of these organisms into starter cultures may therefore, upgrades the nutritional value of foods. Furthermore, selected strains may enhance the general benefits of spontaneous fermentation such as improved protein digestibility and micronutrient bioavailability, and contribute more specifically to biological enrichment through the biosynthesis of vitamins and essential amino acids. Spontaneous food fermentations are neither predictable nor controllable. Pure cultures isolated from mixed populations of traditional fermented foods exhibit a diversity of metabolic activities, which vary even among strains. These include differences in growth rate, adaptation to a particular substrate, ability to degrade antinutritive factors, antimicrobial properties, flavour and quality attributes and competitive growth behaviour in mixed cultures. Single- and mixed-strain cultures are must of the time tested at the pilot scale, prior to their use in

small-scale operations. According to Holzapfel,(2002), the introduction of starter cultures in traditional small-scale fermentations should incorporate considerations for improving processing conditions and product quality through: rapid accelerated metabolic activities (acidification or alcohol production), improved and more predictable fermentation processes, desirable sensory attributes, improved safety and reduced hygienic and toxicological risks. Commercial starter cultures generally originate either from food substrates or from the processes in which they are applied. Environmental conditions, back-slopping, adaptation and the repeated use of specific utensils can contribute to the selection of microbial populations typical of a fermentation process. The selection of suitable starter strains should take into account their interactions in mixed cultures, with consideration for the behaviour of these strains under defined conditions, and within the food substrate. Other factors, which should be considered, include: competitive behaviour, viability and survival, antagonism against pathogens and spoilage microbes, the rate of acid or alcohol production, organoleptic changes, primary metabolites of fermentation, degradation of antinutritive factors, detoxification (Asmahan and Muna, 2009).

Interactions between yeasts and lactic acid bacteria and their respective role in wheat dough have been well established. It is believed that the growth of the various lactic acid bacteria is promoted in co-culture with yeast mainly due to the excretion of specific amino acids and small peptides (Cheirship *et al*., 2003; Paramithiotis *et al*., 2006). The interaction between LAB and yeast is important in the acceptability of the final product, as the production of acid influences the final characteristics of the bread, mostly texture and flavor (Gobbetti *et al*., 1998; Rehman *et al*., 2007). A co-culture of obligate and or facultatively heterofermentative lactobacilli and yeast dominate mature dough. The use of

heterofermentative LAB results in the production of flavoring compounds like ethyl acetate, alcohols and aldehydes, whereas, homo-fermentative LAB produce diacetyl and other carbonyls. However, yeast fermentation results in the production of iso-alcohols only, which may contribute little towards final bread flavor (Rehman *et al.*, 2006).The most typical and well-studied association of LAB and yeasts in sourdoughs is *Lactobacillus sanfranciscensis* and *Candida humilis*. Research has shown that Sourdough bread utilizes many hetero- and homofermentative LAB, as well as yeast, with *Saccharomyces exiguus, Candida krusei, Pichia* norvegensis and *Hansenula anomala* being prevalent at about a 100:1 LAB: yeast ratio, giving the bread its distinct flavour (Gobbetti *et al*., 1998). The most prevalent species of LAB include *Lactobacillus plantarum, Lactobacillus sanfranciscensis* and *Lactobacillus brevis*, (Corsetti *et al*., 2001). It has been shown that volatiles in dough bread are important in the sensory evaluation of the product. The most intense bread-like flavor is related to many compounds including 2-phenylethanol, derived from phenylalanine. bread fermented with *Lactobacillus plantarum* exhibits an unpleasant metallic sour taste, but when supplemented with *S. cerevisiae* the bread has a more aromatic flavour and shows a higher content of aromatic compounds including 2-phenylethanol (Hansen and Schieberle, 2005). LAB are generally regarded as safe (‘‘GRAS’’) and therefore dominant microbes of these fermentations do not appear to be associated with any health risks. These beneficial microorganisms serve to some extent in safeguarding against pathogens and spoilage organisms. Lactic fermented foods in particular are considered to be safe and wholesome. On the other hand, acidification to pH values of less than 4.2 constitutes a major safety concern in fermented foods. Recent observations, however, confirm that a number of metabolites, such as acetic acid (from

heterofermentative LAB), hydrogen peroxide and bacteriocins, produced during the fermentation process, exhibit antimicrobial properties which may contribute to the safety of lactic fermented foods. Organic acids, which show strong antagonistic effects in the undissociated form at lower pH values, are particularly effective in inhibiting Gram-negative bacteria, such as pathogens (De Vuyst and Neysens, 2005)

The predominance of lactic food fermentations in Africa and the contribution of these fermentations to food safety deserve special attention. Low expected turnover, distribution over extensive areas, and sophisticated methods for propagation and preservation are factors which complicate LAB starter culture development. Studies into the application of selected LAB strains for traditional fermentations, however, sug'gest sufficient potential for the use of LAB starters in small-scale food fermentations in Africa (Holzapfel, 2002). The traditional starter kudeme is used as an inoculant in West Africa, for preparing the fermenting cassava dough, agbelima. A stable LAB-enriched starter dough, aflata applied in the preparation of the fermented maize-meal product, kenkey, produces accelerated acidification and contains a natural selection of acid resistant strains. Cabinet and drum-drying of this dough from 54 - 10% moisture content, was shown to enhance the viability of LAB strains thereby indicating the potential for its extended storage and distribution as a starter. Addition of calcium carbonate ($CaCO_3$) (''chalk'') to fermenting substrates increases retention of the metabolic activities of LAB. $CaCO_3$ confers this protective effect primarily through the neutralisation of lactic acid. It is not, however, effective in inhibiting undesirable microbes, such as yeasts and moulds. Fermenting *Lactobaccilus plantarum*, isolated from kivunde, a traditional product of the Western and North-Western Regions of

Tanzania produced safe cyanide levels less than 10 mg/kg during cassava fermentations (Kimaryo *et al*., 2000). Kivunde is typically formed into small balls, (3–5 cm in diameter) and air-dried, thereby preserving viable strains over extended periods. These starter cultures may find application and might serve to improve small-scale fermentations even in rural areas, upon identification and selection of suitable strains. Such approaches will, however, only be successful if the basic principles of good processing practices (GMP) are observed (Holzapfel, 2002)

2.10 The Selected Organisms used in the Study

Fermentation is a traditional biotechnology that has evolved from 'natural' processes in which nutrient availability and environmental conditions select particular microorganisms, to the use of specific starter cultures. Traditionally, the fermenting organisms came from the natural micro-flora or a portion of the previous fermentation. In many cases the natural micro-flora fermentation is chanced and spontaneous, hence could be inefficient, uncontrollable, and unpredictable, in most cases, the starter organisms could be destroyed during preparation of the product prior to fermentation (eg pasteurization). A starter culture which may consist of single or mixed pure strains can however provide particular characteristics that could impact predictable fermentation benefits (Holzapfel, 2002).The association of LAB and yeast in baked foods is well documented (Ali *et al.*, 2009; Byrne, 2009).

2.10.1 The yeast Saccharomyces cerevisiae

Saccharomyces cerevisiae is a species of yeast. It is one of the most useful microorganisms and has been selected for decades for their dough-leavening characteristics. Yeast used in baking is predominately *Saccharomyces cerevisiae*. The yeast produces carbon dioxide that results in dough leavening and contributes to the flavor and crumb structure of bread.

Yeast belongs to the phylum Thalophytes. Yeast is a single cell fungus. It is a 'facultative anaerobe,' which means that it is biologically capable of growth and respiration in the presence and absence of oxygen. Yeasts, especially strains of *Saccharomyces cerevisiae* have long been used for the production of bread, alcoholic beverages, solvents and other chemicals, *Saccharomyces cerevisiae* has been known to man for thousands of years as they are involved in bread leavening and fermentation processes. In the modern bakery, yeasts are used for manufacturing of different kinds of bread and confectionaries. They lack chlorophyll but however need energy to survive. Fermentation and respiration are the two basics ways they obtain energy (Teramoto *et al*., 2005; Corriher, 2001)

Yeast converts the fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand or rise as the carbon dioxide forms pockets or bubbles. When the dough is baked it 'sets' and the pockets remain, giving the baked product a soft and spongy texture. Without fermentation by yeast, a living and natural product, the equivalent would not be obtained of bread (Nasrin *et al*., 2007; Corriher, 2001)

Simple Sugar → Ethyl Alcohol + Carbon Dioxide

$C_6 H_{12} O_6 \rightarrow 2C H_3 CH_2 OH + 2CO_2$

According to Cauvain and Young (2006), the higher the levels of yeast present in the recipe, the faster the rate at which carbon dioxide will be produced. Several investigations have been carried out in different natural and crop-growing environments so as to obtain better knowledge of yeast biodiversity and to define the impact of this on food products. Wild yeast has been isolated from fruits, cereals and some other fermented food products and found to compete favourably with commercially available yeast in dough leavening **(**Ma'aruf *et al*., 2011).Plant materials containing fermentable sugars provides suitable substrate for yeast species. The yeast strains which are popular today are breed for gas production and fermentation speed. Among the studies that has been reported including a Brazilian sugarcane spirit (plant materials) (Maristela *et al*., 2006), and fresh orange fruit and juice (Francisco *et al*., 2002), in crop-growing environment in Cameroon (Marzia *et al*., 2008) and on tropical fruits, flowers and leaves. Raw fruit juice is usually a strongly acidic solution, containing from 10 to 25 percent soluble sugars. Its acidity and high sugar concentration make it an unfavourable medium for the growth of some bacteria but highly suitable for LAB, yeasts and moulds. Raw fruit juice naturally contains many yeasts, moulds, and bacteria, derived from the surface of the fruit (Francisco *et al*., 2002). Varieties of the *Saccharomyces* strains are the most common yeasts in fermented foods and beverages based on fruit and vegetables. *Saccharomyces pombe* and *Saccharomyces cerevisiae* are the dominant yeast in the production of traditional fermented beverages,

especially those derived from maize and millet (Trindade *et al.*, 2002; Ma'aruf *et al*., 2011).

2.10.2 The diversity of lactic acid bacteria

Lactic acid bacteria (LAB) are a rather diverse group of related bacteria with few common properties and the most important is that they produce lactic acid as major metabolic product. Lactic acid bacteria have the property of producing lactic acid from carbohydrates through fermentation (Reddy *et al*., 2008). Lactic acid bacteria have been used to ferment or culture foods for at least 4000 years. These organisms are heterotrophic and generally have complex nutritional requirements because they lack many biosynthetic capabilities. Most species have multiple requirements for amino acids and vitamins. Because of this, lactic acid bacteria are generally abundant only in communities where these requirements can be provided. Lactic acid bacteria are used in the food industry for several reasons. During their growth, they break down the carbohydrate content of the foods by fermention and lactic acid produced lowers the pH. It is this acidification process which is one of the most desirable effects of their growth. The pH may drop to as low as 4.0, low enough to inhibit the growth of most other microorganisms including some common human pathogens, thus, prolonging the shelf life of these foods. LAB consists of bacterial genera within the phylum Firmicutes comprising of about 20 genera. The genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella* are the main members of the LAB (Jay, 2000; Ercolini *et al*., 2001; Holzapfel *et al.*, 2001; Axelsson, 2004;). *Lactobacillus* is the largest of these genera, comprising around 80 recognized species (Axelsson, 2004). The classification of lactic acid bacteria has been based on the

Gram reaction and the production of lactic acid from various fermentable carbohydrates. *Lactobacilli* vary in morphology from long, slender rods to short coccobacilli, which frequently form chains. Typical LAB are Gram-positive, nonsporing, catalase-negative, devoid of cytochromes, anaerobic but aerotolerant cocci or rods that are acid-tolerant and produce lactic acid as the major end product during sugar fermentation (Axelsson,2004). However, under certain conditions, some LAB do not display all these characteristics. Thus, the most profound features of LAB are Gram positive reaction and inability to synthesize porphyrin groups. The inability to synthesize porphyrin (e.g., heme) results in the LAB being devoid of catalase and cytochromes (without supplemented heme in the growth media). Therefore, the LAB does not possess an electron transport chain and rely on fermentation to generate energy (Axelsson, 2004). Since they do not use oxygen in their energy production, LAB grows under anaerobic conditions, but they can also grow in oxygen's presence. They are protected from oxygen by-products (e.g. H_2O_2) because they have peroxidases. These organisms are aerotolerant anaerobes. Because of the low energy yields, lactic acid bacteria often grow more slowly than microbes capable of respiration, and produce smaller colonies of 2–3 mm in diameter. Lactic acid bacteria can grow at temperatures from 5 - 45 ^{0}C and not surprisingly are tolerant to acidic conditions, with most strains able to grow at pH 4.4. The growth is optimum at pH 5.5–6.5 and the organisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates.LAB can be divided into three groups according to the process of sugars utilization (homofermentative, heterofermentative and facultatively heterofermentative). For homofermentative genera (*Pediococcus* sp., *Streptococcus* sp, *Lactococcus* sp. and some lactobacilli), hexoses are

metabolized by enzymes of the glycolytic Embden-Mayerhoff pathway. More than 90 % of substrate is converted to lactic acid during anaerobic metabolism. Homofermentative: produce more than 85% lactic acid from glucose. They ferment 1 mol of glucose to 2 mol of lactic acid, generating a net yield of 2 mol of ATP per molecule of glucose metabolized. Lactic acid is the major product of this fermentation. Some of the *Lactobacillus* spp. of interest for commercial scale lactic acid production such as *Lactobacillus casei* sub specie. are facultatively heterofermentative, producing only lactic acid from glucose under anaerobic or microaerophilic conditions, but lactic and acetic acids, aceton, and carbon dioxide under aerobic conditions (Reddy *et al*., 2008).

Heterofermentative genera (*Weisella* sp., *Leuconostoc* sp. and some *Lactobacilli*) metabolize hexoses via both pathways, Pentose phosphate pathway predominates in the deficiency of fermentable sugars. Heterofermentative: produce only 50% lactic acid. These ferment 1 mol of glucose to 1 mol of lactic acid, 1 mol of ethanol, and 1 mol of CO_2. One mole of ATP is generated per mole of glucose, resulting in less growth per mole of glucose metabolized (Singh *et al*., 2006; Wee *et al*., 2006; Reddy *et al*., 2008) .During this process only 50 % of substrate is converted to lactic acid, the rest is metabolized to acetic acid, formic acid and ethanol.

Lactic acid bacteria which are able to convert starch into lactic acid and other organic acids via their own hydrolytic activity have been described as amylolytic lactic acid bacteria (ALAB). The ability of LAB to ferment starch into organic acids is due to amylase activity. The search for amylolytic lactic bacteria has been justified by the high starch

content of raw materials such as rice, millet, maize. Researchers have reported that amylolytic lactic acid bacteria (ALAB) may account for 65% of isolates from different types of foods, non dairy food environments, and different geographical areas (Damelin *et al*., 1995; Reddy *et al*., 2008).

2.10.3 Some uses of lactic Acid bacteria in food

The representative's genera of LAB used as food are Lactobacillus, Leuconostoc, Pediococcus, and Weissella .*Lactobacillus plantarum, Lactobacillus sanfranciscensis* and *Lactobacillus brevis* are most widely used LAB in dough fermentation. They are generally recognized as safe organisms. Beside this, several species of yeast, *Sacchromyces cerevisiae* and *Candida humilis* are added in dough (De Vuyst and Neysens, 2005).Like other fermented foods produced by mixed micro flora, sensory, health and nutritional properties of baked dough products depend on joint activity of yeast and LAB (Gobbetti, 1998). The yeast is mainly responsible for the leavening, while LAB are the main cause of dough acidification. Although they are known for their role in the preparation of fermented dairy products, they are also used for pickling of vegetables, baking, wine making, curing fish, meats and sausages. Lactic acid bacteria are used to produce cultured foods with improved preservation properties and with characteristic flavours and textures different from the original food (Chatterjee *et al*., 2011)**.**

2.10.3.1 Exopolysaccharides production

Exopolysaccharides (EPS) are polysaccharides secreted by *Lactobacilli*. EPS are able to replace hydrocolloids in gluten-free dough (Tieking and Ganzle, 2005). EPS produced by bread associated *Lactobacilli* favourably influence host of 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 (Tieking and Ganzle, 2005; Patel *et al*., 2012). 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 (Ganzle, 2009). In situ production of homopolysaccharides in sourdough was reported to be more effective than external addition (Galle *et al*., 2012).The presence of certain lactic acid bacteria in sourdough can lead to the production of exopolysaccharides (EPS), some of which are included in the fibre fraction and seem to have beneficial effects on consumers' health by lowering the cholesterol level. Furthermore, EPS can improve the product texture by increasing the viscosity. Levan and dextran are the most studied EPS in the food industry and they can be produced in the dough by some lactic acid bacteria under certain cultural conditions (Tieking and Ganzle, 2005; Tsuda 2013)

2.10.3.2 Flavour development of baked food products.

The flavour of leavened baked goods is influenced by raw materials, dough fermentation, the type of starters, proofing and baking conditions. The ratio between lactic acid and acetic acid is an important factor affecting the aroma of the final bread (Corsetti and Settanni 2007), and it is influenced by the fermenting microorganisms, the fermentation temperature, and the type of flour (Hansen and Schieberle 2005).Fermentation was shown to induce production of flavouring compounds also in gluten-free products. For example, fermentation of sorghum for production of 'towga' generated different flavouring compounds (Mugula *et al*., 2003). Alcohols were produced in high concentration when

fermentation was carried out with *Lactobacillus orientalis* in combination with *Lactobacillus brevis* or *Lactobacillus plantarum*, and diacetyl was produced in significant amounts by *Lactobacillus plantarum* and *Pediococcus pentosaceus*. The production of aldehydes was increased by co-fermentation with *Lactobacillus plantarum* and yeasts. Fermentation with mixed cultures containing *Lactobacillus plantarum* enhanced the diacetyl content in maize fermented meals (Edema and Sanni 2008;Thiele *et al*., 2002; Tieking *et al*., 2003).

2.10.3.3 Safety of lactic acid bacteria.

Lactobacilli and lactococci commonly hold a GRAS status. Japan legally recognises functional foods (Foods for Specified Health Use, FOSHU) (Sanders, 2003). Lethal dose (LD50) of LAB was measured for mice by oral administration and found to be greater than 1011 cfu/kg, depending on the strain (Ishibashi and Yamazaki, 2001) commonly used in the food industry or naturally occuring in raw food materials are resistant to glycopeptide antibiotics such as teicoplanin and vancomycin (Goldstein *et al*., 2000). The term food-grade can be used when the modified microorganism contains such elements not harming the consumer when present in foods. Food-grade cloning systems need to be based on DNA from LAB or other microbes with a long history of safe use in the food industry (De Vos, 1999). Genetically modified LAB can in future be utilized as improved starters in food fermentation and for the safe production of metabolites used as food additives (De Vos, 1999).

2.10.4 *Lactobacillus plantarum* in fermented food

Lactobacillus plantarum is one of about 180 species recognized among the genus *Lactobacillus*. Many *Lactobacillus* species are highly specialized and are found in a restricted number of niches. For example, *Lactobacillus delbrueckii* spp. *bulgaricus* is adapted to the dairy environment and is widely applied in yoghurt manufacture (van de Guchte *et al*., 2006). Whereas other species such as, *Lactobacillus rhamnousus, Lactobacillus reuteri, Lactobacillus gasseri and Lactobacillus acidophilus* are found in the mammalian gastro-intestinal tract and are used as probiotic cultures (Russell and Klaenhammer, 2001; Siezen and Wilson, 2010) .The specie *Lactobacillus iners* is considered the predominant member of the vaginal microbiota (Macklaim *et al*., 2011). In contrast, the ubiquitous *Lactobacillus plantarum* is able to colonize several ecological niches such as meat, fish, vegetables, dairy products (Gardner *et al*., 2001; Ercolini *et al*., 2003; Bringel, 2005) and mammalian gastrointestinal tract (Ahrne *et al*., 1998). The ability to colonize several environments by *Lactobacillus plantarum* could be attributed to large and organised genome of this bacterium. Recent researches such as the complete genome sequencing of *Lactobacillus plantarum* strains WCFS1 (Kleerebezem *et al*., 2003; Siezen *et al*., 2012), ST-III (Wang *et al*., 2011) as well as comparative genomic studies (Siezen and van Hylckama, 2011) have shown interesting data about the genomic architecture of this bacterium. There is a high phenotypic and genotypic diversity as well as a high metabolic versatility and flexibility among *Lactobacillus plantarum* group (Bringel, 2005; Siezen *et al*., 2012; Siezen and van Hylckama, 2011). The large set of genes involved in sugar uptake and utilization as well as the large number of surface bound extracellular proteins is also likely to contribute to the large versatility with its environment

(Kleerebezem *et al*., 2003). Furthermore, it is hypothesized that *Lactobacillus plantarum* chromosome has specific regions, designated life-style adaptation regions, dedicated to the interaction with the environment (Kleerebezem *et al*., 2003).

2.10.5 Amylolytic lactic acid bacteria

Amylolytic lactic acid bacteria (ALAB) have been reported from different tropical amylaceous fermented foods, prepared mainly from cassava and cereals (e.g., maize and sorghum). Strains of *Lactobacillus plantarum* have been isolated from African cassava-based fermented products and the new ALAB species, *Lactobacillus manihotivorans* was isolated from cassava sour starch fermentations in Colombia. *Lactobacillus plantarum*, an amylolytic strain, was isolated and characterized from *burong isda*, a fermented food made from fish and rice in Philippines (Bertolini *et al*., 2001; Demiate *et al*., 2000; Naveena *et al*., 2004; Kostinek *et al*., 2007; Putri *et al*., 2011). Amylolytic strains of *Lactobacillus fermentum* were isolated for the first time from Benin maize sourdough (ogi and mawè) by Agati *et al*., (1998). Sanni *et al*., (2002) , described amylolytic strains of *Lactobacillus plantarum* and *Lactobacillus fermentum* strains in various Nigerian traditional amylaceous fermented foods. The high starch content of the raw material justifies the search for ALAB in fermented starchy food products. Their role has yet to be elucidated since mono- and disaccharides, such as glucose and sucrose, which occur naturally in cereals and cassava, are readily available for lactic acid fermentation. Processing of the raw material to a large extent determines the composition of the microbiota and, in particular, the occurrence of ALAB (Guyot and Morlon-Guyot, 2001a). Amylolytic lactic acid bacteria have repeatedly been isolated from traditional cereal or cassava-based fermented foods. Due to the ability

of their alpha-amylases to partially hydrolyze raw starch (Rodriguez-Sanoja *et al*., 2000), Researchers have established that ALAB can ferment different types of amylaceous raw material, such as corn, potato, or cassava and different starchy substrates (Vishnu *et al*., 2002; Naveena *et al*., 2005) Amylolytic lactic acid bacteria utilize starchy biomass and converts into lactic acid in single step fermentation as illustrated in figures 2.1 and 2.2. Most of the amylolytic lactic acid bacteria are used in food fermentation. Amylolytic lactic acid bacteria are also involved in cereal based fermented foods such as European sour rye bread, Asian salt bread, sour porridges, dumplings and non-alcoholic beverage production. Few of them are used for production of lactic acid in single step fermentation of starch.

2.10.6 Starch-hydrolysing enzymes –An overview

Starch-hydrolyzing enzymes are very common in nature, and can be found in a variety of species throughout animals, plants and microorganisms (Horvathova *et al*., 2001). Different starch-hydrolyzing enzymes show different specificity towards starch and/or different oligosaccharides, and vary in their ability to perform hydrolysis and/or synthesis of alpha -1,4 and alpha -1,6 linkages in starch and/or oligosaccharides (Reddy *et al*.,2008). Many organisms that are known to efficiently utilize starch produce a combination of starch-hydrolyzing enzymes to optimize starch assimilation. It has always been of great interest in the industry to understand the catalytic functions of different starch-hydrolyzing enzymes so that the “best” ones can be used for designed tasks under specific industrial conditions. There are many different ways to categorize amylolytic enzymes, including their substrate specificity, product types, sequence /domain similarities and so on. One commonly accepted method is to categorize amylolytic enzymes by their cleavage action

towards starch or oligosaccharides. Based on this, all amylolytic enzymes can be divided into four major groups (with a few exceptions (van der Maarel *et al.*, 2002), including endoamylases, exoamylases, debranching enzymes, and transferases. Among the four groups, endoamylase and exoamylase groups are the ones that have been studied the most, and many species in the groups have been widely used in industrial applications. Endoamylases are amylolytic enzymes that cleave alpha-1,4 glycosidic linkages in an endo-acting fashion. alpha-amylase (EC 3.2.1.1) is a well-known endoamylase. Alpha-amylase activity generates oligosaccharides with varying lengths with an alpha-configuration. Since alpha-amylase does not cleave alpha-1,6 linkages, its end-products constitute branched oligosaccharides. Conversely, exoamylases attack on starch or oligosaccharides in an exo-acting fashion, which specifically release glucose or maltose from the non-reducing ends (figure 2.3). Based on the specificity towards the glycosidic linkages, exoamylases can be divided into two groups. The first group exclusively cleaves alpha-1,4 glycosidic linkage, such as -amylase (EC 3.2.1.2). The second group cleaves both alpha -1,4 and alpha -1,6 glycosidic linkages, such as glucoamylase (EC 3.2.1.3) (Pandey *et al*., 2000; Horvathova *et al*., 2001). However, glucoamylase cleaves alpha-1,6 linkages at a much slower rate than alpha -1,4 linkages. It is well-known in industrial starch processing that alpha -amylase and glucoamylase attack starch and oligosaccharides in a synergistic fashion, thus optimal starch hydrolysis rate is achieved by incorporating both enzymes (Sivaramakrishnan *et al*., 2006; Fossi *et al*., 2011).Debranching enzymes and transferases have been receiving increasing attention, since under many situations starch hydrolysis cannot go to completion employing only alpha -amylase and glucoamylase activity. The addition of appropriate debranching enzymes and transferases

can increase the efficiency of starch hydrolysis. Debranching enzymes are a group of starch-converting enzymes that exclusively cleave alpha -1,6 glycosidic linkages in amylopectin and pullulan. Pullulan is a olysaccharide with a repeating unit of maltotriose that is alpha -1,6 linked. Based on substrate specificity, there are two major types of starch debranching enzymes: isoamylase (EC 3.2.1.68) and pullulanase type I (EC 3.2.1.41). Isoamylase hydrolyzes alpha -1,6 glycosidic linkages exclusively in amylopectin, while pullulanase hydrolyzes alpha -1,6 linkages in both amylopectin and pullulan. The end-products of both isoamylase and pullulanase are long linear polysaccharides (Pandey *et al*., 2000; Sivaramakrishnan *et al*., 2006).Despite their important role in starch hydrolysis, industrial applications of debranching enzymes are still very limited since the commercially available enzymes lack sufficient thermostability. The last group of amylolytic enzymes are transferases, which cleave an alpha -1,4 linkage of one molecule and then transfers part of the molecule to another one by generating a new glycosidic linkage (Rosell *et al*., 2001) This group of amylolytic enzymes includes amylomaltase (EC2.4.1.25) and cyclodextrin glycosyltransferase (EC 2.4.1.19).Cyclodextrin glycosyltransferase is currently used commercially for production of the sweetener stevioside, which can reduce the bitterness of syrup by increasing its solubility. Amylomaltase was proposed to be used for the production of thermoreversible starch gel, which has the property to be repetitively dissolved upon heating. However, neither the enzyme nor the starch gel is commercially available (van der Maarel *et al.*, 2002). To date, among all the amylolytic enzymes that have been recorded, α -amylase and glucoamylase are the ones that have been studied the most due to their industrial importance in starch processing. The employment of cloning and recombinant DNA techniques made it possible

to study many of those enzymes at the molecular level, and the knowledge has provided valuable insights into designing amylolytic enzymes with industrial-favored characteristics (Wong *et al*., 2002; Siezan and Hylckama, 2011).

2.10.7 The catalytic function and industrial applications of alpha -amylase

Alpha -Amylase (1, 4- alpha -D-glucan glucanohydrolase, EC 3.2.1.1) catalyzes the hydrolysis of alpha -1, 4 glycosidic linkages in starch and oligosaccharides with the release of maltose and glucose. So far, there are 21 different enzyme specificities recorded in alpha -amylase family. Based on their catalytic functions, they can be categorized into two different groups. One group is composed of enzymes that perform starch hydrolysis while the other group is involved in starch-modification or –transglycosylation. Members of apha -amylase family are widely used in the food, chemical, pharmaceutical, detergent, textile and bioethanol industries. Starch-hydrolyzing alpha -amylases with high thermostabilities are extensively used in starch fermentation. They are also employed to remove starch in beer, fruit juices, from clothes and porcelain and pretreat animal feed to improve its digestibility. High thermostability of many alpha-amylases is the key for their production in large-scale fermentation and commercialization. The use of thermostable alpha-amylases has transformed the starch processing industry (Van der Maarel *et al*., 2002) Early starch processing was based on acid hydrolysis. Incubating starch in acidic solution at high temperature degrades starch into glucose monomers. However, the use of acid causes the production of unwanted by-products and is highly corrosive to the equipment. Following the discovery of thermostable alpha-amylase from *Bacillus amyloliquefaciens*, the old method was replaced by the enzyme-mediated hydrolysis of starch. Recently, the

Bacillus amyloliquefaciens alpha -amylase has been replaced by a more thermostable alpha-amylase from *Bacillus stearothermophilus* or *Bacillus licheniformis*. Since the alpha - amylases used in industry are inactivated at a pH lower than 5.9 at the high temperature currently being used, the pH needs to be adjusted to six (6) by the addition of sodium hydroxide (NaOH). New findings showed that α –amylase from *Lactobacillus fermentum* 04BBA19, a starch degrading Lactic acid bacteria showed high thermostable activity at 100^0C (Fossi and Tavea (2013). The commercialization of this enzyme will advance the industry further. However, instead of exclusively employing alpha-amylase with high thermostability, the potential of using starch hydrolysis for production now encourages researchers to search for α -amylase with raw starch-binding and hydrolytic activity. A new generation of starch processing enzymes will likely transform the industry again in the very near future.

