NUTRITIONAL AND BIOCHEMICAL CHARACTERISTICS OF LACTIC ACID BACTERIA-CHALLENGED SORGHUM AND ITS FERMENTATION PRODUCT

BY

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ABSTRACT

Sorghum is one of the major cereals malted for the brewing of beer. The conditions of transport and storage of this cereal predispose it to contamination by microbes thus affecting the quality of the end products. The contamination is usually controlled by treating the sorghum with chemicals, which however alter the chemical constituents of the sorghum. Therefore, the need to seek alternative functional methods of control of microbial contaminants becomes imperative. This study was aimed at investigating lactic acid bacteria as biocontrol agents against microbial pathogens of stored cereals for beer fermentation.

Sorghum were obtained from Bodija market and from the Institute of Agricultural Research and Training, Ibadan. Lactic Acid Bacteria (LAB) were isolated from spontaneously-fermenting sorghum and identified using classical techniques. The abilities of the LAB strains to produce antimicrobials and their antagonistic activity against known cereal pathogens were used to select the best three strains for further work. The selected strains were applied singly and in combination at inoculum concentration of 2.3 x 10^4 cells/mL for five days to challenge sorghum seeds prior to malting and wort production. Sorghum wort was fermented for five days with *Saccharomyces carlsbergensis*. Physiological and nutritional characteristics of the unchallenged and challenged sorghum, and fermentative characteristics of the wort were determined using the European Brewery Convention methods. Data were analysed using ANOVA.

One hundred and twenty seven strains of LAB were isolated and identified as *Lactobacillus plantarum* (32), *Lactobacillus brevis* (31), *Lactobacillus fermentum* (25), *Lactobacillus delbrueckii* (8), *Lactobacillus casei* (12) and *Lactobacillus acidophilus* (19). *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus casei* produced high antimicrobial lactic acid (2.5±0.5g/L, 2.4±0.3g/L and 2.5±0.5g/L respectively) and had high inhibitory activities (17mm, 14mm and 17mm respectively). *Lactobacillus brevis* produced antimicrobial lactic acid with the highest mean concentration of 2.7±0.5g/L from local sorghum but was not used for further work because the inhibitory activity was low when tested against pathogenic organisms. All the LAB produced bacteriocin with antagonistic effects on all the pathogens tested, and *Lactobacillus plantarum* had the highest zone of inhibition (17mm) against *Bacillus subtilis*. All the LAB grew at temperature of 30^{o}C, pH 5.0-5.5, high glucose and peptone concentration (1.5-2.0mg/ml). The malted untreated -

sorghum had 13.2 % protein, 3.0 % crude fat, 1.9 % ash, 1.8 % crude fibre, 42.5 mg/g phytate, 36.0 mg/g tannin, 2.0 mg/g protein inhibitor and 16.0 IoBunits diastatic power. Fermentation of the unchallenged wort (pH 6.2) yielded ethanol content of 2.2 %. With LAB treatment, there was a reduction in protein (12.2 %), crude fat (2.1 %) and crude fibre (1.1%); and significant ($p<0.05$) reduction in antinutrients (phytate 32.7 mg/g, tannin 22.4 mg/g, protease inhibitor 0.0 mg/g and wort pH 4.2). There was also an increase in diastatic power (24.0 IoBunits). The subsequent fermentation produced 4.8 % ethanol. The microbial profiles of the challenged malted sorghum showed a steady decrease in *Bacillus*, *Staphylococcus and Pseudomonas* count compared with the unchallenged where they showed steady increase.

Lactobacillus starter cultures reduced spoilage pathogens, antinutritional factors of sorghum during malting and improved the end products.

Keywords: Lactic acid bacteria, Starter cultures, Fermentation, Wort, Malted sorghum

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Folake Titilayo, Ojo

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CERTIFICATION

I certify that this work was carried out by Folake Titilayo Ojo (Matric No. 119186) under my supervision in the Department of Microbiology, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

This project work is dedicated to God Almighty for His mercies and protection over me throughout the period of the programme.

TABLE OF CONTENTS

Content	Page
Title page … … … … … … … … … …	i
Abstract … … … … … … … … …	ii
Acknowledgements … … … … … … … …	iv
Certification … … … … … … … … …	vi
Dedication … … … … … … … … …	vii
Table of Contents … … … … … … … …	viii
List of Tables … … … … … … … … …	ix
List of Figures … … … … … … … … …	xii
List of Plates … … … … … … … … …	xv
CHAPTER ONE	
Introduction … … … … … … … … …	1
CHAPTER TWO	
Literature Review … … … … … … … …	6
CHAPTER THREE	
Materials and Methods … … … … … … …	37
CHAPTER FOUR	
Results … … … … … … … … …	71
CHAPTER FIVE	
Discussion … … … … … … … … …	176
Conclusion … … … … … … … … …	192
Recommendations … … … … … … … …	194
References … … … … … … … … …	195
Appendices … … … … … … … … …	215

Table **LIST OF TABLES** **Page**

Table 4.1	Biochemical and Physiological characteristics of LAB isolates from fermented cereal gruels	72
Table 4.2	Frequency of occurrence of LAB isolates obtained from the fermented cereal gruels	82
Table 4.3	Hydroly tic enzymes production by the different LAB isolates from gruels of local sorghum and maize varieties	117
Table 4.4	Hydrolytic enzymes production by the different LAB isolates from gruels of typed sorghum and maize varieties	118
Table 4.5	Antimicrobial activity of LAB isolates obtained from gruels of typed sorghum and maize varieties against some indicator strains	120
Table 4.6	Antimicrobial activity of LAB isolates from gruels of local sorghum and maize varieties against some indicator strains	121
Table 4.7	Quality assessment of the sorghum varieties prior to treatment	123
Table 4.8	Total microbial load during the germination of sorghum seed varieties	133
Table 4.9	Moisture content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	136
Table 4.10	Total carbohydrates content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	137
Table 4.11	Diastatic power at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	139
Table 4.12	Protease inhibitor at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	140
Table 4.13	Tannin content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	141
Table 4.14	Phytate content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	143
Table 4.15	α-Amylase activity at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	145

Table 4.16	Gelatinization temperature at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	146
Table 4.17	pH at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	148
Table 4.18	Crude fibre content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	149
Table 4.19	Ash content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	150
Table 4.20	Ether extract at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	152
Table 4.21	Protein content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	153
Table 4.22	Free Amino Nitrogen content at different time intervals of the malted sorghum subjected to challenge by lactic acid bacteria isolates	154
Table 4.23	Yeast cell count during fermentation of wort	156
Table 4.24	Total titratable acidity at different time intervals of wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	157
Table 4.25	Reducing sugar content at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	158
Table 4.26	Total residual sugar content at different time intervals of wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	160
Table 4.27	Free Amino Nitrogen at different time intervals of sorghum wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	161
Table 4.28	pH at different time intervals of wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	162
Table 4.29	Ethanol content at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	165
Table 4.30	Specific gravity at different time intervals of the wort derived from malted	

	sorghum subjected to challenge by lactic acid bacteria isolates	166
Table 4.31	Colour at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	167
Table 4.32	Turbidity at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	168
Table 4.33	Protein content at different time intervals of the wort derived from malted grain subjected to challenge by lactic acid bacteria isolates	169
Table 4.34	Total solids at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	170
Table 4.35	Total soluble solids at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates	172
Table 4.36	Sensory evolution results of the produced fermented sorghum drink samples	175

Figure **LIST OF FIGURES** **Page**

Figure 2.1 Sorghum malting process 21

Figure 3.1 Outline of the process involved in the malting of sorghum 59

Figure 4.1 Effect of glucose on the growth of the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 83

Figure 4.2 Effect of glucose on Amylase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 85

Figure 4.3 Effect of glucose on protease production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 87

Figure 4.4 Effect of glucose on invertase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 88

Figure 4.5 Effect of magnesium sulphate on the growth of different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 90

Figure 4.6 Effect of magnesium sulphate on Amylase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 91

Figure 4.7 Effect of magnesium sulphate on protease production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 93

Figure 4.8 Effect of magnesium sulphate on invertase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 94

Figure 4.9 Effect of pH on the growth of the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 96

Figure 4.10 Effect of pH on the production of protease by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 97

Figure 4.11 Effect of pH on the production of invertase by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 99

Figure 4.12 Effect of pH on the production of amylase by the different LAB isolates obtained

from gruels of local (LV) and typed (TV) sorghum and maize varieties 100
Figure 4.13 Effect of peptone on amylase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 102
Figure 4.14 Effect of peptone on protease production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 104
Figure 4.15 Effect of peptone on invertase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 105
Figure 4.16 Effect of peptone on the growth of the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 106
Figure 4.17 Effect of temperature on the growth of the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 108
Figure 4.18 Effect of temperature on invertase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 109
Figure 4.19 Effect of temperature on protease production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 111
Figure 4.20 Effect of temperature on amylase production by the different LAB isolates obtained from gruels of local (LV) and typed (TV) sorghum and maize varieties 112
Figure 4.21a Electrophoretogam of crude DNA extracted from pure cultures of Lactic acid bacteria isolates (isolates 3, 5, 8, 21 and 23) 114
Figure4.21b Electrophoretogam of crude DNA extracted from pure cultures of Lactic acid bacteria isolates (isolates 19-24) 115
Figure 4.22 Effect of LAB suspension on the plumule length of sorghum seeds during malting at different time intervals 124
Figure 4.23 Effect of LAB suspension on the radicle length of sorghum seeds during malting at different time intervals 125
Figure 4.24 Effect of LAB suspension on the weight of sorghum seeds during malting at different time intervals 126
Figure 4.25 Amylase activity of sorghum seeds during malting of the sorghum varieties at different time intervals 128
Figure 4.26 Protease activity during malting of the sorghum varieties at different time intervals 129

Figure 4.27 Total fermentable sugar at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates 174

Plate **LIST OF PLATES** **Page**

Plate 4. 1 The germination of the different sorghum varieties at different time intervals 130

Plate 4. 2 Improvised fermentors for the fermentation of the wort from the different sorghum varieties. 131

CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

The lactic acid bacteria (LAB) are a group of Gram-positive bacteria, non-motile, non-spore forming, cocci or rods, which produce lactic acid as the major end product of fermentation of carbohydrates. They are the most important bacteria in food fermentations, being responsible for the fermentation of sour bread, sorghum beer, all fermented milks, cassava (to produce *gaari* and *fufu*) and most "pickled" (fermented vegetables) (Thanh *et al.*,2010). Historically, bacteria from the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main species involved. Several more have been identified, but play a minor role in lactic acid fermentations. Lactic acid bacteria taxonomy were reviewed by Axelsson (2004).

Lactic acid bacteria carry out their reactions (the conversion of carbohydrate to lactic acid plus carbon dioxide and other organic acids) without the need for oxygen. They are described as microaerophilic as they do not utilize oxygen. Because of this, the changes that they effect do not cause drastic changes in the composition of the food. Some of the families are homofermentative that is they only produce lactic acid, while others are heterofermentative and produce lactic acid plus other volatile compounds and small amounts of alcohol. *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus pentoaceticus, Lactobacillus brevis* and *Lactobacillus thermophilus* are examples of lactic acid-producing bacteria involved in food fermentations (Than *et al.*, 2010)

The lactic acid bacteria belongs to two main groups-the homofermenters and the heterofermenters. The pathways of lactic acid production differ for the two. Homofermenters produce mainly lactic acid, via the glycolytic (Embden-Meyerhof) pathway. Heterofermenters produce lactic acid plus appreciable amounts of ethanol, acetate and carbondioxide, via the 6-phosphoglucanate/phosphoketolase pathway. The glycolytic pathway is used by all lactic acid bacteria except *Leuconostocs*, group III *Lactobacilli, Oenococci* and *Weissellas*. Normal conditions required for this pathway are excess sugar and limited oxygen (Axelsson, 2004).

Homolactic fermentation

The fermentation of 1 mole of glucose yields two moles of lactic acid:

$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH$

Glucose Lactic acid

Heterolactic fermentation

The fermentation of 1 mole of glucose yields I mole each of lactic acid, ethanol and carbondioxide:

$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH + C_2H_5OH + CO_2$

Glucose Lactic acid Ethanol Carbondioxide

Microbial proliferation has been well established as an indigenous component of the malting and brewing environment (Lowe *et al.,* 2005) with the resultant microflora having both beneficial and detrimental effects on malt quality (Lowe *et al.,* 2005). LAB omnipresent on the surface of malt barley may positively influence the quality and safety of the malt and derived products; this property has been exploited for the biological improvement of the malting and beer process. Contamination of barley and sorghum by mycotoxigenic and active gushing inducing *Fusaria* are of particular concern to both maltsters and brewers.

Biological control methods, involving inoculation with lactic acid bacteria (LAB) and yeast (*Geotrichum candidum*) starter cultures (Thu *et al.,*2011); have shown promise for the control of unwanted bacteria and fungi during malting. Bacterial cultures have also been added to the grain during malting to inhibit the growth of fungi (Lowe and Arendt, 2004). LAB are of particular interest as biopreservation organisms. Lactic acid bacteria have long been known to have the ability to inhibit growth and survival of the normal spoilage microflora and pathogens (Thu *et al.,* 2011). Certain LAB starters (e.g. *Lactococcus lactis, Lactobacillus acidophilus, Streptococcus lactis, Lactobacillus plantarum* and *Pediococcus pentosaceus*) have been found to inhibit the growth and survival of some fungi (Savadogo *et al.,* 2004)

In addition to offering the potential to improve the safety and quality of malt, the use of microbial cultures in steeping is claimed to be easy use, nature-friendly, in-

expensive and not lead to the formation of toxic compounds, nor alter the nutritional quality and palatability properties of the grain product.

The various factors contributing to the antimicrobial activity of LAB are low pH due to the production of organic acids (lactic acid and acetic acid), carbon dioxide, hydrogen peroxide, ethanol, diacetyl, bacteriocins, depletion of nutrients and microbial competition (Laitila *et al.*, 2002). The "starter"culture in malting is a relatively new process that controls indigenous microbial growth, and is both technically and economically feasible (Laitila *et al*., 2002).

Sorghum is a major food crop and is ranked fifth in terms of world cereal grain production after wheat, rice, maize and barley (FAO, 2005). It is drought-tolerant and thus has an advantage over other cereals because it can yield a crop under harsh environmental conditions. Sorghum has similar nutritional value to maize and is consumed as a major source of energy and protein by millions of people in the semi-arid areas of Africa and Asia (Pozo-Insfran *et al.*, 2004). Much sorghum is malted to brew opaque beer in most parts of Africa, including South Africa and European type beer (e.g. larger) and non- alcoholic malt beverages for example 'pito' 'togwa' 'obiolor' and 'kunu-zaki' in several African countries (Taylor and Dewar, 2001). Malting is the limited germination of cereals in moist air, under controlled conditions, with the objective of mobilizing the endogenous hydrolytic enzymes, especially α – amylase of the grain which attacks the α – (1-4) glucosidic bonds in starch molecules (Taylor and Belton, 2002). The malting process also modifies the structure of the grain so that it will be readily solubilized during the brewing process to produce fermentable wort.

1.2 Statement of Problem

In many African countries, sorghum is used for the production of malt and sorghum beer, also known as opaque beer (Pozo-Insfran *et al.*, 2004). It is also used as an ingredient in the preparation of weaning foods, for example 'ogi' in Nigeria and other African countries (Thaoge *et al.*, 2003). The conditions of transportation and storage of this cereal makes it to be susceptible to microbial attack and this affects the quality of the end products of malting and brewing. The germination conditions, especially not turning the grain, encourages entangling of the roots and shoots growing from the grains. This then leads to the formation of matted clumps, which encourages the growth of bacteria and fungi (Briggs, 1998). Heavy microbial

populations may negatively impact on the quality of the malt by causing discolouration of the malt and development of unacceptable off flavours (Noot, 1999). Of more concern is the colonization of the sorghum malt with coliforms and moulds because coliforms are associated with diarrhoeal diseases (Jay, 2000) and moulds have the potential of producing mycotoxins, which are toxic to animals and humans (Gourama and Bullerman, 1995).

In order to avoid potential hazards associated with the high microbial load and the possible presence of mycotoxins in sorghum malts, a method that can prevent or inhibit the growth of potentially harmful microorganisms during the sorghum malting process should be implemented. One of such methods is the use of biological methods including lactic acid bacteria as biocontrol agents to control pathogens of the malting process.

Lactic acid bacteria are important commercially in the processing of meats, dairy products, alcoholic beverages and vegetables. The products include sausages, cured hams, wines, beer, fortified spirits, pickles and saukerkraut (Jay, 1992). The key process in food fermentations is the production of lactic acid by these fermentative bacteria. The preservative effect of LAB during the manufacture and subsequent storage of fermented foods is mainly due to acidic conditions that they create, converting carbohydrates to organic acids (lactic acid and acetic acids) in the foods during development (Holzapfel, 2001)). They also have a competitive role in that their metabolites prevent growth and metabolism of unwanted microorganisms.

A starter may reduce fermentation times, minimize dry matter losses, avoid contamination with pathogenic and toxigenic bacteria and moulds and minimize the risk of incidental microflora causing off flavour and technology (Holzapfel, 2001). Lactic acid bacteria always include organisms that convert lactose to lactic acid. Starter cultures may consist of single or mixed strains.

1.3 Justification for the work

Selection of LAB as starters will help in reducing the problems encountered in brewing because it will improve malt processing, quality and aids utilization of products made from sorghum. LAB as starters alleviate the variations that occur in natural fermentation and thereby also enhance the shelf -life and quality of the end products.

In other to obtain lactic acid bacteria that could be suitable as starter cultures, the organisms must be screened using physiological properties, biochemical

properties, ability to produce antimicrobial, bacteriocin by the isolates. Food fermenting lactic acid bacteria are not to be toxigenic or pathogenic (Lucke, 2000).

However, there is dearth of information on the use of LAB as starters for sorghum improvement during malting. To enhance and maintain the quality and shelf-life of these traditional alcoholic beverages, there is the need to undertake basic studies on product processing aimed at selecting the best starters for the improvement of the alcoholic beverages.

1.4 Aims and Objectives

The present study aims at isolating and characterizing LAB from different fermented cereal gruels as starter cultures for the fermentation of sorghum products.

The steps that would be taken to reach these objectives consist of:

1. Isolation and identification of lactic acid bacteria (LAB) from fermented cereal gruels.
2. Physiological and biochemical studies on the different LAB isolates.
3. Physiological and nutritional studies on different sorghum varieties challenged with the lactic acid bacteria (LAB) isolates.
4. Study of the fermentative characteristics of the wort derived from the test starters- challenged sorghum.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Group Lactic acid bacteria (LAB)

The lactic acid bacteria are a group of Gram bacteria, catalase negative, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. The genera includes *Carnobacterium, Enterococcus, Lactobacillus, Oenococcus, Pediococcus, Streptococcus* and *Tetragenococcus*, (Ercolini *et al.*, 2001). Lactic acid-producing Gram positive bacteria but belonging to the phylum *Actinobacteria* are genera such as *Aerococcus, Microbacterium* and *Propionibacterium* as well as *Bifidobacterium* (Holzapfel *et al.*, 2001). The genera most commonly used in fermentation process as starter cultures include *Lactococcus, Streptococcus, Pediococcus, Lactobacillus, Leuconostoc* and *Carnobacterium* (Holzapfel *et al.*, 2001).

The seemingly simplistic metabolism of LAB has been exploited throughout history for the preservation of foods and beverages in nearly all societies dating back to the origins of agriculture (Miller and Welterstrom, 2000). Domestication of LAB strains passed down through various culinary traditions and continuous passage on foodstuffs has resulted in modern-day cultures able to carry out these fermentations. Today, LAB play a prominent role in the world food supply, performing the main bioconversion in fermented dairy products, meats and vegetables. LAB also are critical for the production of wine, beer, coffee, silage cocoa, sourdough, and numerous indigenous food fermentations (Wood, 1998).

Lactic acid bacteria (LAB) are the most prominent non-pathogenic bacteria that play a vital role in our everyday life, from fermentation, preservation and production of wholesome foods and vitamins to prevention of certain diseases due to their antimicrobial action. Lactic acid fermentation is generally inexpensive often requiring little or no heat in the process, making them fuel-efficient as well (Keith, 1991).

The taxonomy of LAB based on comparative 16S ribosomal RNA (rRNA) sequencing analysis has revealed that some taxa generated on the basis on phenotypic features did not correspond with the phylogenetic relations. Molecular techniques, especially polymerase chain reaction (PCR) based methods, such as rep-PCR fingerprinting and restriction fragment length polymorphism (RFLP) as well as pulse

field gel electrophoresis (PFGE), are regarded important for specific characterization and detection of LAB strains (Gevers *et al*., 2001).

2.2 Classification of Lactic acid bacteria

There are two main hexose fermentation pathways that are used to classify LAB genera. Under conditions of excess glucose and limited oxygen, homolactic LAB catabolise one mole of glucose in the Embden – Meyerhof Parnas (EMP) pathway to yield two moles of pyruvate. Intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid. This process yields two moles ATP per glucose consumed. Representative Homolactic LAB genera include *Lactococcus, Enterococcus, Streptococcus, Pediococcus* and group I *lactobacilli* (Axelsson, 1998). Heterofermentative LAB use the pentose phosphate pathway, alternatively referred to as the pentose phosphoketolase pathway. One mole glucose 6-phosphate is initially dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of CO_2. The resulting pentose – 5 – phosphate is cleaved into one mole glyceraldehydes phosphate (GAP) and one mole acetyl phosphate. GAP is further metabolized to lactate as in homofermentation, with the acetyl phosphate reduced to ethanol via acetyl-CoA and acetaldehyde intermediates. Theoretically, end - products (including ATP) are produced in equimolar quantities from the catabolism of one mole glucose. Obligate heterofermentative LAB include *Leuconostoc, Oenococcus, Weissella*, and group III *lactobacilli* (Axelsson, 2004).

Lactics are classified by the fermentation pathway used to ferment glucose and by their cell morphology. *Lactobacillus* is a very heterogeneous genus, encompassing species with a large variety of phenotypic, biochemical, and physiological properties. Most species of lactobacilli are homofermentative, but some are heterofermentative. The genus has been divided into three major subgroups and over 70 species are recognized. Group I lactobacilli are obligately homofermentative and produce lactic acid as a major end product (>85%) from glucose. They are represented by *L. delbrueckii* and *L. acidophilus*. They grow at 45^{o}C but not at 15^{o}C. Group II, also homofermentative, grow at 15^{o}C and show variable growth at 45^{o}C. Represented by *L. casei* and *L. plantarum*, they can produce more oxidized fermentations for example acetate if O_2 is present. Group III lactobacilli are heterofermentative. They produce lactic acid from glucose, along with CO_2 and ethanol. Aldolase is absent and

phosphoketolase is present. Representative species include *L. fermentum*, *L. brevis* and *L. keferi*. Lactobacilli are often found in dairy products, and some species are used in the preparation of fermented milk products. For example *L. delbrueckii* subsp *bulgaricus* is used in the preparation of yogurt; *L. acidophilus* is used in the preparation of acidophilus milk; *L. helveticus*, as well as *L. delbrueckii* subsp *bulgaricus*, are used to make Swiss, Mozzarella, provolone, Romano, and parmesan cheeses. Other species are used in the production of sauerkraut, silage and pickles. The lactobacilli are usually more resistant to acidic conditions than are other LAB, being able to grow at pH values as low as 4. This enables them to continue to grow during natural lactic fermentations when the pH has dropped too low for other LAB to grow, so they are often responsible for the final stages of many lactic acid fermentations (Axelsson, 2004).

Lactobacillus are rod - shaped organisms that can be either hetero or homofermentative. They are widespread and can be isolated from many plant and animal sources. *Lactobacillus* are more tolerant to acid than the other genera of lactic acid bacteria and this property makes them important in the final phases of many food fermentations when other organism are inhibited by the low pH (Salminen and von Wright ,1993: Canibe *et al.*,2007).

Leuconostoc are ovoid cocci, often in chains. All bacteria of this genus have a heterofermentative mode of metabolism. When grown in media containing sucrose, copious amounts of a slimy polysaccharide (dextran) are produced. Dextran has found uses in medicine as a plasma extender and in biotechnology (Holzapfel *et al.*, 2001).

Pediococcus are cocci often found in pairs and tetrads that are strictly homofermentative. Their habitat is restricted mainly to plants. *Pediococcus cerevisiae* has been used as a starter culture for the fermentation of some sausages with great success. *Streptococci* are cocci in chains that are distinguished from the *Leuconostoc* by their strictly homofermentative metabolism. These organisms can be isolated from oral cavities of animals, the intestinal tract, skin, and any foods that come in contact with these environments while the other genera of lactic pyogenes is a common, troublesome pathogen, causing sore throat and rheumatic fever.

Enterococcus and *Lactococcus* are two recent taxonomic divisions of lactic acid bacteria. These were created to reorganize the large and divergent streptococcus genus into smaller, more related groups of bacteria. Enterococci are gram-positive cocci that form pairs or chains. They are distributed widely in the environment,

particularly in faeces of vertebrates. Strains can grow in the presence of 6.5% NaCl and with 40% bile present (Salminen and von Wright, 1993).

Lactococcus includes strains that are gram positive, possessing spherical cells, occurring in pairs or chains which may be spiral shaped. They have a strictly homofermentative metabolism and are found in dairy and plant products. For centuries, lactic acid bacteria (LAB) have been used to produce fermented food products including pickles, sauerkraut, yoghurt, cheese, butter milk, soy sauce and more. Lactococci differ from other lactic acid bacteria by their pH, salt and temperatures tolerances for growth. The bacterium can be used in single strain starter cultures, or in mixed strain cultures with other lactic acid bacteria such as *Lactobacillus* and *Streptococcus*. Some examples include *Streptococcus thermophilus* along with *Lactobacillus bulgaricus* that are used in the production of yoghurt. Also, *Lactococcus lactis* and *Streptococcus thermophilus* are two strains often used as starter cultures in the production of cheese (Salminen and von Wright, 1993).

2.3 LAB metabolism

2.3.1 Homolactic fermentation

Homofermentative bacteria transform nearly all of the sugars they use, especially glucose into lactic acid. The homofermentative pathway includes a first phase of all the reactions of glycolysis that lead from hexose to pyruvate. The terminal electron acceptor in this pathway is pyruvate which is reduced to lactic acid **(Axelsson, 1998)**. In fermentation, pyruvate is decarboxylated to ethanal, which is the terminal electron acceptor, being reduced to ethanol.

2.3.2 Heterolactic fermentation

Bacteria using the heterofermentative pathway, which includes *Leuconostoc* use the pentose phosphate pathway. In this pathway, NADPH is generated as glucose is oxidized to ribose 5-phosphate. This five-carbon sugar and its derivatives are components of important biomolecules such as ATP, CoA, NAD+, FAD, RNA and DNA. NADPH is the currency of readily available reducing power in cells (NADH is used in the respiratory chain) (De Vuyst and Vandamme, 1994). This pathway occurs in the cytosol. After being transported into the cell, a glucokinase phosphorylates the glucose into glucose 6-P (glucose 6-phosphate). Its destination is completely different from the glucose 6-P in the homofermentative pathway. Two oxidation reactions occur: the first leads to gluconate 6-P and the second, accompanied by a

decarboxylation, forms ribulose 5-P. In each of these reactions a molecule of NADP+ is reduced. Ribulose 5-P can then be epimerized either to ribose 5-P or to xylulose5-P (Axelsson, 1998).

Xylulose 5-P is then cleaved into acetyl-phosphate and glyceraldehydes 3-phosphate. The glyceraldehyde 3-phosphate is metabolized into lactic acid by following the same pathway as in the homofermentative pathway. The acetylphosphate has two possible destinations, depending on environmental conditions. This molecule can be successively reduced into ethanal and ethanol, in which case the molecules of the coenzyme NADP formed during the two oxidation reactions of glucose at the beginning of the heterofermentative pathway, are reoxidized (Axelsson, 1998). This reoxidation is essential for regenerating the coenzymes necessary for this pathway. The final products are then lactate and ethanol. Alternatively, the acetyl-phosphate can produce acetate (acetic acid) through the enzyme acetate kinase. This reaction also yields a molecule of ATP. The final products of this pathway are then lactate and acetate. Bacteria of the genus *Leuconostoc* preferentially produce lactate and ethanol in a slightly aerated environment and lactate and acetate in an aerated environment (Axelsson, 1998).

2.3.3 Pathways involved

The lactic acid bacteria are categorized into' homo' or 'hetero' according to the metabolic routes they use (Embden-Meyerhof or Phosphoketolase pathways) according to the resulting end products (Aguirre and Collins, 1993).

2.3.4 Metabolites of LAB Metabolism

Lactic acid bacteria produce a wide variety of antagonistic compounds (Vandamme and De Vyust, 1994) including antimicrobial proteins or bacteriocins. Bacteriocins are proteins or protein complexes with bactericidal activities directed against species which are closely related to the producer bacterium. Nisin is a bacteriocin and it displays a bactericidal mode of activity and finds exclusive use as biological food preservative in processed cheese and canned foods (Vandamme and De Vyust, 1994). As the lactic acid bacteria are fastidious microorganisms, they require a complex medium to grow and to produce bacteriocin. Lactic acids also inhibit the growth of harmful putrefactive microorganisms through other metabolic products such as hydrogen peroxide, carbon dioxide and diacetyl. The products of lactic fermentation are organic acids, carbon dioxide, hydrogen peroxide, bacteriocins and ethanol (Vandamme and De Vyust, 1994**)**. Lactic acid is the main primary

product formed during sugar metabolism. It is responsible for the characteristic fresh acid taste of many fermented foods. When protein-rich materials such as milk or meat are fermented by lactic acid bacteria, additional aroma and flavour components are produced by the combined action of proteases and peptidases during ripening period. Lactic acid also interferes with metabolic process such as oxidative phosphorylation (Vandamme and De Vyust, 1994).

2.4 Beneficial aspects of LAB metabolism

Lactic acid is the main primary product formed during sugar metabolism and is responsible for the characteristic fresh acid taste of many different foods. The preservative activity of LAB has been observed in some fermented products such as cereals. The lowering of the pH to below 4 through acid production, inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease. For example, LAB have antifungal activities. By doing this, the shelf life of fermented food is prolonged. This is because the sheer overgrowth of desirable edible bacteria in food outcompetes the other non-desirable food spoilage bacteria. Thus LAB fermented foods have lactic acid as the main preservative since lactic acid bacterial growth is accompanied by the production of lactic and acetic acids with decrease in pH and increase in titratable acidity. The process of fermentation usually takes 4-5 days. The bacterial population stabilizes at around 48 hours (Grasson,2002).

2.4.1 LAB in biocontrol and fermentation

2.4.1.1 Biological Control of Foodborne Pathogens

Research has focused on the biological approach to the control and eradication of food- borne pathogens. Commensal bacteria that inhabit the gastrointestinal tract of animals and humans, as well as those involved in food fermentations have been investigated (Grasson, 2002). Scientists developed natural antimicrobial products for the biocontrol of pathogens and have exploited LAB for the competitive exclusion of pathogens and delivery of vaccines and bioactive compounds (Grasson, 2002).

2.4.1.2 LAB in Competitive Exclusion

The gastrointestinal tract of humans and animals contain a complex bacterial ecosystem. Commensal strains of LAB have history of use with the intention of enhancing health in the form of probiotics and controlling human pathogens in farm

animals. Research has demonstrated the capacity of *Lactobacillus* species to control a range of human pathogens including *Escherichia coli*, *Campylobacter jejuni* and *Clostridium perfringes* (Grasson, 2002).

2.4.1.3 LAB as Probiotics

Apart from the importance of LAB in fermented foods, they are also used as probiotics. Probiotics have been described as organisms and substances that contribute to intestinal microbial balance. Probiotics was later defined as a live microbial feed supplement, which is beneficial to the host animal through improving its intestinal microbial balance (Steinkraus, 1995). *Lactobacillus* species has been used as probiotics organisms. In this case, *L. acidophilus* has been used because it was thought to be the dominant *Lactobacillus* in the intestine. However, a wide range of Lactobacilli has been used in probiotic preparations. These include *Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus casei, Lactobacillus brevis, Lactobacillus cellobiosus, Lactobacillus lactis, Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus reuteri* (Steinkraus, 1995; Vinderola *et al.*, 2002)

2.4.1.4 LAB as beneficial microorganisms

LAB are important commercially in the processing of meats, alcoholic beverages and vegetables. The products include sausages, wines, beer, fortified spirits, pickles and sauerkraut (Jay, 1992). Although, LAB have beneficial effects in the food industry, and they can sometimes be a nuisance as contaminants by producing off flavours (Aguirre and Collins, 1993).