2.10.8 Amylolytic enzymes with raw starch-hydrolysing ability

Only about 10% of amylolytic enzymes have been shown to have raw starch-hydrolyzing ability, (Horváthová *et al*., 2001; Reddy 2008; Fosssi and Tavea 2013) and the presence of a distinct Starch Binding Domain (SBD) is essential for this ability. The SBD is always positioned at the C-terminal part of an amylase except for the glucoamylase from *R. oryzae* which contains the SBD at its N-terminus. Starch binding domains from different amylolytic enzymes share significant sequence identity. The similarity among the SBD is more correlated with the species origin rather than the given amylolytic enzyme family, suggesting that this independent structure was attached or removed from these enzymes during evolution (Wong *et al.*, 2002). The SBD, consisting of several α-strand segments

forming an open-sided, distorted α -barrel structure, is responsible for the ability of an amylase to bind and digest the native raw, granular starch. In α -amylases, the SBD may govern the enzyme's thermostability, while in glucoamylase the domain does not appear to participate in thermostability. Although the SBD may behave as an independent module, it was shown to be more stable in the whole enzyme molecule compared to an isolated domain (Southall *et al.,* 1999). Proteolytic cleavage of glucoamylase can separate the SBD from the Catalytic Domain (CD), which causes the CD to be incapable of hydrolyzing raw starch granules. Several strategies have been shown to effectively alleviate protease production during glucoamylase production, including nutrient and pH control, bioreactor design and use of protease inhibitors (Pedersen *et al.,* 2000a; Xu *et al.,* 2000; Bertolin *et al.,* 2003).

2.10.9 Applications of alph-amylase in bread and baking industry

Amylases are among the most important hydrolytic enzymes for all starch based industries, and the commercialization of amylases is oldest with first use in 1984, today, amylases have the major world market share of enzymes. Several different amylase preparations are available with various enzyme manufacturers for specific use in varied industries.

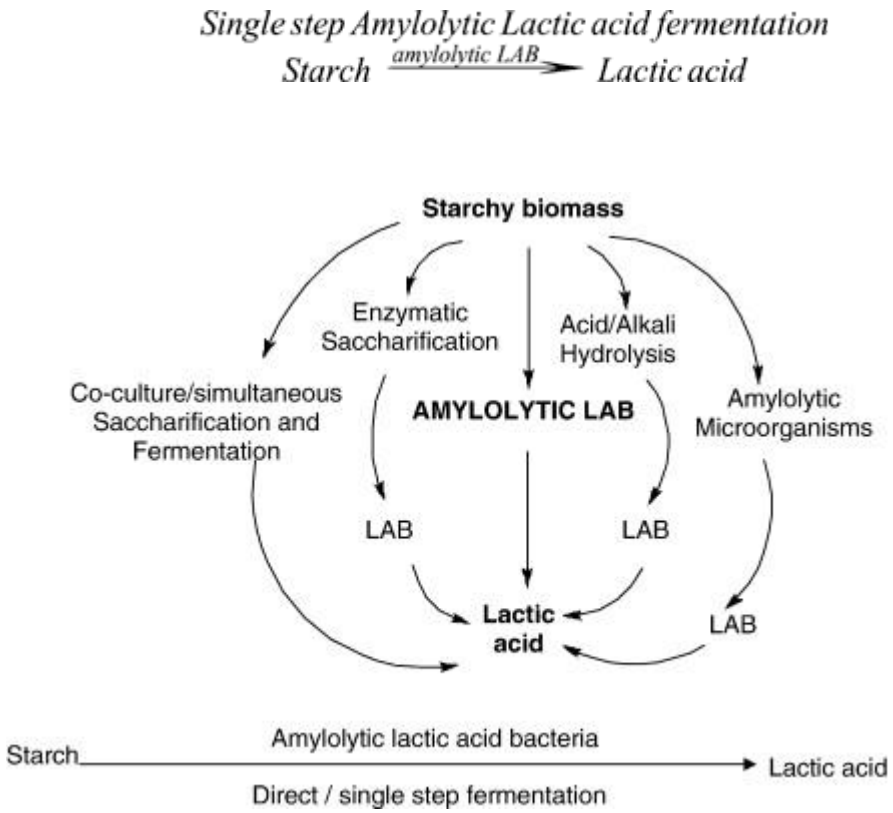


Fig 2.1: Schematic representation of ALAB fermentation. Reddy *et al*., 2008

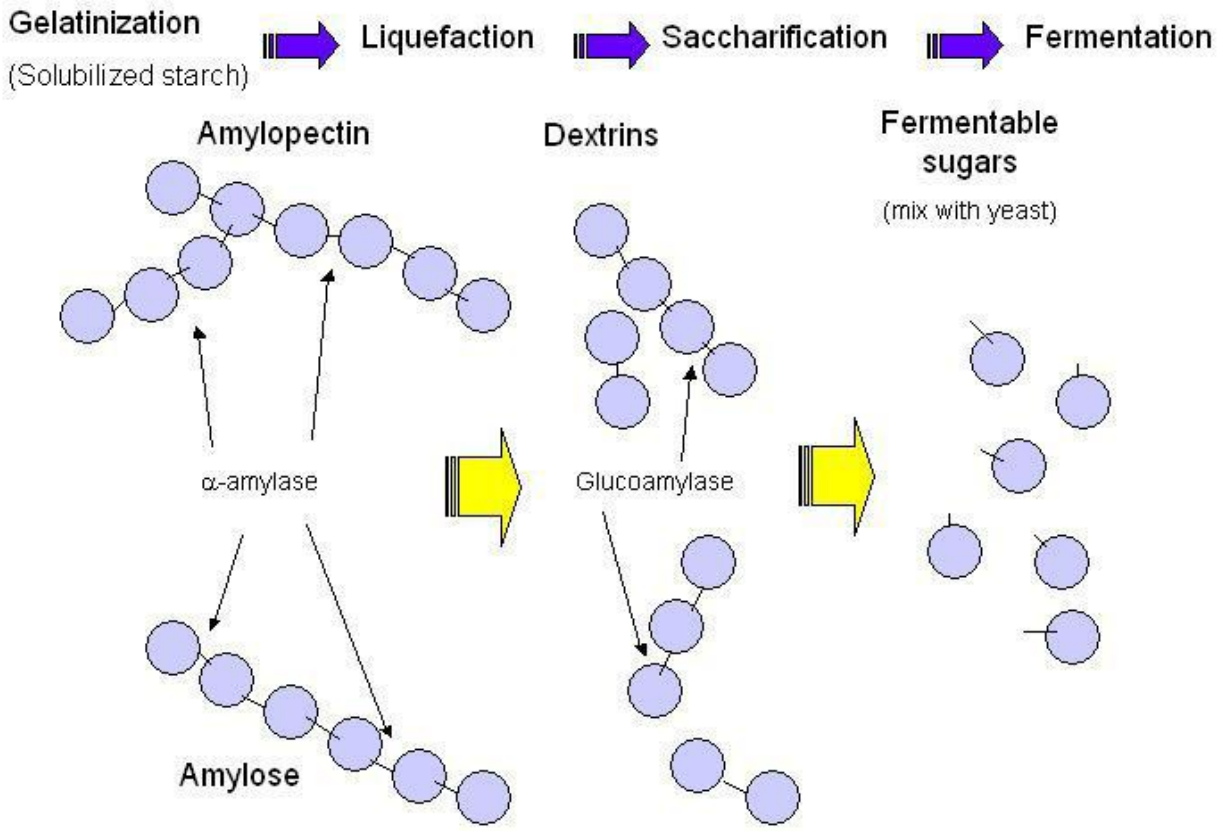


Figure 2.2: Schematic representation of the action of amylases on starch. (Reddy *et al*., 2008)

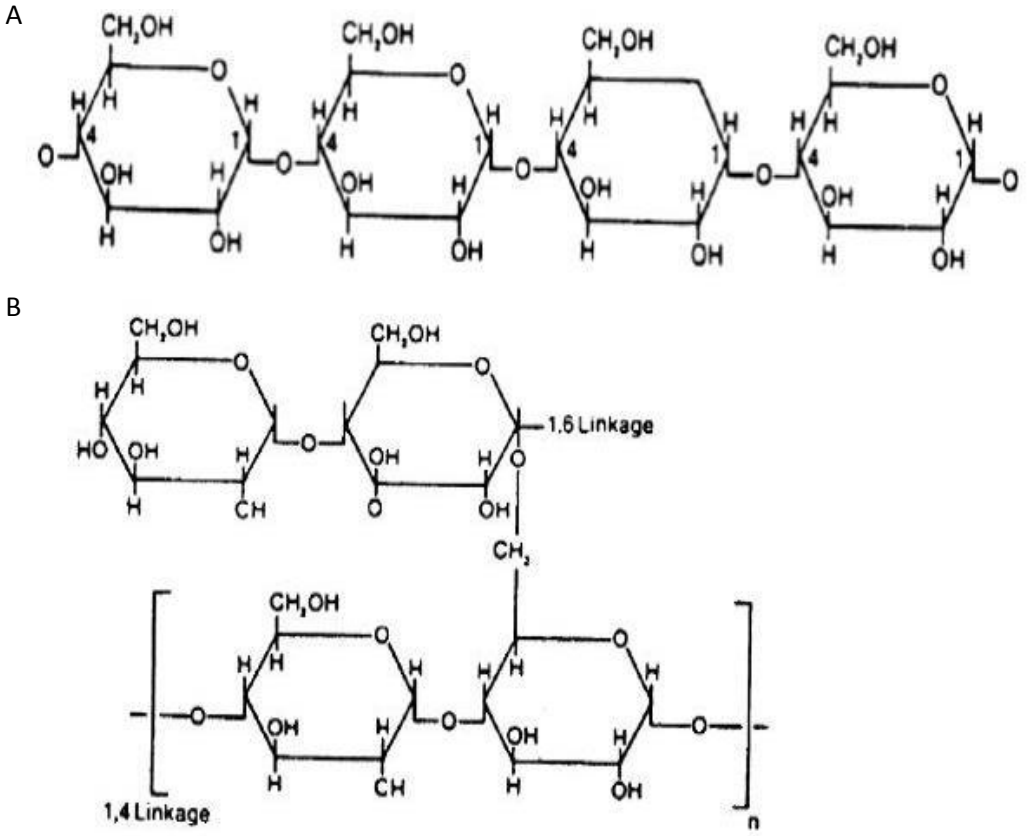


Fig 2.3: Structure of amylose and amylopectin. In amylose, the glucose residues are linked together by α-1, 4 linkage (A). In amylopectin, parallel amylose chains are linked together by two glucose residues at the branch points by α-1, 6 linkage (B). (Reddy *et al.*, 2008

The baking industry has made use of these enzymes for hundreds of years to manufacture a wide variety of high quality products. For decades, enzymes such as malt and microbial alpha-amylases have been widely used in the baking industry. These enzymes were used in bread and rolls to give these products a higher volume, better colour and a softer crumb (Rosell *et al*., 2001; Guy, 2001). It is the malt preparation that has led the way and opened the opportunities for many enzymes to be used commercially in baking.

Today, many enzyme preparations such as proteases, lipases, xylanases, pullulanases, pentosanases, cellullases, glucose oxidases, lipoxygenases are being used in the bread industry for varied purposes, but none had been able to replace alph-amylases. Till date, the alpha-amylases used in baking have been cereal enzymes from barley malt and microbial enzymes from fungi and bacteria. Fungal alpha -amylases have been permitted as bread additives since 1955 in the US and in 1963 in UK after confirmation of their GRAS status. Presently they are used all over the world for industrial applications (Ribotta and Le Bail, 2007). Supplementation of flour with exogenous fungal alpha-amylase having higher activities is common in the present day modern and continuous baking process. Alpha -amylase supplementation in flour not only enhances the rate of fermentation and reduces the viscosity of dough (resulting in improvements in the volume and texture of the product, but also generates additional sugar in the dough, which improves the taste, crust colour and toasting qualities of the bread. One of the new applications of alpha -amylase in the industry has been in retarding the staling of baked products, which reduces the shelf life of these products. Upon storage the crumb becomes dry and firm, the crust loses its crispness and the flavour of the bread deteriorates. All these undesirable changes in the bread are together known as staling (Ribotta and Le Bail, 2007). The importance of

retrogradation of starch fraction in bread staling has been emphasised. A loss of more than US $1 billion is incurred in USA alone every year due to the staling of bread. Conventionally various additives are used to prevent staling and improve the texture and flavour of baked products. Additives include chemicals,small sugars, enzymes/their combinations, milk powder; emulsifiers, monoglycerides/diglycerides, sugar esters, lecithin, etc; granulated fat, anti-oxidant (ascorbic acid or potassium borate), sugars/salts. Recently emphasis has been given to the use of enzymes in dough improvement/as anti-staling agents, e.g. alpha -amylase, branching enzymes and debranching enzymes, maltogenic amylases, beta-amylases amyloglucosidases. Pullulanases and alpha -amylase combination are used for efficient antistaling property (Janecek, 2009). However, a slight excess of alpha-amylases was also used which is undesirable as it causes stickiness in bread. Therefore, a recent trend is to use intermediate temperature stable (ITS) alpha -amylases. They are active after starch gelatinisation and become inactive much before the completion of the baking process. Further, the dextrin with 4-9 degree of polymerization produced by these shows the anti-staling properties. Although a wide variety of microbial alpha -amylases is known, alpha -amylase with 'ITS' property has been reported from only a few microorganisms (Gupta *et al.*, 2003; Janecek, 2009; Fossi and Tavea, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

Products of wet milled cereals (sorghum, millet, maize and acha), cassava tubers and flour, ripe fruits (mangoes, oranges, tomatoes and pineapple), wheat flour and commercial bread were purchased from Samaru and Ahmadu Bello University Community Market in clean low density polythene bags. The samples were taken to the Industrial laboratory of Microbiology Department of the Ahmadu Bello University for determination of physicochemical and proximate composition as well as isolation of the organisms.

3.2 Determination of Physicochemical and Proximate Composition of Flour samples and products

Wheat and cassava flours were analysed for particles size distribution, moisture, Ash, Lipids, protein and carbohydrate contentes (AOAC 2000, AACC. 2000)

3.2.1 Determination of particle size of cassava and wheat flour.

One hundred (100g) of flour was weighed and passed through standard sieves of sizes 425 and 250µm.The flours were shaked for five minutes and the sieved materials collected and weighed. The particle size distribution (PSD) was recorded as percentage of flour sample retained in mesh sieves.

$$PSD = \frac{\text{weight of flour samples retained}}{\text{Total weight of flour samples in all sieves}} \times 10$$

3.2.2 Determination of moisture content

The moisture content of samples was determined as described in the manual of AOAC (2000). Cleaned aluminum dishes were oven dried to a constant weight at 100°C. It was cooled in a dessicator and weighed (W1). Five (5g) of the sample was placed in the weighed dish (W2). The dish containing the sample was kept in an oven for 3hours and then cooled in the dessicator and weighed W3.

The % moisture content was calculated as: $\frac{W2-W3 \times 100}{W2-W1}$

3.2.3 Determination of ash content

Ash content of the samples was determined by incineration according to method No. 08-01 as described in manual of AOAC (2000). Clean crucibles were oven dried, then cooled in the dessicator and weighed (W1). Five (5g) of the sample was placed in the crucibles and weighed (W2). They were transferred into the Muffle Furnace at 550°C temperature until constant ashed, then removed and cooled in the dessicator and weighed (W3).

The % of Ash was calculated as: $\frac{W3-W1 \times 100}{W2-W1}$

3.2.4 Determination of lipids (fat) content

Lipids (fat) content was determined as described in AOAC (2000) manual. Clean 250 ml flasks were oven dried and then cooled in desiccators. Two (2g) of the sample was weight into labelled thimbles (filter paper).The boiling flasks were filled with N-hexane. The soxhlet apparatus were assembled and allowed to reflux for 8h.It was removed and transfered to an oven to dry and cooled in desiccators.

The % of Fat was calculated as: $\frac{\text{Weight of fat} \times 100}{\text{Weight of sample}}$

3.2.5 Determination of protein content

3.2.5.1 Digestion

Protein content of the sample was determined by weighing two (2g) of the sample into a kjeldahl flask. One (1) tablet of catalyst (copper) and 25ml conc. Sulfuric Acid ($H_2SO_{4)}$ were added. The solution was heated in a fume cupboard till solution assumes a green colour. It was allowed to cool and any black particles showing at the mouth and neck of the flask was washed down with distilled water. After cooling, the digest was transferred with several washings into 250ml with distilled water.

3.2.5.2 Distillation:

The digest was steamed through the Markham distillation apparatus for about 15minutes. Before use, a 100ml conical flask containing 10ml of boric acid was placed under a condenser. Ten (10ml) of the digest was pipetted into the body of the apparatus via the small funnel aperture and washed down with distilled water followed by 10ml of 40% NaOH solution. It was then steamed for about 5-7 minutes to collect ammonia sulphate. The receiving flask was removed and the tip of the condenser washed into the flask.

3.2.5.3 Titration:

The solution was titrated in the receiving flask using N/100 (0.01N) hydrochloric acid and the crude protein calculated by multiplying the Nitrogen content by factor F (F_{flour} = 5.70). A blank made up of 10ml 0f 40% NaOH was placed in the distillation set up containing boric acid.The distillate was collected in to a flask and titrated with 0.01N hydrochloric acid.

3.2.6 Determination of carbohydrate (CHO) by difference

This was carried out by difference. Carbohydrate content was obtained by calculations having estimated all other fractions by proximate analysis, That is; 100- (% of moisture + %Ash + %Protein + % fat).

3.3 Isolation and Identification of Yeast

Ten gram each of the wet milled cereals products and fruits samples were suspended in 90 ml of sterile distilled water and manually shaken intermittently for 10 min. Then, 0.1 ml of the resultant mixture was spread on the surface of solidified Potato Dextrose Agar plate supplemented with 0.025g of Chloramphenical (to suppress bacterial contaminants), using an L-shaped glass rod and then incubated at room temperature (30°C) for 5 days. The distinct yeast colonies formed were further sub - cultured on fresh Potato Dextrose Agar. All pure cultures were maintained on Potato Dextrose Agar slants and stored in a refrigerator at 4°C (Amapu *et al*., 2008). Yeast isolates were characterized based on morphological characteristic of yeast colonies grown on PDA agar at 30°C for 48 and 72h.

3.3.1 Microscopic observation of yeast

A drop of lactophenol cotton blue was placed on a clean slide. Using a sterile wire loop, a portion of the colony from the yeast cultures was placed in the drop of the lactophenol blue drop and teased. Then the cover slip was gently placed and observed under the microscope using x10 and x40 objective lens and observed based on cultural characteristics and microscopic morphology.

3.3.2 Identification of yeast isolates using API 20 C AUX kit (BIOMERIUX)

The fermentation pattern of yeast among carbohydrates was determined by the Analytical profile index (API 20 C AUX test kit (France Biomerieux).The strip consist of 20 cupules containing dehydrated substrates of sugars and their derivates .Yeast isolate was inoculated on PDA plates and incubated for 18h. Colonies were picked and a suspension made, whose turbidity matches McFarland Number 2(10^8). Sterile distilled water was added to the honeycombed wells of the trays to create a humid atmosphere. Approximately 100µl of the yeast suspension was transferred into cupules using a micropipette. The inoculated trays were incubated at 30°C and observation made after 24 and 48 h for agglutination. The identification was performed by entering the seven (7) digit numerical profile obtained into the API database version 4.0) (Blomberg, 2000)

3.3.3 True mycelium/pseudomycelium of yeast observation

The presence of pseudomycelium was determined by autoclaving Corn meal agar at 121°C for 15minutes and dispensing into Petri dish plates to solidify at room temperature as described by Blomberg, 2000 .A drop of the suspension obtained from the ampule of API 20C AUX medium was streaked onto the plates across the agar surface (close to one side

of the plate and over the centre).Sterile cover slips were placed over the two point inoculations and incubated for four days at 30°C. The slips were taken out of the Petri dishes and the agar wiped off the back of the slips using sterile cotton wool. The area of the inocula under as well as outside the cover slip was observed for pseudomycelium using a high dry objective (Dashko *et al*., 2014)

3.4 Determination of Growth Conditions of Yeast Isolates

3.4.1 Growth of yeast in glucose

A basal medium composed of 1.0% (^{w}/v) Yeast extracts, 0.75 % (^{w}/v) peptone and 2 % (^{w}/v) agar was in cooperated with 50% (^{w}/v) glucose and was used to grow the yeast isolates as outlined by Safri *et al*., 2011. The media was dispensed into sterile Petri dishes and inoculated with yeast cells. The plates were incubated at 30°C for 48 h and then examined for growth.

3.4.2 Growth of yeast in sodium chloride

The method of Blomberg, 2000 was adapted .Two separate media were prepared one containing 3% (^{w}/v) sodium chloride, 1.0% (^{w}/v) Yeast extract, 0.75 % (^{w}/v) peptone and 2.0 % (^{w}/v) agar and the other containing 10% sodium chloride in addition to the constituents. The media was dispensed into sterile Petri dishes and inoculated with yeast cells.The cultures were incubated at 30°C for 48 h and observed for growth.

3.4.3 Growth of yeast at elevated temperatures

Yeast cultures on PDA were incubated at 30, 37 and 45°C for 48h as described by De Angelis *et al*., 2004. The plates were observed for growth.

3.4.4 Determination of yeast tolerance to ethanol

A 0.1ml of 24h old washed yeast cells were inoculated into Yeast extract broth containing 3 6 and 10% ethanol. The tubes were incubated at 30°C for 48 and 72 h as described by Chiranjeevi *et al*., 2013. Occurrence of turbidity or sedimentation was taken for growth. Yeast extract broth without ethanol was used as control.

3.4. 5 Determination of the fermentation ability of yeast isolates

The fermentation ability of the yeast isolates was determined using oxidation/fermentation (O/F) medim composed of 10.0mg/l glucose and 0.08gm/l bromothymol blue as indicator. Nine (9) ml of the prepared medium was dispensed into duplicate test tubes (twenty four) containing Durham tubes and autoclaved at 121°C for 15minutes. After cooling; 1ml of yeast suspension of McFarland standard 2(10^8 cells/ml) was added to each tube, while the uninoculated tubes served as the control.The tubes were incubated at 30°C for 24h and were observed for colour change and gas production (Chatterjee *et al*., 2011)

3.5 Fermentation Method for Reducing Sugars and Cell Growth of *Saccharomyces cerevisiae*

The method of Abu *et al*., 2005 was adapted for the fermentation methods. Into a two (2) litre flask,50g of soluble starch,10g yeast extract,5g peptone,1g KH_2PO_4, 1g $(NH_4)_2SO_4$,1g $MgSO_4.7H_2O$ (g/l) ,were added and dissolved with one litre distilled water. The pH was adjusted to 4.5 and the media sterilized at 121°C for 15minutes. After cooling, it was inoculated with 1ml (10^8cells/ml) yeast suspension and incubated in a temperature regulated shaker for five (5) days. Sterile syringes were used daily to aseptically transfer

5ml into test tubes and thereafter centrifuged at 4000rpm for 30minutes. Supernatants were collected for reducing sugars and cell growth.

3.6 Propagation of *Saccharomyces cerevisiae*

The procedure of Ameh and Umaru (2000) was adapted for the propagation. The yeast isolates were grown on a basal medium consisting of 2%(^{w}/v)glucose,0.5%(^{w}/v)yeast extract,1%(^{w}/v)peptone,0.1%(^{w}/v) ammonium sulphate and 0.1%(^{w}/v) magnesium sulphate at pH of 5.6.Yeast cells were harvested by centrifugation and rinsing twice with sterile distilled water. The cells were air dried on sterile Millipore filter membrane of pore size 45µm and stored in the refrigerator.

3.7 Isolation and Identification of Lactic Acid bacteria

Ten grams each of the cassava products, wet milled cereals and fruit samples were suspended in 90ml of de Man-Rogosa-Sharpe (MRS) broth. It was incubated anaerobically at 30°C for 48h. Aliquots of 0.1ml was spread on the surface of solidified MRS agar (OXOID) plates and incubated anaerobically at 30°C for 48h. Resultant colonies were purified by successive streaking onto the same agar (MRS agar, OXOID) to obtain pure colonies. Lactic acid bacteria (LAB) isolates were identified using Gram staining, catalase reaction, morphological and physiological characteristics (Amapu *et al*., 2008).

3.7.1 Gram staining

Using a sterile wire loop, a small portion of the bacterial colony was placed on an oil grease free slide and a drop of sterile distilled water added.It was spread evenly and allowed to air dry.The dried slide was heat fixed to allowed the cells stick to the

slide.Crystal violet stain was added to the fixed culture and allowed to stand for one minte.It was then washed off under gentle running tap water. Enough grams' iodine (mordant) was added, allowed to stay for one minute and rinsed with water. Acetone (a decolouriser) was also added and washed off imediatetly with water.Lastely, safranin, a counter stain was added. This was allowed to stand for one minute and thereafter washed off with water.Excess water was drained off and the slide air dried.The slide was viewed under an oil immersion objective (×100) (Ochei and Kolhatkar, 2008)

3.7.2 Catalase test

The method of Ochei and Kolhatkar, 2008 was adapted .Using a wooden applicator stick, a small amount of the colony to be tested was transferred from the agar to the surface of a clean dry glass slide. One drop of 3% hydrogen peroxide was immediately placed on to the organism on the slide and mix properly. The slide was then observed for formation of bubbles indicating the organism is catalase positive.

3.7.3 Screening LAB for amylase production and starch hydrolysis.

Determination of amylase production potentials of LAB isolates was carried out following procedures adapted by Sun *et al*., 2010. Lactic acid bacteria isolates were spot inoculated onto sterile solidified MRS agar fortified with 2% soluble starch and incubated at 30°C for 24h.At the end of the incubation period, plates were flooded with Grams iodine and observed for zones of clearance.

3.7.4 Identification of amylolytic lactic acid bacteria (ALAB) using API 50 CH test kit

The isolates which were Gram positive, catalase negative and exhibited clear zone around each colony were selected for identification using API 50 CH test kit (Biomerieux, France) as described by Lopez *et al*., 2008. This test is made of strips which included 49 different sugars and their derivates in micro-chamber. Bacterial isolates were grown on MRS agar for 18h. Colonies were harvested using swab sticks and suspension made to match McFarland standard number 2(10^8cells/ml). Using a sterile syringe, 4-5 drops of the bacterial suspension were transferred into API 50 CHL medium and mixed thoroughly. Sterile distilled water was added to the honeycombed wells of the trays to create a humid atmosphere. The strips were placed in the incubation tray. The bacterial mixture was then distributed into the cupules. Anaerobic condition in the inoculated cupules was obtained by overlaying the cupules with mineral oil. Trays were incubated at 30°C and observations were made after 24 and 48 h. Evaluation of fermentation of sugars is visual by changing of indicator colour from purple to yellow. The identification of isolates was facilitated by a computer programme API LAB PLUS 4.0 database.

3.8 Determination of Growth Conditions for Lactic acid bacteria

3.8.1 Growth of lactic acid bacteria in sodium chloride

The method of Brandt *et al*., 2004 was adapted. Freshly harvested bacterial cells were streaked on separate sodium chloride medium composed of 3, 6 and 10% NaCl, 1.0% Yeast extract, 0.75% peptone and 2.0% agar each. The plates were incubated at 30°C and examined for growth after 24 and 48h.

3.8.2 Growth of lactic acid bacteria at elevated temperatures

The Amylolitic lactic acid bacteria (ALAB) isolates were inoculated on MRS agar and incubated at 30, 37, 40 and 45°C following the method outlined by Brandt *et al*., 2004. Plates were observed for growth after 24 and 48h.

3.8.3 Heterofermentation test for ALAB

In a flask containing 100ml distilled water, 1g of peptone powder, 1g of Yeast extract and 2.5% glucose were added. It was then properly mixed and nine (9) ml was dispensed into test tubes containing Durham tubes. The mixture was autoclaved at 121°C for 15minutes. After cooling; 1ml of ALAB suspension of McFarland standard 2(10^8 cells/ml) was added and overlaid with mineral oil. The tubes were incubated at 30°C for 24 h and were observed for gas production (Wee *et al*., 2006)

3.9 Fermentation Method for Reducing Sugars and Cell Growth of ALAB

The method of Fossi and Tavea, 2013 was adapted, where the basal medium for ALAB fermentation was composed of (g/l) soluble starch 10, yeast extract 10, Sodium acetate 5, K_2HPO_4 2, MgSO4.7H2O 0.2, and MnSO4.4H2O 0.05. It was dissolved in distilled water, the pH adjusted to 6.5 and one hundred (100) ml of the medium was placed into 150ml flask and autoclaved at 121°C for 15 minutes. After cooling, 1ml of the bacterial suspension (10^8cells/ml) was added. The flasks were incubated in a temperature regulated shaker for five (5) days. Sterile syringes were used daily to aseptically transfer 5ml into test tubes and thereafter centrifuged at 4000rpm for 30minutes.Supernatants were collected for reducing sugars and cell growth determination (Abu *et al*., 2005).

3.9.1 Determination of enzyme activity of ALAB.

The bacterial isolate were grown at 40°C for 70 h in a 50ml basal medium containing 1% (w/v) soluble starch and 0.5% (w/v) yeast extract. The flasks were incubated in a temperature regulated shaker. Sterile syringes were used to aseptically transfer 5ml into test tubes and thereafter centrifuged at 4000rpm for 30minutes.Supernatants were collected for amylase activity as described by Fossi *et al*., 2011.

3.9.2 Assay of amylase activity

The reaction mixture was made up of 1ml of crude enzyme, 0.5ml 0f 1% starch in 0.1ml of 1M citrate phosphate buffer (pH 6.0). The mixture was incubated at 60°C for 1h. Di-nitro salicylic acid (DNS) reagent (1) ml was added and the mixture boiled for 5 minutes in a water bath and 0.4ml distilled water was added to the reaction mixture in a test tube. The blank contained 0.1ml of 1M citrate phosphate buffer (pH 6.0), 0.5ml of 1% starch solution, 0.4ml of distilled water and 1ml of DNS reagent added. Absorbance was taken at 540nm using Jenway UV/VIS spectrophotometer and amount of reducing sugar read from a standard curve prepared from various concentrations of Glucose. The reducing sugar content of the reaction was determined using the modified method as outlined by Fossi *et al*., (2011) .One unit of amylase activity (U) was defined as the amount of enzyme able to hydrolyse a g of soluble starch during 60 min under the experimental condition. Activity was determined at different temperatures, pH and time.

3.9.3 Evaluating the effect of temperature on enzyme activity and stability

The temperature stability was determined by adding 1ml of crude enzyme, 0.5ml of 1% starch and 0.1ml of 1M citrate phosphate buffer (pH 6.0) into test tubes. It was thereafter

incubated in a water bath shaker at temperatures ranging from 27 to 90°C for 60 minutes. Exactly 0.4ml of distilled water and 1ml of DNS reagent was added. Absorbance was taken at 540nm and the activity determined using Jenway UV/VIS spectrophotometer (Adesokan *et al*., 2009).

3.9.4 Evaluating the effect of pH on enzyme activity and reducing sugar

The effect of pH on enzyme activity and reducing sugar was determine by incubating 1ml of crude enzyme, 0.5ml 0f 1% starch in 0.1ml of 1M citrate phosphate buffer pHs 4.5, 5.0, 5.5, 6.0,and 6.5 in a water bath shaker at 30°C for 60 minutes. Exactly 0.4ml of distilled water and 1ml of DNS reagent were added. Absorbance was taken at 540nm using Jenway UV/VIS spectrophotometer (Adesokan *et al*., 2009).

3.9.5 Evaluating the effect of time on enzyme activity and reducing sugar

The effect of time on enzyme activity and reducing sugar was determine by incubating 1ml of crude enzyme, 0.5ml 0f 1% starch in 0.1ml of 1M citrate phosphate buffer (pH 6.0) in a water bath shaker at 30°C for 60 minutes. Exactly 0.4ml of distilled water and 1ml of DNS reagent was added. Absorbance was then taken hourly at 540nm using Jenway UV/VIS spectrophotometer (Adesokan *et al*., 2009).

3.10 Preparation of Standard Curve for Reducing Sugar Determination

Glucose standard was prepared by weighing 0.1g into a flask and diluted with 100ml distilled water.Aliquot of 0.2, 0.4, 0.6, 0.8 and 1.0ml of the stock solution was transferred into test tubes and volume made up to 1ml with distilled water, followed by addition of 1.0ml DNS. It was then boiled for 5minutes and absorbance was measured at 540nm using

Jenway UV/VIS spectrophotometer. A standard curve was drawn by plotting concentration of the glucose against absorbance and used to determine the reducing sugar concentration and enzme activity of the isolates (Abu *et al*., 2005)

3.11 Preparation of Composite Flours

Cassava flour was purchased from Federal Institute of Industrial Research Oshodi (FIIRO) and Samaru market, while wheat flour was purchased from Samaru market. A modified method of Aboaba and Obakpolor, 2010 was used where higher propotions of the cassava flour were added. A 10, 20, 30, 40 and 50 weight portions of cassava flour was mixed with 90,80,70,60 and 50 weight portions of wheat flour to obtain 10/90, 20/80, 30/70, 40/60 and 50/50% of cassava and wheat composite flour respectively.

3.11.1 Determination of dough rising ability of isolates and leavening profile

After the growth of the yeasts in basal medium consisting of 2% (^{w}/v) glucose, 0.5% (^{w}/v) yeast extract,1% (^{w}/v)peptone,0.1% (^{w}/v)ammonium sulphate and 0.1% (^{w}/v) magnesium sulphate of pH 5.0, the culture was centrifuged at 4000rpm for 30minutes. The cell mass deposit was then washed with sterile distilled water and collected into sterile tubes. Dough was prepared by mixing each composite flour (50 g), 4ml of yeast cells (10^6 cells/ml), 4ml ALAB cells (10^8 cells/ml), sugar (2 g), salt (0.5 g) on dry weight basis and water (32ml) in a beaker according to the method specified by Eddy *et al*., 2007.The dough mixture was transferred into measuring cylinders whose sides had been smeared with vegetable oil to prevent the dough from sticking to the sides and covered with foil paper. The dough was incubated at 30°C for four (4) h in an incubator. Controls were made with the same ingredients described above but with commercial yeast and another without any

treatment.The cylinders were incubated at 30°C for four (4) h. The height of the dough was measured from the graduated surface of the cylinder before and after fermentation time.

3.11.2 Determination of dough pH and total titratable acidity

The dough pH was measured as described by Ndife *et al*., 2013.Ten (10g) of dough was weighed into a polyethylene container and 90ml of distilled water added. The mixture was shaken thoroughly and the pH meter electrode was dipped into it. Values were recorded at 0h and subsequently at thirty minutes intervals. The suspension was then poured into a 200ml flask and titrated with 0.1M NaOH, with 0.1ml of 0.5% phenolphthalein as indicator. Total titratable acid was expressed as the amount of NaOH used after the indicator end point colour changed to pink.

% Total titratable acidity was calculated as:

$$\frac{\text{Titre value} \times \text{chemical factor}}{\text{Weight of sample}} \times 100$$

3.11.3 Determination of ALAB viability count

The ALAB viability was determined using the method of Putri *et al*., 2011 where ten (10g) of the fermenting dough was added into 90ml sterile distilled water at thirty minutes interval. After serial dilution, 0.1ml was plated on MRS agar plates and incubated at 30°C under anaerobic conditions for 48h. Plates were observed for growth and resultant colonies counted and recorded.

3.11.4 Determination of yeast viability count

Yeast viability counts were determined following the method of Gerez *et al*., 2009. 0.1ml from the dilutions above was plated out on PDA plates and incubated at 30°C for 48h. Resultant colonies were counted and recorded.

3.12 Preparation of Bread from Composite Flour

The dough was prepared as above (3.11.1). Dough was hand kneaded into ball and divided into equal portions by weight. Each portion was placed into well greased baking pans and left for 1 to proof before transfering into a pre-heated oven and allowed to bake. The baked loaves were allowed to cool and removed from pans. Commercial bakers' yeast leavened bread served as control (Aboaba and Obakpolor, 2010).

3.13 Determination of Organoleptic Properties of Cassava and Wheat Bread

The sensory reponses was carried out by a team of fifteen (15) panelist made of staff and students at room temperature. The parameters used were, colour, texture, taste, aroma and general acceptability. The rating was presented on a 5 point hedonic scales method where 1 = dislike very much and 5 = like very much as described by Eddy *et al* (2007), Aboaba and Obakpolor (2010). The panelists were instructed to rate the attributes indicating their degree of liking or disliking by putting a number as provided in the hedonic scale according to their preference.

3.14 Data Analysis

Statistical analysis were using Statistical Analysis System (SAS) package. One way ANOVA was carried out to determine the quality of performance of the test organisms. Differences among samples were considered significant at $P < 0.05$ using LSD.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical and Proximate Composition of Samples and Products

Proximate composition of wheat and cassava flours is presented in Table 4.1. The result shows that moisture contents ranged from 2.28 - 5.16% for cassava and wheat flour. Wheat flour (Ff) had high values of moisture, fats and protein contents (5.6 7.66 and 3.05% respectively), when compared to other flours, Highest ash content of 1.76% was observed in market cassava flour (Mcf), compared to 0.60% of industrial flour. Highest protein content was obtained in wheat flour, while market cassava had the least. Also, wheat flour had the highest fat content. However there was no significant difference in carbohydrate of flours.Particle size values obtained after sieve analysis were significantly different. Sieve 250µm had the highest percentage (74.47 – 87.24) of flour.However wheat flour (Ff) had the higest (87.24%), market cassava flour had the least (74.47%).While lower percentage was obtained in sieve 425µm.

Result of the proximate composition of wheat/cassava flour blended bread samples are presented in Table 4.2 The proximate composition shows that bread samples had high moisture content ranging from 30.14 - 34.45%, Cb had the least moisture content of 24 .44%, closely followed by sample G, with 28.24%.The Ash content of the bread samples tend to increase with increase in cassava ratio, however, it was observed that Am and Bf had low Ash content of 0.37 and 0.41% respectively. It was equally observed that Cb had the highest Ash content (1.48%). Fat content of the composite bread samples were in the

range of 8.50- 9.05% for industrial and 9.25 – 8.22% for the market cassava. However, the cassava flour substitution did not seem to have an effect on the Fat content. Protein content showed no significant difference between samples Af, Bf and F (7.07 7.05 and7.09% respectively. It reduced as the substitution of cassava flour was increased. Again Cb had the least values for fat and protein.

The carbohydrate contents of the breads increased significantly in line with increase in cassava flour substitution. Sample Em had the highest (64.22%), while F had the least carbohydrate content (43.01%).Generally, Moisture, Fat, protein and carbohydrate contents decrease with increases in cassava flour ratio.

4.2 Morphological and Physiological Characteristics of Yeast Isolates

Pure Colonies of the yeast were smooth, either large or small and raised. The isolates were examined microscopically and the view showed oval shaped cells and some exhibiting budding stage. The isolates all utilized glucose at 5% concentration and tolerated 3% ethanol (Table 4. 3). The isolates all grew at 30 and 37°C.While most of the isolates grew at 45°C, YMz1, YMg, YTm andYMz2 showed weak growth. All isolates grew at 3% NaCl, while most survive at 10%, YMl2, YMz1 and YMg showed weak growth, YMl1 YMz2 and YTm did not grow. Most of the isolates grew in oxidation /fermentation media and produced gas, while others did not produce gas. Morphological and physiological characteristic of Amylolytic Lactic acid bacteria isolates is presented in Table 4.4.The pure colonies were mostly white and few were creamy. The cells were Gram positive, rods, cocci and some in chains.

Table 4.1: Proximate composition (%) of cassava and wheat flours

Sample code	Sieve sizes		Moisture	Ash	Fats	Protein	Carbohydrate
	425µm	250 µm					
Icf	30.34±0.20[a]	82.41±0.81[b]	2.54 ±0.35[b]	0.60±0.08[c]	6.51±0.12[b]	2.59±0.38[b]	88.76±0.80[a]
Ff	13.09±0.16[c]	87.24±0.80[a]	5.16 ±0.29[a]	1.64±0.24[b]	7.66±0.53[a]	3.05±0.28[a]	83.16±0.81[c]
Mcf	18.52±0.27[b]	74.47±0.61[c]	2.28 ±0.22[c]	1.76 ±0.26[a]	6.08±0.28[c]	2.05 ±0.37[c]	87.84±0.80[b]
ANOVA	158193.211	1005.520	13126.264	645.609	2056.483	318.228	993.754
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are means of triplicate determination

Values with different superscripts in the same column are significantly different. Mean values were separated using Duncan's multiple range test (DMRT).

Key: Ff= wheat flour, Icf = industrial cassava flour, Mcf = market cassava flour

Table 4.2: Proximate composition (%) of cassava and wheat flour blended bread

Sample	Moisture	Ash	Fat	Protein	Carbohydrate
Af	30.16 ±0 .41^{e}	0.44 ±0.06^{f}	8.84 ±0.46^{c}	7.07 ±0.12^{a}	51.49 ±0.64^{h}
Am	30.18 ±0.58^{e}	0.37 ±0.11^{g}	8.22 ±0.20^{e}	5.17 ±0.38^{d}	52.46 ±0.68^{g}
Bf	30.14 ±0.40^{e}	0.41 ±0.08^{fg}	9.05 ±0.16^{b}	7.05±0.13^{b}	53.96 ±0.74^{f}
Bm	31.31 ±0.55^{d}	0.58 ±0.24^{e}	8.60 ±0.37^{cd}	5.11 ±0.81^{e}	53.79 ±0.84^{f}
Cf	31.67 ±0.44^{d}	0.67 ±0.03^{d}	8.74 ±0.31^{c}	5.06 ±0.31^{e}	55.62 ±0.35^{e}
Cm	30.45 ±0.20^{e}	0.65 ±0.18^{d}	8.94 ± 0.27^{b}	5.09 ±0.13^{e}	54.27 ±0.63^{f}
Df	32.49 ±0.33^{c}	0.93 ±0.05^{b}	8.44 ±0.40^{d}	3.30 ±0.12^{g}	58.78 ±0.64^{c}
Dm	31.15 ±0.51^{d}	0.90 ±0.10^{b}	8.00 ±0.38^{f}	4.02 ±0.85^{f}	57.90 ±0.88^{d}
Ef	33.15 ±0.39^{b}	0.89 ±0.03^{bc}	8.50 ±0.27^{d}	3.35 ±0.14^{g}	58.71 ±0.50^{c}
Em	34.45 ±0.35^{a}	0.85 ±0.13^{c}	8.25 ±0.22^{e}	4.03 ±0.50^{f}	64.22 ±0.97^{a}
F	28.44 ±0.30^{f}	0.45 ±0.04^{f}	9.87 ±0.40^{a}	7.09 ±0.12^{a}	43.01 ±0.48^{j}
G	28.24 ±0.18^{f}	0.93 ±0.11^{b}	8.94 ±0.38^{b}	6.47 ±0.17^{c}	47.08 ±0.58^{i}
Cb	24.44±0.19^{g}	1.48±0.25^{a}	4.09±0.34^{g}	1.46±0.18^{h}	60.09±0.52^{b}
ANOVA	141.246	322.226	737.257	7376.658	1565.557
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Results are means of triplicate determination

Values with different superscripts in the same column are significantly different. Mean values were separated using Duncan's multiple range test (DMRT).

Key: f= industrial cassava flour, m =market cassava flour, F= Bread with isolates, G= control (Bread with commercial yeast), Cb=commercial bread, Af = 10%, Bf=20%, Cf= 30%, Df=40%, Ef=50%, Am = 10%, Bm =20%, Cm=30%, Dm=40%, Em=50%,

Table 4.3: Morphological and physiological characteristics of yeast isolates

Isolates code	Colonial Morphology	Microscopy	Growth in NaCl (%)		Ethanol Tolerance(%)			Growth in 5% Glucose	Fermentative Ability	Growth at elevated Temperatures(°C)		
			3%	10	3	6	10			30	37	45
YMl1	Smooth/small	Oval	+	-	+	+	-	+	+G	+	+	+
YMl2	Smooth/raised	Oval	+	-	+	+	+	+	+	+	+	+
YPple	Smooth /large	Oval	+	+	+	+	+	+	+	+	+	+
YMz1	Smooth/small	Oval/budding	+	-	+	+	+	+	+G	+	+	+/-
YMg	Smooth/large	Oval	+	+	+	+	-	+	+G	+	+	+/-
YSg1	Smooth/large	Oval	+	+	+	+	+	+	+G	+	+	+
YTm1	Smooth/large	Oval/budding	+	+	+	+	-	+	+G	+	+	+
YOg	Smooth/small	Oval	+	+	+	+	-	+	+G	+	+	+
YMg	Smooth/large	Oval	+	+	+	+	-	+	+G	+	+	+
YSg2	Smooth/large	Oval/budding	+	+	+	+	+	+	+G	+	+	+
YTm2	Smooth/large	Oval	+	-	+	+	-	+	+	+	+	+/-
YMz2	Smooth/large	Oval	+	-	+	+	+	+	+G	+	+	+/-

Key: +/- weak growth, - = No growth, + = Growth, +G= growth with gas production, Y = Yeast, Sg=Sorghum, Ml= Millet, Mz= Maize, Og=orange, Mg=Mango, Le-=Lemon, Li= Lime, Pple=Pineapple, Tm=Tomato.

The isolates grew at ambient, 30, 37 and 40°C temperatures and few grew at 45°C.Growth was observed in 3 and 6% NaCl. Weak growth was observed at 10%. All the isolates were catalase negative. Most isolates were able to produce gas from glucose, which classifies them as heterofermenters, while a few did not produce gas.

Twelve (12) 60% of the isolates were confirmed to be *Saccharomyces cerevisiae*. Other yeast species isolated include *Candida guilliermondi three(3)*15% ,*TrichosporonMucoides two(2)10% Rhodotorula glutinis one(1) 5% Kloeckera sp one(1) 5%* and *Cryptoccocus humicola* one(1)5%.Twelve of the yeast isolates are from fruits while eight are from wet milled cereals. (Figure 4.1)

Figure 4.2: shows the distribution of ALAB isolated from wet milled cereals, fruits and cassava. Results shows 8(57.14%) to be confirmed as *Lactobacillus plantarum*, followed by *Leuconostoc mesenteroides*, 2 (14.29%), while the others belonged to *Lactobacillus fermentum* 1(7.14%), *Lactobacillus* brevis 1(7.14%), *Lactococcus* Lactis 1(7.14%) and *Pediococcus acidolactici* 1 (7.14%).

4.3 Reducing Sugar Utilization by Isolates

Result for reducing sugar utilization by isolates is presented in Figure 4.3 .The graph shows an increase in the reducing sugar from the first day of fermentation. *Lactobacillus plantarum* from maize gave the highest reducing sugar followed closely by *Saccharomyces cerevisiae* from sorghum. *Lactobacillus fermentum* and *Saccharomyces cerevisiae* from pineapple had the lowest respectively.

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Table 4.4: Morphological and Physiological characteristics of Amylolytic lactic acid bacteria isolates

Isolates code	Colonial morphology	Gram reaction	Temperature(°C)					Growth in NaCl (%)			Catalase test	Gas from glucose	Tentative identification
			25±2	30	37	40	45	3	6	10			
AMz_1	White	+rods	+	+	+	+	+	+	+		-	+	*Lactobacillus plantarum*
AMz_2	White	+rods	+	+	+	+	+	+	+	-	-	+	*Lactobacillus plantarum*
AMz_3	creamy	+cocci	+	+	+	+	+	+	+	-	-	-	*Lactococcus Lactis*
AMz_4	White	+rods	+	+	+	+	-	+	+	-	-	+	*Lactobacillus plantarum*
AMz_5	White	+rods	+	+	+	+	+	+	+	+	-	+	*Lactobacillus plantarum*
ACs_1	Creamy	+rods	+	+	+	+	+	+	+	-	-	+	*Lactobacillus plantarum*
ACs_2	White	+cocci	+	+	+	+	+	+	+	-	-	-	*Pediococcus acidolactici*
ACs_3	White	+rods	+	+	+	+	-	+	+	-	-	+	*Lactobacillus plantarum*
ACs_4	creamy	+cocci in chains	+	+	+	+	-	+	+	-	-	+	*Leuconostoc mesenteroides*
ACs_5	White	+rods	+	+	+	+	+	+	+	-	-	-	*Lactobacillus plantarum*
ACs_6	White	+rods	+	+	+	+	+	+	+	-	-	-	*Lactobacillus plantarum*
ASg	creamy	+rods	+	+	+	+	+	+	+	+/-	-	+	*Lactobaicllus Fermentum*
AAc	Creamy	+cocci in chains	+	+	+	+	-	+	+	-	-	+	*Leuconostoc mesenteroides*
ATt	White	+rods	+	+	+	+	+	+	+	-	-	+	*Lactobacillus brevis*

Key: +/- weak growth, + positive, - Negative, A=Amylolytic, Mz=Maize, Cs=cassava, Sg=sorghum, Ac=Acha, Tt=Tomato.

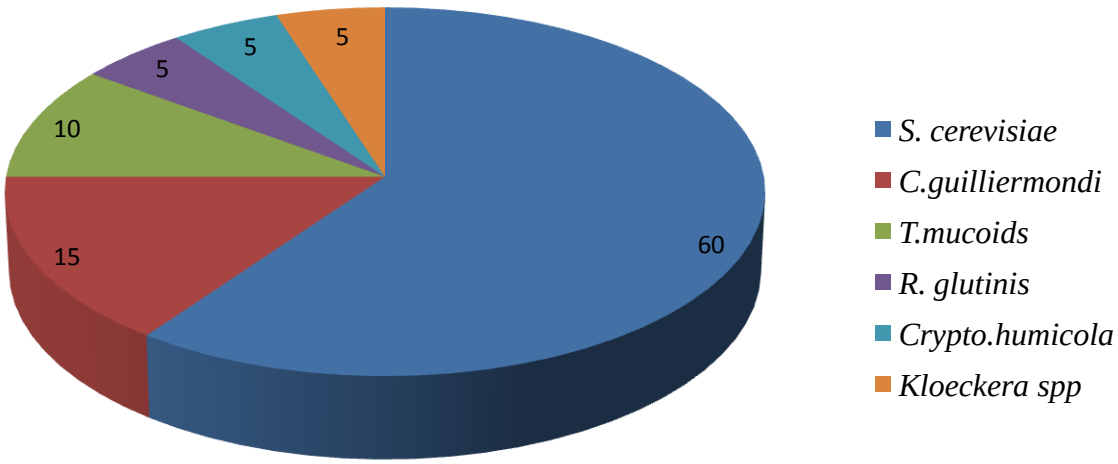


Figure 4. 1: Distribution of Yeast Isolates (%) from Fruits and wet Milled Cereals.

Key: *Saccharomyces cerevisiae, Candida guilliermondi three, Trichosporon Mucoides Rhodotorula glutinis, Kloeckera sp* and *Cryptoccocus humicola.*

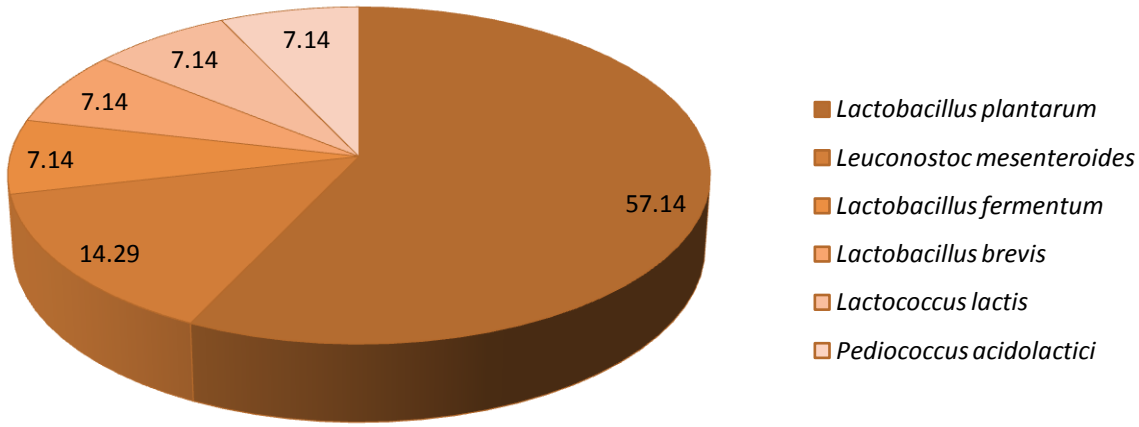


Figure 4.2: Distribution of ALAB isolates (%) from Fruits, Wet Milled Cereals and Cassava

However there was a progressive decrease in the level of reducing sugar with the length of the fermentation period.

Figure 4.4 show the influence of incubation time on co-culture fermentation of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in relation to concentration of reducing sugar. The result shows increase in concentration of reducing sugar (0.72mg/ml with fermentation time. The concentration rose steadily then peaked off at six (6) h.

Effect of pH on reducing sugar concentration is presented in in Figure 4.5. There was a slight drop in reducing sugar production at pH 4-5. It however picked off and maximum concentration was obtained at pH 6.0 (0.69mg/ml.

4.4 Cell Growth Count of Isolates

Cell growth count (g/100ml) for yeast is presented in Figure 4.6. The chart shows an increase in cell growth count from day one with YSg2 isolate having the highest yield and YMl2 was having the least count. Comparatively, ALAB cell growth also had high counts at twenty four (24) h with AMz5 having the highest count and AMz2 the least (Figure 4.7).Generally, cell growth however reduced with fermentation days.

4.5 Effects of Temperature, Time and pH on Enzyme Activity and Stability of Isolates

Figure 4.8 illustrates the effect of temperature on enzyme activity and stability. The result shows optimum activity to be at 37°C for both isolates, with *Lactobacillus plantarum* having the highest activity. The activity was stable at 45 -50°C .There was however a gradual drop in the activity at 51°C, but it remained stable up to 70°C.The activity thereafter dropped with increased temperature (80-90°C

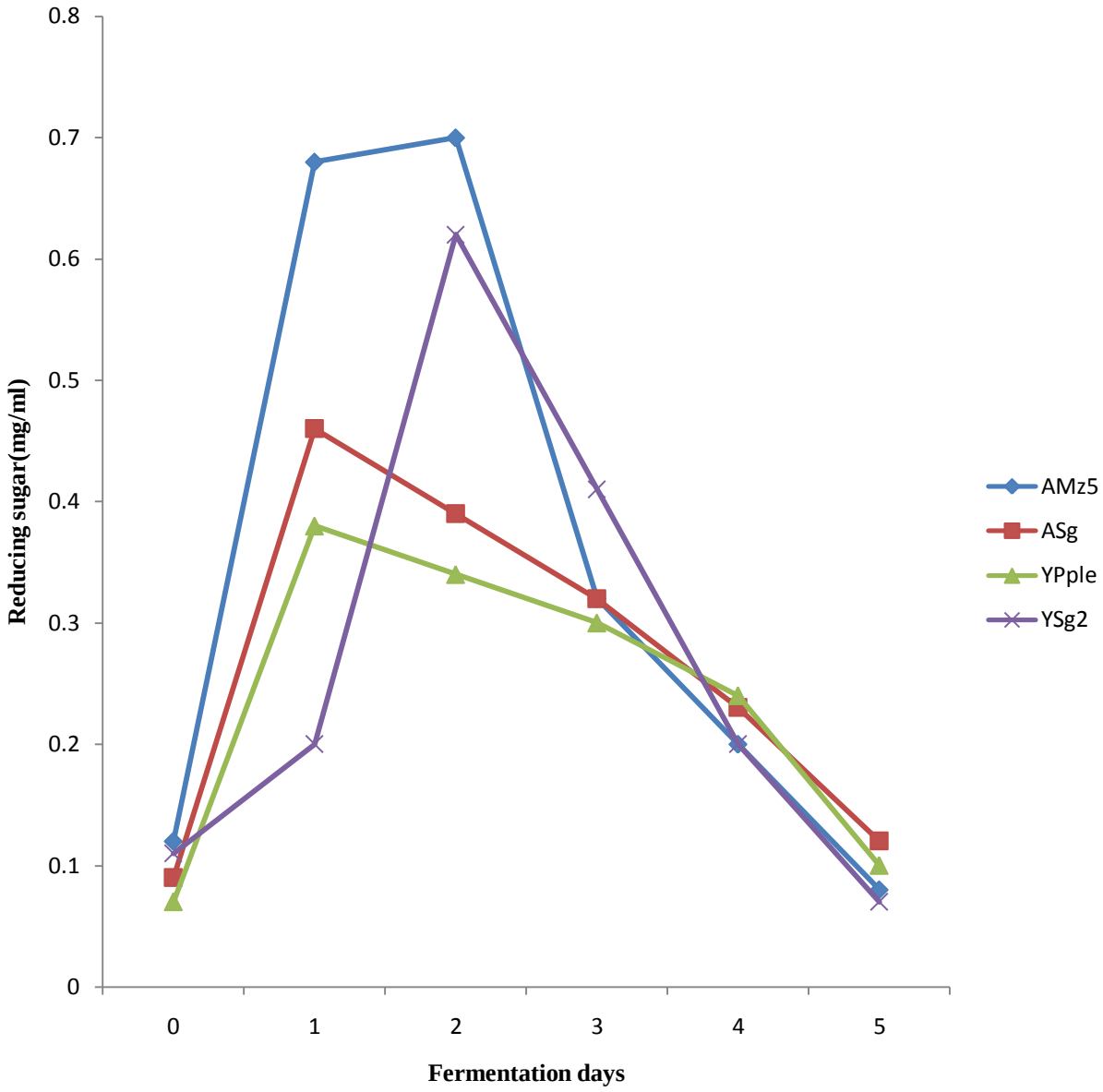


Figure 4.3: Effect of Incubation Days on Reducing Sugar of ALAB and *Saccharomyces cerevisiae*