Lactobacillus spp and *Streptococcus faecum* are beneficial microorganisms, which have been proven to replenish essential microflora and decrease the incidence of gastrointestinal disorders. Beneficial bacteria especially *Lactobacillus spp.* can produce specific antimicrobial substances which have been observed to inhibit the growth of some pathogenic microorganisms. The addition of Lactic culture may be an additional safeguard to establish good manufacturing practices and hazard analysis and critical control point (HACCP) programmes in the control of *Escherichia coli* 0157:H7 in cheese (Yost *et al.*,2002). These beneficial microorganisms are most effective during periods of disease or stress and following antibiotic treatment.

2.4.1.5 LAB and human health

Of interest is the role of LAB in the treatment of people suffering with tumours and immuno - compromised subjects. LAB has been reported to stimulate the

immune system although there are many questions about mechanisms and effective utilization (Wood and Brian, 1992). If this potential is supported in practice, then there are many components to conventional therapies. This may include cost effectively due to their ease of products derived from LAB seem to have relatively low toxicity compared to other treatments (Wood and Brian, 1992).

2.4.1.6 Lactic Acid Bacteria as Starter Culture

Lactic acid bacteria frequently termed "Lactis" are basic starter cultures with widespread use in the dairy industry for cheese making, cultured buttermilk, cottage cheese and cultured sour cream (Jay, 1986). Identifying and providing practical means of using appropriate starter cultures is advantageous due to the competitive role of microorganisms and their metabolites in preventing growth and metabolism of unwanted microorganisms. A strong starter may reduce fermentation time, minimize dry matter losses, avoid contamination with pathogenic and toxigenic bacteria and moulds and minimize the risk of incidental microflora causing off flavour (Holzapfel, 2001). Lactic acid bacteria always include organisms that convert lactose to lactic acid, for example *L. lactis, L. cremoris,* or *L. diacetilactis.* Where flavour and aroma compounds, such as diacetyl are desired, the lactic starter culture will include a heterolactic fermenter such as *Leuconostoc citrovorum, L. diacetilactis* or *L. dextranicum*. Starter culture may consist of single or mixed strains. *Lactococci* generally make up 90% of a mixed dairy starter population and a good starter culture normally converts most of the lactose to lactic acid (Jay, 1992).

Yoghurt is produced with a yoghurt starter culture, which comprises equal cell numbers of *S. thermophilus* and *L. bulgaricus.* The coccus (*Streptococcus thermophilus*) grows faster than the rod (*L. bulgaricus*) and is primarily responsible for acid production, while the rod adds flavour and aroma. The associative growth of the two organisms result in lactic acid production at a rate greater than that produced by either when growing separately and more acetaldehyde (the main volatile flavour component of yoghurt) is produced by *L. bulgaricus* when growing in association with *S. thermophilus* (Board *et a*l.,1995).

2.5. Plasmids

2.5.1 Plasmids of LAB

Most of the relevant properties of LAB (e.g. lactose fermentation, citrate transport, protease production, phage resistance mechanisms are plasmid –encoded (Kuipers *et al*., 2000). Plasmids of LAB do not commonly carry transmissible

antibiotic resistance genes but can take in conjugative transposons and plasmids. Some plasmids, such as those with bacteriocin immunity genes, can integrate into the chromosome (Rauch and deVos, 1992). Plasmid- linked antibiotic resistance therefore poses a hazard. (Kuipers *et al*., 2000) has emphasized the effective use of gene manipulated LAB in the battle against food spoilage and pathogenic bacteria.

2.5.2 LAB and Bacteriocins Production

Bacteriocins are biologically active proteins or protein complexes displaying a bactericidal mode of action exclusively towards Gram-positive bacteria and particular closely related species. Some LAB strains ribosomally synthesize antimicrobial peptides, or bacteriocins targeted to inhibit the other Gram-positive bacteria (O' Sullivan *et al*., 2002). Even though antimicrobial peptides occupy an inhibition spectrum narrower than that of antibiotics (Morency *et al*., 2001), bacteriocins produced by LAB have been reported to permeate the outer membrane of Gram-negative bacteria and induce the inactivation of Gram-negative bacteria in conjunction with other enhancing antimicrobial environmental factors such as low temperature, organic acid and detergents (Alakomi *et al*., 2000).

Bacteriocins produced by LAB are classified into three main groups, I antibiotics being the most documented and industrially exploited. The groups are I antibiotic (class I), non – I antibiotics, small heat stable peptides (class II) and large heat labile protein (class III) (O' Sullivan *et al*., 2002).

2.6 Sorghum

Sorghum is a plant belonging to the tribe of Andropogoneae and the family of Poaceaes. Sorghum is a monocotyledonous plant – the embryo of the grain contains only one cotyledon. This single cotyledon is called the scutellum. Two of the best known species of sorghum are *Sorghum vulgare* and *Sorghum bicolor* (Palmer *et al*., 1989). Sorghum grains vary from red, black and brown to fawn, yellow and white. Polyphenolic materials, which form the pigments, are located mainly in the pericap testa.

Sorghum is the fifth most important cereal crop after wheat, rice, maize and barley in terms of production (FAO, 2005). Total world annual sorghum production (FAO, 2004) is about 60 million tons from cultivated area of 46 millions hectare. Most important producers are the United States, Nigeria, Sudan, Mexico, China, India, Ethiopia, Argentina, Burkina Faso, Brazil and Australia. Burkina Faso is the world leader of sorghum production and consumption per inhabitant (FAO, 2005).

Sorghum plant is drought- resistant and heat -tolerant and is therefore an extremely important commodity that provides necessary food and feed for millions of people living in semi-arid environment worldwide. Investigations showed that sorghum has better malting characteristics than the other cereals tested; maize, millet, rice (Koleoso and Olatunji, 1992).

The physical structure of sorghum kernels are free of hulls or glumes, are ova shaped, weigh 20-30 mg, and may be white, red, yellow, or brown in color. Hand dissected kernels were found to be 7.9% pericarp, 9.8% germ, and 82.3% endosperm, which is both vitreous and opaque (Hoseney, 2004). Some varieties are labeled "bird resistant" due to the bitter tannins that deter birds from consuming the grain prior to harvest (Taylor and Dewar, 2001). Sorghum differs from barley in that the aleurone tissue is a single layer of cells as opposed to three cells (Ogbonna, 1992). Other varieties are labeled food- grade and described by Taylor *et al*. (2006) as a white sorghum developed to produce bland-tasting flour that is suitable for food products because it does not impart "off" colors or flavors. Sorghum starch is chemically similar to that of maize in size and shape. The starch granules vary in shape from almost polygonal, near the outside of the kernel, to almost spherical, towards the center of the kernel (Hoseney, 2004). Starch comprises the greatest portion of the sorghum grain by weight (Daiber and Taylor, 1995). Comparable to barley, starch granules and storage proteins are enclosed in the endosperm cells. However, sorghum starch granules are tightly packed at the peripheral region, giving a steely, vitreous texture, while the inner part is floury (Ogbonna, 1992). Starch gelatinization temperature is 68°-78°C. The prolamin protein portion of sorghum is referred to as kafirin and resembles the maize protein, zein, in amino acid composition (Hornsey, 2004). Lipid composition of sorghum is 2.1%-5.0%, and 75% of the lipids are contained in the germ with the remainder split evenly between the bran and the endosperm (Hoseney, 2004). Currently, sorghum is widely used in beer brewing in Africa due to the greater availability of sorghum versus barley (Igyor *et al*. 2001). In the continent of Africa sorghum has been malted for centuries to be used in products such as baby food and traditional alcoholic and non-alcoholic beverages (Beta *et al*. 1995). Estimates for Southern Africa alone indicate 200,000 tons of sorghum are malted annually and some 3billion liters of sorghum beer are brewed annually (Taylor and Dewar, 2001). In fact, the importation of cereal grains, including barley malt, to Nigeria was banned in 1988. This forced brewers to utilize the sorghum that was

locally available and has caused an increased interest in brewing lager-style beer from malted sorghum (Dewar *et al*. 1997b; Taylor and Dewar, 2001).

Sorghum has been used as an adjunct in beer in the United States as a response to the popularity of paler and more mildly flavored beer (Hooseney, 1994). Sorghum use has been considered in the Mexican brewing industry as an adjunct to replace corn and rice due to lower price and greater availability (Osorio-Morales *et al*. 2000). Sorghum has been used to produce gluten-free beer in several studies (Okafor 1980; Okafor and Aniche, 1986; Owuama and Okafor, 1987; Taylor, 1992; Owuama, 1999a; Igyor *et al*., 2001; Pozo-Insfran *et al.*, 2004). Sorghum malt varies from barley malt in several ways. Physically, sorghum does not contain a husk like barley does, has a higher starch gelatinization temperature, and has less diastatic, β-amylase, and glucanase activities. Therefore the traditional brewing procedures for barley have to be altered to account for the differences between the grains (Pozo-Insfran *et al.*, 2004).

Various types of foods are prepared from sorghum whole kernels, for instance unfermented and fermented breads (chapati, rot, kisra) in Asia and East Africa and steamed foods (couscous), fermented and unfermented porridges and pastes (*ugali, akamu, eko, kamu, koko, ogi*) and alcoholic and non-alcoholic beverages in Africa generally (Chilkunda and Paramahans, 2001). In China, sorghum is the most important ingredient for the production of distilled beverages such as Maotai and Kaoliang, as seen in the 1987 film *Red Sorghum*. In southern Africa, sorghum is used to produce beer, including the local version of Guinness. African sorghum beer is a popular drink primarily amongst the black community for historical reasons. Sorghum beer is known by many different names in various countries across Africa, including 'burukutu', (Nigeria), 'pombe' (East Africa) and 'bil-bil' (Cameroon).

2.7 Traditional African Sorghum Brewing

Sorghum beer is brewed most predominantly in Africa, although Ogbonna (1992) references beer produced in Mexico, India, and Sri Lanka whose success has stimulated awareness of the brewing potential of sorghum.

Traditional sorghum beer production in Africa is commonly referred to as opaque beer and is identified as Bantu beer, kaffir beer, utshwala, joala, busaa, and dolo, depending on the region (Daiber and Taylor 1995). African sorghum beer is

brewed using grain sorghum and undergoes dual fermentation including lactic acid fermentation as well as alcoholic fermentation. The steps in brewing African sorghum beer include malting, mashing, souring, and alcoholic fermentation. The souring of African sorghum beer is done by lactic acid fermentation, and is responsible for the distinct sour taste. Souring may be initiated using yoghurt, sour dough starter cultures, or by spontaneous fermentation (Vander, 1956). Novellie (1962, 1966) stresses that sorghum beer utilizes different ingredients and techniques compared to traditional barley beer and thus bears little resemblance. Opaque sorghum beer is not hopped like conventional beer (Taylor 1992). This beverage is produced in homes and in local villages. Opaque beer is an important source of energy and nutrition for the African people because of the high level of complex carbohydrates and nutrient content (Novellie, 1966; Taylor 1992; Daiber and Taylor, 1995; Kayode *et al.* 2007). The source of fermentable carbohydrate is most commonly malted sorghum or millet (Daiber and Taylor, 1995). Traditionally, sorghum malting occurs outdoors by placing the steeped sorghum grain in thin layers on covered or uncovered floors. Following a 4-6 day germination period, the green malt is dried in thin layers in the sun (Daiber and Taylor, 1995). The traditional brewing process utilizes malted grain sorghum and two different fermentations. The first fermentation is by lactic acid bacteria to produce lactic acid, which provides the characteristic flavor and lowers the pH, thus reducing microbial growth. The second fermentation is by yeast, *Saccharomyces cerevisiae*, to produce alcohol (Watson and Novellie, 1976). A study by Novellie (1966) utilized the following process for sorghum beer production. Processing begins with the souring step by combining sorghum malt and water; a cereal adjunct may be added to the mixture. The mixture undergoes lactic acid bacteria fermentation at 50°C until the pH decreases to 3. The soured mixture is then diluted and boiled. After cooling the mixture to 60°C, additional malt is added and the mixture is mashed for 2 h. During the mashing, not all of the starch is hydrolyzed, which yields a high viscosity beverage, characteristic of opaque beer. The mash is then cooled to 30°C and pitched with *Saccharomyces cerevisiae* (Daiber and Taylor, 1995).

Like other cereal products, sorghum products have poor nutritional value. This is due to their deficiency in lysine, threonine and tryptophan (Chavan *et al.,* 1988) and to the presence of anti-nutritional factors such as tannins and phytates that interact with proteins, vitamins and minerals, thus restricting their bioavailability (Chavan *et*

al., 1988). Various simple techniques have been investigated to improve the protein digestibility and mineral availability of sorghum by reducing its tannin and phytate content. These include malting, fermentation (Chavan *et al.,* 1988) and cooking. Fermentation is widely used traditionally for processing sorghum and fermented products are well accepted and widely used as complementary foods (Tomkins *et al.*, 1988). Their low pH confers the advantage of microbiological safety. The malting improves the nutritional quality of the product while the fermentative improves its safety and digestibility.

2.7.1 Brewing Conventional Beer with Sorghum

A number of the many varieties of sorghum work well as sorghum malt. These varieties possess beneficial qualities for beer brewing, such as good diastatic power, α-and β-amylase activities, and extract recovery (Owuama, 1999b). Brewing beer with malted grain sorghum is best achieved with several modifications to the traditional brewing procedure due to higher starch gelatinization temperature of starch versus barley (Agu 2005). Taylor (1992) reports that the starch gelatinization temperature for sorghum malt starch is 64-68°C, while barley is 55-59°C. Ogbonna (1992) reviews several studies that found that an increase in gelatinization and saccharification temperatures along with the development of decantation mashing procedures improved sorghum beer studies. Igyor (2001) found that increasing the mash temperature to 100°C during decoction produced a better beer with more alcohol and flavor components.

2.7.2 Sorghum Malting

Malt is the major raw material used in the brewing industry. Barley is traditionally the cereal chosen for malting in order to develop enzymes (Kuntz and Bamforth, 2007). In Nigeria, where attempts to cultivate barley have met with little success, the high cost of importing barley malt, in conjunction with the rising demand for European-type larger, has forced the use of local cereals particularly sorghum, as a malting and brewing grain. Sorghum malting is best performed with viable grain, that is not of the tannin containing variety and that has been placed in storage (Daiber and Taylor, 1995). Storage of sorghum for two to three years at 12-23°C gives a higher level of amylases (57-73%); approximately 25% higher compared to newly harvested grains (Ogbonna, 1992; Owuama, 1999b).

Sorghum malting yields high proportions of hydrolytic enzymes such as α-glucosidase, and α- and β-amylases (Owuama, 1999b). Agu (2005) reported that when sorghum grain is malted, sufficient hydrolytic enzymes are produced to extract the sugars and proteins needed for beer production. Initial studies on the malting of sorghum did not employ a definite malting temperature. This lack of consistency slowed the development of sorghum as a source of malt for brewing procedures (Agu, 2005). Enzyme development during germination of sorghum differs from that of barley.

In the germination process of barley, hormonal signals cause the production of endosperm- degrading enzymes in the aleurone layer. Ogbonna (1992) reported that in sorghum, production of α-amylase and carboxypeptidases are produced by the scutellum. Endo-β-glucanase, limit dextrinase, an endo-protease enzyme development occurs in the starchy endosperm. In sorghum malting, α-amylase is produced in embryos of sorghum while β-amylases are activated from latent form in starch endosperm (Owuama, 1999b). Another difference in the malting of barley versus sorghum is evident in the microscopic studies of the endosperm of the malted grain. Malted barley cell walls are degraded extensively whereas sorghum cell walls are left intact except for small portals through which amylolytic and proteolytic enzymes pass to degrade starch and protein reserves (Ogbonna, 1992).

2.7.2.1 Benefits of sorghum malting and the uses of sorghum malt

Sorghum malting results in high levels of amylases, reduces anti-nutritional factors, enhances vitamin content and improves mineral content. It also improves in-vitro digestibility, improves the composition and content of essential amino acids and increases the in -vitro starch digestibility. It has also been established that the use of sorghum malt make grains to be readily solubilized during the brewing process, reduces the viscousity of porridges whilst maintaining their nutrient and energy density and imparts flavour and sweetness to porridge (Taylor and Dewar, 2001).

2.7.3 Sorghum Malting Process

The primary step in brewing is malting, during which barley and sorghum is allowed to germinate under controlled conditions. The main aim of malting is the development of hydrolytic enzymes, especially of the β-amylase group (Dewar *et al.*, 1997b). The secondary aim is the friability of the grains and making a contribution to final flavour. Malting is a form of controlled germination of the grain carried out to activate the enzymes in the grains that are necessary to break down the insoluble

contents into soluble matter. Malting has been defined as a traditional processing technology that could possibly be used to improve the nutritional quality of the protein. (Dewar *et al.*, 1997b). The malting of sorghum grains is similar to the malting of other grains. Like barley malting, sorghum malting involves three main steps: steeping, germination and drying/kilning (Novellie and De Schaepdrijver, 1986). The malting process is shown in figure 2.1

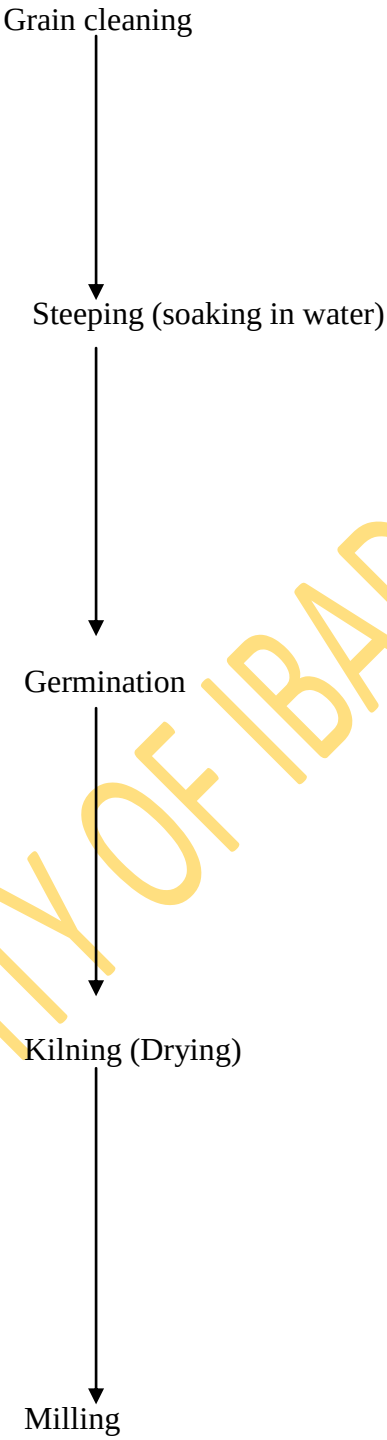

Figure 2.1 Sorghum malting process

Source: Dewar *et al.* (1997b)

2.7.3.1 Steeping

Steeping, the first step of the malting process is the immersion of the grain in water. It is practiced mainly as a means of achieving imbibition of water by the dormant grain and thereby initiating biochemical processes leading to seed germination (Briggs, 1998). Steeping is also carried out to remove clean and broken grains. A moisture of 33 to 35% (wet basis) should be achieved during steeping of sorghum grains (Daiber and Taylor, 1995). The more water that is taken up during steeping, the higher is the resulting malt quality (Dewar *et al*., 1997b).

Factors associated with the grain that affects the rate at which the grains absorb water include: grain structure-softer grains absorb more water than hard grains, and grain size- smaller grains absorb moisture more rapidly (Pitz, 1989). The temperature, time and aeration required for steeping can also affect the rate at which the grains absorb water and are therefore chosen to achieve a good level of hydration in order to produce good malt (Reinikkanen and Carregal, 1991). The optimum steeping temperature required for sorghum grains to reach appropriate water content is 25-30^{o}C and 16-40 hours respectively (Dewar *et al.*, 1997a). Aeration, either by draining the water from the grain periodically (air-resting) or by sparging air through steeping water is necessary for production of good quality malt (Novellie and DeSchaepdrijver, 1986).

During steeping, the grains swell and softens, while the living tissues resume their metabolism (Briggs, 1998). There is a break down of complex carbohydrates and nutrients leach out from the grain into the steep water. Steeping is complete when the white tips of the rootlets emerge, which is known as chitting. At this point, the grains will have swollen one and one-third times of their original size (CSIR, 1999).

2.7.3.2 Germination

Germination normally takes about 6 days. The germination of sorghum occurs rapidly between 20^{o}C and 30^{o}C with an optimum of 25-28^{o}C (Dewar *et al.*, 1997a). The germination phase of sorghum is physiologically very active. Important physiological processes associated with the germination phase are the synthesis of amylases, proteases and other endogenous hydrolytic enzymes (Palmer, 1989). The hydrolytic enzymes migrate from the germ into the endosperm where starch and protein are hydrolyzed to sugars and amino acids respectively. These are then transported into the germ where they are further metabolized by the growing seedling (Priest and Campbell, 1996). During germination the hard endosperm is converted

into a friable malt. Certain conditions impact greatly on the quality of the finished sorghum malt during the germination phase. These include grain moisture content, temperature, length of germination time and oxygen availability (Palmer *et al.*, 1989). Germinating sorghum grains have the tendency to rapidly lose water taken up during steeping. Hence, it is necessary to spray germinating grains at intervals during the germination phase because the higher the level of moisture content (within limits), the higher the resulting malt quality (Palmer *et al.*, 1989). Good humidification could also be maintained by germinating the grain in an atmosphere of near-water saturation (Palmer *et al.*, 1989) or by continous passage of moist air through the malting environment. The germination step is complete when the whole of the endosperm (the storage part of the grain) which naturally sustains the development of the growing embryo or germ (the living part) during germination, has modified (partially attacked by enzymes) (Briggs *et al.*, 1981).

2.7.3.3 Drying/Kilning and Milling

Drying/kilning is the final stage of the malting process. The purpose of drying is to stop the growth of the green malt at the end of the germination process and to produce a shelf –stable product complete with active enzymes by reducing the moisture content and water activity (a_w) (Novellie and De Schaepdrijver, 1986). During this phase, the germinated sorghum grains are dried at temperature of about 50^{o}C for 24 hours. The resultant product has a moisture content of around 10%.

2.8 Lager Beer

Beer is the most consumed alcoholic beverage in the world, and is the most popular beverage behind water and tea (Nelson, 2005). Beer is a beverage of great variety. Most often beer is produced from malted barley, hops, yeast, and water, yet simple changes in the formulation has created 25,000 to 35,000 varieties of beer worldwide. Variations of this simple formula include beer brewed from a variety of grains such as rice, millet, sorghum, barley, and corn depending on the regional staple, and fermented by wild yeasts. Brewing began at home and was followed by small village breweries that eventually led to the modern day large brewery (Papazian, 2003).

2.8.1 Nature and History

According to historians, beer has been produced for centuries. Early records show beer was produced in ancient Babylon about 8,000 years ago. Beer was an important aspect of both the Egyptian and Mesopotamian cultures where barley was

the staple grain (Papazian, 2003). Ancient Sumerian literature, which dates to about 1800 BC, provides a hymn to the Sumerian goddess of brewing that included an ancient recipe for beer (Katz and Maytag, 1991). Historians have argued about the advent of beer and whether ancient cultures developed beer or bread first. Historians have held that ancient cultures abandoned hunter-gatherer practices to grow grain for beer (Braidwood *et al*., 1953). Bamforth (2006) reasons that the adoption of grain production, and subsequent production of beer, makes brewing the world's oldest biotechnology. Ancient beer was subject to wild yeast and bacterial contamination which meant spoilage occurred quite easily. Thus, a majority of the beer consumed was probably sour most of the time (Maytag, 1992). Without modern packaging to prevent spoilage, beer was consumed in the home or village where brewing had recently occurred, and the beer was not widely distributed. While ancient beer was not of the high quality seen today, the demand was great because the brewing process eliminated pathogenic microorganisms commonly found in drinking water (Papazian, 2003).

2.8.2 The Brewing Process

All beers are brewed using a process based on a simple formula. Key to the process is malted grain— mainly barley though other cereals, such as sorghum, wheat or rice, may be added. Malt is made by allowing a grain to germinate after which it is then dried in a kiln and sometimes roasted. The germination process creates a number of enzymes, notably α-amylase and β-amylase, which convert the starch in the grain into sugar (Briggs *et al*., 2004). Depending on the amount of roasting, the malt will take on a dark colour and strongly influence the colour and flavour of the beer. The malt is crushed to break apart the grain kernels, expose the cotyledon which contains the majority of the carbohydrates and sugars, increase their surface area, and separate the smaller pieces from the husks.

2.8.2.1 Initial Steps in the Brewing Process

There are several steps in the brewing process, which include malting, milling, mashing, lautering, boiling, fermenting, conditioning, filtering, and packaging.

Malting is the process where the barley grain is made ready for brewing. Malting is broken down into three steps, which help to release the starches in the barley. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hours. During germination, the grain is spread out on the floor of the germination room for around 5 days. The goal of germination is to allow

the starches in the barley grain to breakdown into shorter lengths (Papazain, 2009**)**. When this step is complete, the grain is referred to as green malt. The final part of malting is kilning. Here, the green malt goes through a very high temperature drying in a kiln. The temperature change is gradual so as not to disturb or damage the enzymes in the grain. When kilning is complete, there is finished malt as a product (Papazian, 2009).

The next step in the brewing process is milling. This is when the grains that are going to be used in a batch of beer are cracked. Milling the grains makes it easier for them to absorb the water that they are mixed with and which extracts sugars from the malt (Papazian, 2009). Milling can also influence the general characteristics of a beer.

Mashing is the next step in the process. This process converts the starches released during the malting stage, into sugars that can be fermented. The milled grain is dropped into hot water in a large vessel known as a mash tun. In this vessel, the grain and water are mixed together to create a cereal mash. The leftover sugar rich water is then strained through the bottom of the mash in a process known as lautering. Prior to lautering, the mash temperature may be raised to about 75°C (165-170 °F) (known as a mashout) to deactivate enzymes. Additional water may be sprinkled on the grains to extract additional sugars (a process known as sparging) (Goldhammer, 2009).

At this point the liquid is known as wort. The wort is moved into a large tank known as a "copper" or kettle where it is boiled with hops and sometimes other ingredients such as herbs or sugars. This stage is where many chemical and technical reactions take place, and where important decisions about the flavour, colour, and aroma of the beer are made. The boiling process serves to terminate enzymatic processes, precipitate proteins, isomerize hop resins, and concentrate and sterilize the wort. Hops add flavour, aroma and bitterness to the beer. At the end of the boil, the hopped wort settles to clarify in a vessel called a "whirlpool", where the more solid particles in the wort are separated out (Hornsey, 2004).

After the whirlpool, the wort then begins the process of cooling. This is when the wort is transferred rapidly from the whirlpool or brew kettle to a heat exchanger to be cooled. The heat exchanger consists of tubing inside a tub of cold water. It is very important to quickly cool the wort to a level where yeast can be added safely. Yeast is unable to grow in high temperatures.

After the wort goes through the heat exchanger, the cooled wort goes into a fermentation tank. A type of yeast is selected and added, or "pitched", to the fermentation tank. When the yeast is added to the wort, the fermenting process begins, where the sugars turn into alcohol, carbon dioxide and other components (Hornsey, 2004).

2.8.2.2 Fermentation

Definition and Process

Fermentation in brewing is the conversion of carbohydrates (glucose, fructose, maltotriose and sucrose) to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions (Hough *et al.*, 1971). The free amino nitrogen (FAN) present in the wort is vital for yeast growth and rapid fermentation. A more restricted definition of fermentation is the chemical conversion of sugars into ethanol.

After the wort is cooled and aerated — usually with sterile air — yeast is added to it through pitching and it begins to ferment. It is during this stage that sugars won from the malt are metabolized into alcohol and carbon dioxide, and the product can be called beer for the first time. Fermentation happens in tanks which come in all sorts of forms, from enormous cylindro-conical vessels, through open stone vessels, to wooden vats (Kunze, 2004). Most breweries today use cylindro-conical vessels, or CCVs, which have a conical bottom and a cylindrical top. The cone's aperture is typically around 60°, an angle that will allow the yeast to flow towards the cones apex, but is not so steep as to take up too much vertical space (Kunze, 2004). CCVs can handle both fermenting and conditioning in the same tank. At the end of fermentation, the yeast and other solids which have fallen to the cones apex can be simply flushed out a port at the apex. Open fermentation vessels are also used, often for show in brewpubs, and in Europe in wheat beer fermentation. These vessels have no tops, which makes harvesting top fermenting yeasts very easy (Kunze, 2004). The open tops of the vessels make the risk of infection greater, but with proper cleaning procedures and careful protocol about who enters fermentation chambers, the risk can be well controlled. Fermentation tanks are typically made of stainless steel. If they are simple cylindrical tanks with beveled ends, they are arranged vertically, as opposed to conditioning tanks which are usually laid out horizontally.

Fermentation methods

There are three main fermentation methods, warm, cool and wild or spontaneous. There may be a secondary fermentation which can take place in the brewery, in the cask or in the bottle.

Brewing yeasts may be classed as "top cropping" (or "top fermenting") and "bottom cropping" (or "bottom-fermenting") (Kunze, 2004). Top cropping yeasts are so called because they form a foam at the top of the wort during fermentation. They can produce higher alcohol concentrations and in higher temperatures, typically 16 to 24 °C (61 to 75 °F), produce fruitier, sweeter, ale-type beers. An example of top cropping yeast is *Saccharomyces cerevisiae*, sometimes called ”ale yeast". Bottom cropping yeasts are typically used to produce lager-type beers, though they can also produce ale-type beers. These yeasts ferment more sugars, creating a dryer beer, and grow well at low temperatures. An example of bottom cropping yeast is *Saccharomyces pastorianus*, formerly known as *Saccharomyces carlsbergensis*.

For both types, yeast is fully distributed through the beer while it is fermenting, and both equally flocculate (clump together and precipitate to the bottom of the vessel) when fermentation is finished. By no means do all top cropping yeasts demonstrate this behaviour, but it features strongly in many English ale yeasts which may also exhibit chain forming (the failure of budded cells to break from the mother cell) which is technically different from true flocculation (Kunze, 2004**)**.

The most common top cropping brewer's yeast, *Saccharomyces cerevisiae*, is the same species as the common baking yeast. However, baking and brewing yeasts typically belong to different strains, cultivated to favour different characteristics: baking yeast strains are more aggressive, in order to carbonate dough in the shortest amount of time; brewing yeast strains act slower, but tend to produce fewer off-flavours and tolerate higher alcohol concentrations (with some strains, up to 22%) (Kunze, 2004**)**.

Warm fermenting

Ale yeasts, such as *Saccharomyces cerevisiae*, ferment at warm temperatures between 15–20 °C (59–68 °F), and occasionally as high as 24 °C (75 °F), though the yeast used by Brasserie Dupont for saison ferments at temperatures (29 °C (84 °F) to 35 °C (95 °F)) (Farmhouse Ales, 2004). Ale yeasts generally form a foam on the surface of the fermenting beer, as during the fermentation process its hydrophobic

surface causes the flocs to adhere to CO_2 and rise; because of this they are often referred to as "top cropping" or "top fermenting"— though this distinction is less clear in modern brewing with the use of cylindro-conical tanks, where the behaviour of lager and ale yeast are quite similar (Bamforth, 2006).

In industrial brewing, to ensure purity of strain, a "clean" sample of the yeast is stored refrigerated in a laboratory. After a certain number of fermentation cycles, a full scale propagation is produced from this laboratory sample. Typically, it is grown up in about three or four stages using sterile brewing wort and oxygen.

Ales are generally ready to drink within three weeks after the beginning of fermentation; however, some brewers will condition an ale for several months. Ales range in colour from very pale to an opaque black. England is best known for its variety of ales. Ale yeasts can be harvested from the primary fermenter, and stored in the refrigerator or freezer

Cool fermenting

While the nature of yeast was not fully understood until Emil Hansen of the Carlsberg brewery in Denmark isolated a single yeast cell in the 1800s, brewers in Bavaria had for centuries been selecting these cold-fermenting lager yeasts by storing ("lager") their beers in cold alpine caves. The process of natural selection meant that the wild yeasts that were most cold tolerant would be the ones that would remain actively fermenting in the beer that was stored in the caves. Some of these Bavarian yeasts were brought back to the Carlsberg brewery around the time that Hansen did his famous work.