Key: A= Amylolytic, Y= yeast, Mz= Maize, Sg= Sorghum, Pple= Pineapple

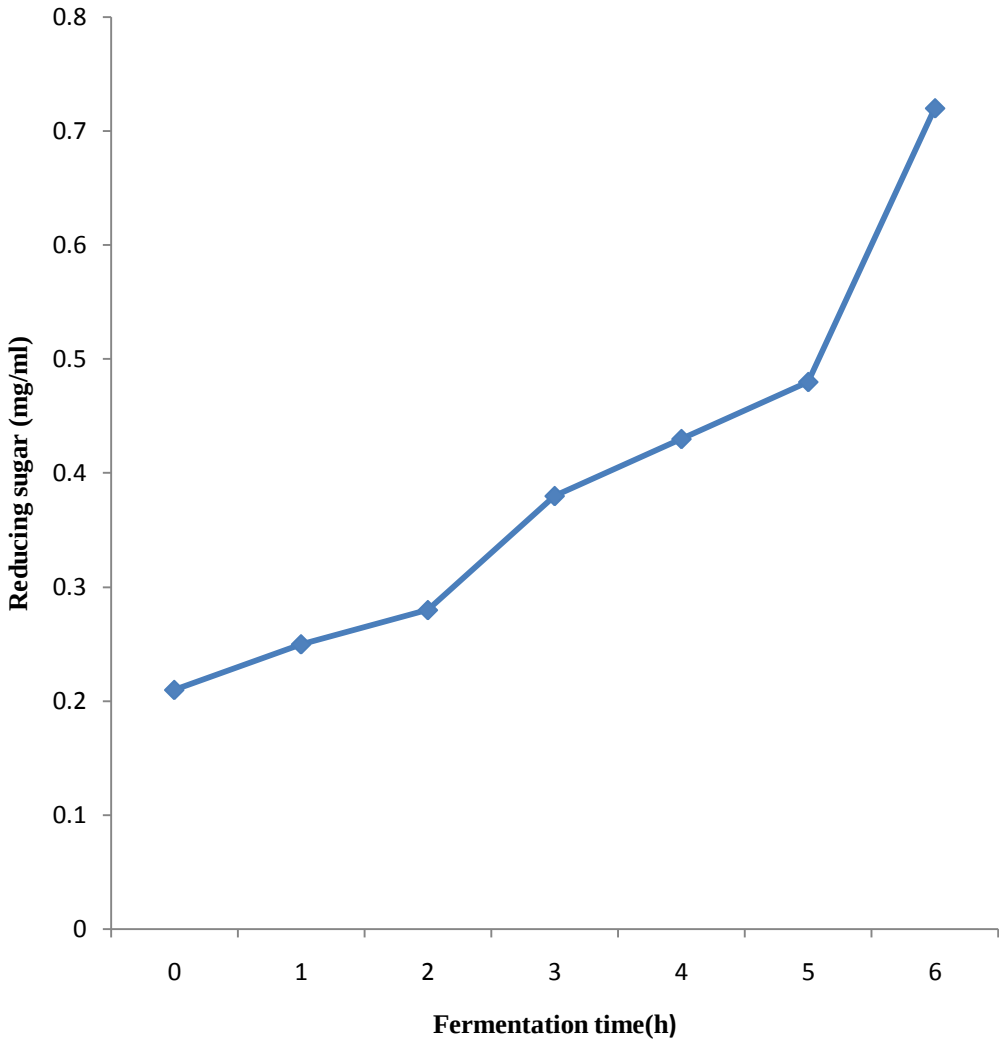


Figure 4.4: Effect of Fermentation Time on Reducing Sugar Concentration during Co - culture of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*

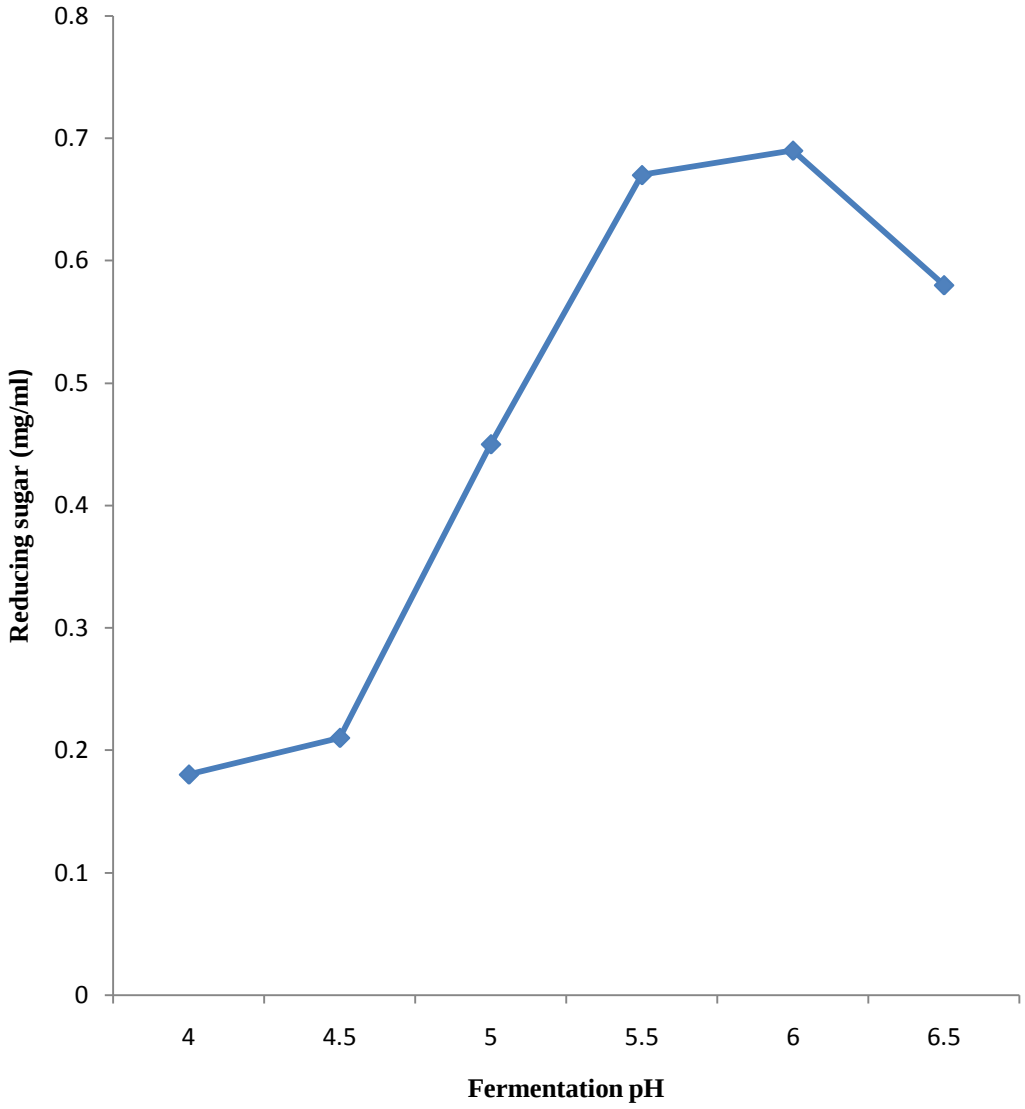


Figure 4.5: Effect of pH on Reducing Sugar Concentration during Co- culture of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*

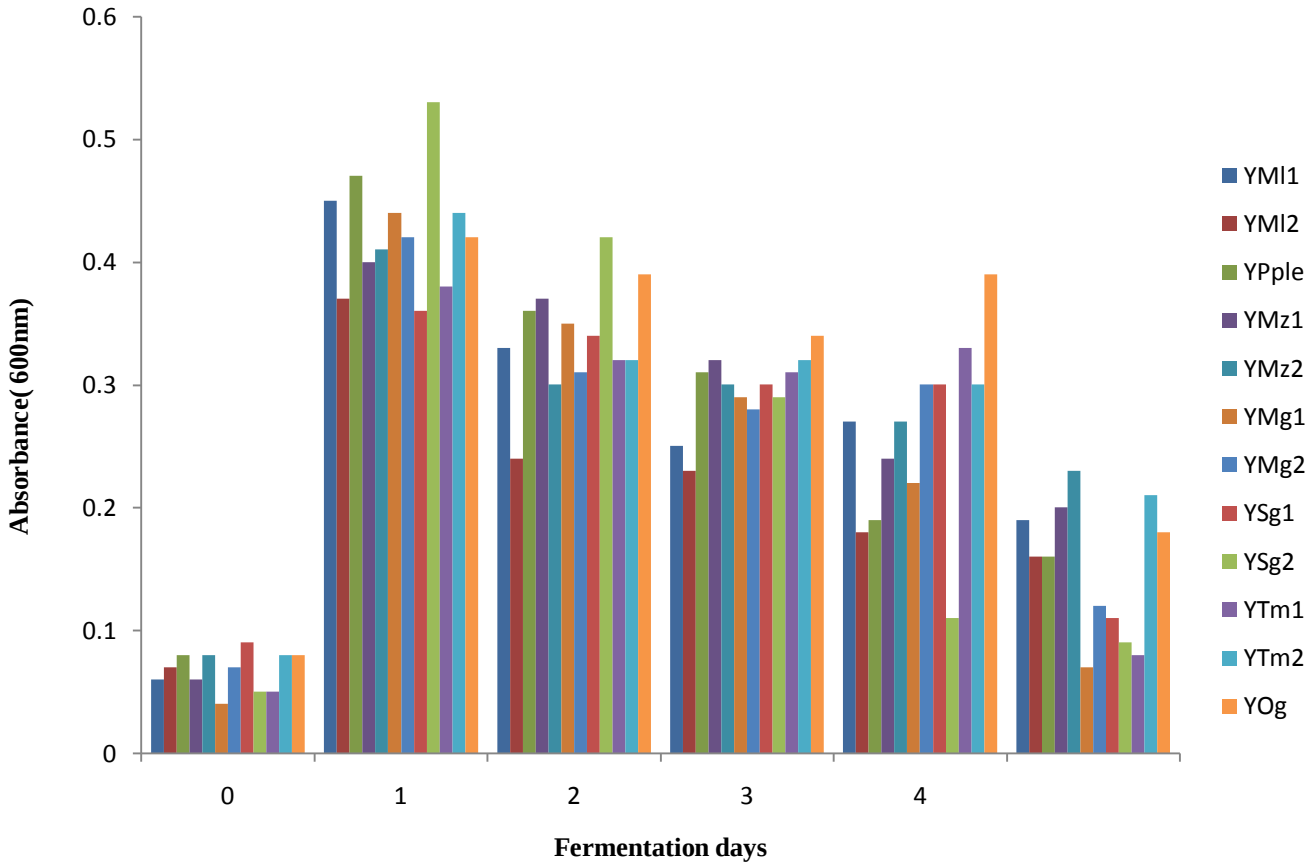


Figure 4. 6: Cell Number Count (g/100ml) of *Saccharomyces cerevisiae* Isolates.

Key: Y = Yeast, Sg = Sorghum, Ml = Millet, Mz = Maize,Og = orange, Mg = Mango, Le = Lemon,

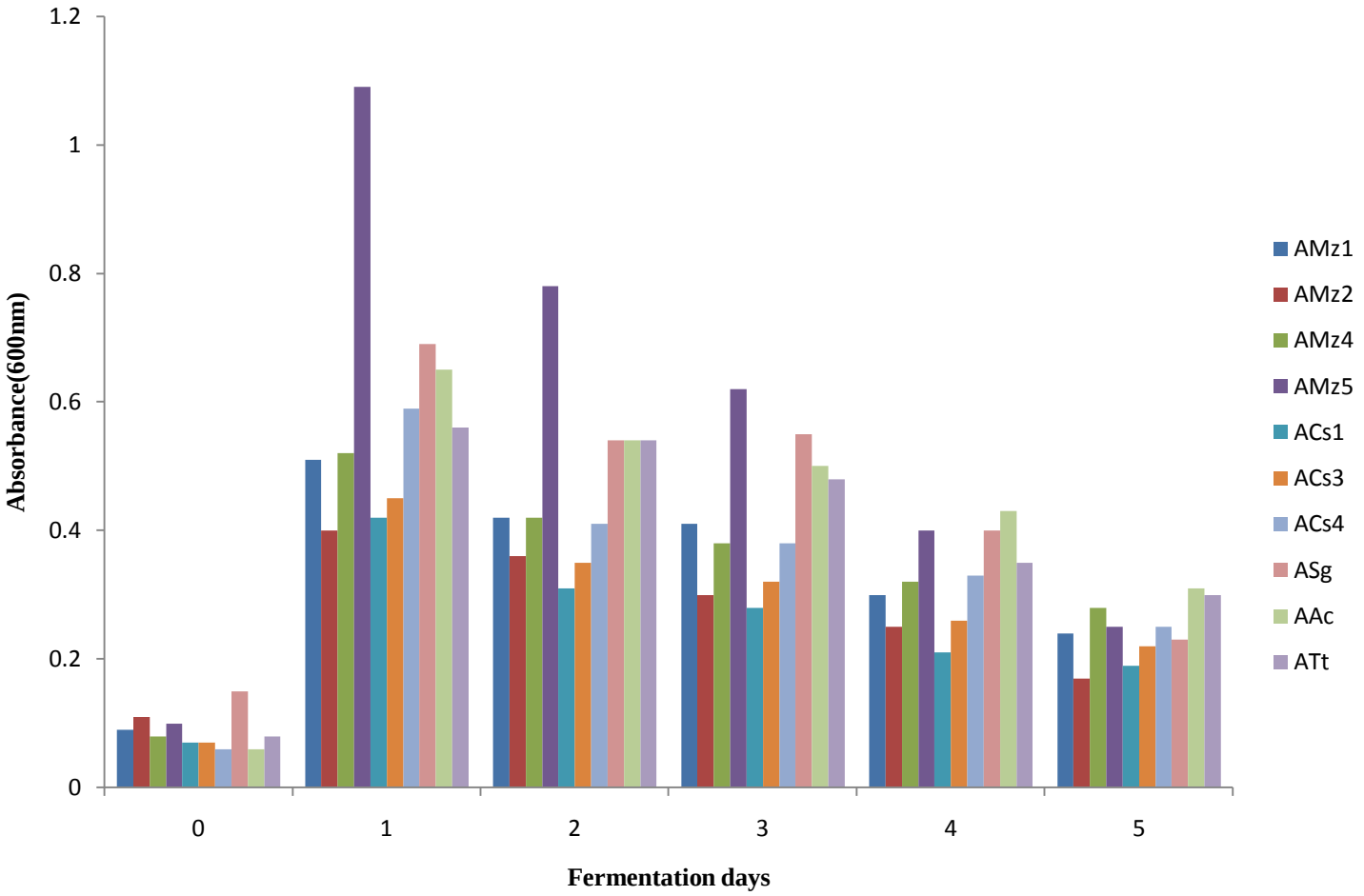


Figure 4.7: Cell Number Count (g/100ml) of Amylolytic lactic acid bacteria (ALAB) Isolates.

Key: A= Amylolytic, Mz = Maize, Cs = Cassava, Sg = Sorghum, Ac = Acha, Tt = Tomato, Li = Lime, Pple = pineapple, Tm = Tomato

Similarly, Figure 4.9 shows the influence of time on co-cultures of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*. Increase in time, as observed, showed higher enzyme activity (1.03U/ml.

The enzyme activity of ALAB is contained in Figure 4. 10. The graph shows a sharp increase in the activity from day one (1) to day five (5) of the fermentation period, the best activity was observed in *Lactobacillus plantarum*. While less activity was observed in *Lactobacillus fermentum*. The activity however decreased with fermentation days.

The enzyme activity in relation to pH is presented in Figure 4.11.There was a gradual increase in activity. It however, reached maximum at pH 6.0 (3.11U/ml. It was generally observed that reducing sugar and enzyme activity of the co cultures increased with increase in pH.

4.6 Dough Rising and Leavening Profiles of Co-culture of Isolates.

The effect of ALAB and Yeast isolates on pure wheat flour was investigated (Figure 4.12) The time taken to achieve maximum dough rise varied.There was a gradual increase in dough volume with time.Control (commercial yeast) had dough rise at 90 minutes (87.5 cm^3) this however decline with time. Dough expansion rate of *Lactobacillus plantarum* (AMz5) and *Saccharomyces cerevisiae* (YSg2) gave a higher dough volume (94cm^3) than the control (87cm).Although the dough continue to increase after four hours of incubation.

Figures 4.13 illustrate the effect of varying proportions of industrial cassava and wheat flour in dough leavening. Evidently, the cassava and wheat flour blends interacted differently resulting in different effects on dough rise. The graphs show a general steady

increase in dough rise. The positive Control blend (Commercial yeast) shows a high dough rise at three hours and thereafter began a gradual decline, while the dough with wild isolates increased steadily up to four hours. There was no substantial difference in dough rise of Wf (wheat flour), 10 and 20% blends. While Figure 4.14 shows the effects of different proportions of market cassava and wheat flour in dough leavening.The 10% dough blend had a higher and continueous dough rise than the positive control, it was closely followed by Wf. Although the dough rise decreased with the ratios of cassava flour added. However, there was no appreciable change in dough rise of negative control (Uninoculated wheat dough). Pure cassava dough (CS) had the least dough rise.

4.7 Change in pH during Dough Fermnetation

Change in pH of market cassava and wheat dough during fermentation is presented in Figure 4.15. There was no significant difference in the initial pH between each experiment. Decline in pH for all the dough was observed after two (2) h. During fermentation, the pH decreased to reach final values of 4.75 - 3.6. However, a steady decline was observed for 10, 20, 30, 40 and 50% dough blends from pH 5.3 - 4.5 at four (4) h.While the Positive control and Wf recorded a sharp drop in pH from 5.3 - 3.9 and 3.7 respectively. The industrial cassava and wheat dough result in Figure 4.16 presents a similar trend. Although a general decline in pH was observed for all the blends, the pH of 10%, Wf, Vf, and positive control were observed to be sharp, while for 20, 30, 40 and, 50%, Cs and negative control blends, the decline was gradual.

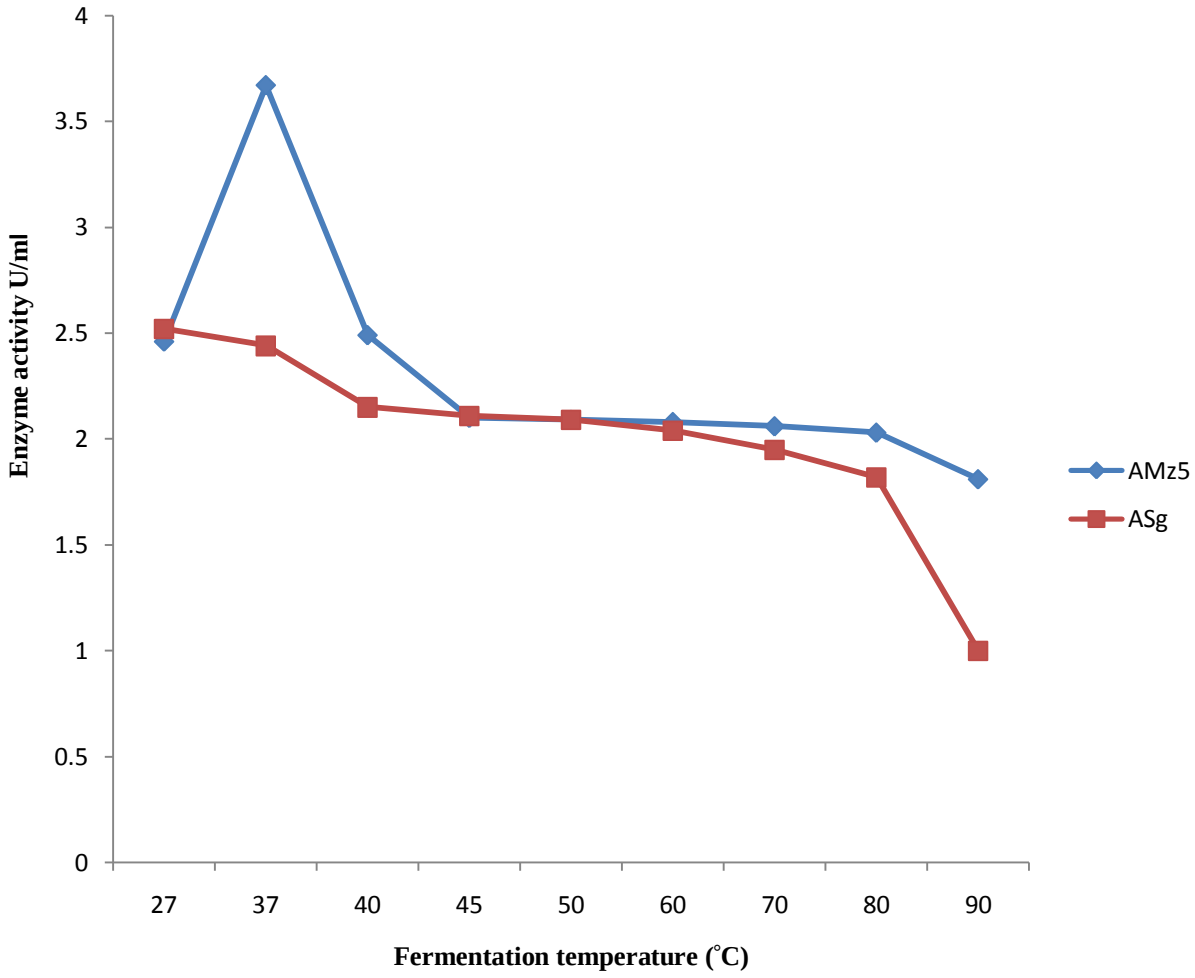


Figure 4.8: Effect of Temperature on Enzyme Stability of ALAB Isolates.

Key: A= amylolytic, Mz= maize, Sg= sorghum

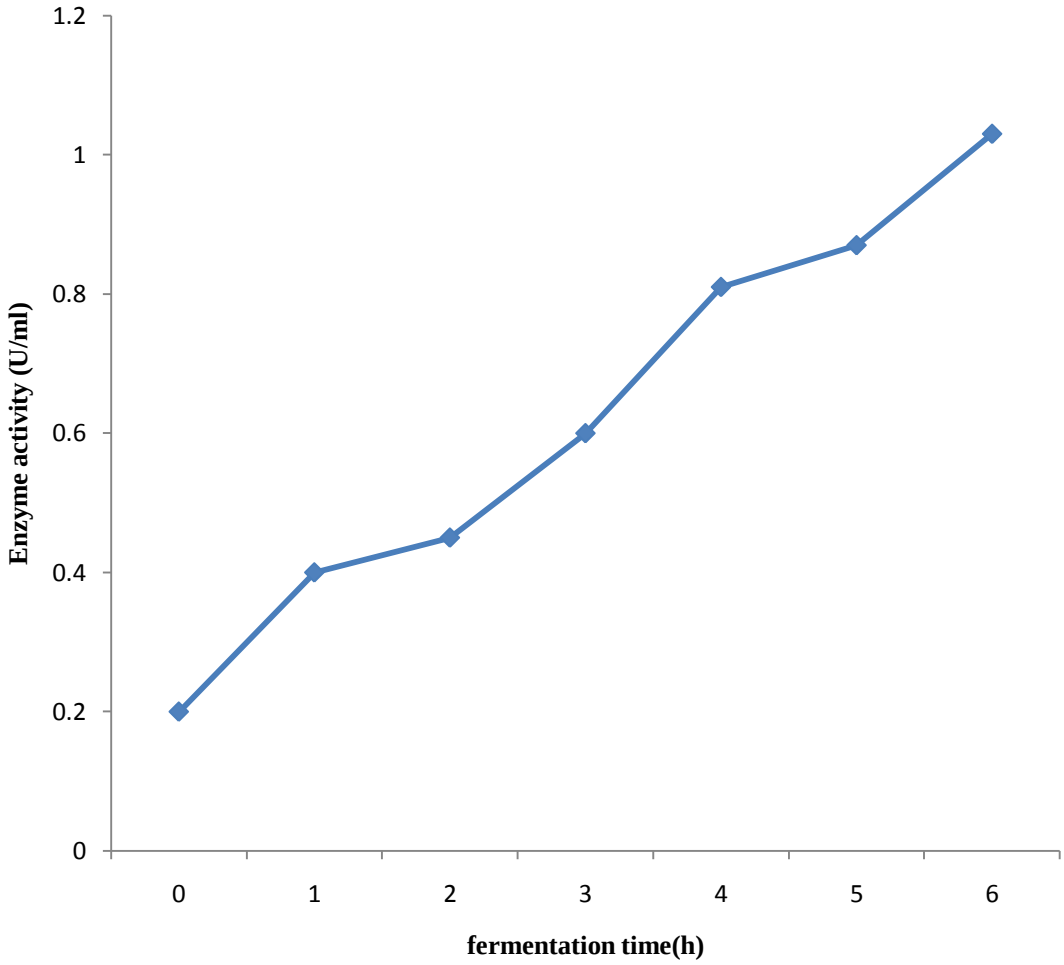


Figure 4.9: Effect of Fermentation Time on the Enzyme Activity of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in co -culture.

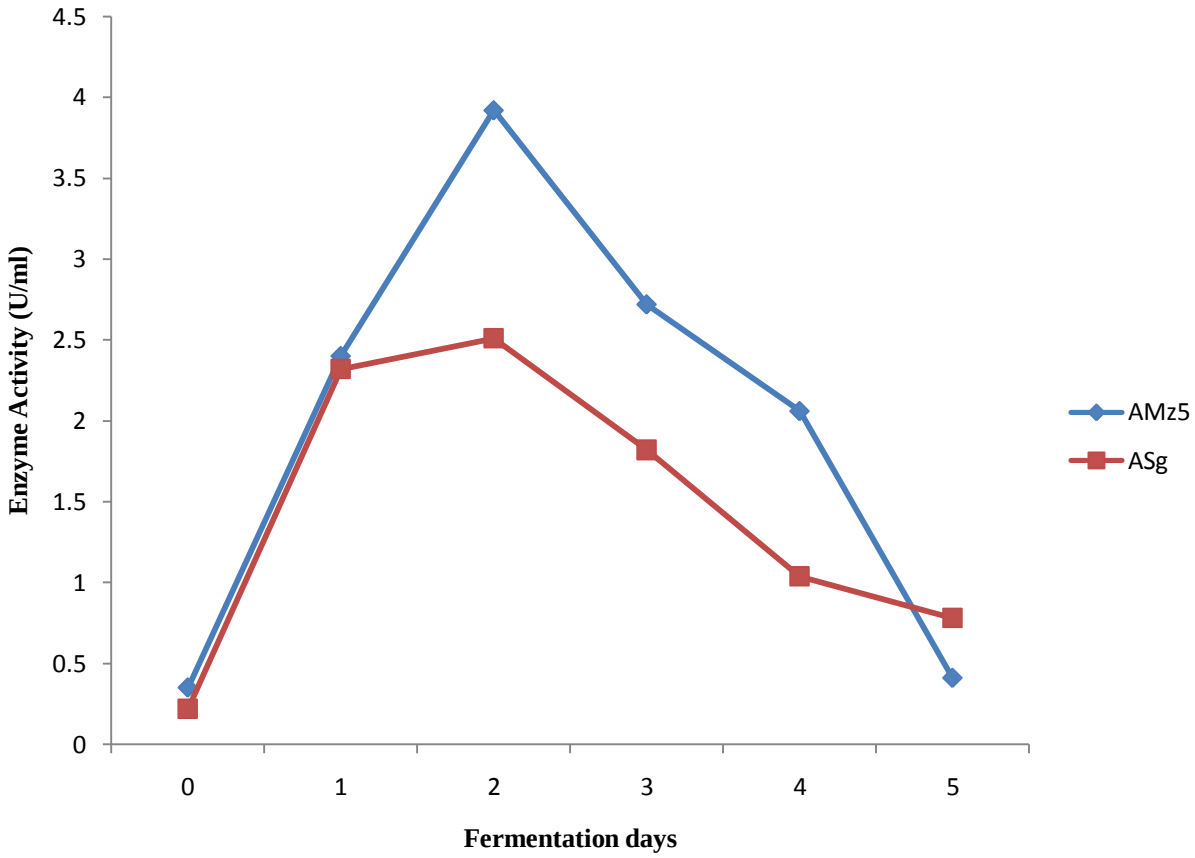


Figure 4.10: The Enzyme Activity of ALAB Isolates from Maize and Sorghum.

Key: A= amylolytic, Mz = maize, Sg = sorghum

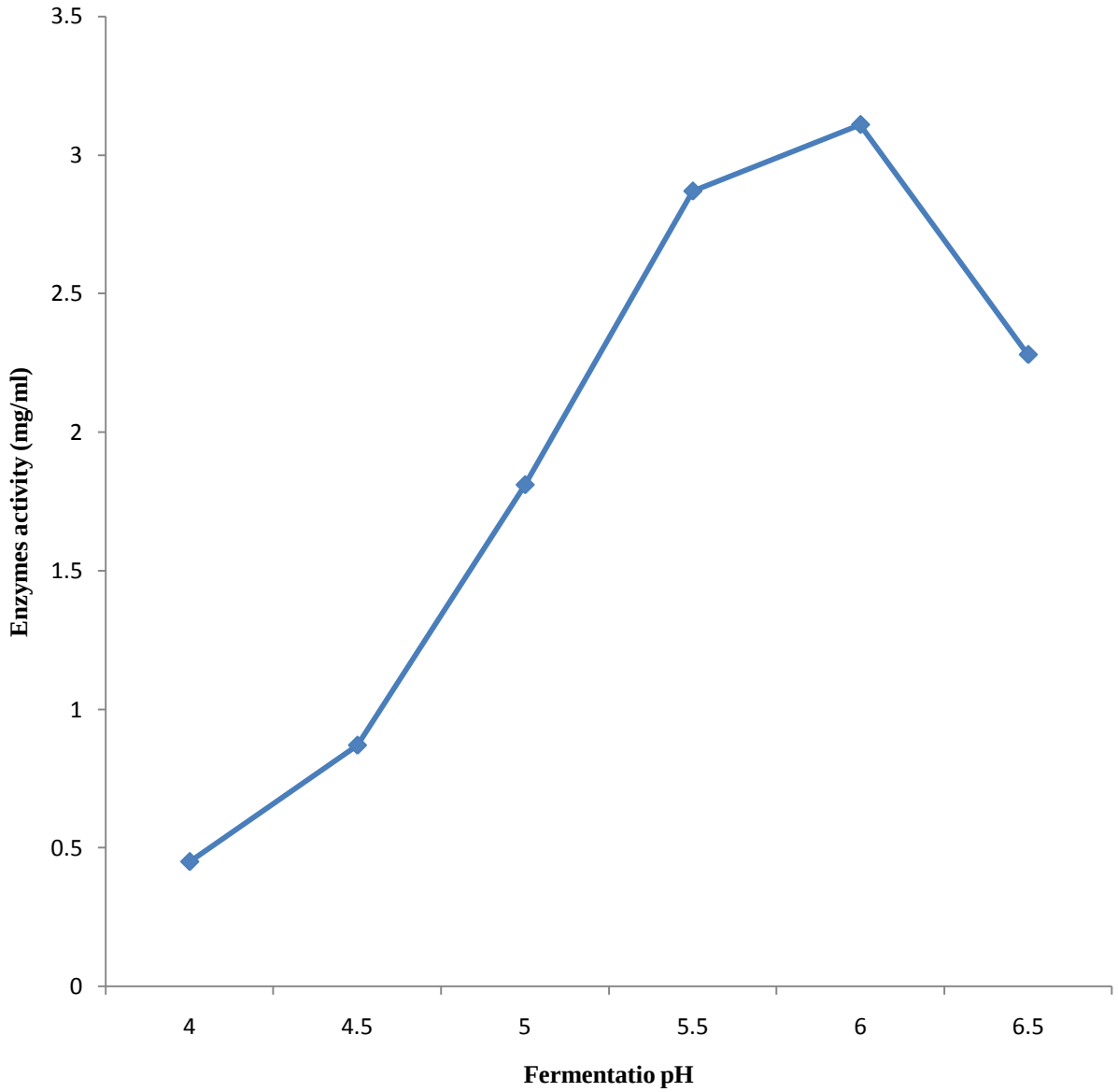


Figure 4. 11: Effect of pH on Enzyme activity of *Lactobacillus plantarum* and *Saccharomyces* in co -culture

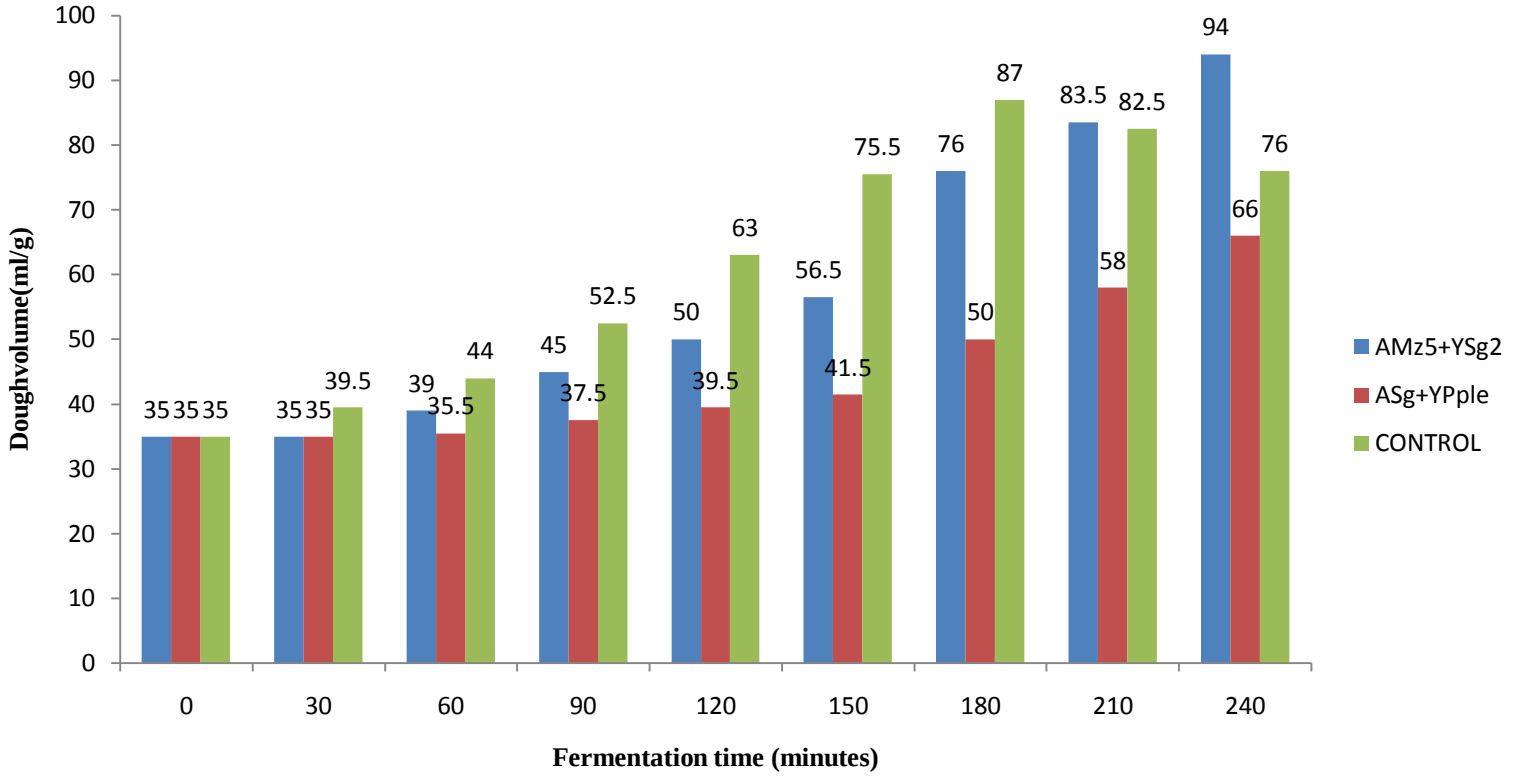


Figure 4.12: Effects of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* Co Fermentation on Wheat Dough Volume.

Key: AMz5 = *Lactobacillus plantarum* from maize, ASg = *Lactobacillus plantarum* from sorghum, YSg2 = *Saccharomyces cerevisiae* from sorghum, YPple = *Saccharomyces cerevisiae* from pineapple, Control = Commercial yeast.

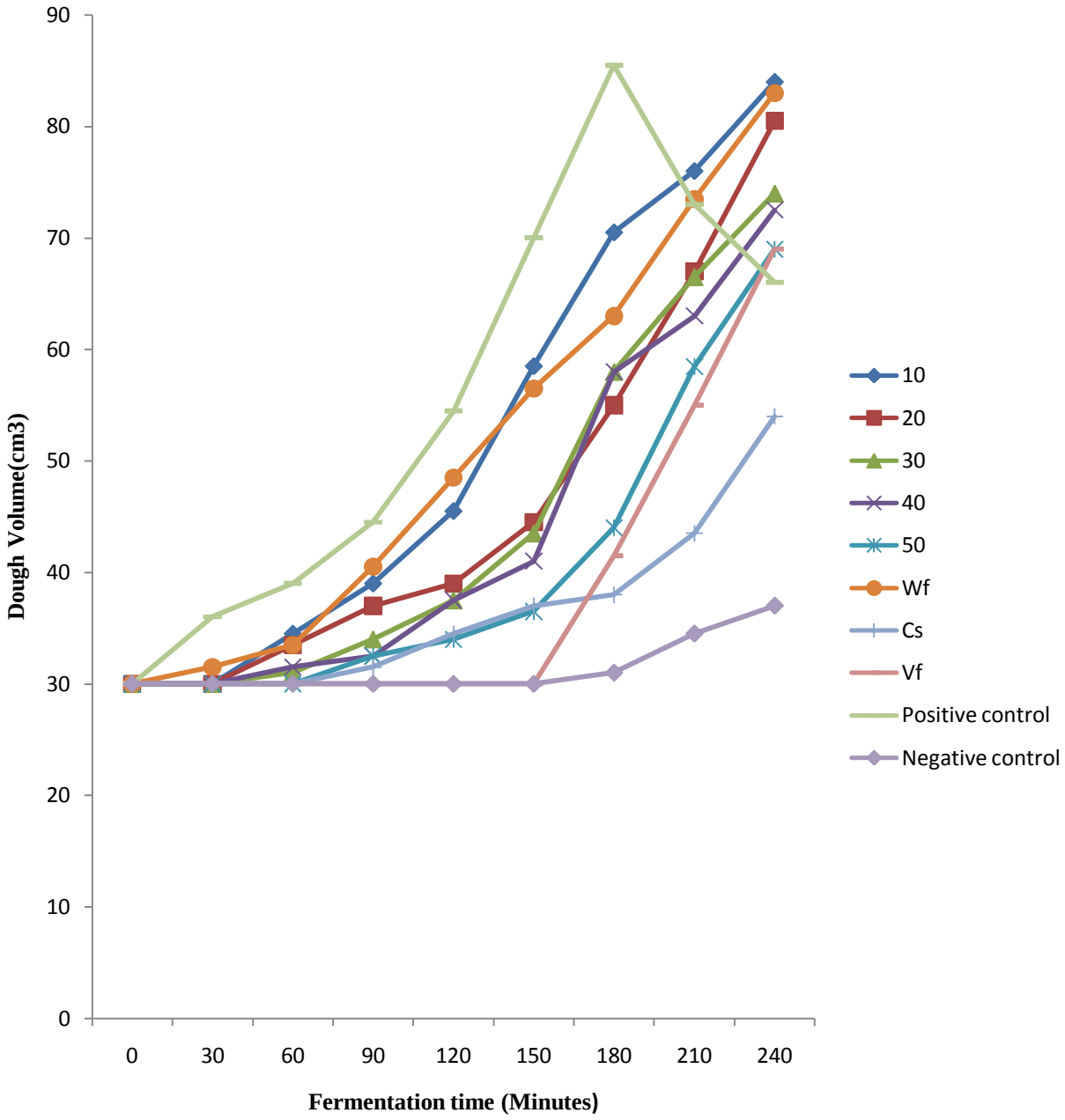


Figure 4. 13: Effect of Varying Proportions of Industrial Cassava and Wheat on Dough Leavening

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates), Cs = cassava dough, Vf = virgin flour dough, Positive control = wheat dough, plus commercial yeast, Negative control = Uninoculated dough

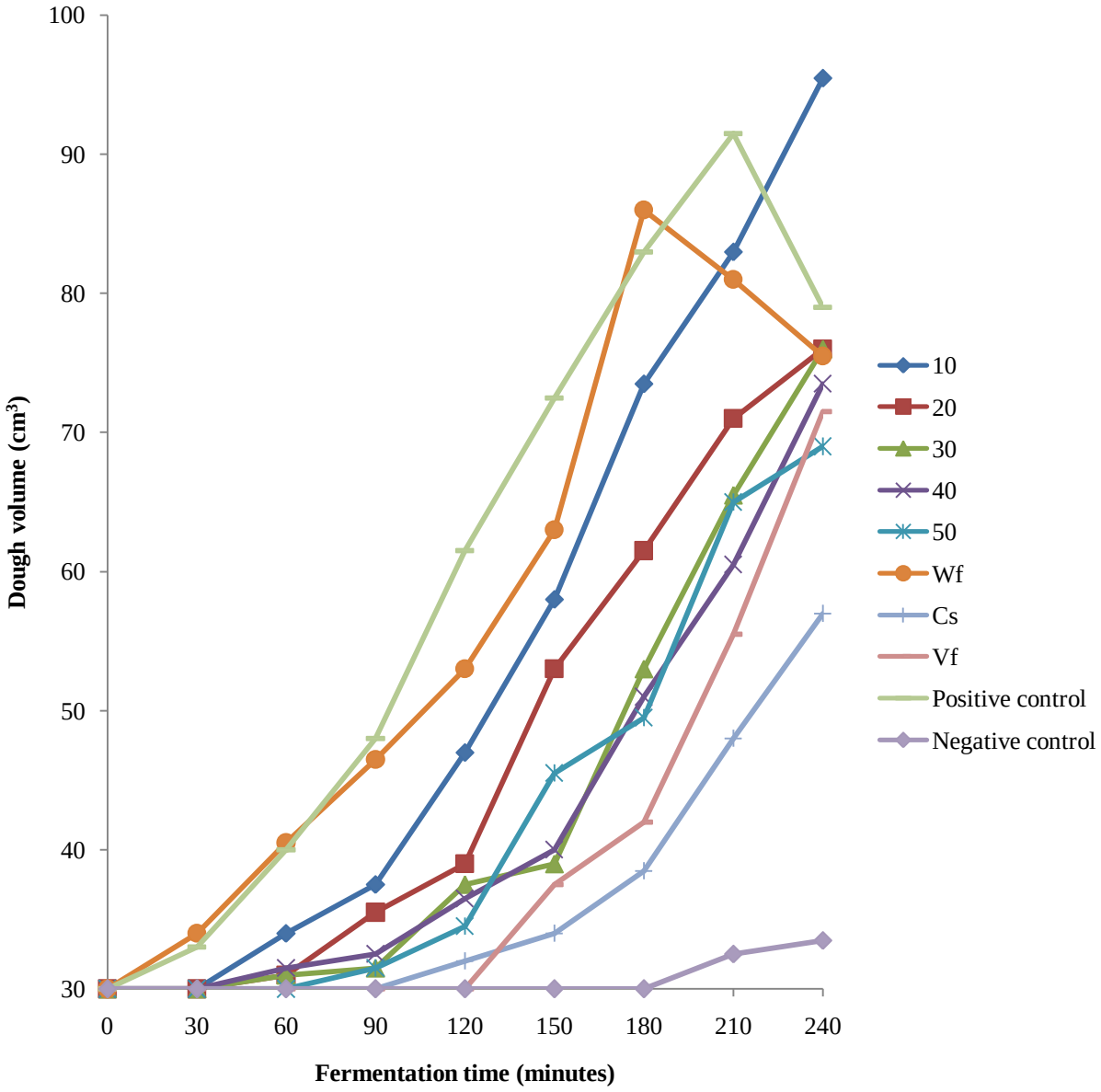


Figure 4.14: Effect of Varying Proportions of Market Cassava and Wheat on Dough Leavening

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = wheat dough (isolates) dough, Cs = cassava dough, Vf = virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

4.8 Viable Counts of Isolates during Dough Fermentation

LAB viable counts were determined during market cassava and wheat dough fermentation and results are presented in Figures 4.17.A continuous increase in LAB counts was observed after four (4) h of fermentation for all the blends except the cassava dough, positive and negative controls, where growth was gradual and seemed to reduce after three hours of incubation.A similar trend was observed in Figure 4.18 for the industrial cassava and wheat dough blend where viable counts increased with fermentation time. Although the increase in viable counts for the two controls was slow, it later peaked off. However the negative control showed no appreciable increase after four hours of fermentation.

The growth of yeast during market cassava and wheat dough fermentation is contained in Figure 4.19. A steady increase in counts was observed for all the blends. However, 10% blend recorded a sharp increase in counts after three hours of fermentation.The positive control competed favourably with 10%, 20% and Wf blends. Yeast growth was not observed for negative control until after two hours of fermentation. There was no appreciable increase during the fermentation period. Similarly, yeast growth during industrial cassava and wheat dough fermentation is presented in figure 4.20. The yeast counts peaked off and increased steadily. Generally, the counts increased with fermentation time.The negative control showed no appreciable increase in counts.

4.9 Total titratible acidity (TTA) of fermenting dough

Dough started with commercial bakers yeast had the highest total titrable acidity of 7.49 ± 0.84^{a}, followed by dough started with isolates with 6.66 ± 0.90^{ab}. However, cassava flour dough had the least TTA value of 3.02 ± 0.23^{d}. Although dough started with isolates (Wf) and 10% had the same ratings. Lower TTA values were obtained in the market cassava and wheat dough than the industrial cassava dough and wheat. It was generally observed that, TTA decreased with increase in cassava flour proportions for both cassava flour types.

4.10 Organoleptic Properties of Product

Table 4.5 shows the sensory responses of cassava and wheat industrial flour blended bread. The mean scores show a decrease as the level of cassava flour substitution increased. AI1 and WIS compared well with PW0 in appearance, taste, texture, aroma and general acceptability for both industrial and market bread blend samples. There was no significant difference ($P \geq 0.05$) in the tastes, appearance, texture ratings of the bread samples of PW0 (100% wheat bread), WIS, 10% and 20% (AI1 and BI2). However, DI4, EI5, and gave low ratings by the panelist for the attributes tested for. This is also reflected in the Duncan range tests result. However, commercial bread (Cb) samples had the least rating. While a similar trend was observed in cassava and wheat market flour blended bread (Table 4.5).AMI and WIS compared favourably with PW0.The ratings for the attributes tested for tend to decrease with increase in cassava flour ratio. Again Cb had the least ratings.

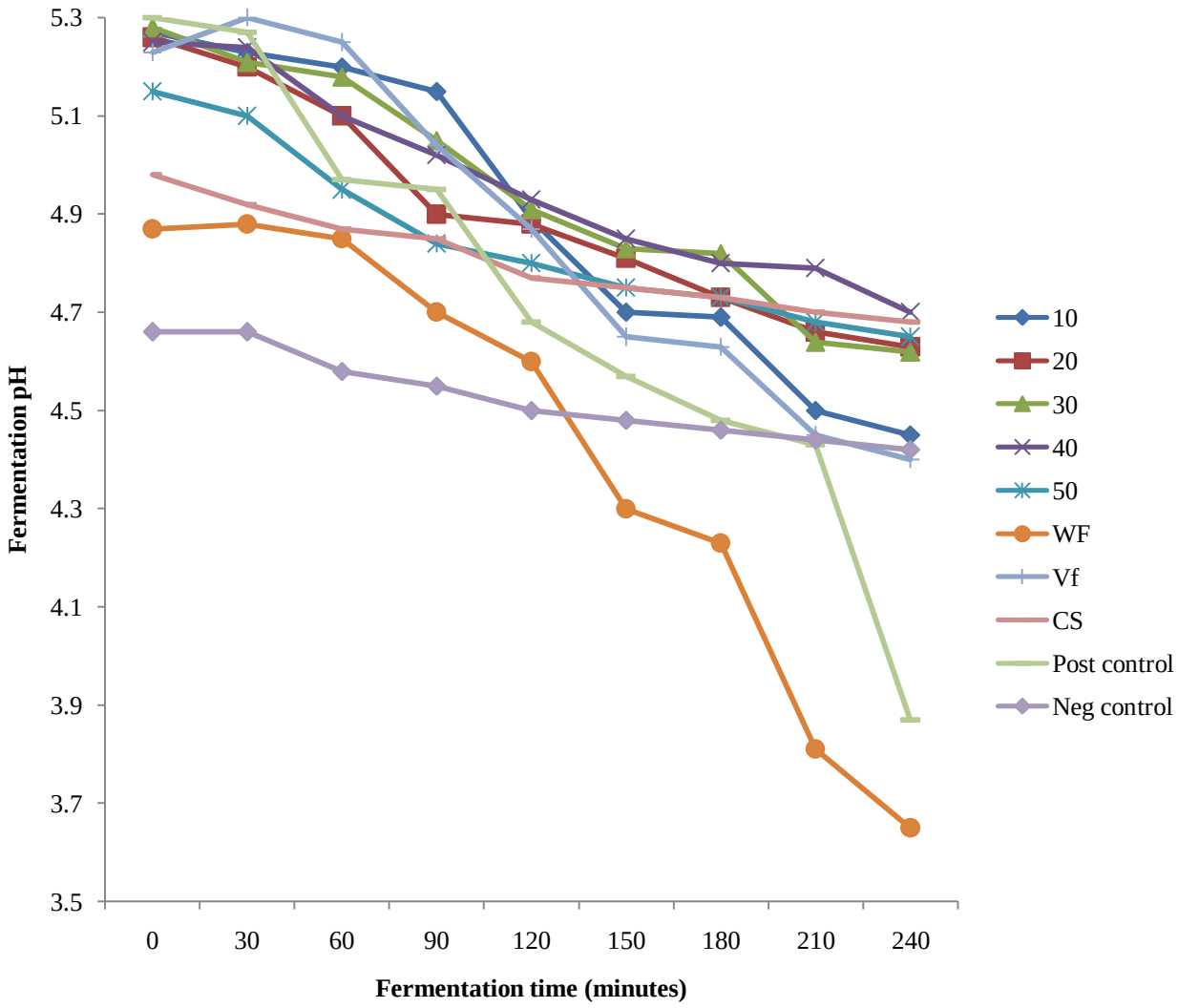


Figure 4.15: Changes in pH during Market Cassava and Wheat on Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava and wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

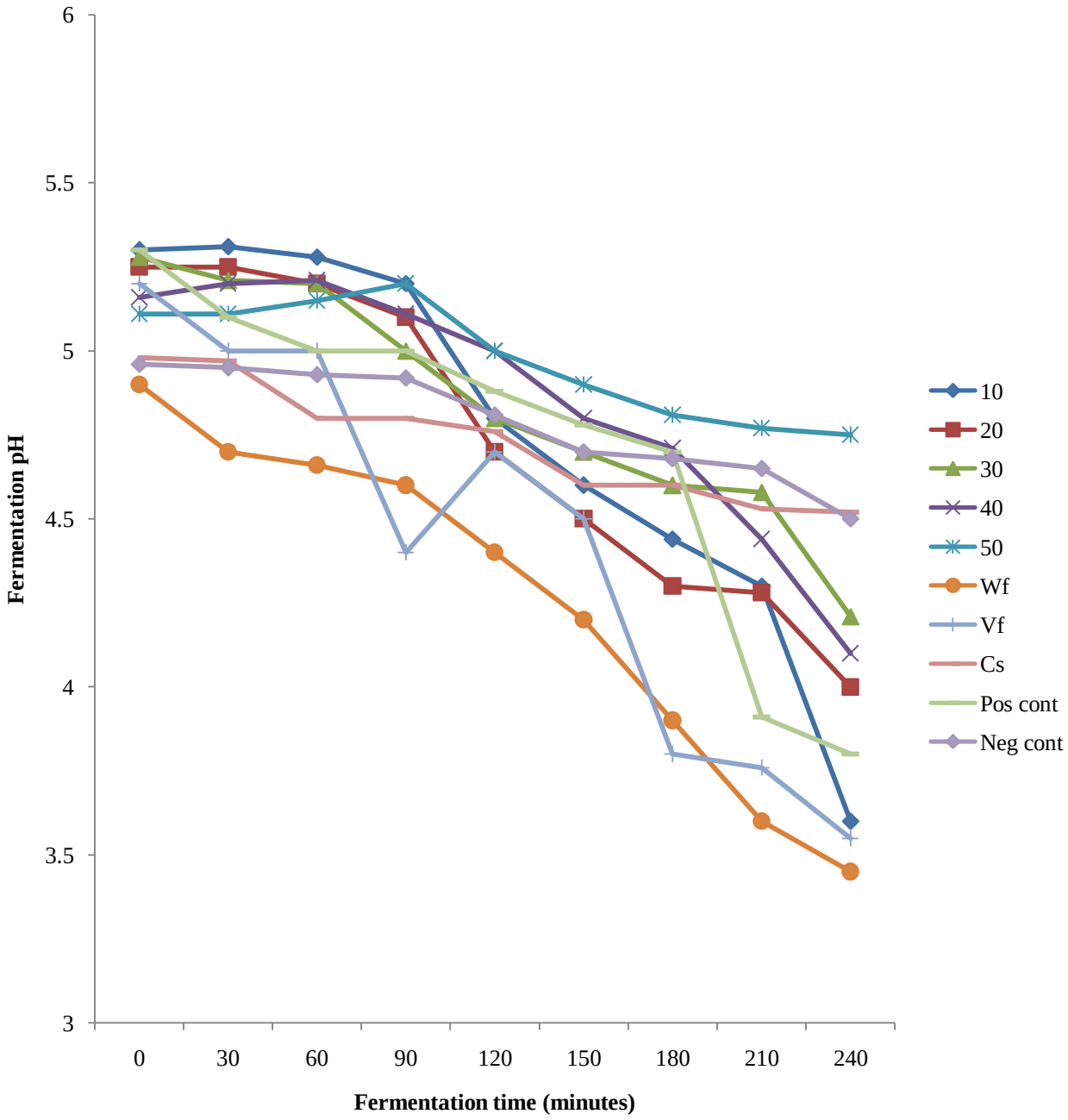


Figure 4. 16: Changes in pH of Industrial Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

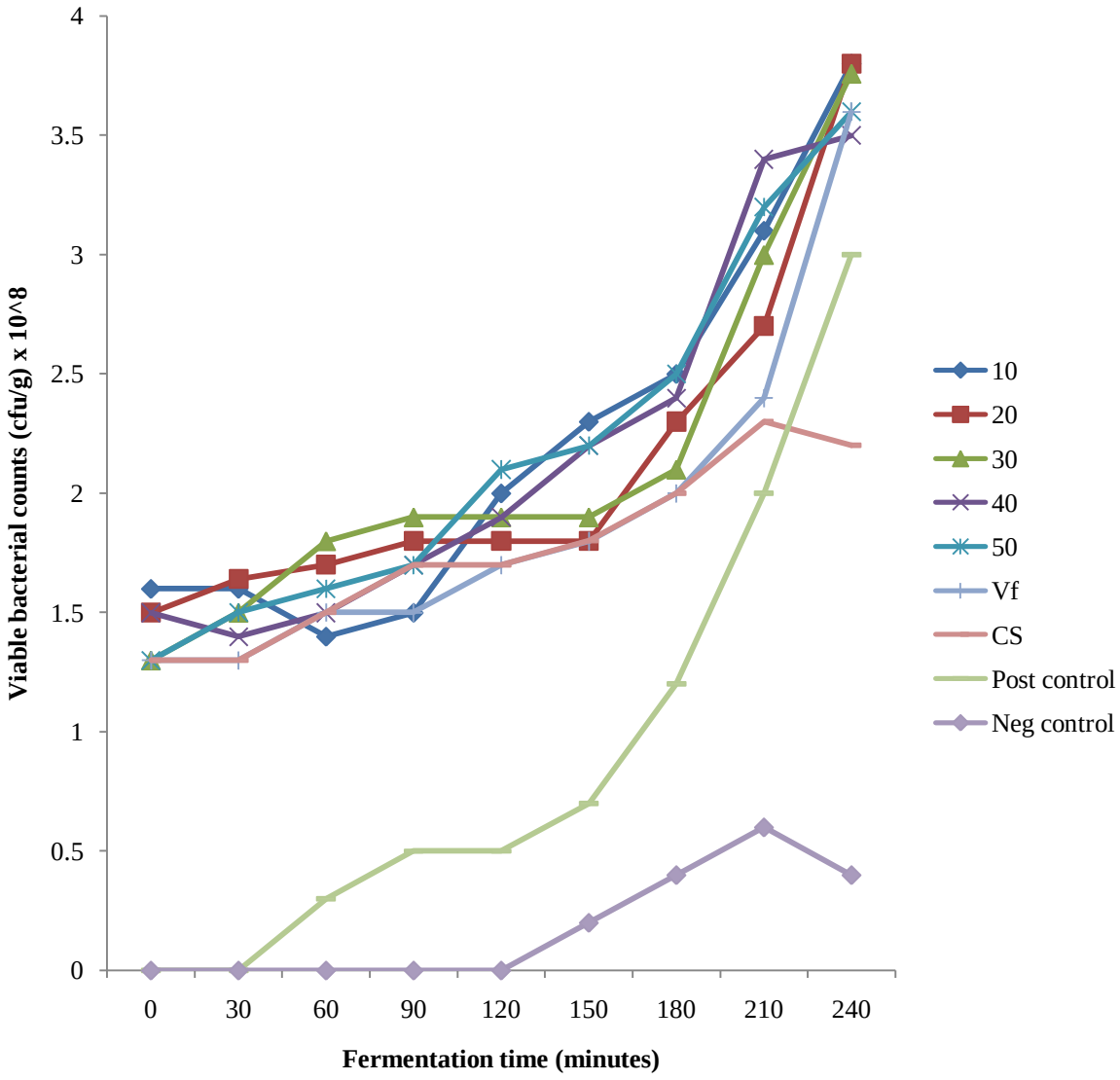


Figure 4.17: Growth of ALAB during Market Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

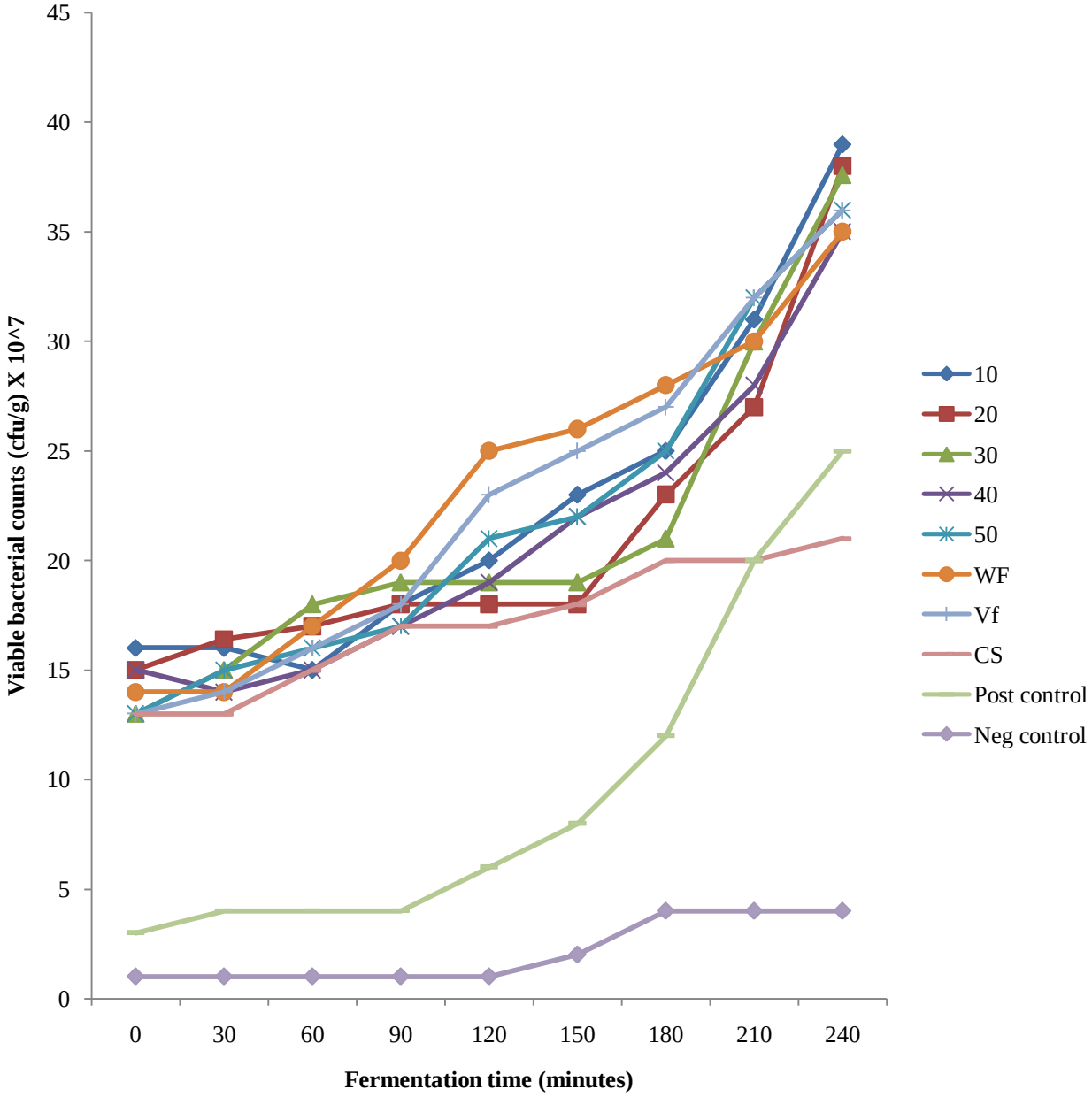


Figure 4.18: Growth of ALAB during Industrial Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