Traditionally, ales and lagers have been differentiated as being either top fermenting or bottom fermenting; however, using modern technology, ale yeasts frequently bottom ferment (Ehrlinger, 2009). The main difference between the two is lager yeast's ability to process raffinose (a trisaccharide composed of the sugars galactose, fructose, and glucose) which means that all sugars are fermented, resulting in a well attenuated beer; ale yeast only cleaves and ferments the fructose portion of raffinose, leaving melibiose, which ale yeast cannot further cleave into two monosaccharides due to its lack of melibiase, so ale remains sweeter with a lower conversion of sugar into alcohol(Ehrlinger, 2009). Raffinose is a minor dry component of Carlsberg barley, but once malted is practically nonexistent (Briggs *et al.*, 2004).

Lager yeast tends to collect at the bottom of the fermenter and is often referred to as bottom-fermenting yeast. Lager is fermented at much lower temperatures, around 10 °C (50 °F), compared to typical ale fermentation temperatures of 18 °C (64 °F). It is then stored for 30 days or longer close to the freezing point. During storage, the beer mellows and flavours become smoother. Sulfur components developed during fermentation dissipate. The popularity of lager was a major factor that led to the rapid introduction of refrigeration in the early 1900s.

Today, lagers represent the vast majority of beers produced, the most famous being a light lager called Pilsner which originated in Pilsen, Czech Republic *(Plzeň in Czech)*. It is a common misconception that all lagers are light in colour—lagers can range from very light to deep black, just like ales.

Lager yeast normally ferments at a temperature of approximately 5°C (40 °Fahrenheit), where ale yeast becomes dormant. Lager yeast can be fermented at a higher temperature normally used for ale yeast, and this application is often used in a beer style known as "steam beer". *Saccharomyces pastorianus* is used in the brewing of lager

Spontaneous fermentation

These beers are primarily brewed around Brussels, Belgium. They are fermented in oak barrels after being inoculated with wild yeast and bacteria while cooling in a Koelship. Wild yeast and bacteria ferment the wort (unfermented beer) in the oak barrels. The beers fermented from yeast and bacteria in the Brussels area are called Lambic beers. These bacteria add a sour flavour to the beer. Of the many styles of beer very few use bacteria, most are fermented with yeast alone and bacterial contamination is avoided. However, with the advent of yeast banks and the National Collection of Yeast Cultures, brewing these beers, although not through spontaneous fermentation, is possible anywhere. Specific bacteria cultures are also available to reproduce certain styles.

Brettanomyces is a genus of yeast important in brewing lambic, a beer produced not by the deliberate addition of brewer's yeasts, but by spontaneous fermentation with wild yeasts and bacteria (Iserentant and Verachtert, 1995).

Secondary fermentation

After initial or primary fermentation, the beer may be transferred into a second container, so that it is no longer exposed to the dead yeast and other debris (also known as "trub") that have settled to the bottom of the primary fermenter. This

prevents the formation of unwanted flavours and harmful compounds such as acetylaldehydes, which are commonly blamed for hangovers (Priest and Campbell, 2006). During secondary fermentation, most of the remaining yeast will settle to the bottom of the second fermenter, yielding a less hazy product (Priest and Campbell, 2006)

Bottle fermentation

Some beers undergo fermentation in the bottle, giving natural carbonation. This may be a second or third fermentation. They are bottled with a viable yeast population in suspension. If there is no residual fermentable sugar left, sugar may be added. The resulting fermentation generates CO_2 which is trapped in the bottle, remaining in solution and providing natural carbonation (Ehrlinger, 2009).

2.9 Factors Affecting Beer Quality

These factors include changes in the quality of raw materials (the use of adjuncts, mycotoxins caused by fungal infections of barley). The technological stresses affecting yeast during the classical brewing process, during propagation, fermentation and storage include, oxidative stress and stress caused by aerobic/anaerobic shift, nutritional and ethanol-induced stress, cold shock, and additional stresses caused by modern change(s) in brewing technology (hydrostatic pressure, CO_2 overpressure, osmotic stress and other stresses occurring during fermentation in cylindro-conical vessels, osmotic stresses that yeast encounters during high-gravity brewing (Sigler *et al*.,2009), mechanical and chemical stress owing to centrifugation and acid washing). The effects of these stresses and/or their combinations may affect the physiological and technological state of the yeast (changes in metabolic pathways, premature aging, apoptotic changes, reduced fermentation vigor, changes in sedimentation (Sigler *et al*., 2009) which in turn may strongly affect the course of wort fermentation as well as the quality of the final product, i.e. beer (flavor, aroma, haze (Sigler *et al*., 2009).

The quality of finished beer may be negatively affected by biological factors such as microbial spoilage (Sigler *et al*., 2009) and also by non-biological interactions of beer components (proline-rich hordeins, polyphenols) influencing beer clarity. These factors may cause adverse changes in beer flavor compounds such as vicinal diketones, esters, aldehydes, organic and inorganic sulfur volatiles, higher alcohols or fatty acids. These and other changes may give rise to off-flavors, decreased foam stability, undesirable haze, gushing, shortening of shelf life, or complete loss of drinking quality.

2.10 Preservation in the Brewing Industry

Biological preservation aims to improve the microbiological safety of foods and beverages through the use of competitive or antagonistic microorganisms or their metabolic products, to prevent or inhibit the growth of undesired microorganisms in foods or beverages (Schillinger *et al*., 1996). Certain lactic acid bacteria (LAB) are widely exploited for biopreservation. The biopreservative actions of LAB are due to their production of antimicrobial compounds which inhibit the growth of other bacteria or fungi. These preserving effects may be due not only to the end products of their fermentative activity such as lactic acid, but also to the formation of small, heat stable inhibitory peptides, referred to as bacteriocins. Among the variety of antimicrobial substances produced by LAB, bacteriocins are one of the most promising natural food preservatives. According to a classical definition, bacteriocins are proteinaceous compounds that are bactericidal to strains closely related to the producer strain (Klaenhammer, 1993). Bacteriocins of LAB are able to inhibit a wide spectrum of beer spoilage organisms, in particular strains of *Lactobacilli, Pediococci* and *Micrococci.* Some bacteriocins, however, appear to inhibit potential food borne pathogens including *Clostridium botulinum, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus* and *Bacillus species.* Bacteriocins of LAB are considered to be safe biopreservatives, since they are assumed to be degraded by the proteases in gastrointestinal tract.

LAB can be found in brewing materials at almost every stage of the malting and brewing process, from the standing barley crop to the finished beverage. LAB play a positive role in the beer manufacturing process by eliminating undesirable microorganisms including beer spoiling LAB or by contribution to wort bioacidification.

To enhance and maintain the quality and shelf- life of these traditional alcoholic beverages, there is the need to undertake basic studies on product processing aimed at selecting the best starters for the improvement of the alcoholic beverages.

2.10 .1 Raw materials for brewing

The raw materials for brewing include starchy grains (malted barley, malted sorghum and maize grits) water, hops and yeast (Charles and Lindren, 1999). The preparation may be in two parts. The two parts are malting and brewing.

Water

Water is the ingredient used in the greatest quantity in brewing. Traditionally, variations in water sources have had a large impact on the characteristics of beer around the world. For example, the soft water in the Pilsen region of the Czech Republic is best suited for light lager production, whereas, the hard water of Dublin, Ireland creates superior dark ales, such as Guinness (Palmer, 2006). The hardness of the water influences pH and other factors such as the stability of enzymes, extractability of grist and hop components, and flocculation of yeast (Bamforth 2006). Palmer (2006) outlines several requirements for brewing water. The important ions that contribute to mash pH are calcium, magnesium, bicarbonate, and sulfate. Sodium, chloride, and sulfate are evaluated for contribution to the taste of the beer. In the United States, water hardness is measured in two ways. Temporary hardness is measured by the amount of bicarbonate, and high levels of bicarbonate, greater than 100ppm, will cause harsh flavors in the final beer. Permanent hardness is determined by the calcium and magnesium levels. Permanent hardness lowers the pH. At certain levels low pH is desirable in all-grain brewing for enzyme reactions (Papazain, 2003). Palmer (2006) reported that the pH of the water is an important parameter. However, for the overall process, the pH of the mash is the more important factor. Papazain (2003) reported the importance of brewing with water at a pH below 8; a pH level above 8 indicates hard water. The water pH affects mash enzyme activity and the extraction of bitter tannins from the grain husks (Palmer, 2006).

Hops

In brewing, hops refers to the flower harvested from the cone of the female plant *Humulus lupulus*. The hardy, climbing, herbaceous perennial plant is grown in all the temperate regions of the world. The cone-shaped flowers are valued by brewers for their resins and oils located in the lupulin glands that impart both bitterness and aroma to the beer (Bamforth, 2006). Hops were not used in most ancient beer production; instead, spices and other plants were used to flavor the beer. In 1079, Saint Hildegard of Germany cited by Bamforth (2006) noted the anti- spoilage properties of hops, and brewers began to take note. Cultivation of hops began in central Europe and spread to Western Europe and Great Britain in the early 1500s (Palmer, 2006). However, hops were not common in beer until the early1800s. Hops work well as a preserving agent in the brewing process because they eliminate

undesirable malt proteins, aid clarification, and stabilize beer flavors. An added benefit is their ease of cultivation, and ability to impart characteristic flavor and aroma (Papazain, 2003). Two components of hop composition are essential to beer production, the essential oils and resins. The oil portion contributes to the aroma characteristic of the final beer (Bamforth, 2006). Hop resins contains alpha acids that contribute to bitterness. The level of alpha acid is unique to each variety of hops. Alpha acids are also referred to as humulones and indicate the bitterness imparted to the beer. Hops are added to the beer during the boiling of the wort. This is necessary to promote the isomerization reaction that renders the alpha and beta acid resins water soluble, as humulinic acid and isohexenoic acid. Once water soluble, these compounds are released into the sweet wort where bitterness is imparted to the wort (Papazain, 2003).

Increased boil -time increases the bitterness imparted to the final beer. The aroma that hops provide to beer is produced by essential oils, which account for 1%-2% of the total dry weight of the cone. These essential oils are volatile and easily lost during the boil (Palmer, 2006). Therefore, hops are added at scheduled intervals during the boil to produce the desired flavor and aroma in the final beer.

Yeast

Yeast is important to beer production to convert the sugars in the wort to alcohol, creating beer. Although, beer production is one of the world's oldest crafts it was not until 1836 that C. Cagniard-Latour theorized that fermentation of sugar was due to yeast. The following year Theodore Schwann recognized the fungal nature of yeast and named the organism *Saccharomyces* (Briggs *et al.*, 1981). Determining the type of yeast is often the first step in beer classification. Beer is categorized into ales and lagers based on the type of yeast used, and traditionally yeast was classified by where it settled in the fermenting vessel. Historically, most of the world used top-fermenting yeasts up until the nineteenth century. Bottom-fermentation yeast was only used by Bavarian brewers. In 1842 a Bavarian monk smuggled these fermentation techniques into Czechoslovakia and the technology began to spread across the globe (Briggs *et al.*,. 1981). Ale yeasts typically floated on the top, whereas lager yeast settled to the bottom. The yeast used in brewing belongs to the genus *Saccharomyces*. The taxonomy of yeast in brewing is classified into ale strains that belong to the species *S. cerevisiae* and typically ferment at warmer temperatures 18-

22°C. Lager strains are categorized as *S. pastorianus* and typically ferment at 6°-15°C. *S. pastorianus* most likely evolved from the merging of *S. cerevisiae* with *S. bayanus*, a yeast commonly employed in wine making (Bamforth, 2006). Merging of these yeasts resulted in the larger and more complex genome of lager strains. *S. uvarum* and *S. carlsbergensis* were used to identify lager strains prior to the genetic technology to identify *S. pastorianus* (Bamforth, 2006). The basic difference between ale and lager strains is the ability to ferment the sugar melibiose; only lager strains can ferment this particular sugar (Bamforth, 2006). When brewing gluten-free products, gluten-free yeast selection is important. Often yeast is propagated in a solution that may contain barley or wheat malt. For gluten-free products, yeast should be propagated using other carbohydrate sources such as molasses.

2.10.2 End products

The brewing process is the step involved in the breakdown of starch and protein complexes present in the grains into sugars and simple proteins and the eventual production of beer. The main aim of brewing is to hydrolyse the malt starch and, if the 'enzyme capacity' is sufficient, some non-malted amylaceous products which are less costly (maize, rice); it is not all hydrolyzed since it is necessary to keep some of the dextrins to give the beer 'body' (Charles *et al*.,1999).

Esters

Chemically, esters are formed when an alcohol combines with an organic acid. They typically impart a fruity aroma and flavor to beer. There are many esters associated with beer fermentation. Two common esters associated with brewing are Ethyl acetate, and Isoamyl acetate. Ethyl acetate produces a fruity character and can be detected at 33 ppm, while isoamyl acetate is responsible for the banana characteristic in German Wheat Beers. It can be detected at 3 ppm. Ester formation is positively correlated to wort gravity, yeast growth, and fermentation temperature (Charles *et al.*, 1999). That is, higher gravity worts, rapid yeast growth, and higher fermentation temperatures increase ester production. Ester formation is reduced by high yeast pitching rates because the yeast will not grow as fast. Also, wort with insufficient oxygen levels favour ester formation.

Fusel Alcohols

These alcohols have a more complex molecular structure than ethyl alcohol. They provide an initial sweetness followed by a harsh after taste. Formed by the metabolism of amino acids, so over modification during malting or mashing can lead to higher fusel alcohol levels. They increase with fermentation temperature, level of amino acids, and wort gravity. Wild yeast can produce very high levels of fusel alcohols. Some yeast strains produce phenolic alcohols that typically have a medicinal flavor; however, the clove like character of German Wheat beers is produced from the phenolic 4-vinyl-guaiacol. Wild yeast can produce phenolic alcohols with very unpleasant flavors (Charles *et al.*, 1999)

Ketones

The two important ketones in brewing are diacetyl and 2, 3-pentanedione. These two ketones are classified together as the vicinal diketone level in beer. Diacetyl has a very low flavor threshold, 10 mg/L (ppm). In fresh beer, low levels of diacetyl may impart a caramel flavour, however, over time it will take on a butter or butterscotch characteristic. Early in a normal fermentation, during the aerobic stage, yeast will produce diacetyl. Later in the anaerobic fermentation stage, yeast reduces diacetyl to levels below the flavor threshold (Charles *et al.*, 1999).

Gram-positive lactic acid bacteria can produce large amounts of diacetyl. Mutant yeast cells can lose their ability to reduce diacetyl, leading to elevated levels of diacetyl. Wort that does not contain sufficient levels of the amino acid valine can lead to higher levels of diacetyl. Fortunately, most all-malt worts contain an over abundance of amino acids (Charles *et al.*, 1999).

Higher temperatures early in the fermentation lead to higher levels of diacetyl and higher temperatures later in the fermentation lead to a greater reduction in diacetyl.

For lager yeast that typically ferment at lower temperatures, some brewers perform a diacetyl rest during the latter fermentation stage called the late krausen phase. To perform a diacetyl rest, slowly raise the fermentation temperature to around 60^{o}F and hold this temperature for two days and then slowly lower the temperature back to the original fermentation temperature. Here, slowly means no more than 5^{o}F per day, otherwise you may shock the yeast.

2, 3-pentanedione

Has a flavor threshold of 1 mg/L (ppm). Produces a flavour similar to honey. Found in some Belgium ales where honey flavors are appropriate for the style. Wort that does not contain sufficient levels of the amino acid leucine can lead to higher levels of 2, 3-pentanedione. Fortunately, most all-malt worts contain an over abundance of amino acids.

Fatty Acids

Tend to add a soapy flavor to beer. They are produced when yeast break down amino acids. They are suppressed by lower fermentation temperatures. Usually the yeast will convert fatty acids to aldehydes then into alcohols

Sulfur Compounds

Hydrogen sulfide production during fermentation can lead to flavors reminiscent of rotten eggs; however, during normal fermentation, hydrogen sulfide is reduced during the fermentation process. The flavor threshold for hydrogen sulfide is 10-35 ppm. Gram-negative bacteria, like *Escherichia coli* can produce large amounts of sulfur compounds. For ales, higher fermentation temperatures tend to suppress sulfur compounds (Charles *et al.*, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Treatment of samples

3.1.1 Sorghum, Maize and Millet samples

Different varieties of maize (*Zea mays*), sorghum (*Sorghum bicolor* and *Sorghum vulgaris*) and millet (*Eleusine coracana*) were purchased locally from Bodija market and the typed varieties were collected from the Seed Production Unit of the Institute of Agricultural Research, Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria and Institute of Agricultural Research (IAR&T), Ibadan Oyo State in Nigeria. The defective seeds were removed and the viable or healthy seeds were stored in sterile polythene bags.

3.1.2 Indicator organisms

The indicator organisms used in this study were obtained from the Environmental Microbiology and Biotechnology Laboratory of the Department of Microbiology, University of Ibadan. The indicator organisms were: *Proteus mirabilis, Bacillus licheniformis, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus.*

3.1.3 Brewery flocs

Brewery floc was obtained from Nigerian Breweries factory in Ibadan and the yeast was isolated and identified using conventional methods.

3.2 Preparation of 'Ogi' from the cereal samples

This was carried out using the method of Odunfa and Adeyele (1985).The cereal grains were cleaned and steeped in tap water for 2days in sterile plastic containers. The water was decanted and the grains wet-milled before sieving with muslin cloth or fine wire-mesh. The pomace was then discarded and the starch suspension was allowed to sediment during which fermentation was carried out for 2-3 days by the natural floral of the grains (Odunfa and Adeyele, 1985). The different types of the cereal gruels prepared are named as follows; white sorghum 'ogi' (WS), White maize 'ogi' (WM), Yellow maize 'ogi' (YM), Millet 'ogi' (MT), Red sorghum 'ogi' (RS) and the typed ones from the Research Institutes are named as follows; Samsorg 41 (S-41), Samsorg 40 (S-40), Ex-Kano (Ex – k) Samsorg 14 (S-14), TZPB –SR –W, ART/98/SW1 (Oloyin) and SUWAN–ISR–Y.

3.3 Culture medium

The medium for the isolation of Lactic acid bacteria was de Mann Rogosa and Sharpe agar (MRS Agar, Oxoid) (De Man *et al.*, 1960). The medium was prepared according to the manufacturer's direction and sterilized by autoclaving at 121^{o}C for 15 minutes.

3.4 Isolation and identification of lactic acid bacteria (LAB)

Lactic acid bacterial strains were isolated from the different fermented cereal gruels using the serial dilution method of Harrigan and McCance (1976). One millilitre of each sample was thoroughly mixed with 9ml of sterile distilled water to give a 10^{-1} dilution. Higher dilutions were plated out and incubated at 37^{o}C using a cool air incubator (DNP-9082 Laboratory incubator, Biofield Medical Instruments England) for 48hours under anaerobic condition using anaerobic jar. After the incubation period, the plates were observed for bacterial growth and representative colonies were randomly selected. Purification of the isolates was done by subculturing into fresh medium (MRS) to obtain pure cultures (Mante *et al*., 2003).

Identification of the isolates was carried out based on their microscopic, macroscopic, cultural, physiological and biochemical characteristics with reference to Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 1986) and an Approach to the classification of Lactobacilli (Rogosa and Sharpe, 1959). The colonies were observed for their morphological features such as size, colour, elevation, opacity, consistency and edges.

3.5 Preservation of pure cultures of LAB isolates

The pure cultures of the organisms were transferred to prepared sterile MRS slants. The organisms were preserved after incubating for 24hrs at 30^{o}C and was stored in the refrigerator at 4^{o}C and MRS broth containing 12 %(v/v) glycerol. The organisms were subcultured every 2 weeks onto fresh agar slants to maintain the viability. The preserved cultures were used for all the various test that were carried out with the isolates (Savadogo *et al*., 2004).

3.6 Characterization of the obtained Isolates

The characterization of the isolates was carried out by employing macroscopic, physiological and biochemical tests according to the methods of Olukoya *et al.*, (1993a); Olsen *et al.*, (1995). Young cultures (between 18 -24 hours) were used for each biochemical test inoculation except otherwise stated.

3.6.1 Morphological Characterization

Macroscopic and microscopic observation of the different colonies as appeared on the MRS agar medium after incubation was done. The elevation, size, degree of growth, opacity, edge and colour of pure cultures of the isolates were examined after 48hours of growth.

3.6.1.1 Microscopic examination

Gram's staining

The pure culture of each isolate was stained as described by Norris and Ribbows (1976). Smears from 24hours old culture were used. A thin smear of the isolate was made on a clean slide and heat –fixed by passing it over a flame. 2drops of crystal violet were added to the smear and it was allowed to stay for 60seconds. The crystal violet was washed off under running tap water and it was then stained with Gram's iodine for solution and then left for another 60 seconds. The iodine was rinsed off by flooding the slide with ethanol for decolourization to take place. Two drops of safranin was added as counter stain for 10seconds and it was then rinsed with tap water. It was allowed to dry and observed using oil immersion objective lens. Gram positive bacteria were characterized by purple colouration while Gram negative cells were stained pink. The staining technique also showed the shapes and arrangement of the isolates.

3.6.2 Biochemical Characterization

3.6.2.1 Catalase Test

This test was done to determine whether the bacteria produce the enzyme catalase, which breaks down hydrogen peroxide to free oxygen and water. A drop of freshly prepared 3% hydrogen peroxide was added to a smear of 24hour culture of the organism on a slide. Effervescence or evolution of gas as white froth indicates a catalase positive reaction (Seeley and Van Demark, 1972). Absence of froth signifies a negative reaction

$$2H_2O_2 \longrightarrow 2H_2O+O_2$$

3.6.2.2 Oxidase Test

This detects the presence or absence in bacteria of certain oxides that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye. A drop of 48hours old culture of the isolate was placed on a Whatman number 1 filter paper that was soaked with the oxidase reagents (1% aqeous tetramethyl-p-phenylene diamine hydrogen chloride). Formation of a very deep purple colouration

within 10seconds indicated a positive reaction while the absence of deep colouration indicated a negative reaction (Seeley and Van Denmark, 1972).

3.6.2.3 Indole Test

Indole test was done to determine the ability of the isolated bacteria to degrade the amino acid tryptophan to indole. Tryptone broth (1%) was prepared and distributed to screw capped tubes and sterilized. After cooling, the tubes were inoculated and then incubated at 37^{o}C for 48hours. 3mls of Kovac's reagent was added to 6mls of the culture fluid. The mixture was thoroughly shaken for even mixing. The mixture was allowed to stand until the reagent rose to the top. After a few minutes, a deep red colour was not observed which indicated a negative reaction. A deep red indicated an indole production (Harrigan and McCance, 1976).

3.6.2.4 Gelatin hydrolysis Test

This was a test for the production of gelatinase. 9mls of gelatin broth (Harrigan and McCance, 1976) were distributed into screw capped tubes and sterilized by autoclaving at 121^{o}C for 10minutes. The test organisms were inoculated into the tubes and incubated at 30^{o}C for 7days. Gelatin hydrolysis was tested for by cooling the tubes in freezer for about 15minutes after which the tubes were observed for gelatin hydrolysis. The tubes in which gelatin hydrolysis did not take place had their gelatin content remaining in solidified state, while a positive reaction was indicated by the gelatin remaining in the liquid state without solidification (Harrigan and McCance, 1976).

3.6.2.5 Starch Hydrolysis Test

Equimolar amount of soluble starch was prepared and added to MRS agar without glucose or meat extract to give a 1% soluble starch agar medium. The medium was sterilized at 121^{o}C for 15mins before being poured into sterile plates and allowed to set. Single streak was made on the plates and incubated at 30^{o}C for 48hours. After incubation, the plates were flooded with Gram's iodine solution. This was done to test for the amylase activity of the isolates. Unhydrolysed starch formed a blue colouration with iodine. Clear zones around the region of growth indicated by the organisms (Seeley and Van Demark, 1972).

3.6.2.6 Casein Hydrolysis

Skim milk agar was prepared by adding (1%, w/v) skim milk to nutrient agar (Harrigan and McCance, 1976). The agar was sterilized at 110^{o}C for10mins. The medium was dispensed into sterile Petri dishes and then left to set. A single line of

streak was then made across the plate with the test isolates and then incubated at 30°C for 2 days. Uninoculated plates served as control. Observation of clear zones along the line of streak indicates a positive result.

3.6.2.7 Nitrate Reduction Test

This test was carried out to detect the ability of the isolates to reduce nitrate compounds, to nitrite and nitrogen gas. Nitrate peptone water consisting of peptone water and 0.1% potassium nitrate (KNO_3) was used. Five millilitre portions of the medium were distributed into screw capped test tube. Each tube contained an inverted Durham tube. The test tubes and their contents were sterilized at 121°C for 15minutes and allowed to cool before being inoculated with the isolates. Uninoculated tubes served as control. The tubes were incubated at 30°C for 4days. The ability of the isolates to reduce nitrate to nitrite, ammonia or free nitrogen was determined by adding to each tube 0.5ml of 1% sulphanilic acid in 5N acetic acid followed by 0.5ml of 0.6% dimethyl-naphthylamine in 5N acetic acid. The development of a red coloration indicated a positive result and the presence of gas in the Durham's tubes indicated the production of nitrogen gas (Payne, 1973).

3.6.2.8 Hydrogen Sulphide Production Test

Modified MRS medium containing 0.2g lead acetate and 0.08g sodium thiosulphate was used to grow the isolates. The medium was sterilized at 121°C for 15minutes in McCartney bottles. It was allowed to cool upright. Agar deeps were then sterilized at 121°C for 15minutes in McCartney bottles. It was allowed to cool upright. Agar deeps were then stabbed with 18hour old cultures and incubated at 30°C for 48hours. Observation of black colouration along the line of streak indicated a positive result. This showed that the organism produced hydrogen sulphide gas from the modified MRS medium (Harrigan and McCance, 1976).

3.6.2.9 Methyl Red Test

Glucose phosphate peptone broth was prepared as described by Harrigan and McCance (1976). 10mls of the broth was dispensed into screw capped tubes and sterilized. The test organisms were inoculated into broth and was incubated at 30°C for 5days. After incubation, few drops of methyl indicator was added to the culture and a red colouration was indicated positive.

3.6.2.10 Voges Proskauer Test (VP)

Cultures were grown in glucose phosphate peptone broth. The tubes were then incubated at 30°C for 5days. After incubation, 1ml of 6% α-napthol solution and 1ml

of 10% sodium hydroxide (NaOH) was added. This is to know whether the organism after producing acid from glucose are capable of producing acetyl methyl carbinol from the acid. The development of a pink colouration within 5minutes is indicated positive.

3.6.2.11 Production of Ammonia from Arginine

The production of ammonia from arginine was tested using the method of Doring (1988). A modified MRS broth (MRS-Arginine broth) without glucose and meat extract, but containing 0.3% Arginine and 0.2% sodium citrate instead of triammonium citrate was used. The MRS broth without arginine was used as a control medium. 18hours old cultures were inoculated into 10ml of each in a test tube and incubated at 30^{o}C for 5days. After incubation, few drops of Nessler's reagent were added to the tubes. Cultures producing yellow or orange colour as compared to that produced by a similarly treated control medium indicated the production of ammonia from arginine.

3.6.2.12 Growth in 4% Sodium Chloride

30mls of MRS broth containing 4 % (w/v) NaCl was dispensed into screw capped tubes and sterilized at 121^{o}C for 15 minutes. After cooling, the tubes were inoculated with the test organisms and incubated at 30^{o}C for 2days (Schillinger and Lucke, 1987). Increased turbidity of medium was recorded as positive for growth. Uninoculated tubes served as control.

3.6.2.13 Growth at different Temperatures

Each of the isolates was streaked on MRS agar plates and incubated at different temperatures at 15^{o}C and 45^{o}C for 48 hours. Growth along the line of streak was recorded as positive (Gibson and Abd-El-Malek, 1945).

3.6.2.14 Growth at different pH

The pH values of the different MRS broth medium was adjusted to pH 3.9 and 9.2 using 0.1N HCL and 0.1N NaOH with the aid of previously calibrated pH meter (Hanna instrument HI96107). Ten milliliters of the medium was distributed into tubes before autoclaving. After autoclaving, the test cultures were inoculated and then incubated at 30^{o}C for 48 hours. Turbidity of the broth compared with the uninoculated controls was used as indicator of growth of the culture.

3.6.2.15 Motility Test

The organisms were grown in MRS broth for 18 hours at 30^{o}C. The 'hanging drop technique' which involves the use of immersion oil around the edge of the

depression of a cavity slide was used. After incubation, few drops of broth were put on a depression of the cavity slide and covered with a cover slip. This was then inverted over the cover slip such that the culture drop is in the centre of the slide depression and was examined under the x40 objective lens of the microscope for movement or otherwise of the cells (Seeley and Van Demark, 1972).

3.7 Sugar Fermentation Test

The fermentation patterns were determined using modified MRS broth from which meat extract and glucose have been omitted (Sharpe *et al*., 1996). The glucose was substituted with equivalent quantity of the test sugars. The various sugars used werelactose,xylose,arabinose,rhamnose,inositol,raffinose,sucrose,glucose,fructose,galactose,mannitol,sorbitol,maltose etc were used. 0.05% (w/v) Bromocresol purple was added as an indicator and as basal medium for acid production by the isolates as signified by colour change. The medium was then dispensed into screw capped tubes (10mls each) and then sterilized with inverted Durham's tube to detect gas production. This was sterilized at 110^{o}C for 10minutes so as not to denature the sugars. After cooling, the tubes were inoculated with the test organisms and incubated at 30^{o}C for 5-7 days. Uninoculated tubes served as control. A change of colour from purple to yellow indicated acid production while gas production was noticed in the Durham's tubes as a result of empty space. No change in colour for negative tests (Harrigan and McCance, 1976).

3.8 Homofermentative and Heterofermentative Test

The semi-solid medium of Gibson and Abd-El-Malek (1945) as modified by Stainer *et al*., (1964) was used. 20mls of the medium was dispensed into McCartney bottles to a depth of about 5cm and sterilized. The mixture was then inoculated with 0.5ml portion of young MRS broth culture of the test organisms. The test organisms were inoculated in duplicate and sterile agar seal poured onto the surface of each bottle. The tubes were incubated at 20^{o}C for 2 days. Production of gas was indicated by gas bubbles or by forcing of the agar seal up the tubes. Uninoculated tubes served as control.

3.9 Determination of lactic acid, hydrogen peroxide and diacetyl production by the LAB isolates.

For these measurements the test organisms were grown anaerobically in MRS broth for 48hrs at 37^{o}C and centrifuged using 80-2 Bench centrifuge at 3000xg for 15mins.The supernatant fluid was used for all the determinations.

3.10.1 Quantitative estimation of lactic acid

To 25ml of the supernatant fluid of the test organisms was added 3 drops of phenolphthalein indicator. This was titrated against 0.1M NaOH in a burette until a pink colour appeared. The titratable acidity was calculated as lactic acid (%,v/v) (Fontaine *et al.*,1993). Each ml of 0.1M NaOH is equivalent to 90.08mg of lactic acid (A.O.A. C., 1990). This was repeated two times and the average titre value of the two readings was taken

$$\text{Titratable acidity} = \frac{\text{ml NaOH X NaOH X M.E X100}}{\text{Volume of sample used}}$$

Ml NaOH =Volume of NaOH used

N NaOH =Normality of NaOH solution

M.E = Equivalence factor (90.08mg)

3.10.2 Hydrogen peroxide Production

To 20ml of diluted sulphuric acid were added to 25ml of the supernatant fluid of the test organisms. Titration was carried out with 0.1M potassium permanganate. Each ml of 0.1M potassium permanganate is equivalent to 1.79mg of hydrogen peroxide. Solution and decolourization of the sample was regarded as the end point (A.O.A.C., 1990).

$$\text{Titratable acidity} = \frac{\text{ml } KMnO_4 \text{ X } KMnO_4 \text{ X M.E X 100}}{\text{ml } H_2SO_4 \text{ X Volume of sample}}$$

ml $KMnO_4$ =Volume of $KMnO_4$

N KMnO =Normality of $KMnO_4$

Ml H_2SO_4 =Volume of H_2SO_4

M.E =Equivalence factor (1.79mg)

3.10.3 Diacetyl Production

This was determined by transferring 25ml of the supernatant fluid of the test organisms into conical flasks and 7.5ml of hydroxylamine solution were used for the residual titration. The flasks were titrated with 0.1M HCl to a greenish-yellow end point using bromophenol blue as indicator (Sanni *et al.*, 1995). The equivalent factor of HCl to diacetyl is 21.5mg (A.O.A.C, 1990). The concentration of diacetyl produced was then calculated according to the method of Food Chemicals Codex (1972).

$$AK = \frac{(s-b)\ X\ (100E)}{W}$$

AK=percentage of diacetyl, b=Amount (ml) of 0.1N HCL consumed in titration sample

E = Equivalence factor (21.5mg)

W =Volume of sample

s =Number of ml of 0.1N HCL consumed in titration of sample

b=Number of ml of 0.1N HCL consumed in titration of blank

3.11 Determination of organism to be used as starters

When the rate of production of lactic acid, hydrogen peroxide and diacetyl by the LAB isolates has been determined, the LAB isolates that produced all the antimicrobial best were selected and further work was carried out on all the isolates.