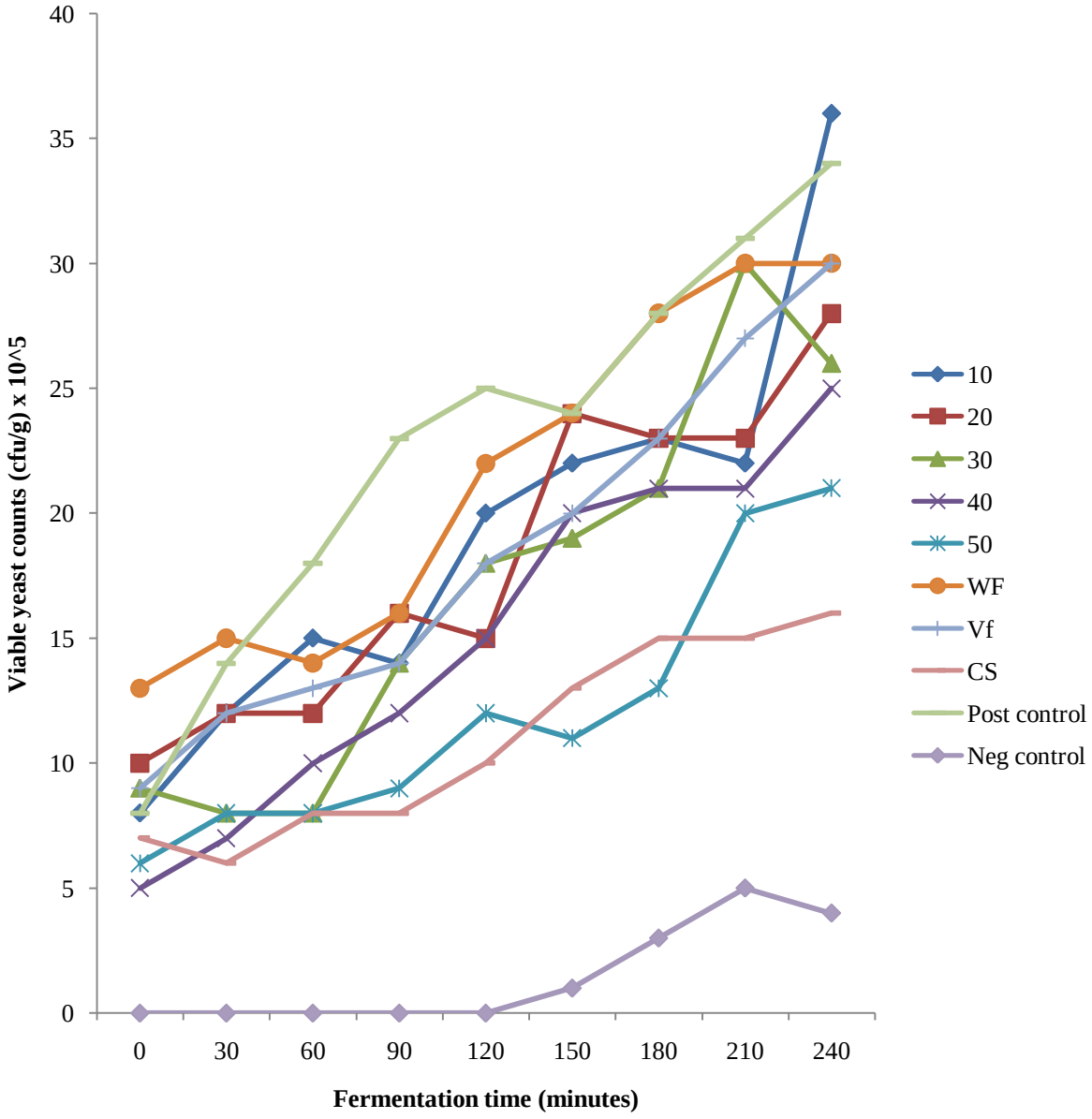


Figure 4.19: Growth of Yeast during Market Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough.

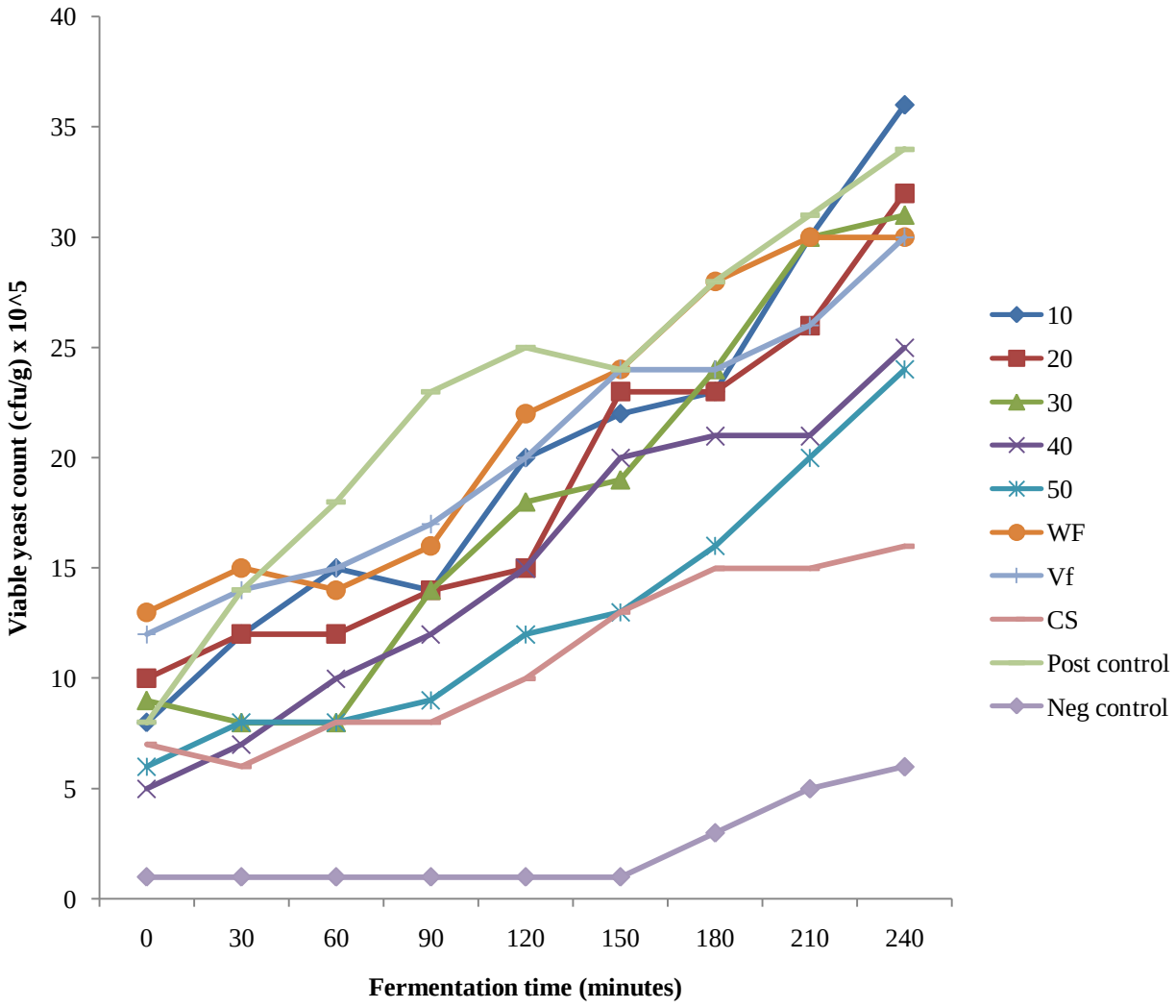


Figure 4.20: Growth of Yeast during Industrial Cassava and Wheat Dough Fermentation.

Key: 10, 20, 30, 40, 50 = Ratios of cassava/ wheat in dough, Wf = Wheat dough (isolates) Cs = cassava dough, Vf =virgin flour dough, Positive control = wheat dough (commercial yeast), Negative control = Uninoculated dough

Table 4.5: Total titratable acid of fermenting cassava and wheat dough samples

	Ind	**Mkt**
Samples	**Mean ± S.E.**	**Mean ± S.E.**
10%	6.51±0.65^{ab}	6.08±0.58^{bc}
20%	5.51±0.66^{bc}	4.99±0.56^{cd}
30%	5.07±0.56^{bc}	5.08±0.56^{bc}
40%	4.69±0.55^{cd}	4.77±0.54^{cd}
50%	4.59±0.57^{cd}	4.48±0.54^{cd}
Wf	6.66±0.90^{ab}	
Vf	4.07±0.32^{cd}	
Cs	3.02±0.23^{d}	
Post cont.	7.49±0.84^{a}	
Neg. cont.	3.73±0.26^{d}	
Total	5.13±0.23	5.08±0.25
F-value	5.652	1.192
p-value	0.000*	0.329

Means in the same column with different superscript are significantly different by ($p < 0.05$) Duncan multiple range test (DMRT)

Key: 10,20,30,40, and 50% = different proportions of cassava and wheat flour in dough. Wf=, dough started with isolates, Vf= virgin flour dough, Cs= cassava dough, Positive control= dough started with commercial yeast, Negative control = uninoculated dough, S.E= Standard Error of Mean, Ind= industrial, Mkt=market

Table 4.6: Sensory Responses of Industrial Cassava and Wheat Flour Blended Bread

S/No	Bread Sample	Appearance	Taste	Aroma	Texture	General Acceptability
1	AI1	4.27 ± 0.18^{ab}	4.33 ± 0.16^{ab}	4.47 ± 0.13^{b}	4.27 ± 0.18^{ab}	4.60 ± 0.11^{b}
2	BI2	3.53 ± 0.13^{bc}	3.60 ± 0.19^{bc}	4.07 ± 0.15^{de}	4.00 ± 0.17^{de}	4.33 ± 0.13^{ab}
3	CI3	3.33 ± 0.13^{bc}	2.67 ± 0.15^{abc}	3.20 ± 0.15^{abc}	2.87 ± 0.13^{ab}	3.67 ± 0.13^{c}
4	DI4	2.67 ± 0.13^{abc}	2.13 ± 0.13^{a}	2.13 ± 0.19^{a}	2.67 ± 0.19^{abc}	3.00 ± 0.17^{abc}
5	EI5	2.00 ± 0.17^{abc}	1.87 ± 0.09^{a}	1.87 ± 0.13^{a}	2.53 ± 0.19^{cde}	2.07 ± 0.21^{abcd}
6	WIS	4.67 ± 0.13^{de}	4.67 ± 0.13^{de}	4.53 ± 0.13^{cde}	4.80 ± 0.11^{d}	4.47 ± 0.13^{cde}
7	PW0	4.20 ± 0.11^{ab}	4.07 ± 0.15^{abc}	4.00 ± 0.17^{abc}	3.93 ± 0.15^{abc}	4.40 ± 0.13^{bc}

Means in the same column with different superscript are significantly different by ($p < 0.05$) Duncan multiple range test (DMRT)

Key: AI1= 10%, BI2=20%, CI3=30%, DI4=40%, EI5=50%, WIS = wheat flour bread, PW0 = control (commercial yeast)

Table 4.7: Sensory Responses of Market Cassava and Wheat Flour Blended Bread

S/No	Bread Sample	Appearance	Taste	Aroma	Texture	General Acceptability
1	AM1	4.27 ± 0.12^{ab}	3.93 ± 0.21^{a}	3.93 ± 0.15^{a}	3.93 ± 0.15^{a}	4.33 ± 0.13^{ab}
2	BM2	3.80 ± 0.20^{cd}	2.93 ± 0.21^{a}	3.00 ± 0.17^{a}	3.07 ± 0.15^{a}	3.13 ± 0.09^{ab}
3	CM3	3.33 ± 0.21^{bc}	2.93 ± 0.21^{ab}	2.73 ± 0.18^{a}	3.00 ± 0.24^{ab}	2.80 ± 0.18^{ab}
4	DM4	3.13 ± 0.26^{c}	2.67 ± 0.16^{abc}	2.60 ± 0.16^{abc}	2.67 ± 0.30^{abc}	2.33 ± 0.21^{ab}
5	EM5	2.87 ± 0.22^{c}	2.40 ± 0.13^{abcd}	2.47 ± 0.13^{abcd}	2.67 ± 0.27^{de}	1.73 ± 0.23^{a}
6	WIS	4.27 ± 0.18^{abc}	3.93 ± 0.15^{a}	4.00 ± 0.14^{a}	4.20 ± 0.18^{ab}	4.33 ± 0.13^{abc}
7	PW0	4.40 ± 0.21^{bc}	3.87 ± 0.19^{ab}	3.87 ± 0.17^{ab}	3.80 ± 0.24^{a}	4.20 ± 0.18^{abc}
8	Cb1	2.21 ± 0.22^{c}	2.94 ± 0.24^{ab}	2.40 ± 0.13^{abcd}	3.04 ± 0.24^{ab}	2.36 ± 0.23^{ab}

Means in the same column with different superscript are significantly different by ($p < 0.05$) Duncan multiple range test (DMRT)

Key: AM1= 10%, BM2=20%, CM3=30%, DM4=40%, EM5=50%, WIS = pure wheat flour bread, PW0 = control (commercial yeast),Cb1= commercial bread

CHAPTER FIVE

5.0 DISCUSSION

Cassava is known to be rich in carbohydrate but low in protein and fat. This is evident in the results obtained, where the values for carbohydrate and protein fall within the standard specifications of 80-90% and 1-3% respectively (Montagnac *et al*., 2009). Nwosu *et al*., (2014) reported carbohydrate content of cassava flour as 82.93%. Values for moisture content falls within the recommended levels of Less than 14% (Ajani *et al*., 2012). It is known that moisture and water activity of a product determine its keeping quality. The ash content of the wheat flour (1.64%) falls outside recommended standard of 1.2%, indicating adulteration. Results show values for Ash in cassava flour obtained from the market to be higher than industrial cassava, reflecting the effect of processing and exposure to dust. The higher the Ash content, the lower the bread making quality. The sieve analysis consisted of dirts and cassava shafts in wheat flour, indicating adulteration. Particle size variation could be as a result of milling processes.This property determines the swelling power of flour (Ndife *et al*., 2011, Nwosu *et al*., 2014).

The chemical composition of the composite flours has been shown to affect both physico-chemical properties and nutritional quality of their products (Eddy *et al*., 2007; Ogunbawo *et al*., 2008; Aboaba and Obakpolor, 2010; Ndife *et al*., 2011. Generally, the proximate values decreased with increasing levels of cassava flour substitutions. Although significant exceptions existed, this may be attributed to processing methods employed. Carbohydrate content in bread samples ranged from 43.01 - 64.22%.These comparable values are reported by Ndife *et al*., 2011, 54.27%.The carbohydrate values

obtained could be due to the high content of carbohydrate in cassava which predominates in the roots (Nweke, 2003).

The proximate values obtained decreased with increasing levels of cassava flour substitutions.Although significant exceptions occured, these may be attributed to processing methods employed. However, there was no significant difference (P >0.05), between the products made from industrial and market cassava flours. Carbohydrate content in bread samples ranged from 43.08 -64.22% .These comparable values are reported by Ndife *et al*., 2011, 54.27%.The carbohydrate values obtained could be due to the high content of carbohydrate in cassava which predominates in the roots (Nweke, 2003).

Food materials have different capacity for retaining moisture which may exist as absorbed water, even at high baking temperature (Eddy *et al*., 2007). The variation could be due to differences in storage and processing methods of the samples. The high moisture content of the bread samples (24.44- 32.49%), when compared to that of flours (2.28% -5.16), may be due to the water added during the baking process (Ajani *et al*., 2012).The range of the moisture content values obtained, is similar to findings by Shittu *et al*., (2007) who reported moisture content of cassava wheat bread within the range of 32-39% ,Alian *et al*., (1997) also reported moisture content of 33–34% from balady and pan bread..

The ash content results indicated that as the substitution of cassava flour was increased, the ash contents of the bread samples equally increased significantly 0.39 – 1.25% (P < 0.05). This may be due to the high ash content of the cassava flour which indicates the high mineral contents in it (Ogunbawo *et al.,* 2008; Aboaba and Obakpolor, 2010).The

ash content obtained is similar to those reported by Banu *et al*., (2012), 0.42 -1.43% and Enriquez *et al*., (2003) reported 0.60 – 0.92%.

Studies have shown the contribution of *Lactobacillus plantarum* enzymes to peptide degradation and amino acid turnover during dough fermentation (Gobetti *et al*., 2005; Loponen *et al*., 2007; Rizzello *et al*., 2007).The result of the protein content showed no significant difference between samples Af, Bf and F(7.07%,7.05% and7.09% respectively. Wheat and cassava are poor sources of protein and that would have resulted in the general low protein content of the samples (Montagnac *et al*., 2009; Eduardo *et al*., 2013).

Fat plays a significant role in the shelf life of food products and as such relatively high fat content as was obtained in these findings could be undesirable in bread products. Fat can promote rancidity in foods, leading to development of unpleasant odour (Agiriga, 2014). Fat content of the composite bread samples were in the range of 8.55 - 10.44% for industrial and 8.22 - 9.25% for the market cassava. The high values could also have been due to the cooking fat that was added during the baking processing.

Fruits, vegetables, and cereals are very important microhabitats for several yeasts and Amylolytic Lactic acid bacteria (ALAB) species. Yeast and ALAB strains were isolated from sugar rich samples such as fruits and starch rich cereals. This findings agree with the works of Ali and Mustafa, (2009), Ma'aruf *et al*., (2011). Colonies formed depend on the ability of the particular microorganism to form capsules or other extracellular matrix material, as well as on the capability of the cells to enter different stages of the life cycle for example, mating, sporulation or pseudohyphal growth as

observed in yeast cells (Vopalenska *et al*., 2005, Chatterjee *et al*., 2011, Dashko *et al*., 2014).

Yeasts and Amylolytic Lactic acid bacteria (ALAB) in their natural habitat can be found living in numerous, miscellaneous and changeable environments, they are also exposed to highly variable habitats. On such diverse ambiences, it can be expected that yeast and ALAB regularly withstand fluctuations in the types and quantities of available nutrients, acidity and osmolarity, as well as temperature of their environment. Traditional, biotechnology such as baking exposes microbial cells to various environmental stresses; this is evident in their ability to grow above ambient temperatures. Although most isolates grew at 35 and 37°C, few grew at 45°C. This is in agreement with the finding of Ma'aruf *et al*., (2011), who reported few growth at the same temperature. Elevated temperatures are known to affect the cell components of microorganisms (Beney and Gervais, 2001, De Angelis *et al*., 2004, Gobbetti *et al*., 2005); however, microorganisms exposed to mild heat predisposed them to acquiring certain degree of thermotolerance, which can affect the viability and vitality of the culture hence the potential for industrial applications. Those microbial strains which were able to survive at these temperatures indicated that they can be used in bread making to speed up the proofing process, increased carbon dioxide production and formation of flavor and aroma may also be enhanced. Temperature can affect the fermentation process and the metabolism of yeast (Hansen *et al.*, 2002). Commercial yeast has optimal reproduction temperature at 28°C or lower. Even though the yeast can ferment well at 32-35°C, its metabolic activity is compromised at high temperatures. Furthermore, the yeast and ALAB ability to tolerate high temperature declines dramatically (Venturi *et al*., 2013, Aguilera *et al*., 2007).

The applications of yeasts and ALAB in baking involve exposure of strains to numerous physical and chemical stresses (Gobbetti *et al*., 2005, Aguilera *et al*., 2010). Variation in the ethanol concentration as observed in this study shows all the yeast isolates utilising 3%, 6% and some 10% ethanol. It is expected that yeast should be able to tolerate some degree of ethanol produced during dough fermentation, which is a normal end product of its fermentation processes. Ethanol is said to have major effects on yeasts, thus it decreases the rates of growth and fermentation as well as its cell viability (Safri *et al*., 2011). The range of ethanol tolerance obtained in the present study was 3-10%, which correlates with the previous reports by Chiranjeevi *et al*., (2013) who observed a tolerance level of 7-12%. Aguilera *et al*., (2007), reported that a microbial strain must be able to grow and yield efficiently from the perspective of the manufacturer, and to tolerate the varieties of uses and abuses encountered during various baking procedures in order to be of commercial value (Goddard, 2008, Aguilera *et al*., 2010)

Sugar is frequently used by ALAB and *Saccharomyces cerevisiae* as a carbon source in dough fermentation. Amylolytic lactic acid bacteria breaks down starch into glucose which provides food for the yeast during the early stages of fermentation giving rise to gas production and ethanol (Reddy *et al.,* 2008, Fossi and Tavea, 2013). Sugar contributes three characteristics to breads; sweetness, color and moisture retention. In this study, all the yeast isolates utilised glucose at 5% and also produced gas from oxidation/fermentation media while most ALAB produced gas from glucose and were referred to as hetero-fermentative (Reddy *et al*., 2008). Lactobacilli are fermentative bacteria. Depending on the metabolic pathway of sugar fermentation, they are divided into homofermentative or heterofermentative group. Some species have the genetic and physiological capability to ferment sugars by both ways and, therefore, they are

referred to as facultative heterofermentative bacteria. Heterofermentative lactic acid bacteria produce 1 molecule of lactate along with 1 molecule of ethanol and 1 molecule of carbon dioxide or acetic acid (O´Toole and Lee, 2006, Williams *et al*., 2011, Vlkova *et al*., 2012). According to Chavan and Jana, 2008, 5-6% sugar gives best dough proof.

Gas in form of Carbon dioxide (CO2) is produced by heterofermentative LAB. It supports anaerobic environment and is toxic to some aerobic food microorganisms, because it precludes the cell membrane to reduce and balance the internal and external pH value (Caplice and Fitzgerald, 1999). Carbon dioxide produced by heterofermentative lactobacilli also improves the capacity of the dough by retaining the gas in it (Gobbetti, 1998).

A number of interesting findings have suggested that the presence of salt in the fermentation process accelerates dough fermentation. Salts main function in baking is to bring out the good flavour and mask the off flavours. It also slows down the growth of yeast hence the rate of fermentation. This allows the yeast to metabolise more sugar catalysed by ALAB in a given period of time (Blomberg, 2000; Wang *et al*., 2011; Toshiyuki and Yasuhide, 2013). The ability of the isolates in this study to grow at 3%, 6% and few at 10% NaCl indicates that there is variability in salt tolerance. These concentrations are said to be highly desired attributes for industrial applications especially baking (Sanni *et al*., 2002).Variability in salt tolerance has also been shown for other *Lactobacillus plantarum* strains. Growth at 8% salt has been reported. In general, salt has commonly been used as an essential secondary ingredient in bread making (Guyot and Guyot 2001a, Toyosaki and Sakane, 2013).

The yeast strains isolated in this study are similar to those isolated by other researchers. Dominant yeasts have been isolated from other indigenous fruit and cereal food products (Lei and Jakobsen, 2004; Nasrin *et al*., 2007). Goddard, (2008) also reported the isolation and characterization of yeast strains belonging to *Candida spp*, and *Saccharomyces cerevisiae* from grapes juice. Maragatham and Panneerselvam, (2011) isolated *Saccharomyces cerevisiae, Saccharomyces spp* and *Zygosaccharomyces spp* from papaya fruits, Likewise *Rhodotorula spp, Cryptococcus spp* were isolated from mango *(Mangifera indica) and* soursop (*Annona muricata L*) (Ma'aruf *et al*., 2011). *Saccharomyces cerevisiae, Candida spp* from *Ogi* made from maize, millet and sorghum (Blandino *et al*., 2003, Bekatorou *et al*., 2006; Glover *et al*., 2009, Ogunbanwo and Ogunsanya, 2012; Ashiru *et al*., 2012). *Saccharomyces cerevisiae* and *Lactobacillus plantarum* were isolated from deteriorating mango fruits by Amapu *et al*., (2008). Plant materials containing fermentable sugars provide suitable substrates for the yeast species *Saccharomyces, Candida, Torula* and *Hansenula*. Although the growth rate of these yeasts is lower than that of bacteria, (Francisco *et al*., 2002)

Lactic acid bacteria (LAB) are considered to be the major bacteria involved in numerous natural fermentation of carbohydrate products, their presence in starchy products have been reported by researchers. *Lactobacillus plantarum* and *Lactobacillus fermentum* have been isolated from amylalecous products (Sanni *et al*., 2002; Reddy *et al*., 2008). LAB which are able to break down starch polymer are described as amylolytic lactic acid bacteria (ALAB).These organisms have the ability to convert starch into mono and disaccharides which are later utilized as carbon sources and subsequently a fermentative substrate either for themselves or other microorganisms especially yeast (Calderon *et al*., 2003). ALAB were screened from LAB isolated by their ability to hydrolysed starch.The clear zone is an indication that starch was

degraded. It has been suggested that the suitable source for isolating ALAB is associated to a source rich in carbohydrate such as cassava-based fermented foods and cereals and less from fruits (Agati *et al*., 1998; Diaz-Ruiz *et al*., 2003).

The predominant ALAB strains isolated in this study were heterofermentative strains, most of them belonging to the *Lactobacillus plantarum* 8(57.14%). *Lactobacillus plantarum* is acid tolerant and able to metabolise and transport carbohydrates. They also dominate fermentation processes of cereals and vegetable (Fred *et al*., 2013) .These finding confirm former investigations, in which the presence of *Lactobacillus plantarum* as a dominant species in cassava fermentation was reported by Ben-Omar *et al*., (2000); Amoa-Awua *et al*., (1996). Other predominant strains belonged to *Leuconostoc spp*. This also agrees with studies on the microbial populations associated with cassava fermentations as reported by Amoa-Awua *et al*., (1996), that there was occurrence of *Lactobacillus brevis* and *Lactobacillus mesenteroides* in cassava fermentations. .

The findings also agree with that of Mugula *et al*., (2003) who isolated ALAB from *togwa*, a Tanzanian food prepared from sorghum, maize, millet and maize-sorghum, the microorganisms were identified as *Lactobacillus plantarum*, *Lactobacillus* brevis, *Lactobacillus fermentum*, *Pediococcus pentosaceus*, *Saccharomyces cerevisiae* and *Candida tropicalis*. Sanni *et al*., (2002) described amylolytic strains of *Lactobacillus plantarum* and *Lactobacillus fermentum* strains in various Nigerian traditional amylaceous fermented foods. Other strains of ALAB have been isolated from African cassava-based fermented products (Nwankwo *et al*., 1989, Corsetti *et. al*., 2001). Amylolytic strains of *Lactobacillus fermentum* were isolated for the first time from Benin maize sourdough (ogi and mawè) by Agati *et al*. 1998). On the other hand,

Chahrour *et al*., (2013) isolated *Lactobacillus fermentum* and *Lactobacillus brevis* from sorghum silage. According to Damelin *et al*. (1995), amylolytic lactic acid bacteria (ALAB) may account for 65% of isolates from different food types and environment.

Carbohydrate are an invaluable energy source and provide structural, storage, informational, recognition, shock absorbing and protective functions to an organism. The ability of microorganism to utilise carbohydrate is an important criterion for identification and classification. From the API 20CAUX and API 50CH profiling results, it evident that yeast and ALAB isolates employ different sugars as their main carbon and energy source. Sucrose, maltose, glucose, and fructose, were fermented, they are the main soluble sugars of cereals and cassava-based fermented foods reflecting adaptation to their habitat. *Lactobacilli* are known to ferment wide range of sugars, such as glucose, fructose, lactose and maltose. Reddy *et al*., (2008) and Gereková *et al*., (2011) reported that plant-derived carbohydrates such as cellobiose, amygdaline and trehalose may be fermented as well. Furthermore, all ALAB (except *Lactococcus lactis, Pediococococcus acidolactici and Leuconostoc mesenteroides*) were able to ferment raffinose,an alpha-galactoside contained in cereals. *Lactobacillus plantarum* strains isolated from fermented Ghanaian maize products were able to ferment raffinose, while strains of *Pediococcus acidilactici* and *Pediococcus. Pentosaceus* were unable to do so. The sugar fermentation profile of the isolates showed that indigestible sugars such as raffinose and stachyose can be fermented by the strains. These sugars possess a-D galactosidic bonds which are resistant to cooking and small-scale processing, but which can be hydrolysed by α-galactosidases produced by a number of moulds and by bacteria associated with fermented foods. A research conducted by Yoon *et al*., (2003) showed that *Saccharomyces cerevisiae* readily

utilised the common monosaccharides: D-glucose, D-fructose, D-mannose, and D-galactose, of the disaccharides, maltose and sucrose were completely utilised. Some strains of heterofermentative lactic acid bacteria were able to convert the pentose sugars xylose and arabinose obtained from the hydrolysis of the hemicellulose components of agricultural and forest product wastes to lactic acid through the pentose phosphate–transaldolase–transketolase pathways (Gereková *et al*., 2011).