3.12 Physiological Characterization of the LAB Isolates (cured and uncured)

3.12.1 Determination of Inoculum size for each isolate

The lactic acid bacteria isolates were grown in MRS broth and it was then incubated at 37^{o}C for 24hrs. 0.1ml of the LAB isolate was then used for the physiological characterization of the isolates.

3.12.1 Effect of different pH on the growth and production of different metabolic enzymes by the isolates.

MRS broth was prepared and 10ml each was dispensed into Erlenmeyer flasks. The pH of each broth was adjusted to 3.9, 5.0, 5.5, 6.0, 7.0, and 9.2 by the addition of either sterile 0.5M KOH or sterile 0.5M HCl from a calibrated syringe. The broth was then sterilized at 121^{o}C for 15mins and was allowed to cool after which the 0.1ml of 24hours old culture of the test organisms were inoculated into the broth. It was incubated at 30^{o}C for 24hrs after which the culture in each flask was centrifuged using 80-2 bench centrifuge at 4000rpm at 4^{o}C for 30mins. The cell-free culture supernatant was then measured using a Spectrophotometer. The metabolic enzymes (amylase, invertase, protease, xylanase) produced by the isolates was also assayed for at the different pH (Schillinger and Lucke, 1989).

3.12.2 Effect of different temperatures on the growth and production of different metabolic enzymes by the isolates.

MRS broth was prepared and dispensed into series of Erlenmeyer flasks. The broth was then sterilized at 121^{o}C for 15minutes and allowed to cool after which the LAB isolates were inoculated into the broth by using 0.1ml of the LAB isolates. It was incubated at different temperatures ranging from 20^{o}C, 30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C, 80^{o}C for 24hours using shake flasks, after which the culture in each flask was centrifuged at 4000rpm at 4^{o}C for 30minutes. The metabolic enzymes (amylase, invertase, protease, xylanase) produced by the isolates was also assayed for at the different temperatures (Gibson and Abd-El-Malek, 1945).

3.12.3 Effect of different carbon sources on the growth and production of different metabolic enzymes by the isolates.

MRS broth was prepared and the Glucose was substituted with equivalent quantity of each of different sugars as carbon sources (glucose, lactose, and raffinose) The MRS broth was then dispensed into screw capped battles and sterilized. The LAB isolates were inoculated into the MRS broth and incubated at 30^{o}C for 24hours after which the culture in each flask was centrifuged. The metabolic enzymes (amylase, invertase, protease, xylanase) produced by the isolates was also assayed for using the different carbon sources.

3.12.4 Effect of Cations and Anions on the growth and production of different metabolic enzymes by the isolates.

MRS broth was prepared and varying concentrations of $MgS0_4$ (0.5-2.0mg/ml) was used in the presence of carbon sources and other component was used for the preparation of the MRS broth. The MRS broth was then sterilized and after cooling, the test organisms were inoculated into the broth and incubated at 30^{o}c for 24 hours after which the culture in each flask was centrifuged at 4000rpm at 4^{o}C for 30 minutes using 80-2 bench centrifuge.

MRS broth was prepared and varying concentrations of Triammonium citrate (0.5-2.0mg) was used in the presence of carbon sources and other components was used for the preparation of the MRS broth. The MRS broth was then sterilized and after cooling, the test organisms were inoculated into the broth and incubated at 30^{o}c for 24hours after which the culture in each flask was centrifuged at 4000rpm at 4^{o}C for 30minutes using 80-2 bench centrifuge. The metabolic enzymes (amylase,

invertase, protease, xylanase) produced by the isolates was also assayed for at the different concentrations of cations and anions (Gibson and Abd-El-Malek, 1945).

3.12.5 Effect of different Nitrogen sources on the growth and production of different metabolic enzymes by the isolates.

MRS broth was prepared and the glucose was substituted with equimolar quantity of different nitrogen sources at varying concentration (urea, yeast extract, and peptone). The MRS broth was then sterilized and after cooling, 0.1ml of the LAB isolates were inoculated into the broth and incubated at 30^{o}C for 24 hours after which the culture in each flask was centrifuged. The metabolic enzymes (amylase, invertase, protease, xylanase) produced by the isolates was also assayed for at the different concentration of the nitrogen sources.

3.13 Extraction of Enzymes

The MRS broth was prepared and 10mls of the MRS broth was sterilized and then inoculated with the LAB isolates and was incubated for 48 hours at 30^{o}C. The broth culture was then centrifuged at 4000g for 30 minutes using cold centrifuge (IEC Centra MP4R; International Equipment Company). The culture supernatant was then separated by filtration and the supernatant was used for all the enzyme assays.

3.13.1 Protease assay

This was carried out using the method of Kunitz (1946). 1% (w/v) casein was prepared in 0.2M Phosphate buffer (pH 7.0). The substrate was heat- denatured at 100^{o}C in a water bath (Model DK-420 Water bath, Lemfield Medical England) for 15minutes and was then allowed to cool before use. To 1ml of the casein solution in tubes was then added 0.5ml of enzyme extract. The solution was then incubated for 1hour at 35^{o}C. After incubation, the reaction was terminated by adding 3ml of cold 10% TCA. The tubes were allowed to stand for 1hour at 4^{o}C to allow undigested protein to precipitate.

Control tubes contain 0.5ml of uninoculated MRS broth and casein 1% incubated for 1hour at 35^{o}C before adding 3mls of cold 10% TCA. The reaction mixtures were then centrifuged at 10,000rpm at 4^{o}C for 15minutes. The optical density reading of the carefully decanted supernatant fluid was then measured with UV/visible JENWAY 632OD Spectrophotometer at 660nm wavelength against a blank containing the control.

One unit of protease activity is defined as the amount of enzyme that releases 1ug tyrosine per ml per minute from 1mg casein under the specified assay conditions.

3.13.2 Amylase assay

Amylase activity of the LAB isolates was determined using 3, 5-Dinitrosalicyclic acid (DNSA) reagent method of Bernfeld (1955). 1ml of culture that was centrifuged (supernatant) was added to 1ml of substrate (1.2% soluble starch in 0.1M Phosphate buffer, pH 6.0). The enzyme substrate reaction was incubated at 30^{o}C for 10minutes. The reaction was stopped by the addition of 0.5M NaOH. Dinitro salicylic acid (DNSA) was added and then boiled at 100^{o}C. The solution was then diluted with 18mls of distilled water. The absorbance was read at 540nm using a UV/visible JENWAY 632OD spectrophotometer. 1ml of uninoculated blank similarly treated was used to set the spectrophotometer at zero.

3.13.3 Invertase assay

Invertase activity of the LAB isolates was determined using DNSA reagent method of Bernfeld (1955) as modified by Giraud *et al* (1991). 1ml of culture supernatant was added to 1ml of a solution containing 1.2% sucrose in 0.1M Phosphate buffer, pH 6.0. The enzyme substrate (sucrose) – reaction was incubated at 30^{o}C for 10minutes. The reaction was stopped by the addition of 5M NaOH. The amount of reducing sugar thus produced was estimated according to Bernfeld (1955) with 3,5 – Dinitrosalicyclic acid. The optical density reading of the carefully decanted supernatant fluid was then measured with UV/visible JENWAY 632OD Spectrophotometer at 540nm wavelength against a blank containing the control.

3.13.4 Xylanase assay

Xylanase activity of the LAB isolates was determined using the method of Khan (1980). The glucose in the MRS broth was substituted with 1g of xylan. The LAB isolates was then subcultured onto the MRS broth and was then incubated for 48hours at 37^{o}C. 0.1ml of culture supernatant was added to 0.5ml of a solution containing 0.1%(w/v) of Birchwood xylan in 0.1M sodium acetate buffer, pH 5.0.The enzyme substrate reaction was incubated in a water bath at 40^{o}C with shaking for 30minutes.1ml of DNSA reagent was added, it was then boiled in a water bath for 5mins for colour development. It was then allowed to cool. The optical density reading of the carefully decanted supernatant fluid was then measured with

UV/visible JENWAY 632OD Spectrophotometer at 540nm wavelength against a blank containing the control.

3.14 Bacteriocin Detection

LAB strains selected as test organisms were propagated in MRS broth (pH 5.5) with reduced concentration of glucose (0.25% w/v) and peptone (0.5% w/v) and then incubated for 72hours at 30^{o}C (Schillinger and Lucke, 1989). The Bacteriocin was extracted by centrifuging the culture (4000g for 30minutes) using cold centrifuge IEC Centra MP4R (International Equipment Company). The supernatant was then adjusted to pH of 6.0-6.5 by using 1M NaOH (to exclude the antimicrobial effect of organic acid) and the solution was treated with 5mg/ml catalase to eliminate hydrogen peroxide (Sigma, Germany) while neutralizing the acid with 0.25 mol of NaOH eliminated that of acid. The cell free supernatant served as the crude bacteriocin.

3.14.1 Detection of Antagonistic Activity of the Crude Bacteriocin

The inhibitory activity of LAB was screened by Agar well diffusion assay (Schillinger and Lucke, 1989). The broth culture of each indicator organism was prepared by inoculating 0.1ml indicator organism to prepared nutrient agar and was incubated for 24hours at 30^{o}C. After incubation, 20ml of nutrient agar was prepared and100µl of the indicator organism was then inoculated into the molten nutrient agar and then poured aseptically into sterile Petri dish. After the agar has solidified, wells of 5mm in diameter were cut into the agar using a 5mm diameter cork borer. Aliquots of 100µl of each bacteriocin dilution were placed in wells in plates seeded with the indicator organism (Takahiro *et al.*, 1991). The plates were then incubated at 30^{o}C in an incubator (DNP-9082 Laboratory incubator, Biofield Medical Instruments, England) for 24hours and the diameters of the inhibition zones were then taken and recorded in (mm) (Schillinger and Lucke, 1989).

3.15 Characterization of Amylase enzyme

The most prominent enzyme which was amylase was characterized based on the highest production by the LAB isolates. The characterization includes:

3.15.1 Effects of different nitrogen sources on amylase production by the LAB isolates

MRS medium was prepared and the glucose was substituted with equivalent quantity with different concentrations i.e 0.5mg/ml, 1.0mg/ml, 1.5mg/ml and 2.0mg/ml of the nitrogen sources (peptone, urea and yeast extract). This was then dispensed into screw- capped bottles and then sterilized at temperature of 121^{o}C for

15minutes. After cooling, 0.1ml of 24hours old culture of the LAB isolates were inoculated into it and incubated at 30^{o}C for 24 hours. Growth was observed through increased turbidity. To study the effects of peptone, urea and yeast extract on enzyme production, culture fluids from the above were centrifuged at 10,000 rpm for 15 minutes (Lealem and Gashe, 1994). The cell- free supernatant fluid served as the crude enzyme. This was then assayed for amylase using DNSA method of Bernfeld (1955).

3.15.2 Effects of different carbon sources on amylase production by the LAB isolates

MRS medium was prepared and the glucose was substituted with equivalent quantity with different concentrations (0.5-2.0mg/ml) of the carbon sources (glucose, lactose and raffinose). This was then dispensed into screw capped bottles and then sterilized. After cooling, the organisms were inoculated into it and incubated at 30^{o}C for 24 hours. Growth was observed through increased turbidity. To study the effects of peptone, urea and yeast extract on enzyme production, culture fluids from the above were centrifuged at 10,000 rpm for 15 minutes at 4^{o}C (Lealem and Gashe, 1994). The cell free supernatant fluid served as the crude enzyme. This was then assayed for amylase using DNSA method of Bernfeld (1955).

3.15.3 Effects of different pH on the production of amylase by the LAB isolates

MRS broth was prepared and 20mls each was dispensed into Erlenmeyer flasks. The pH of each flask was adjusted using 0.1M phosphate buffer to adjust the pH of the medium to 3.0, 3.9, 4.0, 5.0, 5.5, 6.0, 7.0 and 9.2 with the aid of a previously calibrated pH meter (Hanna instrument HI96107). This was then dispensed into screw capped bottles and then sterilized at 121^{o}C for 15 minutes. After cooling, the organisms were inoculated into it and incubated at 30^{o}C for 24 hours. Growth was observed through increased turbidity. To study the effects of pH on enzyme production, culture fluids from the above were centrifuged at 10,000 rpm for 15 minutes at 4^{o}C (Lealem and Gashe, 1994). The cell free supernatant fluid served as the crude enzyme. This was then assayed for amylase activity using DNSA method of Bernfeld (1955).

3.15.4 Effects of different temperatures on amylase production by the LAB isolates

MRS broth was prepared in Erlenmeyer flasks. 20mls of the broth was dispensed into different flasks, each flask was sterilized at 121^{o}C for 15 minutes and was then allowed to cool and the test isolates were then inoculated into the broth and incubated at different temperatures ranging between 20^{o} C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C and 80^{o}C for 24 hours using shake flasks. Growth was observed through increased turbidity. To study the effects of temperature on enzyme production, culture fluids from the above were centrifuged at 10,000 rpm for 15 minutes at 4^{o}C (Lealem and Gashe, 1994). The cell- free supernatant fluid served as the crude enzyme. This was then assayed for amylase using DNSA method of Bernfeld (1955).

3.16 Extraction and analysis of Plasmid DNA of LAB Isolates

Plasmid extraction was carried out using a slight modification of the method described by Ehrenfeld and Clewell (1987). Pure isolates were inoculated on MRS broth and incubated. The grown cells were harvested and suspended in 200µl of solution A (100 mM glucose-50 mM Tris hydrochloride (pH 8)-10 mM EDTA) containing 10 mg of lysozyme per ml and 10µg/ml mutanolysin and incubated for 30 min at 37°C in an incubator. 400µl of freshly prepared 1% sodium dodecyl sulfate in 0.2 N NaOH was added and the samples were mixed by inverting tubes. 600µl of a 15% potassium acetate solution (pH 4.8) was added and the samples were mixed by vortexing. After incubating on ice for 5 minutes, the debris was removed by a 5-minute centrifugation in a centrifuge (Model 5415R; Eppendorf). The supernatant was removed and extracted once with a phenol-chloroform mixture (1:1) and precipitated with an equal volume of isopropanol. The plasmid DNA was then dissolved in 100µl of TE buffer.

Electrophoresis of the DNA was carried out on a 1.0% agarose gel in a 0.5X concentration of Tris-Borate-EDTA (TBE) buffer. Agarose gel was prepared by boiling 0.8g of agarose powder in 100mls of 0.5X TBE buffer. After boiling, the solution was allowed to cool and 10µl of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 minutes and the comb was removed. 20µl of the plasmid DNA samples were then loaded into the wells after mixing with 2µl of bromophenol blue. A DNA molecular weight marker was also

loaded into one of the wells. The gel was thereafter electrophoresed in a horizontal tank at a constant voltage of 60V for 1 hour 30 minutes.

After electrophoresis, plasmid DNA bands were viewed by fluorescence of bound ethidium bromide under a short wave ultraviolet light transilluminator and the photograph were taken using a digital camera

3.17 Quality Assessment and Proximate Analysis of Malted and Unmalted Sorghum

Quality assessment of the sorghum was also carried out and this include :1000 corn weight, germinative capacity, germinative energy, total nitrogen, total protein, moisture content, ash content, crude fat, total carbohydrate, soluble protein

3.17.1 1000 Corn Weight

In determining the 1000 corn weight, 500g of cleaned samples of each variety was taken and divided into portions of 40g **(**Okolo and Ezeogu, 1996**)**. Half corns and foreign materials were removed by hand. Each portion of the cleaned samples was weighed. The number of grains in each lot was countered by hand. The moisture content of each lot was also determined. The formula below was then used to calculate the 1000 corn weight:

G = W*1000 (100-M)/N*100

Where:

G = weight of 1000 grains of sorghum (in g)

W = weight of "cleaned" lot of sorghum taken (in g)

M = moisture (in %)

N = number of grains in the "cleaned" lot taken

Average of two lots was taken for each variety of sorghum.

3.17.2 Moisture content of unmalted sorghum

Moisture content was determined by finely milling 20.0 g of unmalted sorghum using Disc Mill (Buhler, Braunschweig, Germany), which was set at 0.2 mm. The sample was mixed thoroughly and 5.0 g was immediately placed in a clean, moisture dish, which had been tarred to 0.001 g. The dish was covered and weighed to 0.001 g. The lid was then removed and together with the dish (which contained the sample) was placed in an oven, which had been preheated to 105^{o}C for 3 hrs. The dish was then covered with the lid and the set removed from the oven, placed in a dessicator and cooled to room temperature for 25mins. The dish and content were

then reweighed on an analytical balance. Triplicate determinations were carried out for each variety. The moisture content was calculated as a percentage of the initial weight of sample. Moisture of the malted sorghum was also determined using the same method (Okolo and Ezeogu, 1996).

3.17.3 Germination Energy

Two lots of cleaned 500 sorghum grains were obtained (Okolo and Ezeogu, 1996**)**. Each lot of 500 grains was transferred into a funnel standing in tap water (to ensure complete flooding of the grains) at 20 ^{o}C. The water was removed after steeping for 3 hours. The grains were covered with whatmans No. 4 filter papers and the funnel itself covered with a glass plate. The steeping was repeated for 2 hours after 20 hours from the beginning of the test. The grains were again covered with filter paper and the funnel with glass plate. After 72 hours from the beginning of the test, the funnels were emptied and the number of non-germinated grains counted. Average result of the two counts (of the lots) after 72hours was obtained. The formula below was used to calculate the Germination (EBC, 1997).

Energy (GE):

Germinative Energy (GE) = (500-N)/5

Where:

N = number of non-germinated grains after 3 consecutive days.

NB: results is reported as = a % (Schonfield method 3 days).

This method was applied to each of the unmalted sorghum varieties.

3.17.4 Germination Capacity

This is an attempt to quantify the percentage of viable grains within a sample. 200 uniform sized and clean grains were handpicked and steeped in 500ml beaker containing 200ml of 0.75% hydrogen peroxide (H_2O_2) solution and incubated for two (2) days at a temperature of 21^{o}C **(**Okolo and Ezeogu, 1996**)**. At the end of the 2 days, the grains were strained and steeped again in 200ml of H_2O_2 also at 21^{o}C for further 24 hours (EBC, 1997). The germinated grains were then counted and the germinative capacity calculated using the formula:

Germinative Capacity = (200-n)/2,

Where:

N = grains that did not show

3.17.5 Total Nitrogen/ Protein: Kjeldahl Method

Finely milled 0.1 g of sorghum was weighed in a small weighing disk, and transferred quantitatively into a dry Kjeldahl flas**k** (A.O.A.C, 1980). 10 g of catalyst mixture (a tablet) consisting of TiO_2, $CuSO_4.5H_2O$ and K_2SO_4 were added. The content was thoroughly mixed by gently adding 25mls of conc. H_2SO_4 and some anti-bumping agent. The flask was then heated. The heating continued for 45 minutes after the solution turned bright green. The Kjeldahl flask and contents were then cooled to room temperature. Two drops of methyl red indicator was added to Erlenmeyer flask into which had been pipetted 20ml of 0.1N H_2SO_4. The flask was connected to the distillation apparatus such that the outlet tube of the condenser dipped beneath the H_2SO_4 in the receiving Erlenmeyer flask. After Kjeldahl flask and content had been cooled to room temperature, 150 ml of distilled water was cautiously added and mixed. The solution was cooled to below 25^oC. Anti-bumping agent was added to prevent bumping. 100 ml of 0.1N NaOH was then added slowly until two distinct layers were formed. The Kjeldahl flask was connected to the distillation apparatus soon after the addition of NaOH solution. The flask was heated smoothly for 5 minutes and then strongly until liquid began to distil. Distillation continued until Kjeldahl flask begun to bump. The excess H_2SO_4in the receiving flask was titrated with standardized 0.1 N NaOH, using methyl red as indicator. Simultaneously with the test, a blank determination on reagents was carried out in which 20ml instead of 25ml of 0.1N H_2SO_4 was added and without the addition of the test sample. This method was used for all malted and unmalted sorghum varieties. The Total Nitrogen/Protein content in the dry samples were then calculated and expressed as a percentage (EBC, 1997).

3.17.6 Moisture Content

Five grams (5g) of the sample (flour) was transferred to previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105 ^oC for 5hrs. The dish was removed and placed in a desiccator to cool and weighed. The dished was re-placed in the oven and heated, cooled and weighed. This stage was repeated until constant weight was obtained. The loss in weight, which represents the moisture, was reported and expressed as a percentage. This was done for each variety in triplicates.

3.17.7 Crude Fat

The dried sample obtained from the moisture determination was transferred to 22 x80 mm paper thimble (A.O.A.C, 1980). A small ball of cotton wool of glass wool was placed into the thimble to prevent loss of the sample. Anti bumping granules was added to the previously dried (air oven at 100^{o}C) 250 mm round bottom flask and weighed accurately. 150 ml of petroleum spirit B.P. 60-80 ^{o}C was added to the flask and apparatus assembled. A quick fit condenser was connected to the soxhlet extractor and refluxed and evaporated on a steam bath. The flask and fat/ oil was heated for 30min in an oven (Memmert oven, Type: ULE 60, Germany) at 103 ^{o}C. The flask and contents were cooled to room temperature in a desiccator. The flask was weighed accurately and the weight of oil/fat collected was determined. This was then expressed as the percentage.

3.17.8 Ash Content

In determining the ash content, 2g of dried sample was transferred to a previously ignited and weighed crucible and placed in muffle furnace that was preheated to 600 ^{o}C for 2 hours. The crucible was removed and cooled in a desiccator. Crucible was allowed to cool and then weighed. Weight was expressed as a percentage. This was repeated for all varieties and also for both malted and unmalted samples. The difference between the final weight and the initial weight of the crucible gave the ash content which was expressed as a percentage of the initial weight (A.O.A.C, 2002). Each sample was repeated in duplicates.

The percentage ash content was calculated as follows:

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where, W1= Weight of crucible

W2 =Weight of crucible + sample before ashing

W3 =Weight of crucible + sample after ashing

3.17.9 Estimation of Crude Protein of both unmalted and malted sorghum

Digestion

Kjeldahl method of nitrogen/protein determination was used (A.O.A.C., 1980). For the protein digestion, 2 g of the dried sample and a half of selenium based catalyst tablet and a few anti-bumping H_2SO_4 was added and the flask shaken

thoroughly to ensure the entire sample was wet. The flask was placed on a digestion burner and heated slowly until bubbling ceased and the resulting solution was clear. The flask was then made to cool to room temperature. The digestion sample solution was transferred into a 100 ml volumetric flask and made up to the mark.

Distillation

The distillation set up was flushed for 10 min. The condenser was treated so as to carry over all liquid in the condenser. 25 ml of 2% boric acid and 2 drops of mixed indicator were put into a 250 ml conical flask. Water was drained from the steam trap and the stopcork left open. The conical flask and its content were placed under the condenser in such a position that the tip of the condenser is completely immersed in solution. 10 mls of the digested sample was pipetted into the steam jacket. 20 ml of 40% NaOH was then added to the decomposition flask. The funnel stopcork was closed to allow the liberated ammonia into the collection flask. The stopcock on the steam trap was shut to force steam through the decomposition chamber. There is a colour change of the boric acid to bluish green as soon as it comes into contact with ammonia. Distillation was continued for 5 min after which the receiving flask was lowered so that the condenser tip is just above the liquid. The end of the condenser was washed with a little distilled water and distillation was continue for 30 sec and the process discontinued by removing the burner from the steam generator (A.OA.C, 1980)

Titration

The distillate was then titrated with 0.1 N HCl solutions. The acid addition continued to run until the solution was colourless. A similar procedure was carried out on the blank. The % nitrogen was then calculated from the titre value obtained using the formula below:

$$\% \text{ Nitrogen} = \frac{C\ (0.1N\ HCl)}{W}$$

Where,

x = mls of standard acid used

w = the amount of sample taken

Percentage Crude protein was then calculated as,

% Crude protein = % Nitrogen × 6.25 (A.O.A.C, 1980)

3.18 Preparation of Inoculum

The lactic acid bacteria culture was subcultured onto fresh MRS agar slants and it was then incubated at 37^{o}C for 48hrs. After incubation, a slant culture of each organism was covered with 9ml of sterile distilled water. The mixture was then shaken to disperse the cells. The dilution was made up to $10^{6.}$ 1ml was then taken from the suspension and it was pipetted into the dilution tubes. Appropriate dilutions were then made in order to get the right concentration for counting in a cell counting chamber (Rodriguez *et al.*, 2003).

3.18.1 Determination of Inoculum size

The inoculum size was determined by taking dilution 10^{3}. Haemocytometer was used to count the number of cells in the suspension. The haemocytometer was cleaned and also the coverslip using 70% ethanol. A loopful from each of the lactic acid bacteria suspension was taken and loaded onto the haemocytometer. It was then covered with coverslip. The excess liquid was removed carefully using cotton wool. The cells were then viewed under the microscope using 20 X objective. The numbers of cells in the squares were then counted and this was done five times and the average was then taken. The volume of the suspension that occupies one primary square is 0.1mm^{3} (1.0mm^{2} X 0.1mm) or 1.0 X10^{-4}mL (Rodriguez *et al*., 2003).

3.18.2 Application of Inoculum of LAB isolates to the sorghum samples

The sorghum samples were screened by hand to remove broken or damaged kernels, stones and other foreign materials since damaged grains cause microbial infection during germination. The sorghum seeds (800grams) were steeped in 3000mls of sterile water using clean buckets and the lactic acid bacteria isolates were inoculated into the steeping water prior to malting. The lactic acid bacteria that were used as starter cultures were: *L. plantarum*, *L. fermentum* and *L. casei*. The organisms were used singly and also in combination. An inoculum size of 2.3 X 10^{4} cells/mL was used to treat the steeped sorghum grains (Haikara and Laitila, 1995).The grains were steeped for 24hrs to raise the moisture level to and activate the metabolic processes of the dormant grains. The steeping was completed after 24 hrs when the white tips of the rootlets emerged.

3.19 Estimation of microbial load in steeped sorghum water

At each interval, that is, every 24hrs, the sample from the steeped water was taken to monitor the profile of organisms. The water was diluted up to 10^{6} dilution. Dilutions 10^{4} and 10^{5} were plated out using the pour plate method (Harrigan and

McCance, 1976). The organisms that were enumerated include: *Bacillus, Pseudomonas, Staphylococcus, LAB, Yeasts* and *moulds.*

3.20 Germination of Sorghum

The red sorghum and white sorghum varieties (800g) were germinated on trays after steeping with the LAB isolates. Following steeping, the grains were then spread to a depth of approximately 1cm trays and incubated at 30^{o}C for 5days. Light watering was done at 12hrs interval and the grains were also being turned to ensure uniform aeration and temperature and also to ensure that the roots and the shootlets does not entangle to one another. After 5days, the germination process was terminated. This process is shown in figure 3.1.

3.21 Kilning of Malted sorghum

The malted grains were then dried using the oven at 50^{o}C for 24hrs.The moisture content was brought to about 4%. The rootlets and the shootlets were rubbed off through sieve and the malt was stored in airtight polythene bags and was kept in the desiccators until when required for further analyses (Demuyakor and Ohta, 1993).

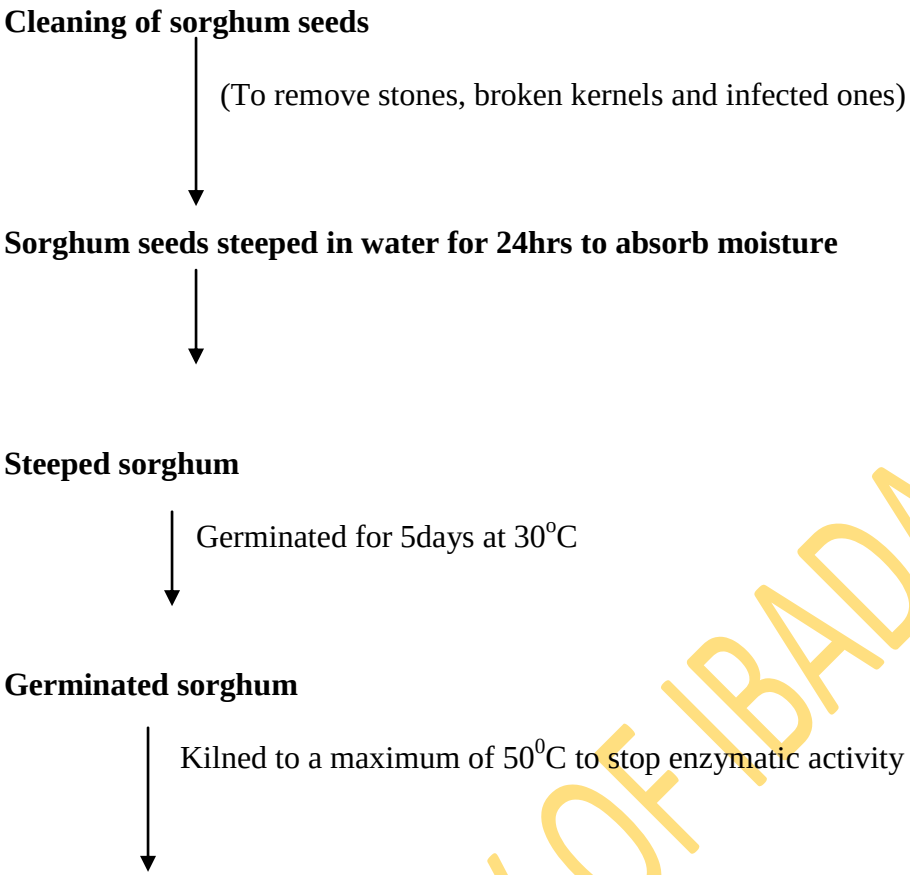

Figure 3.1 Outline of the processes involved in the malting of sorghum

3.22 Physico-chemical and nutritional analyses of malted sorghum samples challenged with Lactic acid bacteria

3.22.1 Measurement of the plumule and radicle length of sorghum

The length of the plumule and radicle of the germinated seeds were taken at random and were measured at intervals. Measurement was done by using a previously calibrated vernier caliper and the readings were recorded in centimeters (cm). The mean of the seeds were then calculated. This was done for both experimental and control seed samples.

3.22.2 Measurement of the weight of seeds

The weights of 50 seeds were taken at random and were then weighed at intervals using a calibrated weighing balance. The weight was taken two times and the mean weight was recorded in grams.

3.22.3 Measurement of pH

Ten grams (10g) of each malted sorghum was weighed in triplicates at intervals into a dry mortar and then ground into paste using pestle. 50mls of distilled water was added to the paste and mixed thoroughly to form slurry. The resulting suspension was decanted and the pH was determined using a previously calibrated digital pH meter (Hanna instrument). Readings were taken in triplicates and the mean value was calculated (Oyewole, 1990).

3.22.4 Determination of Moisture Content of malted sorghum

This was done according to A.O.A.C method (A.O.A.C., 1980). Five grams of each sample was weighed in triplicates into a pre-weighed porcelain crucible. The weight of the sample with the crucible was recorded. The crucibles containing the samples were placed in a pre-heated oven (Gallenkamp) dryer at 100^{o}C for 24hrs, after which they were removed and cooled to room temperature in desiccators. The final constant weight subtracted from the initial weight gave the moisture content, which was expressed in percentage.

3.22.5 Determination of Crude Fibre content of malted sorghum

Percentage crude fiber content determination was carried out using the gravimetric method of A.O.A.C (2002). Two grams of malted sorghum sample was weighed and carefully transferred into a clean sterile crucible. One hundred and fifty

mls of 0.128M H_2SO_4 previously heated in the reagent heating system was added to each sample. Few drops of octane was further added to the mixture which was boiled for thirty minutes and filtered through a Buchner funnel with the aid of a sunction pump. The residue was washed with hot deionised water until it was free of acid and 150mls of 0.22M KOH solution and few drops of octane was added to the residue in 400mls beaker.

The mixture was heated for thirty minutes with constant stirring, the content of the beaker was filtered through Buchner funnel and washed several times until it was free of KOH .The residue was further washed three times with acetone and transferred to a porcelain crucible and dried at 130^{o}C for 2hrs.This was cooled in a dessicator and weighed. The residue was transferred to a weighed dish and ignited in a muffle furnace at 500^{o}C for 3hrs. The dish and its content were cooled and weighed. The loss in weight represented the crude fibre content which was expressed as percentage of the original sample.

3.22.6 Total Carbohydrate Content of Malted Sorghum

The total carbohydrate contents were determined by difference (Bradford, 1976).

Carbohydrate content = (100(total dry weight) - moisture content + protein +fat +ash +crude fiber)

3.22.7 Gelatinization Temperature of Sorghum

Determination of gelatinization temperature of the malted sorghum was determined using a modification method of Ott (1987). Two grams of the sample was weighed into a 50ml graduated beaker containing 10ml distilled water and stirred to disperse. The beaker was heated in a water bath with continual string. A thermometer was inserted and used to determine the temperature of the waterbath. The temperature was regulated to between 60^{o}C and 100^{o}C using a regulator (Erweka Regulator, W. Germany). The temperature and time at which a gel is formed on cooling of the starch paste refers to the gelatinization temperature.

3.22.8 Enzymatic activities determination during the germination of sorghum

Ten gram of the malted sorghum was taken at intervals and was weighed and ground with pestle and mortar. Thereafter, 50mls of distilled water was added to the paste. The mixture was filtered using muslin cloth. The filtrate then served as crude enzyme which was used for the enzyme assay.

3.22.12 Protease assay

This was carried out using the method of Kunitz (1946). 1% (w/v) casein was prepared in 0.2M Phosphate buffer (pH 7.0). The substrate was heat- denatured at 100^{o}C in a water bath for 15minutes and was then allowed to cool before use. To 1ml of the casein solution in tubes was then added 0.5ml of enzyme extract. The solution was incubated in a water bath for 1hour at 35^{o}C. After incubation, the reaction was terminated by adding 3ml of cold 10% TCA. The tubes were then allowed to stand for 1hour at 4^{o}C to allow undigested protein to precipitate.

Control tubes contain 0.5ml of uninoculated MRS broth and casein 1% incubated for 1hour at 35^{o}C before adding 3mls of cold 10% TCA. The reaction mixtures were then centrifuged at 10,000rpm at 4^{o}C for 15minutes. The optical density reading of the carefully decanted supernatant fluid was measured with UV/Visible JENWAY 632OD Spectrophotometer at 660nm wavelength against a blank containing the control.

3.22.13 Amylase assay

Amylase activity of the LAB isolates was determined using 3, 5-Dinitrosalicyclic acid reagent method of Bernfeld (1955). One ml of culture that was centrifuged (supernatant) was added to 1ml of substrate (1.2% soluble starch (analar) in 0.1M Phosphate buffer, pH 6.0). The enzyme- substrate mixture was incubated in a waterbath set at 30^{o}C for 10minutes. The reaction was stopped by the addition of 0.5M Na0H. DNSA was added and boiled at 100°C. Thereafter, the solution was diluted with 18mls of distilled water and the absorbance read at 540nm using a UV/Visible JENWAY 632OD Spectrophotometer. One ml of uninoculated blank similarly treated was used to set the Spectrophotometer at zero.

3.23 α-Amylase Dextrinizing Power (DP) of malted sorghum

The method of Palmer (1997) was used. The crude substrate was prepared by mixing 1% starch with excess β-amylase dissolved in 20mM sodium acetate buffer containing 10mM $CaCl_2$ (pH 5.7). Five grams of milled malt of each sample was prepared by extracting in 80mls of extraction buffer (20mlM sodium acetate buffer pH 5.7). The mixture was shaken for 30mins and then centrifuged at 2,000rpm for 10mins at 4^{o}C (Superspeed Refrigerated DU). The supernatant was removed and diluted appropriately (X20), before 0.5ml of the enzyme extract was used in duplicate for assay. Substrate (0.5ml) was mixed with 0.5ml of the diluted enzyme and the mixture was allowed to digest for 5mins at 25^{o}C. Diluted iodine solution (10ml) 90.254g/l iodine in 4g/l potassium iodide) was added to the digest to stop the enzyme reaction. The colour of the iodine- dextrin complex was determined using a spectrophotometer at 565nm. Digests containing no substrate or no enzyme were also examined with iodine at 565nm.

The α- amylase Dextrinizing Power (DP) measured in Dextrin Units was calculated using the formula:

(DU) = A565nm (absorbance) units X 2 X Dilution

Where A (absorbance) units= A_{565} substance control – A_{565} Assay value.

3.24 β – Amylase (Diastatic Power –DP) of malted sorghum

This was determined with the Institute of Brewing (IOB) methods of analysis as described by American Society of Brewing Chemist (Anonymous, 1958). 25g of finely ground malt was mixed with 550ml of 0.5% Sodium chloride and allowed to stand for 2.5hrs at 20^{o}C with shaking at 20minutes interval. At the end of 2.5hours, the mixture was filtered using Whatman No.1 filter paper.

3.25 Antinutritional Analyses of Challenged Malted Sorghum

The antinutritional factors such as phytate, tannins and protease inhibitors was determined.

3.25.1 Estimation of phytic acid

Phytic acid was estimated by using the method of Davies and Reid (1979). One gram of malted sorghum was ground and extracted with HNO_3 a procedure

accompanied with continous shaking, the mixture was filtered and the filtrate made up to suitable volume with 20 mls of water. To 1.4ml of the filtrate was added 1ml of ferric ammonium sulphate solution (21.6mg in 100ml water), the content mixed and placed in a boiling water bath for 20mins.The content was later cooled and 5ml of isoamyl alcohol added and mixed. To this, 0.1ml ammonia solution was added, shaken thoroughly and centrifuged at 3000rpm for 10mins.The alcoholic layer was separated and the colour intensity was read at 465nm against amyl alcohol blank after 15mins. Sodium phytate standards were run along with the sample. The results were expressed as mg phytic acid/100g dry weight.

3.25.2 Estimation of Polyphenols content

Polyphenol substances were estimated by Folin- Denis method (A.O.A.C, 1984). About 200mg defatted sorghum sample was taken in a 250ml round bottomed flask and 100ml of 1% HCL in methanol was added. The contents were refluxed for 2hrs, cooled, filtered and the volume made up to 100ml with acid-methanol after few washings. 0.2ml of extract was taken and 7.5ml of water and 0.5ml of Folin-Denis reagent were added and mixed. To this, 1ml of saturated sodium carbonate solution was added and volume was measured at 760nm after 30mins. The results were calculated as mg tannic acid equivalents/g sample and expressed as mg/100 g dry weight.

3.25.3 Determination of protease inhibitors

Protease inhibitors expressed as Trypsin Inhibitory Activity was determined by the method of Roy and Rao (1971). The activity of the enzyme trypsin was assayed using casein as substrate and inhibition of this activity was measured in the malted sorghum extracts. 5grams of defatted, pulverized sorghum was treated with 50mls 0f 0.05M sodium phosphate buffer pH 7.5 and 50mls of distilled water. The samples were shaken for 3hours and centrifuged at 700 X 4rpm for 30mins at 15^{o}C. The supernatants were diluted in such a way that there was an inhibition between 40 and 60% of control enzyme activity. One trypsin unit is defined as an increase of 0.01 absorbance units at 280nm in 20min for 10ml reaction mixture under conditions

described and Trypsin Inhibitory Activity (TIA) as number of Trypsin Units Inhibited (TUI) and expressed as units /100g dry weight.

3.26 Fermentative characteristics of the wort derived from the test starters challenged sorghum

The fermentative characteristic of the wort was carried out in 3 stages: (1) Pre-fermentation stage (2) During fermentation (3) Post-fermentation. Samples were being taken at every stage of the fermentation process.

3.26.1 Production of Wort

This was carried out using the single infusion method (Hough *et al.*, 1971). 600g of sorghum malt was used to produce the wort and 200g of maize grits was used as adjunct and it was dissolved in 3000mls of water. The grains were milled using a hammer milling machine before it was used to prepare the wort. The milled sorghum malt was weighed into 3000mls of water and the temperature was maintained at 65^{o}C throughout the process. The mixture was then cooled and the supernatant from the spent grains were then removed by filtration. The resultant supernatant is the wort.

3.26.2 Wort Analyses

(a) Determination of total fermentable sugar of the wort

The total fermentable sugar (glucose, sucrose and maltose) of the wort produced from the sorghum was determined at intervals. Alcohol extract was prepared and clarified. Glucose, sucrose and maltose from the clarified alcoholic extract were separated by paper chromatography and estimated by phenol sulphuric acid method (McKelvy and Lee, 1969). The sugars on the strips were eluted with water and their concentrations estimated colourimetrically by using the modified phenol sulphuric acid method of Dubois *et al.* (1956). The results were expressed as g/100 dry weight.

(b) pH of the wort

The pH of the wort was determined at intervals. It was determined at the 3 stages of the fermentation process. The pH was determined using a previously calibrated pH meter (Hanna Instrument HI 8521) that was inserted into the wort and gently stirred until a stable pH reading were displayed.

(c) Total Residual Sugar

Distilled water (9mls) was added to the supernatant and vortexed. 0.2ml was then pipetted into a test tube and 0.8ml of distilled water,0.5ml of phenol and 2.5mls of concentrated H_2SO_4 was added and vortexed. The sample was then allowed to cool and the absorbance read using a spectrophotometer at wavelength of 490nm. A standard curve was made and it was from 0.01g of 1ml of glucose (Dubois *et al*., 1956).

Calculation:

% sugar= (Abs –Intercept) X Dilution factor X Volume

Weight of sample X Slope X10000

Abs=Absorbance

Dilution factor=depends on aliquot taken for assay

V=Volume

Slope=Slope of the standard curve

(d) Reducing Sugar

1ml of the wort was added to and mixed well with 1ml of the standard starch substrate and incubated at 30^{o}C for 5mins. The reducing sugar produced was determined by adding 2mls of DNSA reagents (Bernfeld, 1955).The mixture was heated in a boiling water bath for 5mins and it was cooled under running tap water. Thereafter, 20mls of distilled water was added. The absorbance was then read at 540nm with the aid of UV/Visible JENWAY 632OD spectrophotometer.

(e) Wort Titratable Acidity

20mls of the wort was pipetted into a clean flask and titrated against 0.1M NaOH using two drops of phenolphthalein indicator. The titer volume was multiplied by 0.09 to give percentage total titratable acidity (Vasconcelos *et al.,* 1990).This was done at intervals during the fermentation process. The acidity was expressed as g lactic acid/100g sample.

%TTA =100/VT X N

Where VT= Volume of sample titrates

N=Concentration

(f) Total Solids (TS) and Total Soluble Solids (TSS)

Brix measures the amount of dissolved solids, indicating the amount of sugar in wort and beer. It also indicates the rate of fermentation. These were determined by using a hand refractometer. The results were expressed as (% and Brix^{o}).

(g) Colour

Colour of the wort was determined using a Colorimeter. The colour was checked using UV spectrophotometer using (EBC, 1998) absorbance set at 430nm.

(h) Wort Protein Content

Kjedahl method of nitrogen/protein determination was used (A.O.A.C., 1980). For the protein digestion, 2 g of the dried sample and a half of selenium based catalyst tablet and a few anti-bumping H_2SO_4 was added and the flask shaken thoroughly to ensure the entire sample was wet. The flask was placed on a digestion burner and heated slowly until bubbling ceased and the resulting solution was clear. The flask was then made to cool to room temperature. The digestion sample solution was transferred into a 100 ml volumetric flask and made up to the mark. Two milliliters aliquot of each solution was then read at 540nm.

(i) Free Amino Nitrogen (FAN)

This was determined using Ninhydrin assay method of the Institute of brewing. Wort samples were diluted 100 times with distilled water. Exactly 1.0 ml of colour reagent (100g/l $NaHPO_4$, 5g/l ninhydrin and 3g/l fructose) was added to 2.0ml of the diluted sample in a glass tube with a screw cap. The sample was placed in a water bath (100^{o}C) for exactly 16mins. After cooling in a water bath (20^{o}C) for 20mins, 5.0 ml of a dilution reagent (2g KIO_3 in 1 liter H_2O/EtOH (600: 400, v/v) was added to the sample and thoroughly mixed. The absorbance of the sample was measured at 570nm. A blank (distilled water) and a glycine standard solution (10.72mg/L) were also analysed following the same procedure. A colour correction was included by taking into account the absorbance caused by coloured compounds (EBC, 1998).

(j) Viable Count

Samples were being plated out at intervals using yeast extract glucose agar. The yeast cells were being counted to check the viability of the cells.

3.26.3 Studies of fermentative characteristics

1. Preparation of the Yeast Inoculum

The yeast *(Saccharomyces uvarum*) that was used for the fermentation of the wort was maintained on yeast extract glucose agar (YEGA). It was then transferred into yeast extract glucose broth and incubated at 25^{o}C.The yeast cell samples were taken at 4hours interval and the number of cells present per ml of broth was determined using a Neubauer Hemocytometer.

2. Pitching of the yeast and fermentation of wort

The fermentation was carried out using a laboratory bioreactor and it was done by inoculating 2800mls of the wort with 200mls of the yeast. A bottom fermenting yeast *Saccharomyces uvarum* was used for the fermentation. The yeast was pitched at the rate of 7.0 X 10^4 yeast cells /mL and was then left to ferment for 5days at 30^{o}C without agitation. Samples were being taken on a daily basis from the fermenting wort for analyzing them for pH, specific gravity, yeast cell count, free amino nitrogen, colour and ethanol content. After fermentation, the yeast was separated from the beer by filtration using Millipore filter to remove the yeasts that have settled at the bottom. The beer was then sterilized by boiling.

3. Analyses of fermentation product

(a) Total sugar

Distilled water (9mls) was added to the supernatant and vortexed. 0.2ml was then pipetted into a test tube and 0.8ml of distilled water,0.5ml of phenol and 2.5mls of concentrated H_2SO_4 was added and vortexed. The sample was then allowed to cool and the absorbance read using a spectrophotometer at wavelength of 490nm.A standard curve was made and it was from 0.01g of 1ml of glucose.

Calculation:

% sugar= (Abs –Intercept) X Dilution factor X Volume

Weight of sample X Slope X10000

Abs=Absorbance

Dilution factor=depends on aliquot taken for assay

V=Volume

Slope=Slope of the standard curve

(b) pH

The pH of the wort was determined at intervals. It was determined at the 3 stages of the fermentation process. The pH determined using a previously calibrated pH meter (Hanna Instrument HI 8521) that was inserted into the wort and gently stirred until a stable pH reading were displayed.

(c) Ethanol Content and Specific Gravity

The percentage alcohol by volume and the specific gravity were determined by using wine and beer hydrometer. Specific gravity measures the density of the liquid wort and beer. Brewers utilize this measurement to indicate the amount of sugar in solution and to determine the rate of fermentation. Specific gravity was measured using a triple scale hydrometer. Approximately 237 ml of wort or beer was placed into a plastic cylinder (included with the hydrometer). The hydrometer was placed in the liquid within the column and gently spun to prevent the hydrometer from sticking to the side. The liquid level was read at eye level for all three scales. The alcohol content by volume was calculated.

Alcohol by volume is calculated as follows**:**

(Original specific gravity - final specific gravity) × 105 = % Alcohol by volume

Papazain (2003).

(d) Total solids

Brix measures the amount of dissolved solids, indicating the amount of sugar in wort and beer. It also indicates the rate of fermentation. These were determined by using a hand refractometer. The results was expressed as (%)

(e) Viable Counts and Yeast Count

Samples were being plated out at intervals using yeast extract glucose agar. The yeast cells were being counted to check the viability of the cells.

(f) Turbidity

Turbidity was determined using Spectrophotometer. The turbidity was checked using absorbance set at 540nm (A.O.A.C., 1990).

(g) Sensory Evaluation

Sensory evaluation of the fermented sorghum drink was carried out by a panel of ten untrained judges in the Department of Microbiology, U.I. Each panelist was requested to compare each of the samples with the control samples that were not challenged with the lactic acid bacteria (LAB) on the basis of appearance, taste, flavour or aroma, colour and overall acceptability of the product. The panelists were asked to score each characteristics on a Nine-point Hedonic scale (Larmond, 1977). Ratings ranged from '1' to '9' where '1' corresponds with dislike extremely and '9' with like extremely. The results obtained were subjected to analysis of variance using one way ANOVA. Differences between the means were separated using Duncan's multiple range test according to Duncan (1955).

CHAPTER FOUR

4.0 RESULTS

4.1 Isolation and characterization of lactic acid bacteria

A total of 127 strains of lactic acid bacteria (LAB) were isolated from the local and typed fermented cereal gruels. The lactic acid bacteria were identified as *Lactobacillus plantarum* (32), *Lactobacillus brevis* (31), *Lactobacillus fermentum* (25), *Lactobacillus delbrueckii* (8), *Lactobacillus casei* (12) and *Lactobacillus acidophilus* (19).

The lactic acid bacteria that were identified in the typed sorghum cereals were also the same with the ones identified in the local cereals.

The results of the biochemical tests showed that all the isolates were gram positive, catalase negative, non-spore forming and non-motile rods that existed singly or in chains. Nitrate reduction, hydrogen sulphide and Voges Proskauer tests were negative. Some strains hydrolysed starch. They were able to form acid from glucose and able to grow at 37^{o}C. They also tested positive to methyl red, starch hydrolysis and amylase production, negative for gelatin and casein hydrolysis. The isolates were facultative anaerobes and were fermentative (Table 4.1).

The frequency of occurrence of the LAB isolates is presented in Table 4.2. *Lactobacillus plantarum* was the most frequently occurred strain (25%) while *Lactobacillus delbrueckii* was the least occurred (6%).

4.2 Physiological studies on the LAB isolates

4.2.1 Effect of glucose on the growth of, and enzymes production by, the isolates

The growth of all the LAB isolates from local varieties (LV) increases with increased glucose concentration except for isolate WS_{10} (*Lactobacillus acidophilus* SL_2) which shows no difference in its response to the different glucose concentration. The least growth at 0.5 mg/ml of glucose was observed in isolate YM_{11} (*Lactobacillus casei* MZL_2) while the most favoured growth was observed in isolate WS_{10} (*Lactobacillus acidophilus* SL_2). Also, for the typed varieties (TV), the growth of the isolates increased with increase in glucose concentration with the exception of isolate

S/N	Isolates code	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Methyl Red Test	VP Test	Indole Test	Gelatin Hydrolysis Test	Casein Hydrolysis Test	Starch Hydrolysis Test	Nitrate Reduction Test	NH_3 from Arginine	Growth at 15^0C	Growth at 45^0C	pH 3.9	pH 9.2	Growth in 4% NaCl	Homo/Hetero fermentative test
1.	WS_1	+	rod	-	-	+	-	-	-	-	+	+	-	+	+	+	+	+	HE
2.	WS_2	+	rod	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	HE
3.	WS_3	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	(+)	+	+	+	HE
4.	WS_4	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	-	+	+	+	HE
5.	WS_5	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	HE
6.	WS_6	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE
7.	WS_7	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	+	+	+	+	HE
8.	WS_8	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	+	+	+	+	HE
9.	WS_9	+	rod	-	-	+	-	-	-	-	+	+NG	-	+	(+)	+	+	+	HM
10.	WS_{10}	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	-	+	+	+	HM
11.	WS_{11}	+	rod	-	-	+	-	-	-	-	+	-	-	+	+	+	+	+	HE
12.	WS_{12}	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HM
13.	WS_{13}	+	rod	-	-	+	-	-	-	-	+	-	-	+	-	+	+	+	HM
14.	WS_{14}	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HM
15.	WS_{15}	+	rod	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	HE
16.	WM_1	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	-	+	+	+	HE
17.	WM_2	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	HE
18.	WM_3	+	rod	-	-	+	-	-	-	-	+	+NG	-	(+)	+	+	-	+	HM
19.	WM_4	+	rod	-	-	+	-	-	-	-	+	+	+	(+)	+	+	+	+	HM
20.	WM_5	+	rod	-	-	+	-	-	-	-	+	-	-	(+)	+	+	+	+	HE
21.	WM_6	+	rod	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	HM
22.	WM_7	+	rod	-	-	+	-	-	-	-	+	+	-	+	-	+	+	+	HE
23.	WM_8	+	rod	-	-	+	-	-	-	-	+	+	-	+	+	+	+	+	HE

24.	WM_9	+	rod	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	HE
25.	WM_{10}	+	rod	-	-	+	-	-	-	-	+	+	+	-	+	+	+	+	HM
26.	YM_1	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	+	+	+	+	HM
27.	YM_2	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE
28.	YM_3	+	rod	-	-	+	-	-	-	-	+	(+)NG	-	+	+	+	+	+	HE
29.	YM_4	+	rod	-	-	+	-	-	-	-	+	+NG	+	-	+	+	+	+	HE
30.	YM_5	+	rod	-	-	+	-	-	-	-	+	+NG	-	-	+	+	+	+	HE
31.	YM_6	+	rod	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	HE
32.	YM_7	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HE
33.	YM_8	+	rod	-	-	+	-	-	-	-	+	+	+	-	+	+	+	+	HE
34.	YM_9	+	rod	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	HE
35.	YM_{10}	+	rod	-	-	+	-	-	-	-	+	+	+	-	+	+	+	+	HM
36.	YM_{11}	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	HM
37.	YM_{12}	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE
38.	YM_{13}	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HE
39.	YM_{14}	+	rod	-	-	+	-	-	-	-	+	-	-	-	+	+	+	+	HM
40.	YM_{15}	+	rod	-	-	+	-	-	-	-	+	+	-	+	-	+	+	+	HM
41.	YM_{16}	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE
42.	YM_{17}	+	rod	-	-	+	-	-	-	-	+	+	-	-	+	+	+	+	HM
43.	YM_{18}	+	rod	-	-	+	-	-	-	-	+	-	+	(+)	+	+	+	+	HE
44.	YM_{19}	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE
45.	YM_{20}	+	rod	-	-	+	-	-	-	-	+	-	-	(+)	+	+	+	+	HE
46.	YM_{21}	+	rod	-	-	+	-	-	-	-	+	+	-	+	+	+	+	+	HE
47.	YM_{22}	+	rod	-	-	+	-	-	-	-	+	+	+	-	+	+	+	+	HE
48.	MT_1	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HE
49.	MT_2	+	rod	-	-	+	-	-	-	-	+	+	-	(+)	(+)	+	+	+	HM
50.	MT_3	+	rod	-	-	+	-	-	-	-	+	-	-	+	-	+	-	+	HM
51.	MT_4	+	rod	-	-	+	-	-	-	-	+	+	-	(+)	+	+	+	+	HE
52.	MT_5	+	rod	-	-	+	-	-	-	-	+	-	-	+	-	+	+	+	HM
53.	MT_6	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE

54.	MT_7	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	-	+	HE
55.	MT_8	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HE
56.	MT_9	+	rod	-	-	+	-	-	-	-	+	-	-	+	(+)	+	-	+	HM
57.	MT_{10}	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	HM
58.	MT_{11}	+	rod	-	-	+	-	-	-	-	+	-	+	(+)	+	+	+	+	HE
59.	MT_{12}	+	rod	-	-	+	-	-	-	-	+	-	-	-	+	+	+	+	HM
60.	MT_{13}	+	rod	-	-	+	-	-	-	-	+	-	-	+	+	+	-	+	HM
61.	MT_{14}	+	rod	-	-	+	-	-	-	-	+	+	-	-	(+)	+	+	+	HE
62.	MT_{15}	+	rod	-	-	+	-	-	-	-	+	-	-	-	+	+	+	+	HM
63.	MT_{16}	+	rod	-	-	+	-	-	-	-	+	-	-	+	-	+	-	+	HM
64.	MT_{17}	+	rod	-	-	+	-	-	-	-	+	+	+	+	-	+	+	+	HE
65.	RS_1	+	rod	-	-	+	-	-	-	-	+	+NG	+	+	-	+	+	+	HE
66.	RS_2	+	rod	-	-	+	-	-	-	-	+	(+)NG	+	+	(+)	+	+	+	HM
67.	RS_3	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	HE
68.	RS_4	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	HE
69.	RS_5	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	-	+	HM
70.	RS_6	+	rod	-	-	+	-	-	-	-	+	-	-	-	+	+	+	(+)	HM
71.	RS_7	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	-	+	HE
72.	RS_8	+	rod	-	-	+	-	-	-	-	+	-	+	-	+	+	+	+	HE
73.	RS_9	+	rod	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	HE
74.	RS_{10}	+	rod	-	-	+	-	-	-	-	+	-	-	+	-	+	+	+	HM
75.	RS_{11}	+	rod	-	-	+	-	-	-	-	+	-	-	+	+	+	-	+	HE
76.	RS_{12}	+	rod	-	-	+	-	-	-	-	+	-	+	+	+	+	-	+	HE
77.	RS_{13}	+	rod	-	-	+	-	-	-	-	+	-	+	(+)	+	+	+	+	HE

S/N	Isolates code	Gram reaction	Cellular morphology	Catalase test	Methyl Red Test	Oxidase test	VP Test	Indole Test	Gelatin Hydrolysis Test	Casein Hydrolysis Test	Starch Hydrolysis Test	Nitrate Reduction Test	NH_3 from Arginine	Growth at 15^0C	Growth at 45^0C	pH 3.9	pH 9.2	Growth in 4% NaCl	Homo/Hetero fermentative test
1.	S-41$_1$	+	Rod	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	HM
2.	S-41$_2$	+	rod	-	+	-	-	-	-	-	+	+	+	-	+	-	+	+	HM
3.	S-41$_3$	+	rod	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	HM
4.	S-41$_4$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
5.	Ex-K$_1$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	-	+	+	HM
6.	Ex-K$_2$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
7.	Ex-K$_3$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	+	-	+	HM
8.	Ex-K$_4$	+	rod	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	HM
9.	Ex-K$_5$	+	rod	-	+	-	-	-	-	-	+	+	-	+	-	(+)	+	+	HM
10.	Ex-K$_6$	+	rod	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	HE
11.	Ex-K$_7$	+	rod	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	HE
12.	Ex-K$_8$	+	rod	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	HM
13.	Ex-K$_9$	+	rod	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	HM
14.	Ex-K$_{10}$	+	rod	-	+	-	-	-	-	-	+	+	(+)	-	+	+	(+)	+	HM
15.	Ex-K$_{11}$	+	rod	-	+	-	-	-	-	-	+	+	-	+	-	-	+	+	HM
16.	Ex-K$_{12}$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	+	+	+	HM
17.	Ex-K$_{13}$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
18.	Ex-K$_{14}$	+	rod	-	+	-	-	-	-	-	+	+	+	-	+	+	+	+	HM
19.	S-40$_1$	+	rod	-	+	-	-	-	-	-	+	-	-	-	+	-	+	+	HM
20.	S-40$_2$	+	rod	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	HM
21.	S-40$_3$	+	rod	-	+	-	-	-	-	-	+	-	-	+	-	+	+	+	HM
22.	S-40$_4$	+	rod	-	+	-	-	-	-	-	+	-	-	+	-	+	+	+	HM
23.	S-40$_5$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
24.	S-40$_6$	+	rod	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	HE
25.	S-40$_7$	+	rod	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	HM

26.	S-40$_8$	+	rod	-	+	-	-	-	-	-	+	+	-	+	(+)	+	+	+	HM
27.	S-40$_9$	+	rod	-	+	-	-	-	-	-	+	+	+	-	(+)	+	+	+	HM
28.	S-14$_1$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	+	+	+	HM
29.	S-14$_2$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
30.	S-14$_3$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
31.	S-14$_4$	+	rod	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	HE
32.	S-14$_5$	+	rod	-	+	-	-	-	-	-	+	-	-	+	-	-	+	+	HE
33.	S-14$_6$	+	rod	-	+	-	-	-	-	-	+	-	-	+	-	+	+	(+)	HM
34.	S-14$_7$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HM
35.	S-14$_8$	+	rod	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	HE
36.	S-14$_9$	+	rod	-	+	-	-	-	-	-	+	+(NG)	-	+	-	-	+	+	HM
37.	S-14$_{10}$	+	rod	-	+	-	-	-	-	-	+	+(NG)	-	+	-	+	+	+	HM
38.	S-14$_{11}$	+	rod	-	+	-	-	-	-	-	+	+(NG)	+	+	-	+	+	+	HM
39.	S-14$_{12}$	+	rod	-	+	-	-	-	-	-	+	-	-	-	+	+	+	+	HM
40.	S-14$_{13}$	+	rod	-	+	-	-	-	-	-	+	+(G)	-	+	-	+	+	+	HM
41.	S-14$_{14}$	+	rod	-	+	-	-	-	-	-	+	+(G)	-	+	-	+	+	+	HM
42.	S-14$_{15}$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	+	+	+	HM
43.	S-14$_{16}$	+	rod	-	+	-	-	-	-	-	+	-(G)	+	+	-	+	+	+	HE
44.	S-14$_{17}$	+	rod	-	+	-	-	-	-	-	+	+	-	+	-	+	+	+	HM
45.	ART$_1$	+	rod	-	+	-	-	-	-	-	+	+	-	-	+	+	+	+	HM
46.	ART$_2$	+	rod	-	+	-	-	-	-	-	+	+	-	+	-	+	+	+	HM
47.	ART$_3$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	+	+	+	HE
48.	ART$_4$	+	rod	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	HE
49	TZPB$_1$	+	rod	-	+	-	-	-	-	-	+	-	+	+	-	+	+	+	HE
50.	TZPB$_2$	+	rod	-	+	-	-	-	-	-	+	+	+	+	-	+	+	+	HM

S/N	Motility test	Glucose	Lactose	Mannitol	Galactose	Sucrose	Inositol	Arabinose	Xylose	Raffinose	Sorbitol	Fructose	Maltose	Probable Identity
1.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. plantarum*
2.	-	+	+	-	+	+	-	+	+	-	+	+	+	*L. delbrueckii*
3.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. casei*
4.	-	+	+	-	+	+	-	+	-	-	+	+	+	*L. acidophilus*
5.	-	+	+	+	+	+	-	+	+	-	+	+	+	*L. casei*
6.	-	+	+	-	+	+	-	+	+	+	+	+	+	*L. acidophilus*
7.	-	+	+	-	+	+	-	+	+	(+)	+	+	+	*L. plantarum*
8.	-	+	+	-	+	+	-	+	+	+	+	+	+	*L. plantarum*
9.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
10.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. brevis*
11.	-	+	+	-	+	+	-	+	+	+	+	+	+	*L. fermentum*
12.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. casei*
13.	-	+	+	+	+	+	-	+	+	(+)	-	+	+	*L. plantarum*
14.	-	+	+	-	+	+	-	+	+	+	-	+	+	*L. acidophilus*
15.	-	+	+	(-)	+	+	-	+	-	-	+	+	+	*L. casei*
16.	-	+	+	+	+	+	-	+	+	(+)	+	+	+	*L. plantarum*
17.	-	+	+	-	+	+	-	+	-	-	+	+	+	*L. delbrueckii*
18.	-	+	+	+	+	+	-	+	-	-	-	+	+	*L. acidophilus*
19.	-	+	+	+	+	+	-	+	-	-	+	+	+	*L. acidophilus*
20.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. acidophilus*
21.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
22.	-	+	+	+	+	+	-	+	-	+	+	+	+	*L. brevis*
23.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. acidophilus*
24.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. fermentum*
25.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*

26.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
27.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. acidophilus*
28.	-	+	+	+	+	+	-	+	-	-	+	+	+	*L. acidophilus*
29.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. acidophilus*
30.	-	+	+	+	+	+	-	+	-	-	+	+	+	*L. delbrueckii*
31.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. fermentum*
32.	-	+	+	-	+	+	-	+	+	+	-	+	+	*L. brevis*
33.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. plantarum*
34.	-	+	+	-	+	+	-	+	-	-	-	+	+	*L. delbrueckii*
35.	-	+	+	-	+	+	-	+	+	(+)	-	+	+	*L. fermentum*
36.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
37.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
38.	-	+	+	+	+	+	-	+	-	-	-	+	+	*L. casei*
39.	-	+	+	-	+	+	-	+	-	-	-	+	+	*L. acidophilus*
40.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
41.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
42.	-	+	+	+	+	+	-	+	-	+	+	+	+	*L. casei*
43.	-	+	+	+	+	+	-	+	+	+	(+)	+	+	*L. brevis*
44.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
45.	-	+	+	-	+	+	-	+	-	-	-	+	+	*L. delbrueckii*
46.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
47.	-	+	+	+	+	+	-	+	+	+	(+)	+	+	*L. brevis*
48.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. fermentum*
49	-	+	+	+	+	+	-	+	+	+	(+)	+	+	*L. brevis*
50.	-	+	+	+	+	+	-	+	-	-	-	+	+	*L. casei*