Starchy substances constitute the major part of the human diet. They are synthesized naturally in a variety of plants. Some plant examples with high starch content are corn, acha, rice, sorghum, wheat, cassava. Similar to cellulose, starch molecules are glucose polymers linked together by the alpha-1, 4 and alpha-1, 6 glucosidic bonds, as opposed to the beta-1, 4 glucosidic bonds for cellulose. In order to make use of the carbon and energy stored in starch, it must first be broken down to smaller assimilable sugars, with the help of the enzyme amylases, which are the residual or reducing sugar, also called fermentable sugars (Negrulescu *et al.,* 2012). The isolates were tested for their ability to break down starch which is a major component of cassava and wheat flour. The results of ANOVA carried out to compare mean values of reducing sugar shows a linear decrease. The total sugar contents decreased significantly ($P < 0.05$) throughout the fermentation period. This is due to a maximum break down of starch substrates to reducing sugars by amylolytic enzymes produced by the fermenting ALAB (Songré-Ouattara *et al*., 2009). There was significant difference ($P < 0.05$) and a linear increase in amylase production was observed when incubation periods was extended to five (5) days and thereafter declined. The effect of time also revealed that twenty (24) hours was optimum for most of the isolates (Rosell *et al*., 2001; Nout and Aidoo, 2002; Miloski *et al*., 2008; Sivakumar *et al*., 2012). Production of reducing sugar was rapid when *Lactobacillus plantarum and Saccharomyces cerevisiae* were co- cultured

(0.72mg/ml) within six hours, than in single cultures (0.68mg/ml) within twenty four hours. Increase in reducing sugar content is a confirmation of starch degradation, while the decrease is as a result of utilization of available simple sugars by the fermenting organisms. Co-metabolism between LAB and yeasts is common in many foods, enabling the utilization of substances that are otherwise non fermentable (for example starch) and thus increasing the microbial adaptability to complex food ecosystems. It has been suggested that the proliferation of yeasts in foods is favoured by the acidic environment created by LAB while the growth of bacteria is promoted by the presence of yeasts, which deplete complex nutrients and growth factors which may provide growth factors, such as, vitamins and soluble nitrogen compounds. The association of LAB and yeasts during fermentation may also contribute metabolites, and have been shown to impart taste and flavour to foods (Gobbetti 1998; Paramithiotis *et al*., 2006; Malherbe *et al*., 2007; Ali and Mustafa 2009).

The cell number counts/optical density of both ALAB and *Saccharomyces* isolates decreased with fermentation days. This could be as a result of accumulation of metabolic waste products in the fermentation medium which affects growth rates and cell viability (Kostinek *et al*., 2007).

An increase in temperature from 37- 90°C showed a linear pattern in amylase production up to 45°C and remained stable up to70°C. Fossi *et al*., 2011 recorded a high thermostable activity at 70°C of *Lactobacillus fermentum 04BBA19*. Mathewson (2000) reported that α amylases from bacterial origin are more thermal stable than from other sources. The progressive decline might have resulted from denaturation or decomposition of amylase as a result of interaction in the culture medium

(Gangadharan *et al*., 2006; Kostinek *et al*., 2007, Panda *et al*., 2008; Sener *et al*., 2007). The stability of the enzyme at high temperature reveals that it can be used for starch hydrolysis in industrial processes especially baking (Rosell *et al*., 2001; Fossi *et al*., 2011).

An important indicator of an efficient fermentation process is pH value. There is a correlation between fermentation and acidity because the yeast and bacteria in a fermentation medium produce acetic acid, lactic acid, and other weak organic acids (Kostinek *et al*., 2007; Reddy *et al*., 2008). This characteristic is an important metabolic activity of microorganisms for dough preparation and bakery industry. The effect of pH on co cultures of *Lactobacillus plantarum and Saccharomyces cerevisiae* revealed that there was maximum reducing sugar and amylase production (0.69mg/ml and 3.11U/ml respectively) at pH 6.0.This is comparable to results obtained by Rosell *et al*., (2001) who recorded highest activity also at pH 6.0.It is similar to the range obtained by Fossi *et al*., (2011) who reported 4.0-7.0, which is the pH of many foods.

An important part of this research is focused on obtaining a starter culture that would significantly contribute to an improvement in product quality of wheat/cassava bread.In this study, the leavening ability of the isolates was investigated in wheat flour. Results from this study shows that co culture of *Lactobacillus plantarum* (AMz5) and *Saccharomyces cerevisiae* (YSg2) had a higher dough volume than the control (commercial bakers yeast), indicating that they are better dough leaveners. According to Holzapfel, (2002) a suitable starter culture must interact in a food matrix. The starter culture must grow, survive, compete and improve a fermentation process. The

leavening potentials of these co cultures were further investigated in wheat/cassava flour.

The interaction rates of the different wheat/cassava dough blends to the starter cultures indicated that dough volume of the blends decreased with increase in cassava flour ratio. This might be due to the inability of a total hydrolysis of cassava starch. A report by Giraud *et al*., (1994) while working with an amylolytic strain of *Lactobacillus plantarum*A6, observed a weak attack on cassava starch granules, but resulted in faster acidification of the fermentation process. Acid and enzymes produced during cassava fermentation were observed to promote important starch granule damage (Marcon *et al*., 2006). Also, a research by Putri *et al*., (2011) revealed that, fermentation with *Lactobacillus amylopylus NBRC 15881*, an amylolytic strain, changed cassava starch molecular structure, resulting in a better swelling and solubility power. Findings by Ogunbanwo *et al*., 2008; Oladunmoye *et al*., 2010; Abdelghafor *et al*., 2011; Nuno *et al*., 2011; Viswanathan and Ho, (2014) revealed that non gluten flours adversely affected dough rising due to the absence of gluten protein. There was no significant difference ($P < 0.05$) among three of the formulations (10%, 20%, and Wf) .This implies that the cassava ratios probably did not have an adverse effect on the dough rising, also the isolates fermented the simple sugars such as glucose fructose, sucrose derived from flour and those added as ingredients, resulting in evolution of carbondioxide in the fermenting dough (Kostinek *et al*., 2007; Shittu *et al*., 2007).The non appreciable change in dough volume of the negative control (Vf -uninoculated) shows that there was little activity. The Lag phase of growth, inocula size, rate of carbondioxide production had been reported to contribute to good dough fermentation **(**Aboaba and Obakpolor, 2010; Ma'aruf *et al*., 2011; Putri *et al*., 2011; Hussein *et al*., 2013). However, the slower dough fermentation observed in this study could be

attributed to the fact that commercial yeast are more refined, concentrated, contain preservatives and fast active ingredients than the isolates (Ma'aruf *et al*., 2011). According to research, some bakers have argued that living yeast, when compressed into cakes contributes a more unique flavour to bread (Azmuda *et al*., 2006).Although longer fermentation time has been found to improve degree of fermentation. Breads baked with long fermentation times have been reported to have larger pores due to the enhanced gas production by the yeast (Antonio *et al*., 2001; Mustafa *et al*., 2009).

Amylolytic lactic acid bacteria and yeast play a key role in dough fermentation as starter cultures. This is attributed to their ability to ferment carbohydrates into useful metabolites such as simple sugars, organic acids and alcohol. In this study, pH, total titratable acid and viable counts of LAB and yeast were investigated in dough fermentation. Results obtained showed that there is a correlation between pH and viable counts of yeast and LAB. It also showed that increase in fermentation decreased pH, while viable counts increased. This suggests that, as the cells multiply, carbohydrates are broken down and used up. There was therefore accumulation of organic acids (lactic acid, acetic acid) which resulted in change in pH values and total titratable acids. An acidic environment naturally favours fermentative organisms (Calderon *et al*., 2002; Corsetti and Settani, 2007). Reports by Corsetti *et al*., (2000); Thiele *et al*., (2002) ;Clarke *et al*., (2004); Rehman *et al*., (2006) and Gobbetti *et al*., (2005), show that lactic acid bacteria and yeast fermentation creates optimum pH for the activity of endogenous factors which improves texture changes. This also contributes directly to bread flavour, especially, through the synthesis of acetic acid, and thus increases the dough volume. A study conducted on mixed commercial starters containing *Lactobacillus brevis* and *Saccharomyces cerevisiae* (baker's yeast), fermented at 30°C

for 20 hours, showed reduced yeast growth and ethanol production in dough but more acetic acid and lactic acid production. The decrease in pH has been reported to be mainly due to the production of lactic acid and acetic acid (Meignen *et al*., 2001; Rehman *et al*., 2006). It can be concluded that pH increase is growth associated. During the fermentation period, there was considerable production of organic acids.This could be due to the metabolism of different carbohydrates in the dough and organic acids produced can vary based on the fermenting organisms (Meignen *et al*., 2001; Sadeghi *et al*., 2008). Results obtained shows that cassava proportions has effect on organic acids produced. Lactic acids production are considered the main reason for pH decrease in dough (Rehman *et al*., 2006) .Total titratable acids had been used to measure fermentation activity and flavour of fermented products (Abegaz *et al*., 2004; Sadeghi *et al*., 2008)

Fermented foods in particular are considered to be safe and wholesome. On the other hand, acidification to pH values of less than 4.5 has a desirable effect (Odunfa and Oyewole, 1998; Abegaz *et al*., 2004; Mustafa *et al*., 2009).The dough pH, values obtained in this study ranged from 5.3 to 3.45 for both blended types. A number of metabolites, such as acetic acid (from heterofermentative LAB), hydrogen peroxide and bacteriocins,produced during the fermentation process, exhibit antimicrobial properties which may contribute to the safety of fermented foods as affirmed by researchers (Kostinek *et al*., 2007;Gerez *et al*., 2009.

The development of organism in a fermenting food depends on pH, incubation temperature, moisture, composition of the food matrix and gas concentration. Mixed cultures of LAB and yeast vary in composition in dough, where yeast acts as leavening

agent, while LAB contributes to the flavour of the bread. In this study, it was observed that the viable counts of LAB and yeast differ considerably among the blends. A continuous increase of the fermenting organisms after four hours of fermentation was observed. The viable counts for LAB ranged between 3.50 $x10^8$ to 3.80 x 10^8 cfu/g, while viable counts for yeast ranged between 2.1$x10^6$ to 3.6 x 10^6 cfu/g. Microbial population of LAB and yeast are expected to increase during the first stage of fermentation provided favourable growth conditions are met (Asmahan and Muna, 2009).This encouraging result shows the efficiency of pure starter cultures. The results obtained agree with findings by De Vuyst and Neyens, (2005) who reported that in a good bakery practice, dough should contain metabolically active LAB at 10^8 to 10^9 cfu/g and yeast at 10^6 to 10^7 cfu/g. Hammes *et al*., (2005) revealed that in mature doughs, LAB ranged from 1 $x10^9$ to 3 $x10^9$ cfu/g and yeasts from 1 $x10^6$ to 5 $x10^7$ cfu/g. In addition, Corsetti *et al*., (2001) found the ratio of yeasts to LAB in sourdough bread ranged from about 1:1000 to 1:10. Low yeast count may probably be due to inhibitory effect of the fermentation. The low counts observed in Cs may not be unconnected to the inability of the organisms to completely break down cassava starch (Giraud *et al*., 1994; Reddy *et al*., 2000).

Bread made from composite flours has to be subjectively assessed to determine its quality and acceptance using the Hedonic testing. There was no significant difference ($P < 0.05$) in the tastes of the bread samples of PW0 (100% wheat bread), 10 and 20% (BI2 and BM2).This indicates that taste in these formulations does not depend on fermenting organism, but on the ingredients added. However, this trend changed at 30% (CI3 and CM3) substitution of cassava flour. Aroma is related to taste and the level of aroma influences taste. The mean scores of aroma and taste obtained were comparable to that of control. The baking conditions (temperature and time variables),

state of the bread components (such as fibers, starch, gluten) and the amounts of absorbed water during dough mixing, all contribute to the final texture of breads (Gomez *et al*., 2003).The texture scores however showed a decrease with cassava flour content. Texture was highly rated in WIS (bread made from wheat, but started with isolates) samples. Raw material, when subjected to biological, physical and chemical processes, change its state and are manifested as expansion of materials, change in solubility and textural strength .This therefore affects the quality of the product (Oladunmoye *et al*., 2010; Kadam *et al* 2012). Studies had also revealed that lactic acid fermentation influences the structure and starch granules porosity by acidic and enzymatic hydrolysis, promoting starch granule damage (Bertolini *et al*. 2001; Putri *et al*., 2011)

The interaction between ALAB and yeast in dough had been found to primarily influence texture and flavour of bread (Rehman *et al*., 2007; Di Cogno *et al*., 2002).The result shows that the use of *Lactobacillus plantarum and Saccharomyces cerevisiae* as starter culture greatly improved the quality of bread produced. This is evident in the good tastes and aroma rating, implying that compounds such as organic acid, alcohols, aldehydes, and carbonyls which impacted appealing flavour to the dough were produced (Thiele *et al*., 2002; Rehman *et al*., 2006). The sensory evaluation also revealed that the most favoured bread sample was WIS (bread prepared with wheat).It was preferred over control because of its taste, aroma, flavour. It was awarded the highest scores for most of the sensory attributes and hence adjudged the best. Bread samples AI1, BI2, CI3 AM1, BM2 and WIS were generally accepted considering the high ratings, while Cb1 was least accepted. Previous reports had indicated that bread made with 10%, 20% composite flours were widely accepted by consumers (Sobowale *et al*.,2007; Ogunbanwo *et al*., 2008; Abdelghafor *et al*., 2011 ; Eduardo *et al*.,2013).

CHAPTER SIX

6. 0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

This study provides an insight to the fact that Amylolitic Lactic acid bacteria and Yeast are the main organisms involved in cereals, fruits and cassava fermentation. Out of the abundance of these organisms, *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were isolated and identified based on morphological and physiological characterised. Attributes such as high enzyme activity and reducing sugar yield, utilization of sugars, thermostable enzymes and osmotolarence to stresses were considered suitable traits for baking industries application.

To obtain a starter culture that would be able to improve the product quality of wheat/cassava dough, the following characteristics were considered; utilization of carbohydrates, production of carbondioxide, growth at elevated temperatures, ethanol and sodium chloride tolerance. *Lactobacillus plantarum* (AMz5) and *Saccharomyces cerevisiae* (YSg2) showed great potentials for these attributes. These organisms also showed good leavening ability when co cultured in wheat dough. Their leavening potentials were later investigated in wheat/cassava flour blends. The results obtained were impressive. Although the dough expansion rates were slow during the first few hours, the volumes still increased after three hours of fermentation and were comparable to control (dough with commercial yeast).

The Federal Government of Nigeria reintroduction of the wheat/cassava bread initiative, aimed at increasing the production of variety of cassava based bakery products and conserve foreign exchange, was vehemently resisted and criticized.

Researchers and research institutes have over the years come up with the fact that bread can be made from wheat substituted with other flours. This study out of many further confirmed this possibility. Bread produced from 10%, 20% composite flours are reported to be widely accepted by consumers. Results from this study reveal that bread produced from 10% and 20% wheat/cassava rated higher than control (bread produced with commercial yeast) and commercial bread in all the attributes tested for. There are good opportunities for the use of cassava flour in bread production in Nigeria.

6. 2 CONCLUSION

The values for proximate composition of flours falls within recommended standard excecpt the ash content which had higher values. The proximate values for samples decrease with increase in cassava flour proportion. However, cassava flour proportion did not affect the fat contents.

Saccharomyces cerevisiae and *Lactobacillus plantarum* are dominant microorganisms in fruits, cereals and cassava fermentation. Their osmotolerance to stresses and ability to ferment wide range of sugars which are the main soluble sugars of cereals and cassava-based fermented foods are desirable attributes for baking industries.

The maximum reducing sugar and amylase production at pH 6.0 and at 24 hours and the stability of the enzyme at high temperature (70°C) shows that the isolates can be used in industrial processes especially baking.

The association of *Saccharomyces cerevisiae* from sorghum and *Lactobacillus plantarum* from maize also shows they have better leavening potentials than control

(commercial bakers yeast) and can be developed into an industrial starter cultures on a larger scale**.**

Bread produced with the isolates rated higher in all the attributes tested for, than those produced with commercial yeast and routine bread, suggesting promising applications in the production of bread and other fermented starch-containing foods.

Consumer acceptance is necessary since they are the ultimate deciders of wheat/cassava policy implementation, although introducing a new product is normally accompanied by lot of challenges, especially for individuals used to the traditional taste of wheat bread.

6.3 RECOMMENDATIONS

1 High quality cassava Flour (HQCF) should be of special interest to bakeries in rural areas of the developing countries. This will contribute further to the development of a new market channel for cassava producers and processing plants and reduce partial or full dependence on wheat importation

2 There should be continuous effort to isolate and characterize amylolytic lactic acid bacteria and yeast from locally available raw materials.This will enhance the opportunities for selecting and adapting starter cultures for food and industrial applications.

3 The performance of amylolytic lactic acid bacteria and yeast could further be assessed to establish their dominance in fermentation by validating the desired

traits (Starch hydrolysis and utilization, Osmotolerance, thermostable enzyme) expressed for product quality impact.

4 The use of amylolytic lactic acid bacteria as a functional starter culture could provide an alternative to the use of traditional or industrial process by combining amylase production and acidification using the same strain, which would improve control of fermentation, and lead to products of more regular quality.

5 Public enlightenment on the benefits of the wheat flour supplemented with cassava flour would help to improve the acceptability of wheat/cassava bread.

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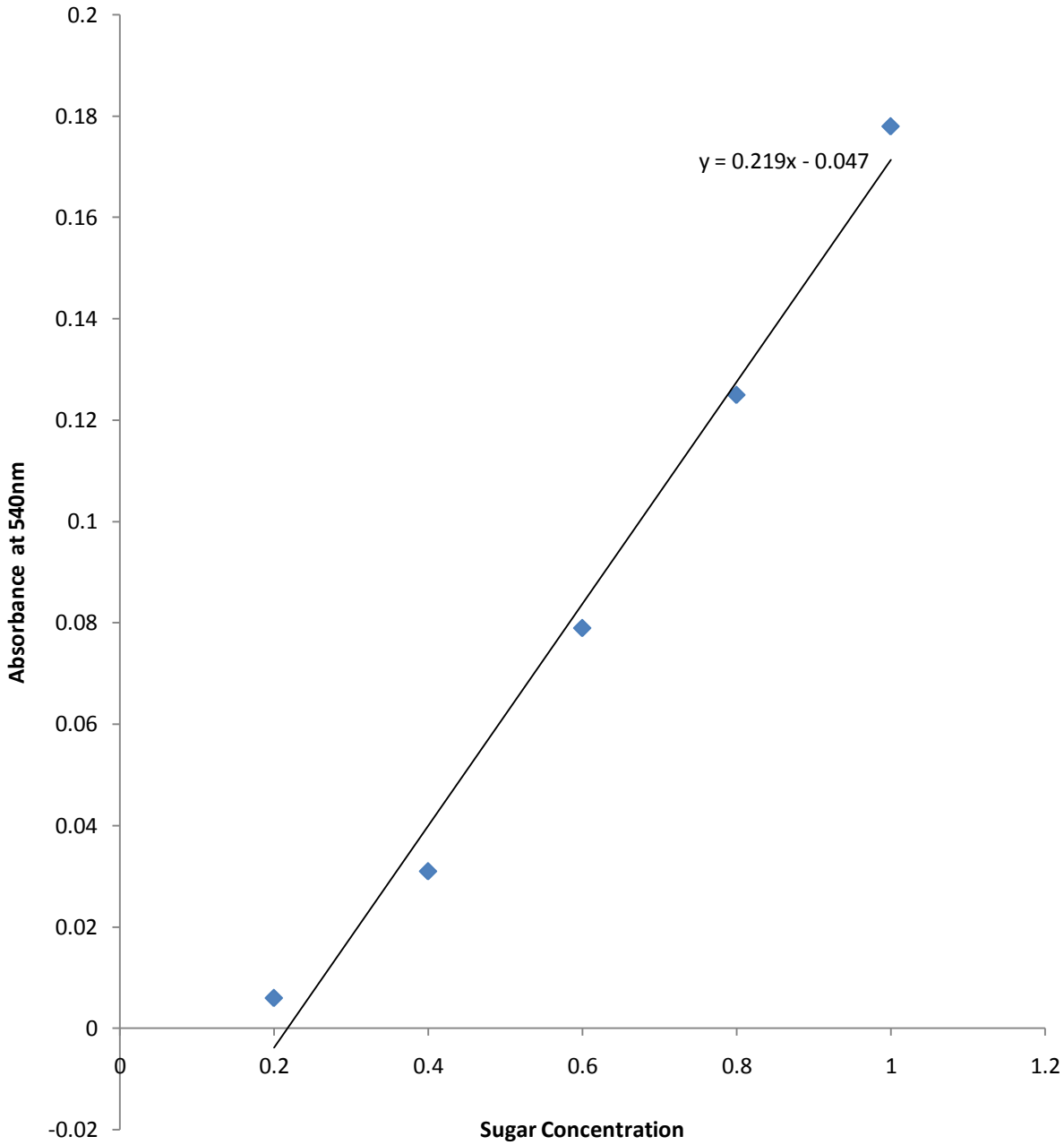
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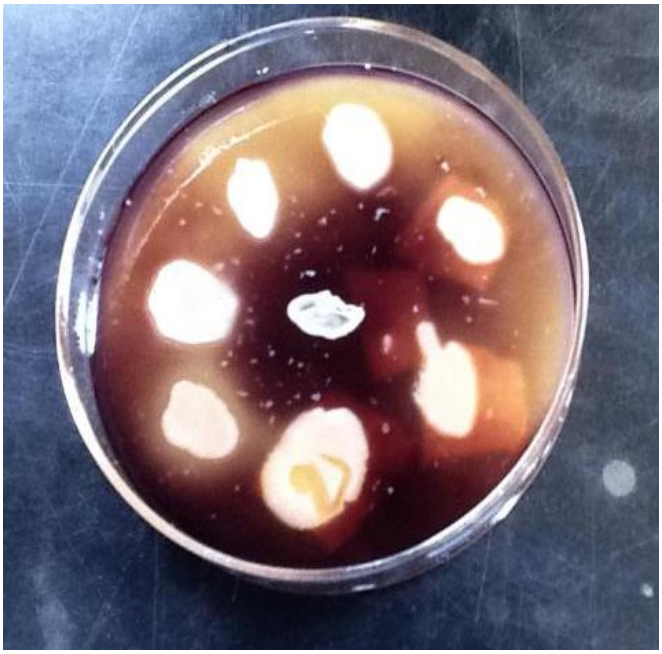
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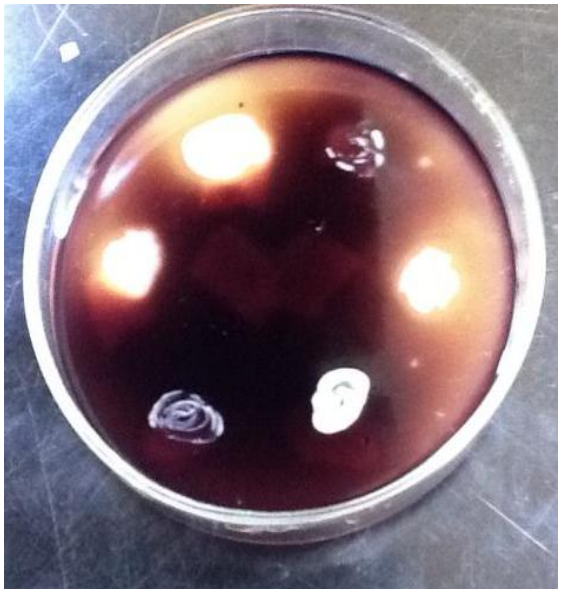
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APPENDIX



Appendix I: Glucose standard curve of reducing sugar.





Appendix II: Lactic acid bacteria isolates exhibiting starch hydrolysis and enzyme activity.

Appendix III: Carbohydrate fermentation profile of Yeast isolates using API 20C AUX kit

	O	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MIZ	RAF	HPH	Inference
YSg1	-	+	-	+	-	+	+	+	+	+	+	+	+	n	n	+	+	+	+	+	+	*Trichosporon. mucoides*
YOg1	-	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	*Candida.guilliermondi*
YMg1	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	*Trichosporon.mucoides*
YLe	-	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	-	+	*Candida.guilliermondi*
YMl1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	*Saccharomyces.cerevisiae*
YLi	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	*Candida guilliermondi*
YMl_2	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	*Saccharomyces.cerevisiae*
YPpl	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	*Saccharomyces.cerevisiae*
YMz1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces.cerevisiae*
YMg2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	*Saccharomyces.cerevisiae*
YSg2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	*Saccharomyces.cerevisiae*
YOg2	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	*Rhodotorula.glutinis*
YTm	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+	*Sacchromyces.cerevisiae*
YOg2	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+	*Saccharomyces.cerevisiae*
YMg3	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	*Saccharomyces.cerevisiae*
YSg3	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	+	*Saccharomyces.cerevisiae*
YOg3	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	*Kloeckera sp*
YTm	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	*Saccharomyces.cerevisiae*
YMl3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Cryptoccocus.humicola*
YMz2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	*Saccharomyces.cerevisiae*

Key: n = Non conclusive, Y = Yeast, Sg=Sorghum, Ml= Millet, Mz= Maize, Og=orange, Mg=Mango, Le-=Lemon, Li= Lime, Pple=Pinapple, Tm=Tomato.GLU=D-Glucose, GLY=Glycerol, 2KG=Calcium 2-keto-gluconate, ARA=L-arabinose, XYL=D-xylose,ADO=Adonitol,XLT=Xylitol,GAL=D=galactose,INO=inositol,SOR=sorbitol,MDG=methyl-α-D-glucopyranoside,NAG=N-acetyl-glucosamine,CEL=D-cellobiose,LAC=D-Lactose,MAL=D-Maltose,SAC=D-sucrose,TRE=D-trehalose,MLZ=D-melezitose,RAF==D-raffinose,HPH=pseudomycelium

Appendix IV: Carbohydrate fermentation profiles of LAB isolates using API 50 CH Kit.

	0	GLY	ERY	DARA	LARA	RIB	DXYL	LXYL	ADO	MDX	GAL	GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN	SOR	MDM	MDG	NAG	AMY	ARB	ESC	SAL	CEL	MAL	LAC	MEL	SAC	TRE	INU	MLZ	RAF	AMD	GLYG	XLT	GEN	TUR	LYX	TAG	DFUC	LFUC	DARL	LARL	GNT	2KG	5KG
AMz_1	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	n	-	-	+	+	n	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-	-	-	-	-	n	+	+
AMz_2	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-	-	-	-	-	n	-	-	-
AMz_3	-	-	-	+	-	n	-	-	-	-	-	n	n	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-		-	-	+	-
AMz_4	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-
AMz_5	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-	-	-	-	-	-	+	+	+
ACs_1	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	n	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	+	-	-	-	-	+	-	+	-	-
ACs_2	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
ACs_3	-	-	-	-	n	+	-	-	-	-	+	+	+	+	+	n	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	n	-	-	-	+	+	-	-	-	-	n	-	+	-	-
ACs_4	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	n	-	-
ACs_5	-	-	-	-	+	n	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+	-	-	+	-	-	-	+	+	-	-	-	-	n	-	+	+	-
ACs_6	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
ASg	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	n	-	-	-	n	-	-	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+
ATt	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	-	n	-	n
AAc	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	-	+	n	-	-	-	-	+	-	+	+	-

Key:n=Non-conclusive,A=Amylolytic,Mz=Maize,Cs=cassava,Sg=sorghum,Ac=Acha, Tt=Tomato,GLY=Glycerol,ERY=Erythritol,DARA=D-Arabinose,LARA=L-Arabinose,RIB=D-Ribose,DXYL=D-Xylose,LXYL=L-Xylose,ADO=D-Adonitol,MDX=Metyl-βD-Xylopyranoside,GAL=D-Galactose,GLU=Glucose,FRU=Fructose,MNE=D-Mannose,SBE=L-Sorbose,RHA=L-Rhamnose,DUL,=Dulcitol,INO=Inositol,MAN=D-Mannitol,SOR=D-Sorbitol,MDM=Metyl-αD-Mannopyranoside,MDG=Metyl-α D-Glucopryanoside,NAG=N-Acetylglucosamine,AMY=Amygdaline,ARB=Arbutine.ESC=Esculine,SAL=Salicine,CEL=D-Celibiose,MAL=D-Maltose,LAC=D-lactose,MEL=D-Melibiose,SAC=D-Saccharose,TRE=D-Trehalose,INU=Inuline,MLZ=D-Melezitose,RAF=D-Raffinose,AMD=Amidon,GLYG=Glycogene,XLT=Xylitol,GEN=Gentiobiose,TUR=D-Turanose,LYX=D-Lyxose,TAG=D-Tagatose,DFUC=D-Fucose,LFUC=L-Fucose,DARL=D-Arabitol,LARL=L-Arabitol,GNT=Potassiumgluconate,2KG=Potassium2-cetogluconate,5KG=Potassium5-cetogluconate.

Appendix V: Mean values of reducing sugar (mg/ml) of Yeast isolates.