S/N	Motility test	Glucose	Lactose	Mannitol	Galactose	Sucrose	Inositol	Arabinose	Xylose	Raffinose	Sorbitol	Fructose	Maltose	Probable Identity
1.	-	+	+	+	+	+	-	-	-	+	-	+	+	*L. fermentum*
2.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. fermentum*
3.	-	+	+	+	+	+	-	-	-	+	+	+	+	*L. brevis*
4.	-	+	+	+	+	+	-	-	-	+	+	+	+	*L. brevis*
5.	-	+	+	+	+	+	-	-	(+)	+	-	+	+	*L. fermentum*
6.	-	+	+	-	+	+	-	+	-	-	-	+	+	*L. brevis*
7.	-	+	+	+	+	+	-	-	-	-	-	+	+	*L. brevis*
8.	-	+	+	+	+	+	-	-	+	-	+	+	+	*L. brevis*
9.	-	+	+	+	+	+	-	-	+	-	+	+	+	*L. plantarum*
10.	-	+	+	-	+	+	-	-	+	+	-	+	+	*L. acidophilus*
11.	-	+	+	-	+	+	-	-	+	+	-	+	+	*L. casei*
12.	-	+	+	+	+	+	-	+	-	+	+	+	+	*L. plantarum*
13.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
14.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
15.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. fermentum*
16.	-	+	+	+	+	+	-	-	-	-	-	+	+	*L. brevis*
17.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. fermentum*
18.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
19.	-	+	+	-	+	+	-	+	-	-	-	+	+	*L. delbrueckii*
20.	-	+	+	-	+	+	-	-	+	+	+	+	+	*L. acidophilus*
21.	-	+	+	(+)	+	+	-	+	+	+	+	+	+	*L. casei*
22.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. casei*
23.	-	+	+	+	+	+	-	-	+	-	-	+	+	*L. brevis*
24.	-	+	+	+	+	+	-	+	-	-	-	+	+	*L. brevis*
25.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*

26.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
27.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. brevis*
28.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. fermentum*
29.	-	+	+	-	+	+	-	-	+	-	-	+	+	*L. fermentum*
30.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. brevis*
31.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. fermentum*
32.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. brevis*
33.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. fermentum*
34.	-	+	+		+	+	-	+	+	-	-	+	+	*L. brevis*
35.	-	+	+		+	+	-	-	-	-	-	+	+	*L. delbrueckii*
36.	-	+	+		+	+	-	+	-	+	-	+	+	*L. casei*
37.	-	+	+		+	+	-	+	-	-	-	+	+	*L. brevis*
38.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. fermentum*
39.	-	+	+	-	+	+	-	-	-	-	-	+	+	*L. acidophilus*
40.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
41.	-	+	+	+	+	+	-	+	+	-	+	+	+	*L. brevis*
42.	-	+	+	-	+	+	-	-	-	(+)	+	+	+	*L. acidophilus*
43.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. fermentum*
44.	-	+	+	+	+	+	-	-	+	+	-	+	+	*L. brevis*
45.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. brevis*
46.	-	+	+	-	+	+	-	-	-	+	-	+	+	*L. fermentum*
47.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. brevis*
48.	-	+	+	-	+	+	-	+	-	+	-	+	+	*L. fermentum*
49	-	+	+	+	+	+	-	+	+	-	+	+	+	*L. plantarum*
50.	-	+	+	+	+	+	-	+	-	(+)	+	+	+	*L. plantarum*
51.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. brevis*
52.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
53.	-	+	+	+	+	+	-	+	-	+	+	+	+	*L. brevis*
54.	-	+	+	+	+	+	-	-	+	+	-	+	+	*L. brevis*
55.	-	+	+	+	+	+	-	+	+	-	-	+	+	*L. fermentum*

56.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
57.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. casei*
58.	-	+	+	+	+	+	-	+	-	-	+	+	+	*L. brevis*
59.	-	+	+	+	+	+	-	+	-	-	-	+	+	*L. acidophilus*
60.	-	+	+	-	+	+	-	+	-	+	-	+	+	*L. plantarum*
61.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. brevis*
62.	-	+	+	+	+	+	-	+	-	-	+	+	+	*L. acidophilus*
63.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
64.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. brevis*
65.	-	+	+	(+)	+	+	-	-	-	-	(+)	+	+	*L. brevis*
66.	-	+	+	+	+	+	-	-	-	-	+	+	+	*L. acidophilus*
67.	-	+	+	(+)	+	+	-	+	-	+	-	+	+	*L. fermentum*
68.	-	+	+	+	+	+	-	+	-	+	-	+	+	*L. fermentum*
69.	-	+	+	-	+	+	-	-	-	-	-	+	+	*L. delbrueckii*
70.	-	+	+	(+)	+	+	-	-	-	(+)	-	+	+	*L. acidophilus*
71.	-	+	+	-	+	+	-	+	(+)	+	-	+	+	*L. fermentum*
72.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. fermentum*
73.	-	+	+	-	+	+	-	+	+	+	-	+	+	*L. brevis*
74.	-	+	+	+	+	+	-	+	+	+	+	+	+	*L. plantarum*
75.	-	+	+	-	+	+	-	+	-	+	+	+	+	*L. fermentum*
76.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. fermentum*
77.	-	+	+	+	+	+	-	+	+	+	-	+	+	*L. brevis*

Table 4.2: FREQUENCY OF OCCURRENCE OF LAB ISOLATES OBTAINED FROM THE FERMENTED CEREAL GRUELS.

LAB ISOLATES	NUMBER OF ISOLATES	FREQUENCY OF OCCURRENCE (%)
Lb. plantarum	32	25
Lb. acidophilus	19	15
Lb. brevis	31	24
Lb. casei	12	10
Lb. delbrueckii	08	06
Lb. fermentum	25	20
TOTAL	127	100%

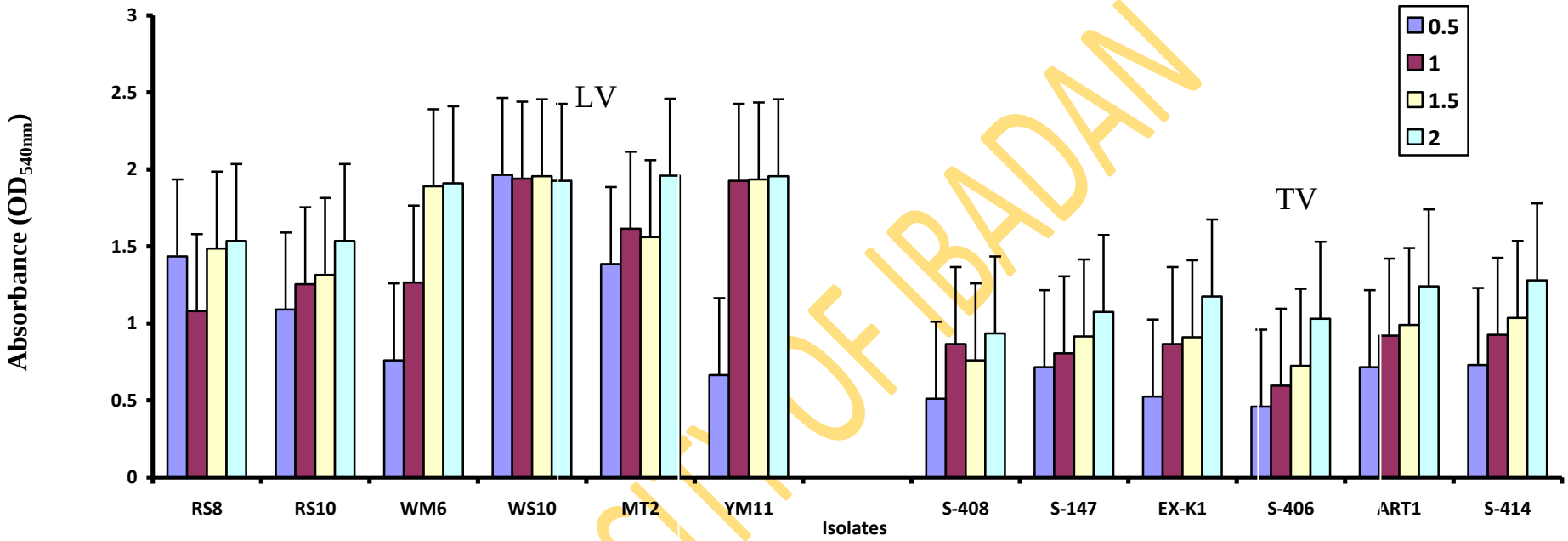

Fig 4.1: Effect of Glucose on the growth of the different LAB isolates obtained from gruels of local (LV)and typed(TV) sorghum and maize varieties

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

S-40(*Lactobacillus plantarum* ST1) in which a decrease was observed at glucose concentration of 1.5 mg/ml (0.80 OD) and later increased at 2.0 mg/ml. The most favoured growth was observed in isolate S-41_4 (*Lactobacillus acidophilus* ST1) at glucose concentration of 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml. Generally, the growth of LAB isolates obtained from gruels of local sorghum and maize varieties were most favoured at the different glucose concentrations used when compared with the isolates obtained from the typed cereals with glucose concentration of 2.0 mg/ml being the best concentration for all the isolates (Figure 4.1).

Increase in glucose concentration from 0.5 mg/ml to 1.0 mg/ml increased amylase production in all the LAB isolates from local varieties (LV) except for isolate WM_6 (*Lactobacillus casei* MZL_1). Amylase production increases with increase in glucose concentration with isolates MT_2 (*Lactobacillus plantarum* MTL_2) and YM_{11} (*Lactobacillus casei* MZL_2). The highest amylase production was observed at 1.0 mg/ml glucose concentration in isolates RS_8 (*Lactobacillus fermentum* SL_2), RS_{10} (*Lactobacillus fermentum* SL_1) and WS_{10} (*Lactobacillus acidophilus* SL_2) and at 2.0 mg/ml glucose concentration in isolates RS_8 (*Lactobacillus fermentum* SL_2), WM_6 (*Lactobacillus fermentum* SL_2) and MT_2 (*Lactobacillus plantarum* MTL_2). For the typed varieties (TV), amylase production by all the isolates increased as the glucose concentrations increased from 0.5 mg/ml to 1.0 mg/ml and drastically decreased at 1.5 mg/ml and thereafter increased at 2.0 mg/ml. Generally, glucose concentration of 1.0 mg/ml favoured amylase production in most isolates obtained from local varieties while 2.0 mg/ml favoured amylase production most in all the isolates obtained from typed varieties except isolate S-41_4 (*Lactobacillus acidophilus* ST1) (figure 4.2).

For the local varieties (LV), it was observed that increase in glucose concentration from 0.5 mg/ml to 2.0 mg/ml favoured the production of protease by the LAB isolates except WS_{10} (*Lactobacillus acidophilus* SL_2) which had a reduction in protease at glucose concentration of 2.0 mg/ml (1.00 Units/ml). In all the isolates obtained from local varieties, isolate MT_2 (*Lactobacillus plantarum* MTL_2) had the least production at the lower glucose concentration (0.5 mg/ml) and also the highest protease production at the highest glucose concentration (2.00 Units/ml) at 2.0 mg/ml respectively. The least protease production and the highest protease production at glucose concentrations 0.5 mg/ml and 1.0 mg/ml respectively were recorded in isolate

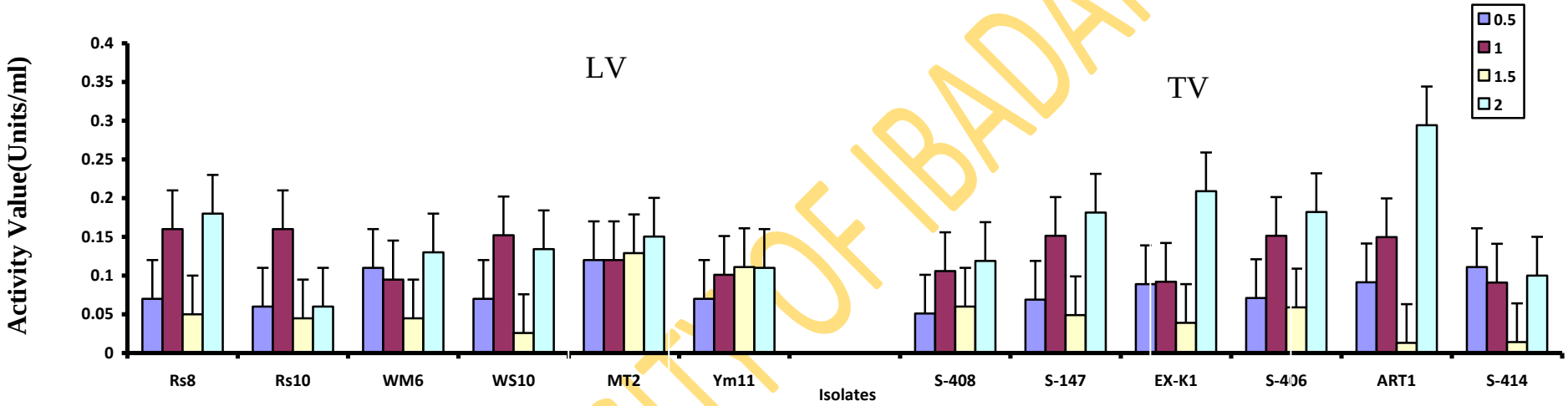

Figure 4.2: Effect of Glucose on Amylase production by the different LAB isolates obtained from gruels of local (LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

MT_2 (*Lactobacillus plantarum* MTL_2) while isolate WS_{10} (*Lactobacillus acidophilus* SL_2) also from local varieties shows little or no difference in protease production as glucose concentration increases. For the typed varieties (TV), there was no difference in the production of protease enzyme by all the isolates except for isolate Ex-K_1 (*Lactobacillus casei* ST_1) which had highest protease production (1.60 Units/ml) at glucose concentration of 0.5 mg/ml and the least production (0.50 Units/ml) by S-41_4 (*Lactobacillus acidophilus* ST_1). Overall, glucose concentration of 2.0 mg/ml favoured protease production by the LAB isolates obtained from both the local varieties and the typed varieties with the exception of isolate Ex-K_1, which had optimum protease production at 0.5 mg/ml of glucose concentration (figure 4.3)

Figure 4.4 shows the effect of glucose on invertase production by the different LAB isolates obtained from gruels of local and typed sorghum and maize varieties. It was observed that increase in glucose concentration led to an increase in invertase enzyme production by the LAB isolates obtained from both the local and the typed varieties. For the local varieties, glucose concentration of 0.5 mg/ml and 1.0 mg/ml favoured the production of invertase in isolate YM_{11} (*Lactobacillus casei* MZL_2) with values of 0.70 Units/ml and 0.86 Units/ml respectively. 0.5 mg/ml glucose produced better invertase than 1.0 mg/ml. The least invertase production at glucose concentration of 0.5 mg/ml was observed in RS_8 (*Lactobacillus fermentum* SL_2) with value of 0.52 Units/ml. Also for the typed varieties, at glucose concentration of 2.0 mg/ml, invertase was produced most in Ex-K_1 (*Lactobacillus casei* ST_1) with value of 0.90 Units/ml and the least was found in ART_1 (*Lactobacillus delbrueckii* MZT_2) with value of 0.59 Units/ml. At glucose concentration of 1.5 mg/ml, Ex-K_1 was found to produce invertase most (0.75 Units/ml) while ART_1 (*Lactobacillus delbrueckii* MZT_2) the least (0.58 Units/ml). For both local and typed varieties, invertase production by the LAB isolates increases as glucose concentration increases. Invertase production by all the isolates were favoured with increased glucose concentration except for isolate ART_1 (*Lactobacillus delbrueckii* MZT_2) obtained from typed varieties.

4.2.2 Growth and enzymes production by the LAB isolates at different concentration of Magnesium sulphate

Increase in Magnesium sulphate concentrations favoured the growth of the different LAB isolates obtained from gruels of both local varieties (LV) and typed

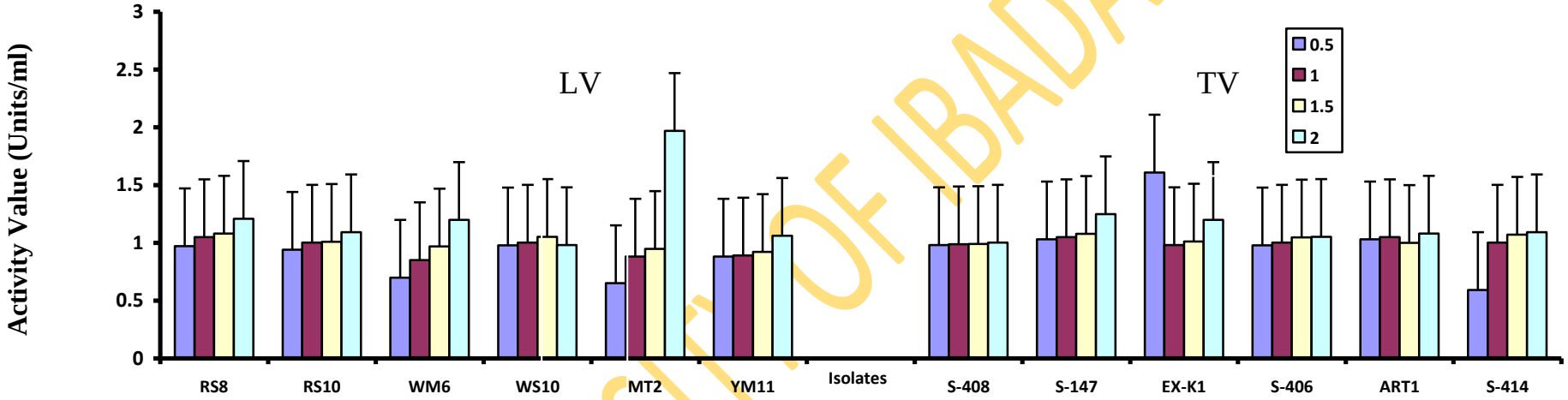

Figure 4.3: Effect of Glucose on Protease production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_5$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

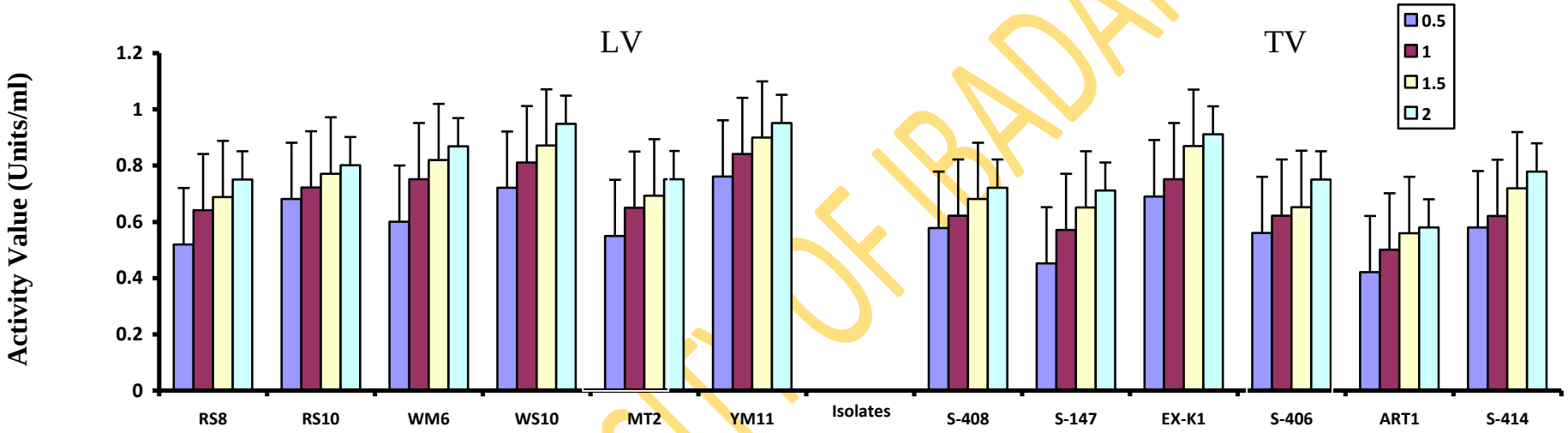

Figure 4.4: Effect of Glucose on Invertase production by the different LAB isolates obtained from gruels of local (LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

varieties (TV) but isolates from local varieties were most favoured. As the concentration of Magnesium sulphate increases, growth increases except for isolate MT_2 (*Lactobacillus plantarum* MTL_2) where growth decreases at 1.0 mg/ml of $MgS0_4$ concentration and later increased as concentration increases. Similarly, the growth of typed varieties isolates also increases as $MgS0_4$ increases but the local varieties were most favoured. Isolate RS_8 (*Lactobacillus fermentum* SL_2) from local varieties and isolate S-41_4 (*Lactobacillus acidophilus* ST1) from typed varieties had the least growth at the least Magnesium sulphate (0.5 mg/ml) concentration while the highest growth at the highest concentration of Magnesium sulphate (2.0 mg/ml) was observed in isolate RS_8 (*Lactobacillus fermentum* SL_2), WS_{10} (*Lactobacillus acidophilus* SL_2), MT_2 (*Lactobacillus plantarum* MTL_2) and YM_{11} (*Lactobacillus casei* MZL_2) of local varieties. In all, the optimum concentration of Magnesium sulphate that favoured the growth of the LAB isolates from both local and typed varieties was 2.0 mg/ml (figure 4.5).

Increase in Magnesium sulphate concentrations favoured amylase production in the LAB isolates obtained from local varieties (LV) (figure 4.6). Isolate RS_8 (*Lactobacillus fermentum* SL_2) produced the least amylase (0.40 Units/ml) at Magnesium sulphate concentration of 0.5 mg/ml while the highest amount (0.55 Units/ml) of amylase was by isolate MT_2 (*Lactobacillus plantarum* MTL_2). At 2.0 mg/ml of Magnesium sulphate concentration, isolates RS_{10} (*Lactobacillus plantarum* SL_1) and MT_2(*Lactobacillus plantarum* MTL_2) recorded the highest amylase production of 0.60 Units/ml while the least production was recorded in isolate RS_8(*Lactobacillus fermentum* SL_2) (0.41 Units/ml). For the typed varieties (TV), Magnesium sulphate concentration of 2.0 mg/ml favoured the production of amylase most in isolates S-40_8 (*Lactobacillus plantarum* ST1) (0.57 Units/ml) followed by S-14_7 (*Lactobacillus delbrueckii* ST1) (0.53 Units/ml) while ART_1 (*Lactobacillus delbrueckii* MZT2) produced the least quantity (0.37 Units/ml). At Magnesium sulphate of 1.0 mg/ml concentration, the production of amylase was highest in isolate S-40_8 (0.52 Units/ml) and the least was recorded in isolate ART_1 (*Lactobacillus delbrueckii* MZT2) (0.28 Units/ml). The Magnesium sulphate concentrations favoured the production of amylase most in isolates S-40_8 (*Lactobacillus plantarum* ST1) and S-14_7(*Lactobacillus delbrueckii* ST1). Overall, 2.0 mg/ml of Magnesium sulphate concentration increased amylase production of the LAB isolates obtained from both local and typed sorghum and maize varieties.

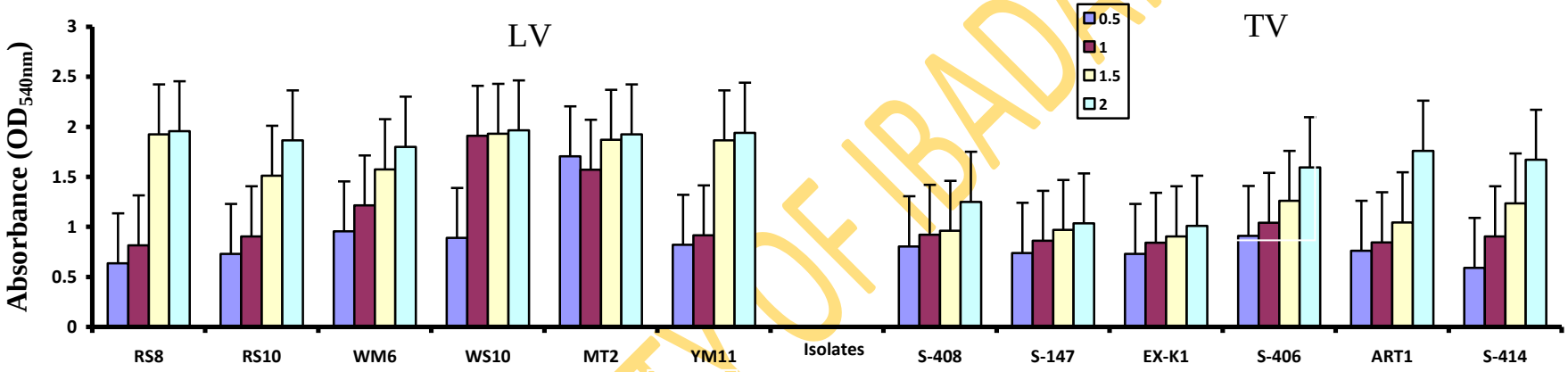

Figure 4.5: Effect of Magnesium sulphate on the growth of different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

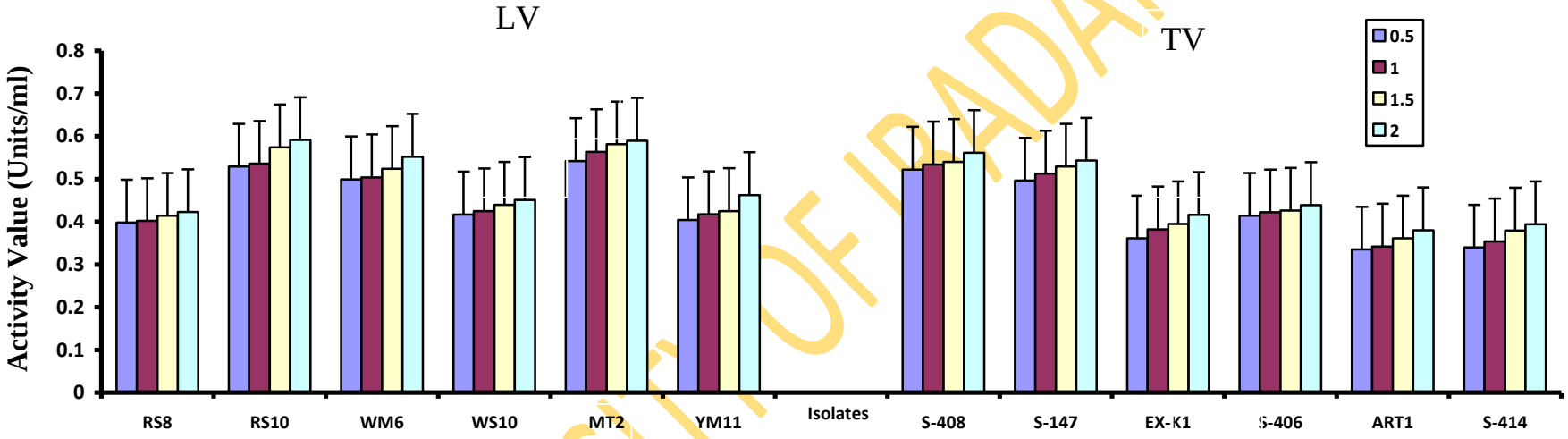

Figure 4.6: Effect of Magnesium sulphate on Amylase production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

The effect of Magnesium sulphate on protease production by the LAB isolates obtained from both local (LV) and type (TV) of sorghum and maize varieties is as shown in figure 4.7. From the figure, isolate RS_{10} (*Lactobacillus plantarum* SL_1) of local varieties (LV) recorded the highest protease production at all concentrations of Magnesium sulphate while isolate ART_1 (*Lactobacillus delbrueckii*MZT2) of typed varieties (TV) shows the least protease production. Generally, protease production by all the isolates increases as the concentration of Magnesium sulphate increases.

Figure 4.8 shows the effect of Magnesium sulphate on invertase production by the different LAB isolates obtained from gruels of local and typed sorghum and maize varieties. Invertase production by all the LAB isolates increased as Magnesium sulphate concentration increases. At all concentrations of Magnesium sulphate, invertase production by isolate RS_{10} (*Lactobacillus plantarum* SL_1) was the highest while the least production at all concentrations was recorded in isolate YM_{11} (*Lactobacillus casei* MZL_2) both of local varieties (LV). For typed varieties (TV) isolates, invertase production was most favoured at different concentrations of Magnesium sulphate by isolates S-40_8(*Lactobacillus plantarum* ST1) and S-14_7 (*Lactobacillus delbrueckii* ST1) while there was little or no difference in the quantities produced by the other isolates.

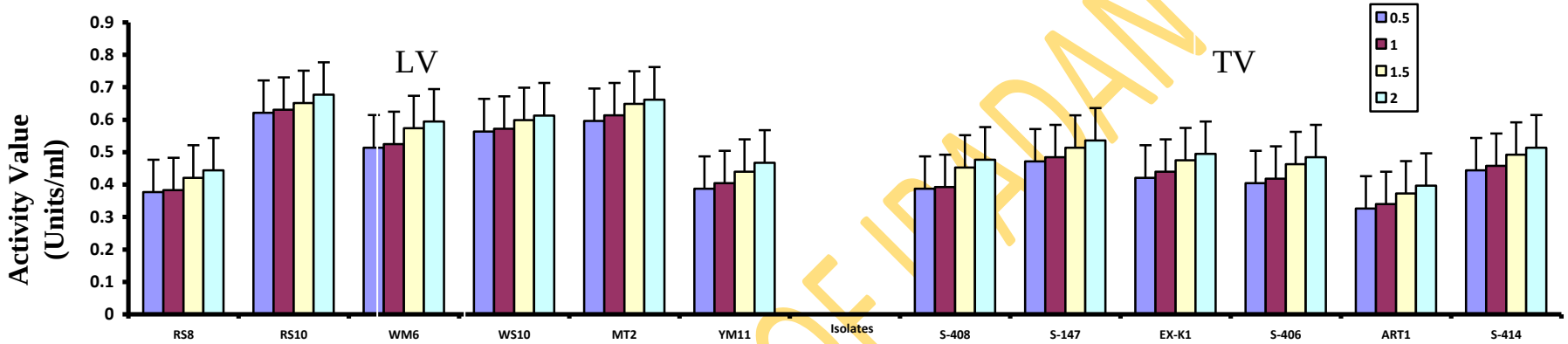

Figure 4.7: Effect of Magnesium sulphate on Protease production by the different LAB isolates obtained from gruels of local (LV)and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

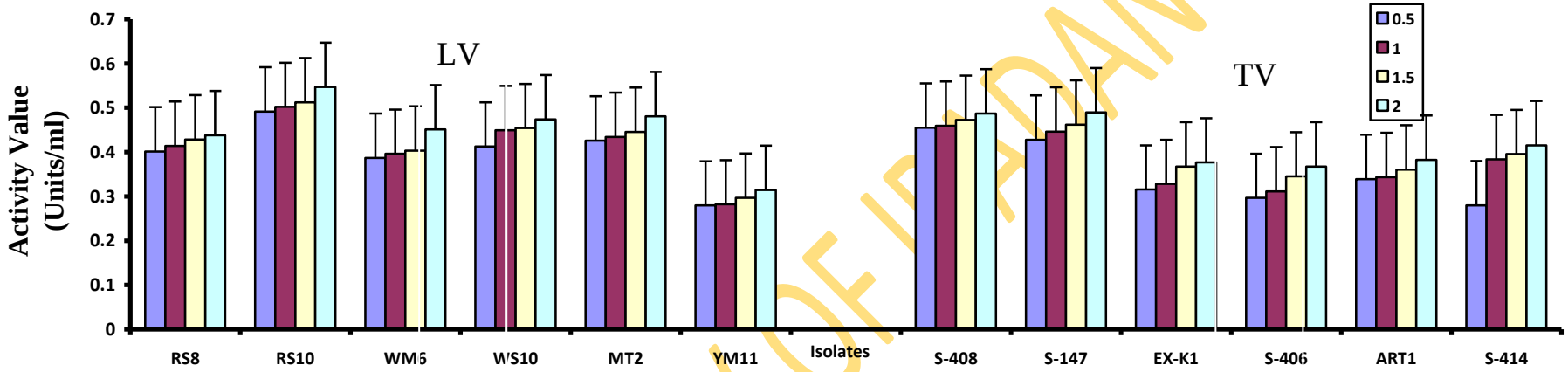

Figure 4.8: Effect of Magnesium sulphate on Invertase production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

4.2.3 Growth and enzymes production by the LAB isolates at different pH

The effect of pH on the growth of the different LAB isolates obtained from gruels of local and typed sorghum and maize varieties is shown in figure 4.9. All the isolates from both local varieties (LV) and typed varieties (TV) gruels had the highest growth at pH 9.2 except isolate RS_8 (*Lactobacillus fermentum* SL_2). At pH 3.9, isolates WS_{10} (*Lactobacillus acidophilus* SL_2) and YM_{11} (*Lactobacillus casei* MZL_2) had the highest growth while the highest growth rate at pH 5.0 was recorded in isolate ART_1 (*Lactobacillus delbrueckii* MZT_2) of typed varieties. At all pH used except 9.2, isolate ART_1 (*Lactobacillus delbrueckii* MZT2) of typed varieties had the highest growth compared to the other isolates while the least growth was recorded by isolate YM_{11} (*Lactobacillus casei* MZL_2). In general, pH of between 5.0 – 9.2 supported the growth of the LAB isolates but pH 9.2 was found to be the best pH for the growth of the LAB isolates obtained from both local and typed sorghum and maize varieties.

For the local varieties (LV), isolate MT_2 (*Lactobacillus plantarum* MTL_2) produced the highest quantity of protease (2.40 Units/ml) at pH 6.0 and WM_6 (*Lactobacillus casei* MZL_1) produced the least (1.00 Units/ml) (figure 4.10). pH 5.5 and 6.0 favoured protease production most in isolates WS_{10} (*Lactobacillus acidophilus* SL_2), MT_2 (*Lactobacillus plantarum* MTL_2) and YM_{11} (*Lactobacillus casei* MZL_2). At all the pH ranges used, isolate RS_8 (*Lactobacillus fermentum* SL_2) produced the least protease with highest protease (1.48 Units/ml) being produced at pH 5.0 and lowest (1.10 Units/ml) at pH 9.2. At pH 3.9, highest protease production (2.15 Units/ml) was recorded by isolate YM_{11} (*Lactobacillus casei* MZL_2) and the least was observed in isolate RS_8 (*Lactobacillus fermentum* SL_2) with value of 1.10 Units/ml. Also for the typed varieties (TV), isolate Ex-k_1 (*Lactobacillus casei* ST1) produced protease best at the different pH ranges while isolate S-41_4 (*Lactobacillus acidophilus* ST1) produced the least. At pH 6.0, isolates Ex-k_1 (*Lactobacillus casei* ST1), S-40_6 (*Lactobacillus fermentum* ST2), ART_1 (*Lactobacillus delbrueckii* MZT2), S-41_4 (*Lactobacillus acidophilus* ST1) and S-40_8 (*Lactobacillus plantarum* ST1) produced protease most and S-14_7 (*Lactobacillus delbrueckii* ST1) at pH 5.5. In all the LAB isolates, pH 5.5 and 6.0 was found to be the best pH that favoured protease production by the isolates obtained from both local and typed sorghum and maize varieties.

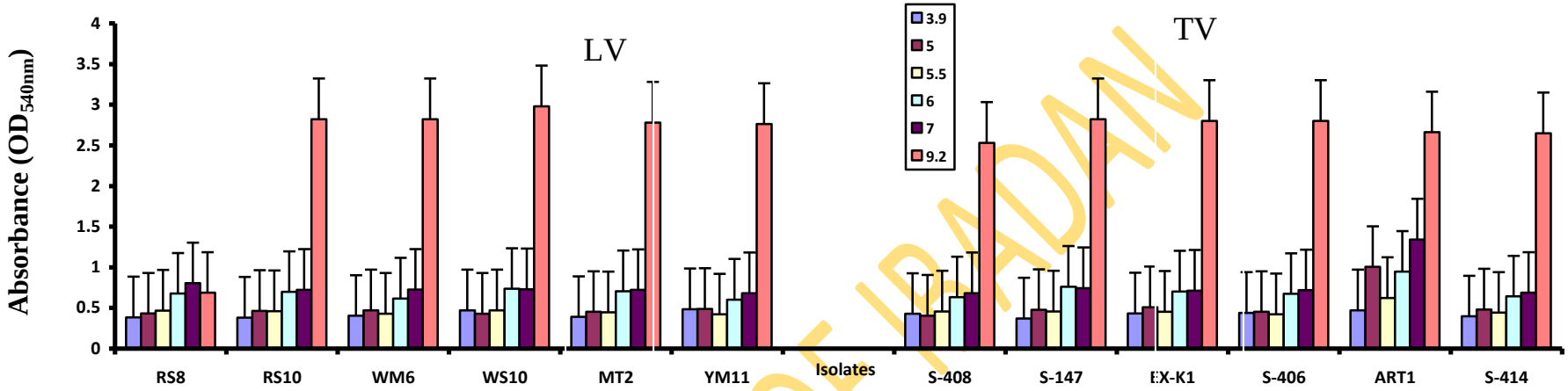

Figure 4.9: Effect of pH on the growth of the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

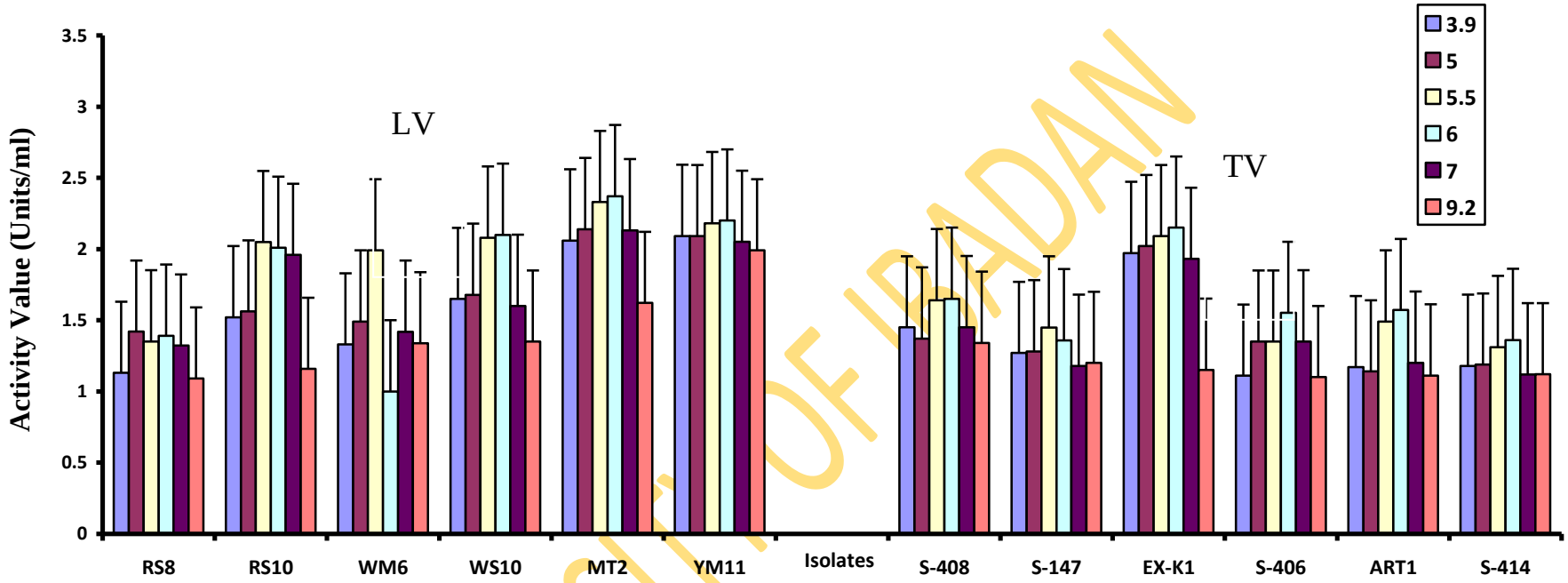

Figure 4.10: Effect of pH on the production of protease by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

The effect of pH on the production of invertase by the LAB isolates obtained from gruels of local and typed sorghum and maize varieties is as shown below (figure 4.11). For the local varieties (LV), pH 3.9, isolate RS_8 (*Lactobacillus fermentum* SL_2) produced the highest invertase (1.30 Units/ml) while isolate RS_{10} (*Lactobacillus plantarum* SL1) produced the least invertase (0.70 Units/ml). As pH increases from 3.9 to 6.0, invertase production by local varieties isolates increased and later decreased till pH 9.2 except isolate WS_{10} (*Lactobacillus acidophilus* SL_2) which increased up to pH 5.0 and decreased thereafter. pH 6.0 favoured invertase production in all the isolates from local varieties except isolates WS_{10} (*Lactobacillus acidophilus* SL_2) and YM_{11} (*Lactobacillus casei* MZL_2). Also for the typed varieties (TV), isolate S-41_4 (*Lactobacillus acidophilus* ST1) recorded the highest (1.60 Units/ml) invertase production at pH 6.0 while isolate S-40_6 (*Lactobacillus fermentum* ST2) had the least invertase (0.72 units/ml) production. It was observed that pH 6.0 favoured invertase production in all the isolates from typed varieties except isolate Ex-k_1 (*Lactobacillus casei* ST1) which best production occurred at pH 9.2. Overall, pH 5.5 and 6.0 was the optimum pH that favoured invertase production in isolates obtained from both local and typed varieties.

For the local varieties (LV), the highest production of amylase (4.30 Units/ml) by isolate RS_8 (*Lactobacillus fermentum* SL_2) was recorded at pH 7.0 while the least production (1.90 Units/ml) was recorded by isolate WS_{10} (*Lactobacillus acidophilus* SL_2) at pH 3.9. For isolate RS_{10} (*Lactobacillus plantarum* SL_1), highest amylase production (3.80 Units/ml) was observed at pH 6.0 and the least (2.70 Units/ml) at pH 5.5. Amylase production increased from pH 3.9-6.0 in isolate WS_{10} (*Lactobacillus acidophilus* SL_2) and later decreased at pH 7.0 thereafter, increased at pH 9.2. Similarly, isolate YM_{11} (*Lactobacillus casei* MZL_2) increased in amylase production as pH increased to 5.5 and decreased at pH 6.0 and later increased from pH 7.0 to 9.2. Isolate MT_2 (*Lactobacillus plantarum* MTL_2) had its optimal amylase production (3.70 Units/ml) at pH 3.9 and production decreased thereafter. For the typed varieties (TV), the optimum production of amylase (4.60 Units/ml) by isolate S-41_4 (*Lactobacillus acidophilus* ST1) was recorded at pH 6.0 while the least production (2.20 Units/ml) was observed at pH 7.0. Isolate S-40_8 (*Lactobacillus plantarum* ST1) produced the lowest quantity of amylase (1.70 Units/ml) at pH 5.5 and the maximum amylase production (3.50 Units/ml) was at pH 5.0. All the LAB isolates obtained

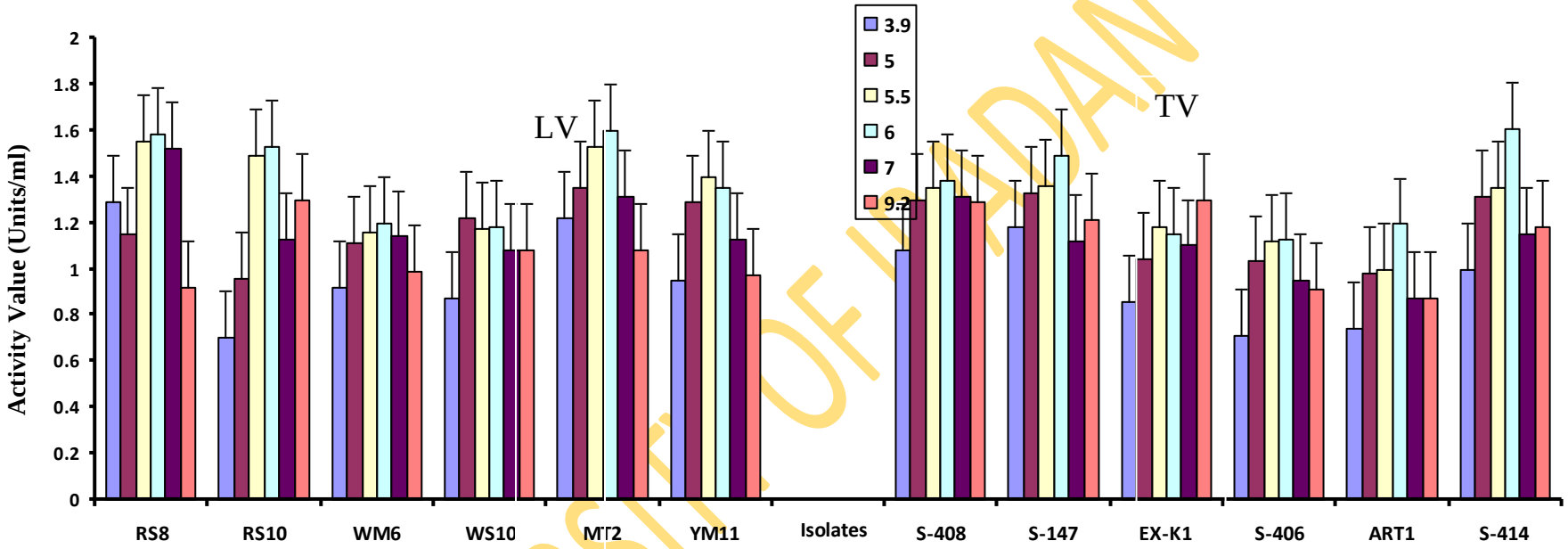

Figure 4.11: Effect of pH on the production of Invertase by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

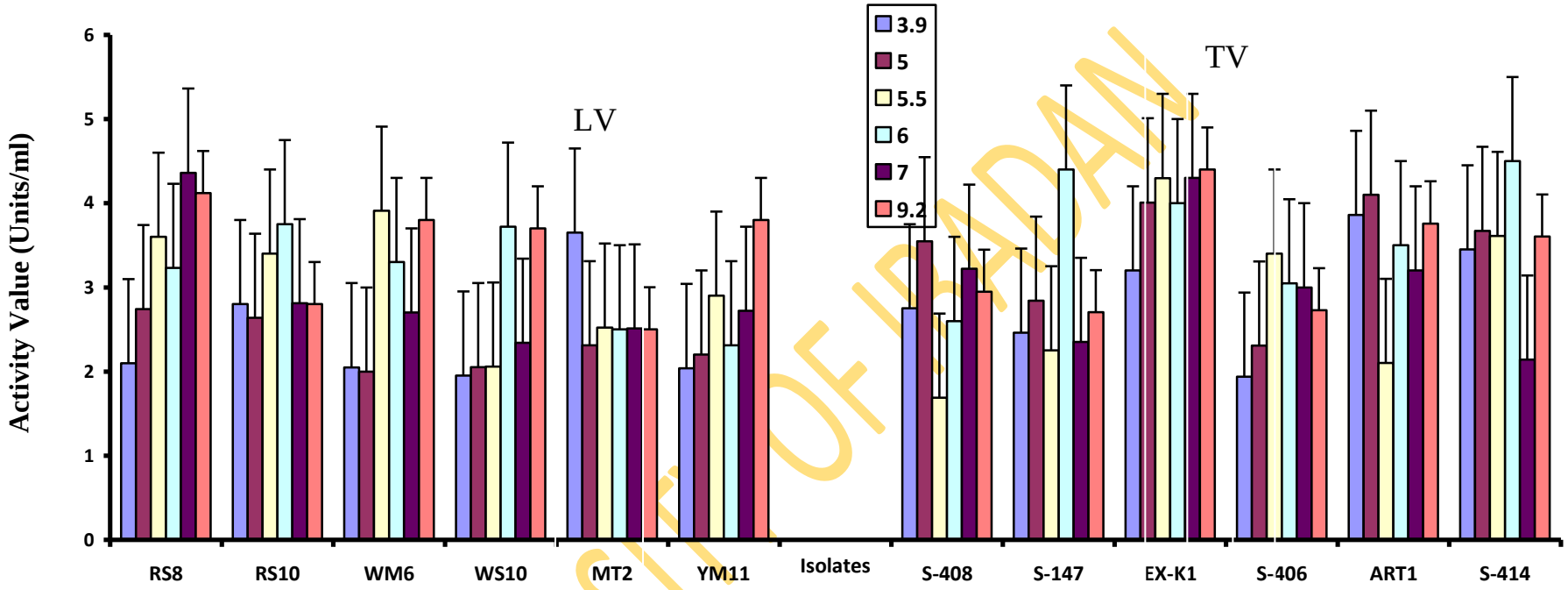

Figure 4. 12: Effect of pH on the production of Amylase by the different LAB isolates obtained from gruels of local (LV)and typed(TV) sorghum varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

from both local and typed varieties were able to produce amylase between the pH range of 5.0-9.2 except isolate MT_2 (*Lactobacillus plantarum* MTL_2) which produced amylase best at pH 3.9 (figure 4.12).

4.2.4 Growth and enzymes production by the LAB isolates at different concentrations of peptone

For figure 4.13, increase in peptone concentrations from 0.5 mg/ml to 2.0mg/ml increased amylase production in all the LAB isolates obtained from local varieties (LV). Isolate MT_2 (*Lactobacillus plantarum* MTL_2) produced the highest amylase at the different peptone concentrations followed by isolate RS_{10} (*Lactobacillus plantarum* SL_1) while the lowest amylase production was observed in isolate YM_{11} (*Lactobacillus casei* MZL_2). For the typed varieties (TV), peptone concentration of 2.0 mg/ml favoured the production of amylase most in isolate S-40_6 (*Lactobacillus fermentum* ST2) (0.60 Units/ml) while Ex-k_1 (*Lactobacillus casei* ST1) produced the least amylase (0.49 Units/ml). At peptone concentration of 0.5 mg/ml concentration, amylase production was highest in isolate S-40_6 (*Lactobacillus fermentum* ST2) (0.57 Units/ml) and the least was recorded in isolates S-14_7 (*Lactobacillus delbrueckii* ST1) and Ex-k_1 (*Lactobacillus casei* ST1) with the same valueof0.48Units/ml.

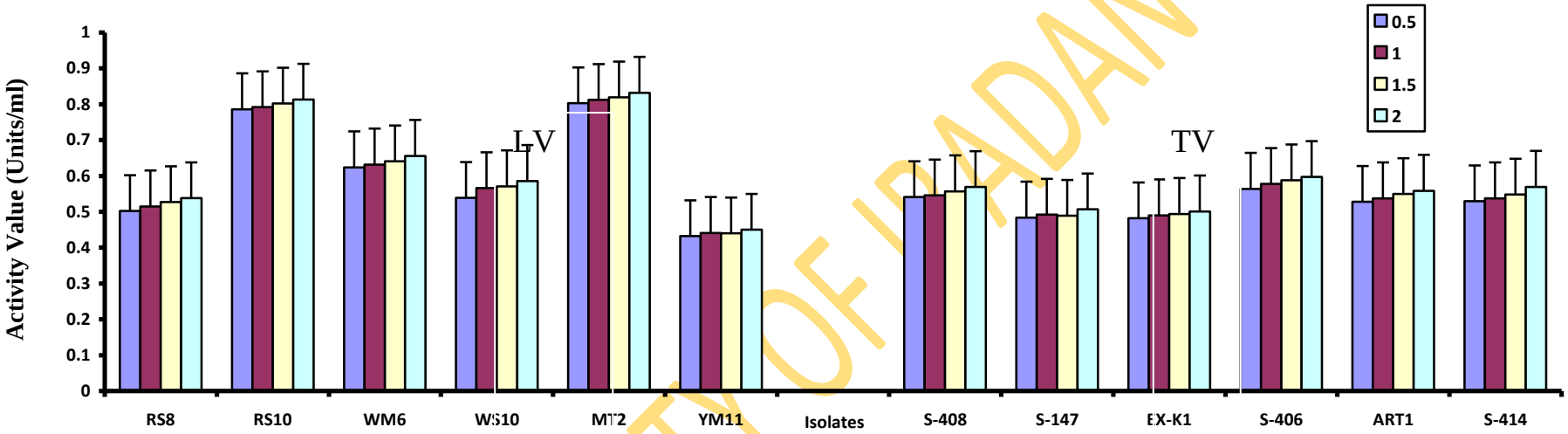

Figure 4.13: Effect of Peptone on Amylase production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

The effect of peptone on protease production by the different LAB isolates obtained from gruels of local and typed sorghum and maize varieties is as shown in figure 4.14. Isolate RS_{10} (*Lactobacillus plantarum* SL_1) of local varieties (LV) recorded the highest protease production at all the different concentrations of peptone while isolate YM_{11} (*Lactobacillus casei* MZL_2) produced the least. Also for the typed varieties (TV), protease production increased with increase in peptone concentrations with the exception of isolate Ex-k_1 (*Lactobacillus casei* ST1) in which there was a reduction in protease production at peptone concentration of 1.5 mg/ml and it increased at 2.0 mg/ml. Isolate S-40_8 (*Lactobacillus plantarum* ST1) was favoured most for protease production at the different peptone concentrations while the least was recorded in isolate ART_1 (*Lactobacillus delbrueckii* MZT2). In all, the addition of peptone favoured the production of protease by all the LAB isolates from both typed and local sorghum and maize varieties.

The effect of peptone on invertase production increases as the concentration of peptone increases from 0.5 mg/ml to 2.0 mg/ml by all the LAB isolates from both varieties (figure 4.15). Isolates RS_{10} (*Lactobacillus plantarum* SL_1) of local variety and isolate S-40_8 (*Lactobacillus plantarum* ST1) of typed variety were most favoured while the least production was recorded in isolate RS_8 (*Lactobacillus fermentum* SL_2) of local variety. Generally, the LAB isolates from both local and typed sorghum and maize varieties were able to utilize peptone at the different concentrations for invertase enzyme production.

Increase in peptone concentrations favoured the growth of the different LAB isolates obtained from gruels of both local varieties (LV) and typed varieties (TV). Isolates RS_8 (*Lactobacillus fermentum* SL_2) and WS_{10} (*Lactobacillus acidophilus* SL_2) from local varieties had the highest growth at the highest concentration of peptone (2.0 mg/ml) while the lowest growth was recorded by WS_{10} (*Lactobacillus acidophilus* SL_2) with optical density of 0.80 at the lowest peptone concentration of 0.5 mg/ml. Similarly, the growth of the LAB obtained from typed varieties (TV) also increases as peptone concentration increases. Isolates S-41_4 (*Lactobacillus acidophilus* ST1) was observed to grow best at peptone concentration of 2.0 mg/ml (1.55 OD) while isolate S-40_8 (*Lactobacillus plantarum* ST1) recorded the least growth of 1.20 OD. At peptone concentration of 1.0 mg/ml, S-41_4 (*Lactobacillus*

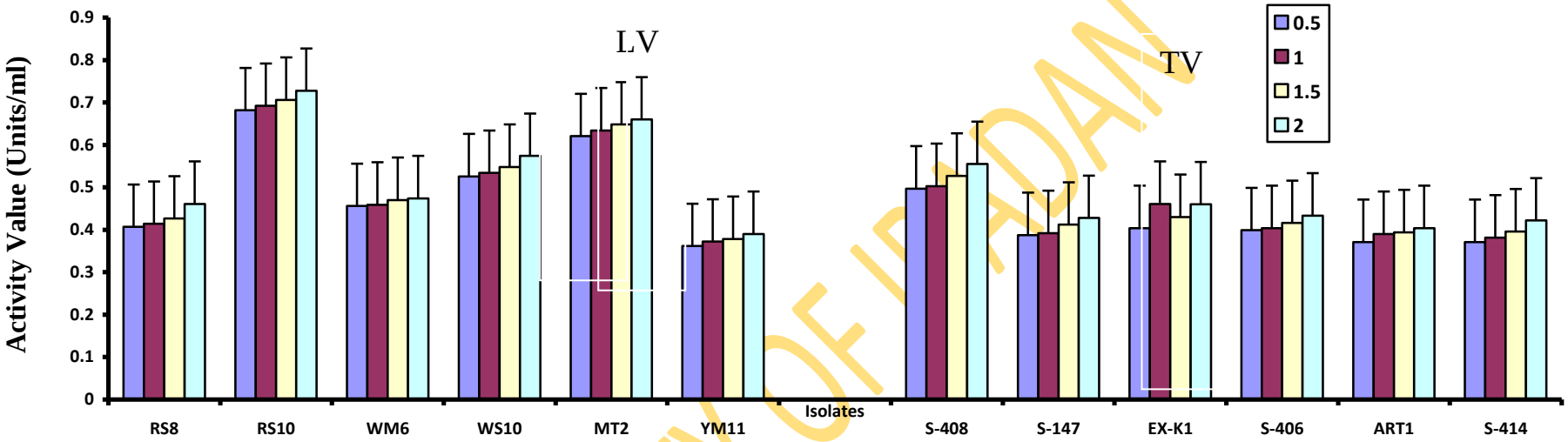

Figure 4.14: Effect of Peptone on Protease production by the different LAB isolates obtained from gruels of local (LV)and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

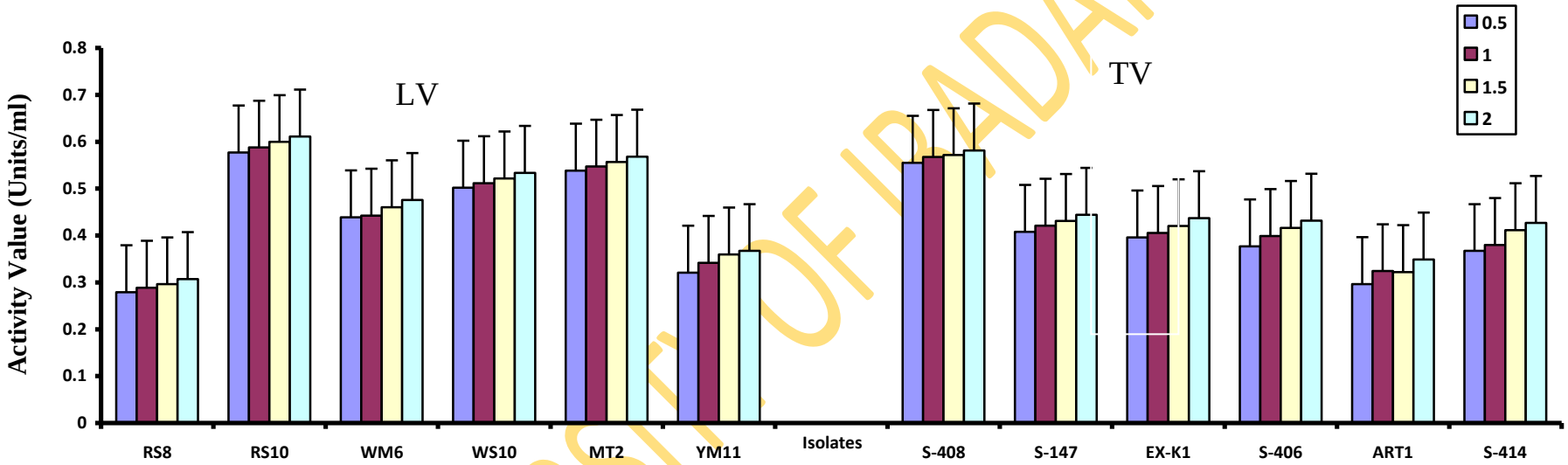

Figure 4.15: Effect of Peptone on Invertase production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

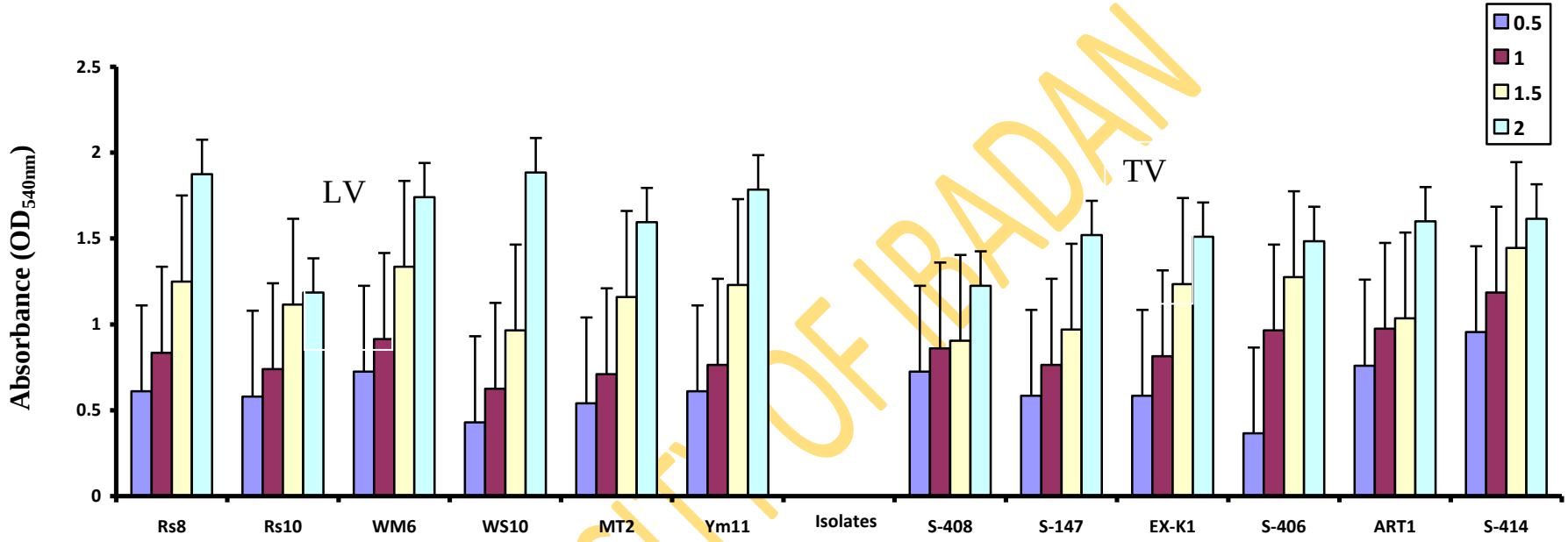

Figure 4.16: Effect of Peptone on the growth of the different LAB isolates obtained from gruels of local (LV)and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

acidophilus ST1) also had the best growth (1.20 OD) with S-14$_7$ (*Lactobacillus delbrueckii* ST1) having the lowest growth (0.70 OD). Overall, the LAB isolates obtained from both local and typed sorghum and maize varieties were able to grow at the different peptone concentrations (figure 4.16).

4.2.5 Growth and enzymes production by the LAB isolates at different temperatures

As temperature increases from 20^{o}C to 30^{o}C, growth increases and thereafter decreases (figure 4.17). For all isolates from local varieties (LV), no growth was observed at temperature of 70^{o}C and 80^{o}C while for typed varieties (TV), there was a drastic reduction in growth. At all the temperature used, isolates from the local varieties were most favoured when compared with isolates from typed varieties. Generally, the temperature of 30^{o}C favoured the growth of LAB isolates obtained from both local and typed varieties while temperature above 40^{o}C to 50^{o}C resulted in a decrease in growth of the isolates. Therefore, the optimum growth temperature for all the LAB isolates was 30^{o}C.