DAYS	YML1	YML2	YPPLE	YMZ1	Y MZ2	YMG1	YMG2	YSG1	YSG2	YTM1	YTM2	YOG
0	0.11±0.02	0.08±0.02	0.09±0.01	0.11±0.01	0.09±0.01	0.08±0.02	0.09±0.01	0.12±0.02	0.16±0.01	0.12±0.01	0.10±0.01	0.10±0.01
1	0.35±0.01	0.42±0.02	0.42±0.02	0.34±0.04	0.43±0.01	0.30±0.01	0.48±0.02	0.58±0.02	0.55±0.05	0.58±0.01	0.49±0.01	0.40±0.01
2	0.25±0.01	0.36±0.01	0.36±0.01	0.25±0.05	0.47±0.02	0.38±0.03	0.30±0.01	0.38±0.02	0.30±0.01	0.38±0.01	0.40±0.07	0.31±0.02
3	0.23±0.03	0.29±0.01	0.29±0.01	0.21±0.02	0.38±0.02	0.28±0.02	0.28±0.02	0.23±0.01	0.23±0.01	0.23±0.02	0.40±0.07	0.22±0.02
4	0.23±0.02	0.18±0.02	0.18±0.01	0.15±0.05	0.38±0.02	0.22±0.02	0.23±0.03	0.21±0.01	0.21±0.01	0.10±0.02	0.35±0.01	0.12±0.02
5	0.18±0.02	0.18±0.02	0.18±0.02	0.11±0.00	0.22±0.02	0.11±0.01	0.15±0.05	0.12±0.03	0.12±0.06	0.05±0.02	0.19±0.01	0.07±0.02
TOTAL	0.22±0.08	0.25±012	0.25±0.12	0.20±0.11	0.33±0.14	0.23±0.11	0.26±0.13	0.26±0.19	0.26±0.15	0.24±0.12	0.32±0.14	0.21±0.12

Results are means of triplicate determination

Appendix VI: Mean values of reducing sugar (mg/ml) of ALAB isolates

DAYS	AMZ1	AMZ2	AMZ4	AMZ5	ACS1	AC13	ACS4	ASG	AAC	ATT
0	0.09±0.03	0.05±0.01	0.12±0.04	0.23±0.01	0.08±0.03	0 .0±0.01	0.07±0.01	0.10±0.00	0.11±0.01	0.06±0.01
1	0.26±0.02	0.34±0.03	0.43±0.01	0.55±0.02	0.43±0.06	0.37±0.01	0.30±0.01	0.51±0.08	0.27±0.01	0.15±0.07
2	0.36±0.01	0.39±0.01	0.41±0.01	0.46±0.01	0.34±0.01	0.48±0.00	0.36±0.01	0.44±0.02	0.33±0.04	0.30±0.02
3	0.24±0.04	0.24±0.02	0.35±0.01	0.25±0.01	0.23±0.01	0.35±0.01	0.32±0.01	0.28±0.02	0.24±0.01	0.21±0.01
4	0.16±0.01	0.19±0.01	0.20±0.01	0.19±0.01	0.13±0.01	0.24±0.02	0.22±0.01	0.19±0.01	0.15±0.07	0.17±0.01
5	0.08±0.03	0.06±0.03	0.11±0.01	0.10±0.01	0.07±0.01	0.08±0.03	0.09±0.02	0.08±0.01	0.14±0.01	0.08±0.01
TOTAL	0.20±0.10	0.21±0.13	0.27±0.14	0.29±0.05	0.21±0.14	0.27±0.15	0.22±0.11	0.26±0.17	0.20±0.09	0.16±0.09

Results are means of triplicate determination

Appendix VII: Mean values of amylase (U/ml) of ALAB isolates.

DAYS	AMZ1	AMZ2	AMZ4	AMZ5	ACS1	ACS3	ACS4	ASG	AAC	ATT
0	0.12±0.01	0.10±0.01	0.13±0.01	0.21±0.01	0.10±0.00	0.09±0.01	0.11±0.01	0.10±0.01	0.19±0.01	0.16±0.21
1	0.44±0.01	0.45±0.03	0.51±0.01	1.10±0.01	0.38±0.01	0.42±0.01	0.53±0.01	0.64±0.02	0.50±0.02	0.73±0.14
2	0.36±0.01	0.38±0.01	0.47±0.02	0.74±0.02	0.40±0.01	0.38±0.01	0.45±0.01	0.48±0.01	0.40±0.03	0.56±0.21
3	0.28±0.04	0.31±0.01	0.31±0.04	0.47±0.05	0.38±0.01	0.34±0.02	0.41±0.01	0.34±0.02	0.34±0.04	0.39±0.01
4	0.21±0.01	0.22±0.01	0.21±0.02	0.32±0.01	0.25±0.01	0.31±0.01	0.32±0.01	0.18±0.01	0.19±0.02	0.27±0.03
5	0.11±0.01	0.08±0.01	0.10±0.01	0.16±0.01	0.19±0.02	0.24±0.04	0.21±0.01	0.05±0.01	0.08±0.01	0.17±0.01
TOTAL	0.25±0.13	0.26±0.14	0.29±0.17	0.50±0.34	0.28±0.12	0.29±0.12	0.34±0.15	0.30±0.22	0.28±0.15	0.38±0.22

Results are means of triplicate determination

Appendix VIII: Cell number count (g /100ml) of yeast isolates

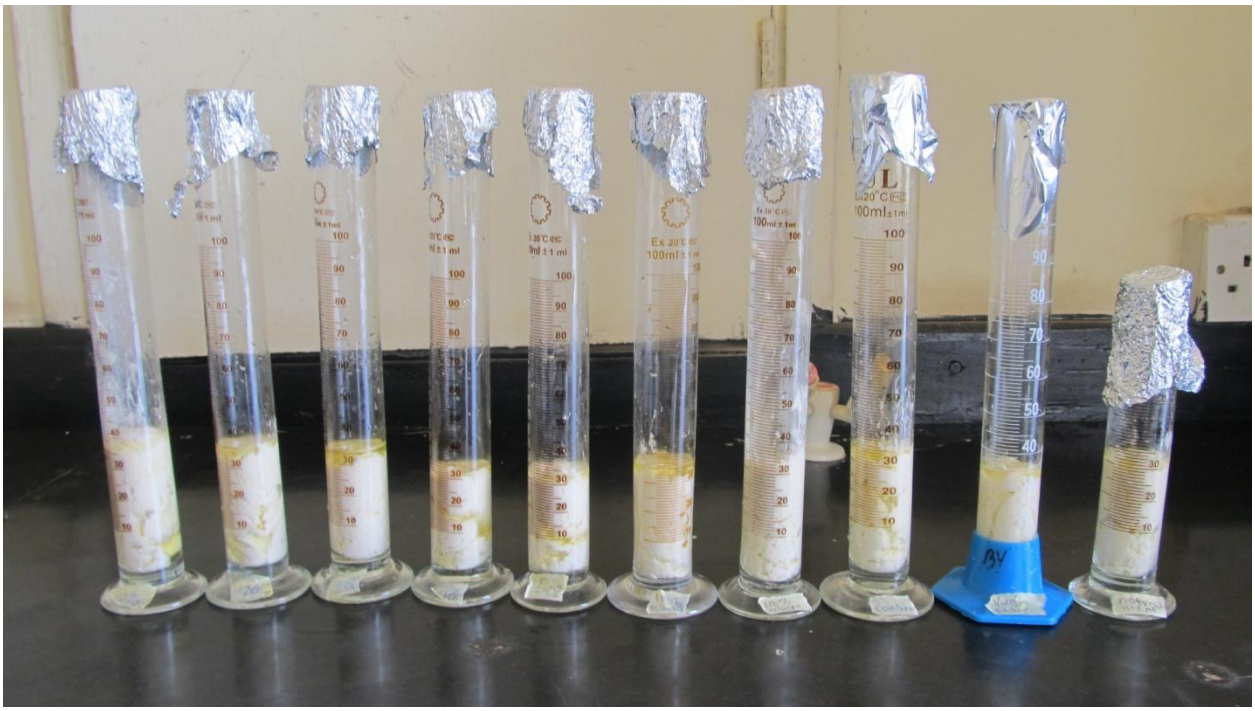
	Ml1	Ml2	Pple	Mz1	Mg1	Sg1	Tm1	Og	Mg2	Sg2	Tm2	Mz2
Days												
0	0.06	0.07	0.08	0.06	0.04	0.09	0.05	0.07	0.07	0.05	0.08	0.08
1	0.46	0.37	0.47	0.40	0.44	0.36	0.38	0.42	0.42	0.53	0.44	0.41
2	0.33	0.24	0.36	0.37	0.35	0.34	0.32	0.39	0.31	0.42	0.32	0.30
3	0.25	0.23	0.31	0.32	0.29	0.30	0.31	0.34	0.29	0.29	0.32	0.30
4	0.27	0.18	0.19	0.19	0.22	0.30	0.33	0.33	0.30	0.11	0.30	0.27
5	0.19	0.16	0.16	0.14	0.07	0.11	0.08	0.18	0.12	0.09	0.18	0.23

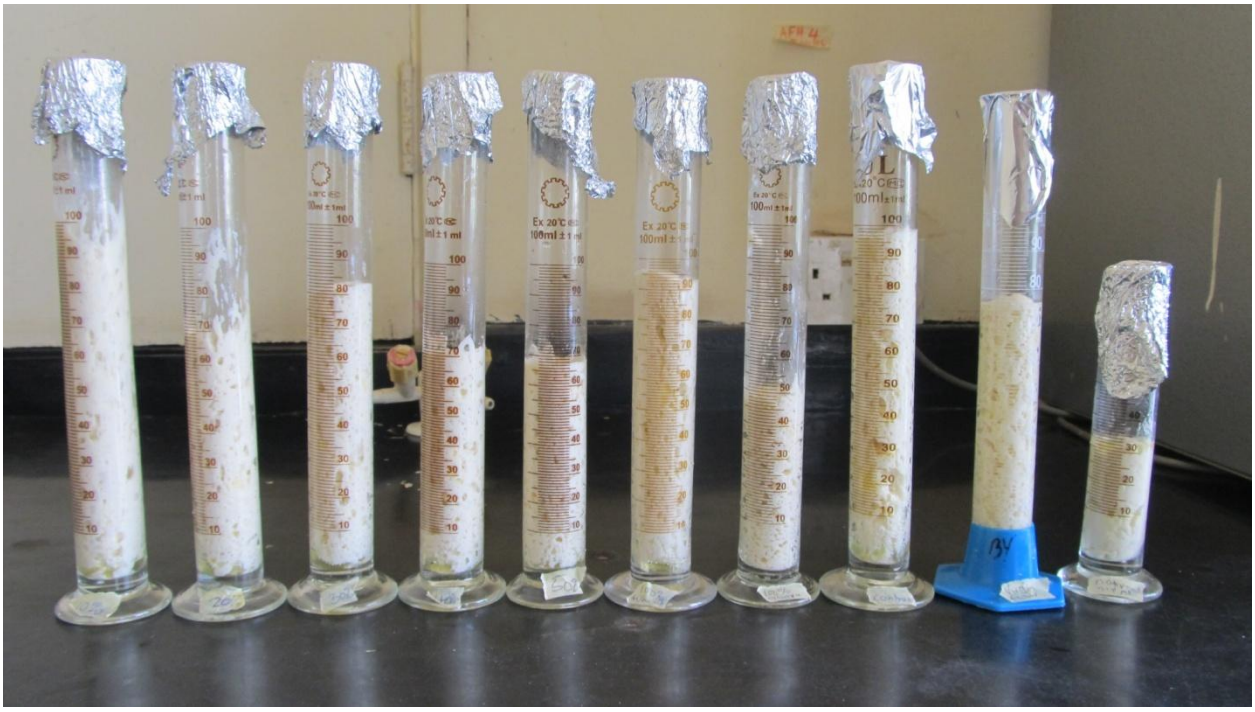
Results are means of triplicate determination.

Appendix IX: Cell number count (g /100ml) of ALAB isolates

	AMz1	AMz2	AMz4	AMz5	ACs1	ACs3	ACs4	ASg	AAc	ATt
Days										
0	0.09	0.11	0.08	0.10	0.07	0.07	0.06	0.15	0.06	0.08
1	0.51	0.40	0.52	1.09	0.42	0.43	0.59	0.69	0.65	0.56
2	0.42	0.36	0.42	0.78	0.31	0.35	0.41	0.54	0.53	0.58
3	0.41	0.30	0.38	0.66	0.28	0.32	0.38	0.55	0.50	0.48
4	0.30	0.25	0.32	0.40	0.21	0.32	0.33	0.40	0.43	0.35
5	0.24	0.17	0.28	0.25	0.19	0.22	0.25	0.23	0.31	0.30

Results are means of triplicate determination





Appendix X: Leavening profiles of cassava and wheat dough before and after fermentation. (From left to right 10%, 20%, 30%, 40%,50%,wheat flour, cassava flour, positive control, virgin flour and negative control

Appendix XI: Correlation of pH and ALAB viable counts for Market cassava and Wheat Dough Fermentation.

	10	*20*	*30*	*40*	*50*	*WF*	*CS*	*Post control*	*Neg control*	*pH10*	*pH20*	*pH30*	*pH40*	*pH50*	*pHWF*	*pHCS*
10	1															
20	0.941829	1														
30	0.930888	0.976015	1													
40	0.971629	0.91386	0.942471	1												
50	0.976604	0.936509	0.958795	0.984692	1											
WF	0.913305	0.881289	0.891021	0.903683	0.950056	1										
CS	0.880037	0.821156	0.877239	0.946602	0.947217	0.929189	1									
Post control	0.956204	0.942618	0.965525	0.98773	0.986148	0.924158	0.95684	1								
Neg control	0.863912	0.757296	0.775642	0.919643	0.882163	0.778983	0.907023	0.903924	1							
pH10	-0.93791	-0.81548	-0.84617	-0.94361	-0.95715	-0.93779	-0.94509	-0.92289	-0.89087	1						
pH20	-0.83291	-0.76782	-0.82157	-0.877	-0.90641	-0.9477	-0.96975	-0.89329	-0.81265	0.938604	1					
pH30	-0.91156	-0.81382	-0.86408	-0.93737	-0.95722	-0.95299	-0.96902	-0.92796	-0.86024	0.985872	0.974872	1				
pH40	-0.85283	-0.77718	-0.81829	-0.87311	-0.91076	-0.96358	-0.9501	-0.88628	-0.80041	0.95619	0.985947	0.972532	1			
pH50	-0.77494	-0.71524	-0.78649	-0.82419	-0.86575	-0.92686	-0.94036	-0.84597	-0.7452	0.908906	0.9881	0.951918	0.983203	1		
pHWF	-0.97769	-0.90843	-0.92896	-0.99137	-0.98633	-0.92658	-0.95202	-0.98051	-0.91578	0.969526	0.907929	0.959349	0.911828	0.859559	1	
pHCS	-0.84093	-0.75625	-0.81169	-0.86391	-0.91218	-0.9472	-0.94113	-0.87273	-0.79741	0.956646	0.976597	0.977002	0.985151	0.978544	0.89792	1
pH +ve control	-0.92314	-0.89691	-0.91128	-0.90476	-0.95198	-0.98127	-0.90972	-0.9237	-0.76509	0.940537	0.928693	0.944383	0.960798	0.919932	0.934935	0.942974
pH Neg control	-0.82408	-0.74621	-0.80381	-0.85991	-0.89671	-0.95246	-0.95126	-0.87281	-0.78621	0.94383	0.984811	0.967856	0.995531	0.990333	0.89257	0.986491

The informative part of the correlation table above is the portion in black. A very strong negative correlation exists between pH and viable bacterial count. This implies that decrease in pH over time leads to increase in viable bacterial count over the same period. Figures 16 and 18 graphs depict this graphically/pictorially.

Appendix XII: Correlation of pH and Yeast Viable count for Market cassava and Wheat Dough Fermentation

	10	*20*	*30*	*40*	*50*	*WF*	*CS*	*Post control*	*Neg control*	*pH10*	*pH20*	*pH30*	*pH40*	*pH50*	*pHWF*	*pHCS*
10	1															
20	0.915453	1														
30	0.723178	0.879393	1													
40	0.865221	0.973148	0.912741	1												
50	0.811173	0.843408	0.938616	0.874202	1											
WF	0.793976	0.926833	0.960802	0.960001	0.915581	1										
CS	0.852337	0.949822	0.925906	0.970807	0.875075	0.972919	1									
Post control	0.788721	0.887205	0.900257	0.948451	0.895366	0.907341	0.883055	1								
Neg control	0.793697	0.813981	0.900627	0.811651	0.918106	0.893429	0.888356	0.776017	1							
pH10	-0.83003	-0.94865	-0.96144	-0.9716	-0.92676	-0.98725	-0.97581	-0.90565	-0.8818	1						
pH20	-0.78749	-0.92977	-0.93957	-0.97175	-0.87837	-0.93858	-0.93011	-0.97985	-0.80373	0.938604	1					
pH30	-0.80622	-0.93958	-0.97487	-0.97379	-0.93812	-0.97546	-0.95065	-0.95449	-0.8582	0.985872	0.974872	1				
pH40	-0.8184	-0.94425	-0.92014	-0.99132	-0.86729	-0.94696	-0.95821	-0.96412	-0.78955	0.95619	0.985947	0.972532	1			
pH50	-0.7559	-0.90084	-0.89728	-0.96125	-0.83465	-0.8989	-0.89997	-0.97784	-0.73124	0.908906	0.9881	0.951918	0.983203	1		
pHWF	-0.88521	-0.93526	-0.95243	-0.93065	-0.96585	-0.94914	-0.94815	-0.88287	-0.93637	0.969526	0.907929	0.959349	0.911828	0.859559	1	
pHCS	-0.78476	-0.91823	-0.91987	-0.98067	-0.8831	-0.95287	-0.93463	-0.97447	-0.78544	0.956646	0.976597	0.977002	0.985151	0.978544	0.89792	1
pH +ve control	-0.89622	-0.931	-0.87588	-0.96495	-0.90872	-0.91528	-0.93221	-0.9351	-0.79422	0.940537	0.928693	0.944383	0.960798	0.919932	0.934935	0.942974
pH Neg control	-0.77422	-0.91364	-0.92131	-0.9774	-0.86118	-0.93421	-0.94146	-0.96686	-0.77374	0.94383	0.984811	0.967856	0.995531	0.990333	0.89257	0.986491

The informative part of the correlation table above is the portion in black. A very strong negative correlation exists between pH and viable yeast count. This implies that decrease in pH over time leads to increase in viable yeast count over the same period. Figure 15 and 19 graphs depict this graphically/pictorially.

Appendix XIII: Correlation of pH and ALAB counts for Industrial cassava and Wheat Dough Fermentation

		10	*20*	*30*	*40*	*50*	*wf*	*Vf*	*Cs*	*pos cont*	*Neg cont*	*pH10*	*pH20*	*pH30*	*pH40*	*pH50*	*pHWF*	*pHCS*
	1.000																	
10	0.974	1.000																
20	0.932	0.974	1.000															
30	0.898	0.953	0.986	1.000														
40	0.922	0.972	0.992	0.993	1.000													
50	0.939	0.961	0.970	0.981	0.985	1.000												
wf	0.968	0.976	0.988	0.965	0.976	0.973	1.000											
Vf	0.987	0.983	0.964	0.942	0.956	0.965	0.983	1.000										
Cs	0.911	0.954	0.918	0.900	0.906	0.880	0.913	0.939	1.000									
pos cont	0.979	0.968	0.935	0.881	0.906	0.895	0.962	0.969	0.935	1.000								
Neg cont	0.882	0.942	0.992	0.984	0.983	0.949	0.964	0.924	0.885	0.894	1.000							
pH10	-0.944	-0.983	-0.973	-0.961	-0.966	-0.950	-0.962	-0.976	-0.961	-0.945	-0.953	1.000						
pH20	-0.973	-0.970	-0.936	-0.892	-0.914	-0.905	-0.953	-0.981	-0.950	-0.982	-0.894	0.971	1.000					
pH30	-0.976	-0.987	-0.938	-0.917	-0.934	-0.935	-0.950	-0.982	-0.962	-0.967	-0.897	0.980	0.982	1.000				
pH40	-0.927	-0.973	-0.994	-0.987	-0.987	-0.967	-0.980	-0.967	-0.941	-0.931	-0.985	0.984	0.943	0.949	1.000			
pH50	-0.900	-0.903	-0.912	-0.853	-0.877	-0.847	-0.920	-0.928	-0.876	-0.930	-0.890	0.927	0.962	0.903	0.917	1.000		
pHWF	-0.976	-0.977	-0.977	-0.944	-0.966	-0.963	-0.992	-0.985	-0.898	-0.966	-0.948	0.961	0.967	0.957	0.967	0.941	1.000	
pHCS	-0.950	-0.958	-0.928	-0.883	-0.925	-0.911	-0.947	-0.930	-0.878	-0.950	-0.889	0.895	0.914	0.925	0.906	0.844	0.951	1.000
pH +ve control	-0.973	-0.916	-0.881	-0.832	-0.848	-0.871	-0.933	-0.947	-0.867	-0.967	-0.826	0.888	0.940	0.925	0.871	0.871	0.934	0.897
pH Neg control	-0.911	-0.922	-0.963	-0.970	-0.970	-0.986	-0.969	-0.943	-0.828	-0.868	-0.950	0.915	0.873	0.885	0.954	0.849	0.956	0.881

The informative part of the correlation table above is the portion in black. A very strong negative correlation exists between pH and viable yeast count. This implies that decrease in pH over time leads to increase in viable yeast count over the same period. Figures 15 and 19 graphs depict this graphically/pictorially.

Appendix XIV: Correlation of pH and Yeast counts for Industrial cassava and Wheat Dough Fermentation

		10	*20*	*30*	*40*	*50*	*Wf*	*Vf*	*Cs*	*Pos cont*	*Neg cont*	*pH10*	*pH20*	*pH30*	*pH40*	*pH50*	*Phwf*	*pHCS*
	1.000																	
10	0.996	1.000																
20	0.965	0.956	1.000															
30	0.982	0.972	0.970	1.000														
40	0.955	0.934	0.911	0.951	1.000													
50	0.896	0.917	0.877	0.865	0.837	1.000												
Wf	0.882	0.879	0.848	0.840	0.860	0.921	1.000											
Vf	0.989	0.988	0.979	0.976	0.938	0.909	0.847	1.000										
Cs	0.892	0.869	0.867	0.873	0.921	0.848	0.955	0.859	1.000									
Pos cont	0.970	0.961	0.948	0.973	0.966	0.839	0.813	0.967	0.843	1.000								
Neg cont	0.785	0.808	0.759	0.725	0.712	0.952	0.908	0.791	0.820	0.669	1.000							
pH10	-0.944	-0.951	-0.933	-0.913	-0.905	-0.951	-0.873	-0.967	-0.877	-0.899	-0.887	1.000						
pH20	-0.973	-0.966	-0.966	-0.962	-0.965	-0.896	-0.860	-0.984	-0.899	-0.970	-0.780	0.971	1.000					
pH30	-0.976	-0.977	-0.943	-0.953	-0.948	-0.909	-0.841	-0.988	-0.865	-0.947	-0.810	0.980	0.982	1.000				
pH40	-0.927	-0.936	-0.923	-0.903	-0.882	-0.985	-0.917	-0.945	-0.890	-0.872	-0.935	0.984	0.943	0.949	1.000			
pH50	-0.900	-0.888	-0.940	-0.891	-0.912	-0.873	-0.867	-0.920	-0.896	-0.917	-0.767	0.927	0.962	0.903	0.917	1.000		
pHWF	-0.976	-0.973	-0.965	-0.950	-0.936	-0.944	-0.948	-0.970	-0.936	-0.933	-0.870	0.961	0.967	0.957	0.967	0.941	1.000	
pHCS	-0.950	-0.932	-0.905	-0.941	-0.948	-0.870	-0.916	-0.920	-0.946	-0.893	-0.810	0.895	0.914	0.925	0.906	0.844	0.951	1.000
pH +ve control	-0.973	-0.968	-0.965	-0.984	-0.906	-0.836	-0.820	-0.963	-0.843	-0.957	-0.696	0.888	0.940	0.925	0.871	0.871	0.934	0.897
pH Neg control	-0.911	-0.929	-0.883	-0.864	-0.821	-0.977	-0.950	-0.901	-0.856	-0.836	-0.937	0.915	0.873	0.885	0.954	0.849	0.956	0.881

The informative part of the correlation table above is the portion in black. A very strong negative correlation exists between pH and viable yeast count. This implies that decrease in pH over time leads to increase in viable yeast count over the same period. Figures 16 and 20 graphs depict this graphically/pictoriall





Appendix XV: Cassava and wheat bread Samples after baking

Appendix XVI: Means showing effect of time on dough rising of composite flours.

Time	0 mins		30 mins		60 mins		90 mins	
Composite (%)	WIC	WMC	WIC	WMC	WIC	WMC	WIC	WMC
10	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	34.50 ±0.50^{b}	34.00±1.00	39.00±1.00^{bc}	37.50±2.50^{ab}
20	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	33.50±0.50^{bc}	31.00±1.00	37.00±1.00^{c}	35.50±2.50^{b}
30	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	31.00±1.00^{d}	31.00±1.00	34.00±1.00^{d}	31.50±1.50^{b}
40	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	31.50±0.00^{cd}	31.50±1.50	32.50±0.50^{de}	32.50±2.50^{b}
50	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	30.00±0.00^{d}	30.00±0.00	32.50±0.50^{de}	31.50±1.50^{b}
Wf	30.00±0.00	30.00±0.00	31.50±0.50^{b}	34.00±1.00	33.50±1.50^{bc}	40.50±6.50	40.50±2.50^{ab}	46.50±8.50^{a}
Cs	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	30.00±0.00d	30.00±0.00	31.50±1.50^{e}	30.00±0.00^{b}
Vf	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	30.00±0.00d	30.00±0.00	30.00±0.00^{e}	30.00±0.00^{b}
Control	30.00±0.00	30.00±0.00	36.00±1.00^{a}	33.00±3.00	39.00±1.00a	40.00±5.00	44.50±3.50^{a}	48.00±4.00^{a}
Untreated	30.00±0.00	30.00±0.00	30.00±0.00^{c}	30.00±0.00	30.00±0.00d	30.00±0.00	30.00±0.00^{e}	30.00±0.00^{b}

Time	120 mins		150 mins		180 mins		210 mins		240 mins	
Composite (%)	WIC	WMC	WIC	WMC	WIC	WMC	WIC	WMC	WIC	WMC
10	45.50±2.50^{b}	47.00±2.50^{bc}	58.50±1.50b	58.00±1.50^{bc}	39.00±1.00^{bc}	37.50±2.50^{ab}	76.00 ±4.00^{a}	83.00±2.00^{b}	84.00 ±4.00^{a}	95.50±1.50^{a}
20	39.00±1.00^{c}	39.00±1.00^{cd}	44.50±0.50c	53.00±3.00^{cd}	37.00±1.00^{c}	35.50±2.50^{b}	67.00±1.00^{b}	71.00±3.00^{c}	85.50±2.50^{ab}	76.00±1.00^{bc}
30	37.50±2.50^{cd}	37.50±2.50^{cd}	43.50±0.00^{cd}	39.00±3.00^{ef}	34.00±1.00^{d}	31.50±1.50^{b}	66.50±1.50^{b}	65.50±0.50^{cd}	74.00±1.50^{bc}	76.00±2.00^{bc}
40	37.50±2.50^{d}	36.50±1.50^{d}	41.00±1.00^{cd}	40.00±0.00^{ef}	32.50±0.50^{de}	32.50±2.50^{b}	63.00±2.00^{bc}	60.50±2.50^{de}	72.50±0.50^{cd}	73.50±0.50^{bc}
50	34.00±1.00^{d}	34.00±1.00^{cd}	36.50±1.50^{de}	45.50±2.50^{de}	32.50±0.50^{de}	31.50±1.50^{b}	58.50±0.50^{cd}	65.00±3.00^{cd}	69.00±1.00^{a}	69.00±1.00^{c}
Wf	48.50±8.50^{ab}	53.00±2.50^{cd}	56.50±3.50^{b}	63.00±5.00^{b}	40.50±2.50^{ab}	46.50±8.50^{a}	73.50±1.50^{a}	81.00±1.00^{b}	83.00±1.00^{a}	75.50±0.50^{bc}
Cs	34.50±0.50^{d}	32.00±2.00^{d}	37.00±1.00^{de}	34.00±2.00^{f}	31.50±1.50^{e}	30.00±0.00^{b}	43.50±1.50^{e}	48.00±0.00^{f}	54.00±1.00^{e}	57.00±1.00^{d}
Vf	30.00±0.00^{d}	30.00±0.00^{d}	30.00±0.00^{e}	37.50±2.50^{ef}	30.00±0.00^{e}	30.00±0.00^{b}	55.00±1.00^{d}	55.50±2.50^{e}	69.00±1.00^{cd}	71.50±6.50^{bc}
Control	54.50±2.00^{a}	61.50±5.50^{a}	70.00±5.00^{a}	72.50±2.50^{a}	44.50±3.50^{a}	48.00±4.00^{a}	73.00±1.00^{a}	91.50±3.50^{a}	66.00±4.00^{d}	79.00±1.00^{a}
Untreated	30.00±0.00^{d}	30.00±0.00^{d}	30.00±0.00^{e}	30.00±0.00^{f}	30.00±0.00^{e}	30.00±0.00^{b}	34.50±0.50^{f}	32.50±2.50^{g}	37.00±1.00^{f}	33.50±3.50^{e}

Values are means of duplicate readings. Means with different superscripts in the same column are significantly different. Mean values were separated using Duncan multiple range test (DMRT).

Key: Wic = wheat/ industrial cassava, Wmc=wheat/market cassava. Wf=wheat flour, Cs=cassava flour, Vf= virgin flour