At temperature of 20^{o}C, isolates RS$_{10}$ (*Lactobacillus plantarum* SL$_1$), MT$_2$ (*Lactobacillus plantarum* MTL$_2$) of local varieties (LV) and S-40$_8$ (*Lactobacillus plantarum* ST$_1$) of typed varieties (TV) had the highest invertase production while most isolates had their best production at temperature of 30^{o}C except isolate YM$_{11}$ (*Lactobacillus casei* MZL$_2$). Generally, for all LAB isolates obtained from both varieties, maximum invertase production was observed at 30^{o}C while production decreases thereafter except for isolates WS$_{10}$ (*Lactobacillus acidophilus* SL$_2$) and YM$_{11}$ (*Lactobacillus casei* MZL$_2$) of local varieties where invertase production later increased at 50^{o}C and thereafter decreased till 80^{o}C. Increase in temperature from 20^{o}C to 60^{o}C had little or no effect on invertase production by isolates RS$_8$ (*Lactobacillus fermentum* SL$_2$) and WM$_6$ (*Lactobacillus casei* MZL$_1$) of local varieties. Similarly, increase in temperature from 20^{o}C to 50^{o}C had no significant effect on invertase production by isolates Ex-K$_1$ (*Lactobacillus casei* ST1) and S-41$_4$ (*Lactobacillus acidophilus* ST1) of typed varieties. The least production of invertase at the least temperature (20^{o}C) and at the highest temperature of 80^{o}C was observed in isolate S-40$_6$ of typed varieties. In all, the optimum temperature for invertase production by the LAB isolates obtained from both local and typed sorghum and

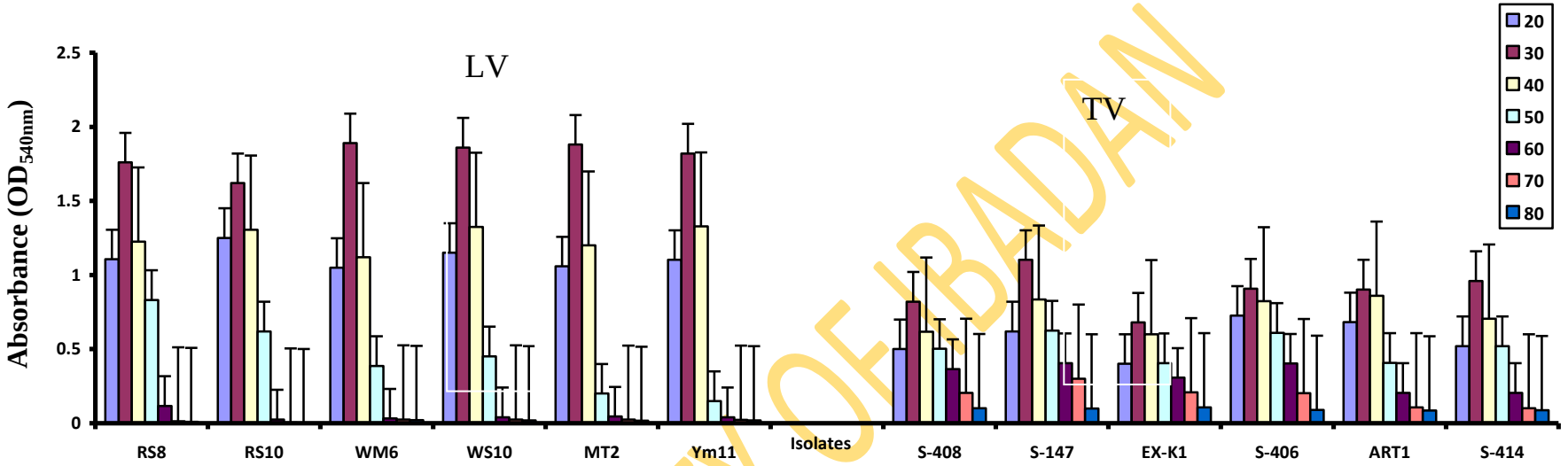

Figure 4.17: Effect of Temperature on the growth of the different LAB isolates obtained from gruels of local (LV)and typed (TV)sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

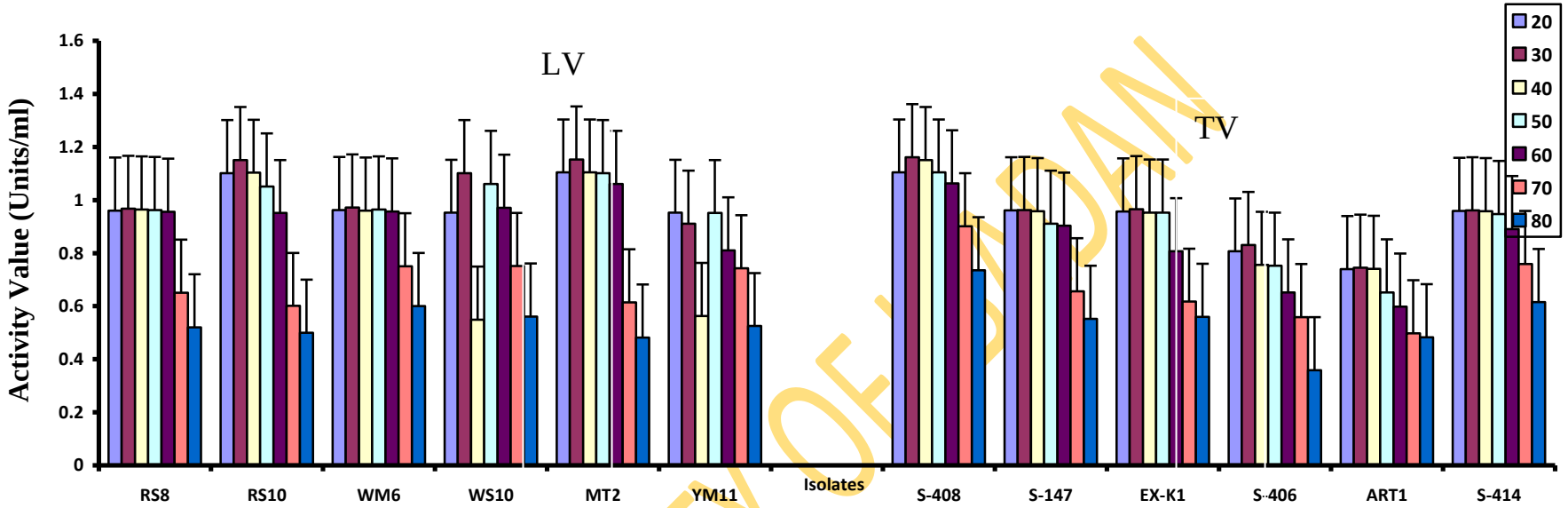

Figure 4.18: Effect of Temperature on Invertase production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

maize varieties was 30°C except YM_{11} (*Lactobacillus casei* MZL_2) which was favoured most by temperature of 20°C (figure 4.18).

For the local varieties (LV), isolate MT_2 (*Lactobacillus plantarum* MTL_2) produced protease most (0.48 Units/ml) at temperature of 20°C while isolate WS_{10} (*Lactobacillus acidophilus* SL_2) produced the least (0.30 Units/ml) (figure 4.19). At 30°C, all the LAB isolates had their maximum protease production with the highest production (0.55 Units/ml) recorded by isolate RS_{10} (*Lactobacillus plantarum* SL_1) and least production (0.31 Units/ml) recorded by isolate WS_{10} (*Lactobacillus acidophilus* SL_2). At all temperatures, isolate RS_{10} (*Lactobacillus plantarum* SL_1) recorded the highest protease production while the least production was observed in isolate WS_{10} (*Lactobacillus acidophilus* SL_2). Also, for the typed varieties (TV), isolate S-406 (*Lactobacillus fermentum* ST2) recorded the highest protease (0.39 Units/ml) while isolate $S\text{-}14_7$ (*Lactobacillus delbrueckii* ST1) produced least concentration of protease (0.28 Units/ml) at 30°C. At 50°C, isolate $S\text{-}40_8$ (*Lactobacillus plantarum* ST1) was found to produce protease best (0.32 Units/ml) followed by isolates $Ex\text{-}K_1$ (*Lactobacillus casei* ST1) and $S\text{-}41_4$ (*Lactobacillus acidophilus* ST1) with value of (0.30 Units/ml) and ART_1 (*Lactobacillus delbrueckii* MZT_2) had the least (0.20 Units/ml). Generally, temperatures of 30°C to 40°C favoured protease production by isolates from both local and typed varieties. Thus, optimum temperature for protease production was 30°C.

As the incubation temperature increased from 20°C to 30°C, amylase production increased and later decreased till 80°C by all the LAB isolates from both local and typed varieties (figure 4.20). For the isolates from local varieties (LV), amylase production by isolate RS_{10} (*Lactobacillus plantarum* SL_1) was most favoured at all temperatures followed by isolate MT_2 (*Lactobacillus plantarum* MTL_2) while isolate WS_{10} (*Lactobacillus acidophilus* SL_2) had the least production. For the typed varieties (TV) isolate *Lactobacillus plantarum* ST1 ($S\text{-}40_8$) produced the highest amount of amylase enzyme at all temperatures followed by *Lactobacillus acidophilus* ST1 ($S\text{-}41_4$) and the least was observed by *Lactobacillus delbrueckii* MZT2 (ART_1). Overall, amylase production was optimum at temperature of 30°C for all the isolates with highest enzyme production of 0.68 Units/ml and 0.60 Units/ml respectively by isolate RS_{10} (*Lactobacillus plantarum* SL_1) of local varieties and isolate $S\text{-}40_8$

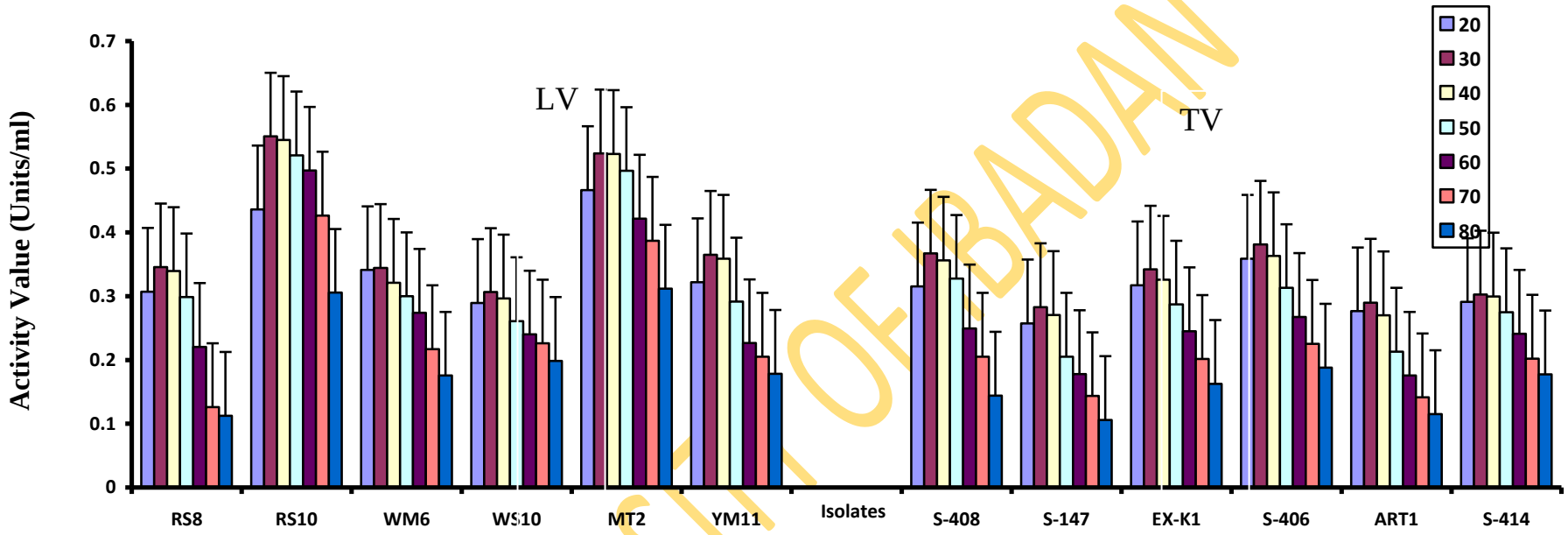

Figure 4.19: Effect of Temperature on Protease production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

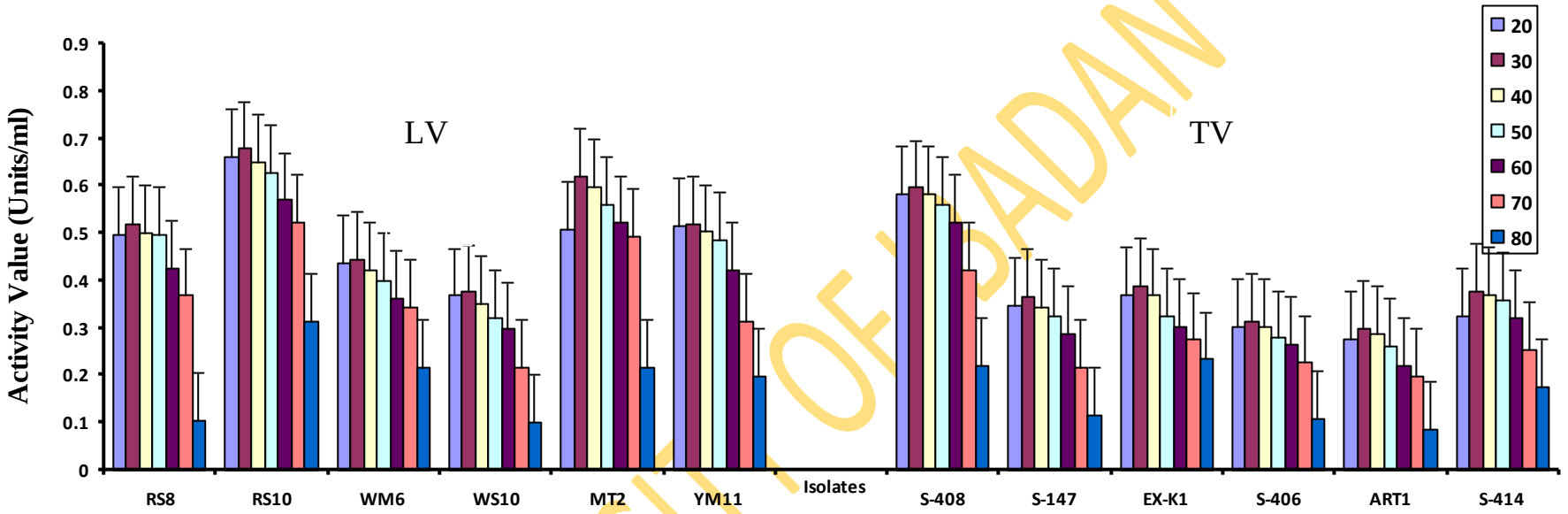

Figure 4.20: Effect of Temperature on Amylase production by the different LAB isolates obtained from gruels of local(LV) and typed(TV) sorghum and maize varieties.

KEY

RS_8	-	*L. fermentum*SL_2	$S\text{-}40_8$	-	*L.plantarum*ST1
RS_{10}	-	*L. plantarum*SL_1	$S\text{-}14_7$	-	*L. delbrueckii*ST1
WM_6	-	*L. casei*MZL_1	$Ex\text{-}K_1$	-	*L. casei*ST1
WS_{10}	-	*L. acidophilus*SL_2	$S\text{-}40_6$	-	*L. fermentum*ST2
MT_2	-	*L. plantarum*MTL_2	ART_1	-	*L. delbrueckii*MZT2
YM_{11}	-	*L. casei*MZL_2	$S\text{-}41_4$	-	*L. acidophilus*ST1

(*Lactobacillus plantarum*ST1) of typed varieties. The LAB isolates from local varieties were able to produce amylase better at the various temperature ranges when compared with the LAB isolates obtained from typed varieties.

4.2.6 Plasmid profile of selected LAB isolates

Figures 4.21a and 4.21b shows the electrophoretogram of crude DNA extracted from pure cultures of Lactic acid bacteria isolates. The result of the DNA extraction showed that not all of them possess plasmid. When the plasmids of the LAB isolates was removed in the process of curing for those that had plasmids, they had a different behaviour in terms of their physiological characters. The LAB isolates did not perform very well unlike when they were in their uncured form.

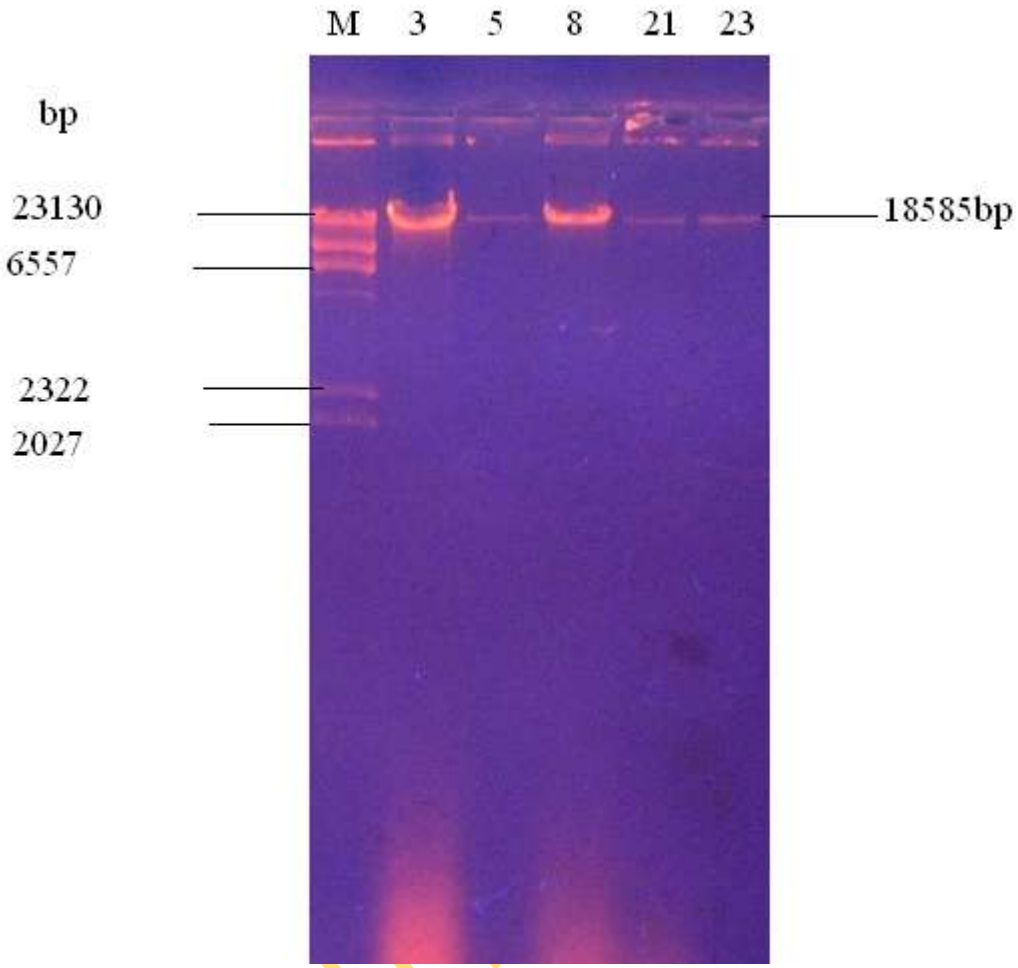

Figure 4.21a: Electrophoretogram of crude DNA extracted from pure cultures of Lactic acid bacteria isolates (isolates 3, 5, 8, 21 and 23).
M=DNA marker (Hyperladder1) Lane3=*Lactobacillus fermentum*ST2: Lane 5=*Lactobacillus acidophilus*ST1: Lane 8=*Lactobacillus delbrueckii*MZL$_2$: Lane21=*Lactobacillus casei*MZL$_1$:Lane 23=*Lactobacillus brevis*MZT1.The DNA samples were separated on 1% Agarose gel.

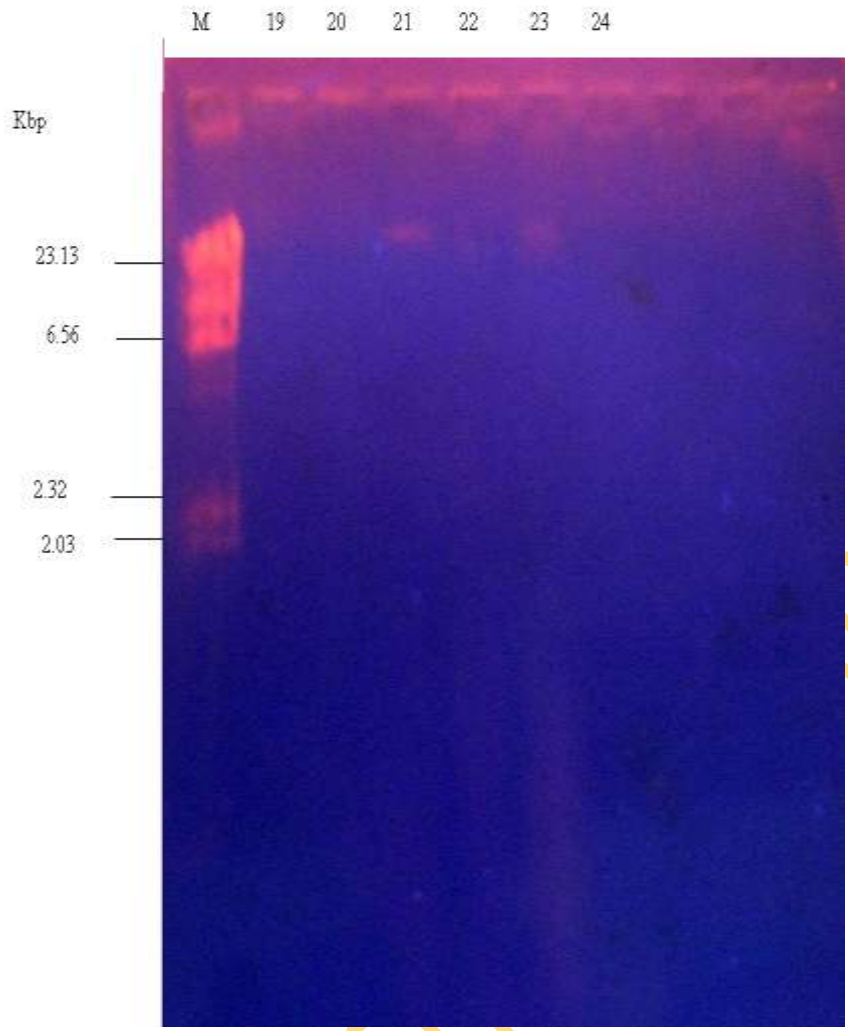

Figure 4.21b: Electrophoretogram of crude DNA extracted from pure cultures of Lactic acid bacteria isolates (isolates 19-24).
M=DNA marker(Hyperladder1): Lane 19= *Lactobacillus plantarum*ST3: Lane 20= *Lactobacillus delbrueckii*MZL$_1$: Lane 21=*Lactobacillus casei*MZL$_1$: Lane 22=*Lactobacillus delbrueckii*MZT2 :Lane23=*Lactobacillus brevis*MZT1: Lane 24=*Lactobacillus acidophilus*SL$_2$. The DNA samples were separated on 1% Agarose gel.

4.2.7 Production of selected hydrolytic enzymes by the LAB isolates

Table 4.3 shows the hydrolytic enzymes production by the different LAB isolates from gruels of local sorghum and maize varieties. *Lactobacillus delbrueckii* MZL_1 produced the highest amylase with value of 1.88 ± 0.01 Units/ml while *Lactobacillus casei*MZL_2 produced the least amylase with value of 0.29 ± 0.01 Units/ml. Invertase enzyme was produced most by *Lactobacillus delbrueckii*MZL_1 with value of 1.96 ± 0.07 Units/ml while *Lactobacillus fermentum*MZL_1 had the lowest invertase with value of 1.23 ± 0.02 Units/ml. *Lactobacillus acidophilus*MTL_1 produced protease enzyme most with value of 0.32 ± 0.02Units/ml while *Lactobacillus casei*MZL_1 and *Lactobacillus delbrueckii*MZL_1 produced the lowest protease value of 0.23 ± 0.03 Units/ml and 0.23 ± 0.01 Units/ml respectively. The production of xylanase enzyme was most pronounced in *Lactobacillus delbrueckii*MZL_1 with xylanase activity value of 1.37 ± 0.22 Units/ml while *Lactobacillus plantarum*SL_1 produced the least xylanase with the value of 0.60 ± 0.03 Units/ml. Invertase enzyme was produced most by the LAB isolates followed by amylase and xylanase with protease being the least.

Table 4.4 shows the hydrolytic enzymes production by the different LAB isolates from gruels of typed sorghum and maize varieties. *Lactobacillus plantarum* ST2 produced the highest amylase with value of 1.96 ± 0.01Units/ml while *Lactobacillus fermentum*ST1 recorded the least amylase with value of 1.77 ± 0.01 Units/ml. *Lactobacillus fermentum*ST1 produced invertase most (1.95 ± 0.01 Units/ml) while *Lactobacillus plantarum*ST1 produced the lowest invertase with value of 1.64 ± 0.02 Units/ml. The optimum protease production was observed in *Lactobacillus plantarum*ST1 with the value of 0.30 ± 0.02 Units/ml while *Lactobacillus brevis* MZT_1 produced the least protease (0.21 ± 0.03 Units/ml). Xylanase was best produced by *Lactobacillus delbrueckii*ST1 with xylanase activity value of 1.18 ± 0.03 Units/ml while *Lactobacillus acidophilus*ST2 produced the lowest xylanase with the value of 0.54 ± 0.03 Units/ml. Amylase enzyme was produced most by the LAB isolates obtained from typed sorghum and maize varieties followed by invertase while protease was produced least.

Table 4.3: Hydrolytic enzymes production by the different LAB isolates from gruels of Local sorghum and maize varieties

LAB Isolates	Enzymes (Units/ml)			
	Amylase	**Invertase**	**Protease**	**Xylanase**
***Lb. fermentum* SL2**	0.36±0.01*	1.63±0.01	0.24±0.07	1.35±0.01
***Lb. acidophilus* MTL1**	1.22±0.00	1.79±0.02	0.32±0.02	0.92±0.02
***Lb. plantarum* SL1**	0.95±0.01	1.61±0.01	0.27±0.01	0.60±0.03
***Lb. brevis* MZ2**	1.43±0.07	1.86±0.02	0.24±0.02	0.75±0.02
***Lb. delbrueckii* MZL1**	1.16±0.01	1.92±0.02	0.31±0.02	1.12±0.06
***Lb. casei* MZL1**	0.60±0.01	1.53±0.01	0.23±0.03	1.28±0.17
***Lb. acidophilus* SL2**	0.43±0.02	1.85±0.03	0.31±0.02	0.87±0.22
***Lb. brevis* SL1**	0.40±0.01	1.42±0.07	0.29±0.07	0.65±0.03
***Lb. fermentum* MZL1**	1.52±0.02	1.23±0.02	0.26±0.07	1.02±0.12
***Lb. delbrueckii* MZL1**	1.88±0.01	1.96±0.07	0.23±0.01	1.37±0.22
***Lb. plantarum* MTL2**	0.89±0.07	1.45±0.07	0.26±0.02	0.70±0.01
***Lb. casei* MZL2**	0.29±0.01	1.70±0.01	0.28±0.02	0.79±0.06

***Mean values ± SEM**

L = Local variety, MZ = Maize, S = Sorghum, Lb = *Lactobacillus*

Table 4.4: Hydrolytic enzymes production by the different LAB isolates from gruels of typed sorghum and maize varieties

LAB	**Enzymes** (Units/ml)			
Isolates	**Amylase**	**Invertase**	**Protease**	**Xylanase**
***Lb. fermentum* ST1**	1.77±0.01*	1.95±0.01	0.25±0.07	0.79±0.012
***Lb. plantarum* ST1**	1.94±0.01	1.64±0.02	0.30±0.02	0.70±0.02
***Lb. delbrueckii* ST1**	1.93±0.07	1.92±0.01	0.25±0.07	1.18±0.03
***Lb. plantarum* ST2**	1.96±0.01	1.83±0.02	0.26±0.00	0.56±0.01
***Lb. casei* ST1**	1.88±0.03	1.75±0.03	0.29±0.10	0.55±0.06
***Lb. fermentum* ST2**	1.91±0.07	1.78±0.01	0.24±0.07	1.10±0.03
***Lb. delbrueckii* MZT2**	1.80±0.03	1.90±0.01	0.27±0.01	0.83±0.22
***Lb. brevis* MZT1**	1.92±0.07	1.87±0.07	0.21±0.03	0.96±0.05
***Lb. acidophilus* ST1**	1.84±0.02	1.74±0.07	0.28±0.01	0.75±0.01
***Lb. plantarum* ST3**	1.85±0.00	1.82±0.02	0.23±0.03	0.85±0.02
***Lb. acidophilus* ST2**	1.95±0.01	1.87±0.01	0.26±0.02	0.54±0.03
***Lb. acidophilus* ST3**	1.91±0.07	1.94±0.01	0.29±0.01	0.71±0.01

***Mean values ± SEM**

T = Typed variety, MZ = Maize, S = Sorghum, Lb = *Lactobacillus*

4.2.8 Antimicrobial activity profiles of the selected LAB isolates

Table 4.5 shows the antimicrobial activity of LAB isolates obtained from gruels of typed sorghum and maize varieties against some indicator strains. *Lactobacillus plantarum*ST1 had the highest inhibition against *Proteus mirabilis* with zone of inhibition of +13mm while *Lactobacillus fermentum*ST1, *Lactobacillus fermentum*ST2 and *Lactobacillus acidophilus* ST1 had the least inhibition of the same value of +7mm against *Proteus mirabilis*. *Lactobacillus plantarum*ST2 and *Lactobacillus plantarum*ST3 had the same highest zone of inhibition against *Bacillus licheniformis* with zone of inhibition of +17mm and the lowest zone of inhibition was observed in *Lactobacillus fermentum* ST2, *Lactobacillus acidophilus* ST1 and *Lactobacillus acidophilus*ST3 with zone of inhibition of +7mm. *Lactobacillus plantarum* ST1 had the highest zone of inhibition against *Pseudomonas aeruginosa* with zone of inhibition of +17mm while the lowest zone of inhibition was observed in *Lactobacillus acidophilus*ST2 against *Pseudomonas aeruginosa* with value of +8mm. *Lactobacillus acidophilus*ST1 did not inhibit *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Lactobacillus delbrueckii*MZT2 also did not inhibit *Bacillus subtilis*. Overall, the highest zone of inhibition was +17mm while the lowest was +7mm.

Table 4.6 shows the antimicrobial activity of LAB isolates obtained from gruels of local sorghum and maize varieties against some indicators strains. *Lactobacillus plantarum* MTL_2 had the highest zone of inhibition against *Proteus mirabilis* with a zone of inhibition of +17mm while *Lactobacillus brevis*SL1 and *Lactobacillus acidophilus* SL_2 had the same lowest inhibition against *Proteus mirabilis* with a zone of inhibition of +7mm. *Lactobacillus fermentum* SL_2, *Lactobacillus casei*MZL_1 and *Lactobacillus casei* MZL_2 had the same highest zone of inhibition against *Bacillus licheniformis* with the value of +17mm while *Lactobacillus acidophilus*SL_2 being the least with zone of inhibition of +5mm. *Lactobacillus fermentum*SL_2 did not inhibit *Staphylococcus aureus.* *Lactobacillus plantarum*SL_1 had the highest zone of inhibition against *Pseudomonas aeruginosa* with zone of inhibition of +17mm while *Lactobacillus brevis*SL_1 produced the lowest zone of inhibition of +7mm against *Pseudomonas aeruginosa*. *Lactobacillus brevis* SL_1, *Lactobacillus brevis* MZ_2, *Lactobacillus delbrueckii* MZL_1 and *Lactobacillus delbrueckii* MZL_2 did not inhibit *Bacillus cereus.*

Table 4.5: Antimicrobial Activity of LAB Isolates obtained from gruels of typed sorghum and maize varieties against some indicator strains

LAB Isolates	Indicator Organisms/Zone of Inhibition (mm)						
	Proteus mirabilis	*Bacillus licheniformis*	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus cereus*
Lb. fermentum ST1	+7*	+11	+10	+9	+12	+12	+12
Lb. plantarum ST1	+13	+9	+10	+9	+11	+17	+17
Lb. delbrueckii ST1	+12	+11	+8	+9	+12	+10	+17
Lb. plantarum ST2	+13	+17	+13	+10	+7	+11	+13
Lb. casei ST1	+9	+8	+10	+9	+9	+10	+11
Lb. fermentum ST2	+7	+7	+9	+8	+7	+13	+10
Lb. delbrueckii MZT2	+11	+8	-	+15	+12	+9	+17
Lb. brevis MZT1	+10	+8	+12	+11	+10	+10	+7
Lb. acidophilus ST1	+7	+7	+17	+17	-	-	+11
Lb. plantarum ST3	+11	+17	+9	+17	+10	+9	+14
Lb. acidophilus ST2	+11	+9	+8	+7	+9	+8	+9
Lb. acidophilus ST3	+10	+7	+10	+9	+8	+13	+17

Key: + = Inhibition, – = No inhibition

T = Typed variety, S = Sorghum, MZ = Maize, Lb = *Lactobacillus*

Table 4.6: Antimicrobial Activity of LAB Isolates obtained from gruels of local sorghum and maize varieties against some indicator strains

LAB Isolates	Indicator Organisms/Zone of Inhibition (mm)						
	Proteus mirabilis	*Bacillus licheniformis*	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus cereus*
Lb. brevis SL1	+7*	+10	+9	-	+17	+7	-
Lb. brevis MZ2	+11	+9	+17	+10	+7	+9	-
Lb. plantarum SL1	+11	+13	+17	+17	+15	+17	+15
Lb. plantarum MTL2	+17	+8	+7	+17	+9	+11	+13
Lb. delbrueckii MZL1	+12	+10	+8	+9	+17	+10	-
Lb. delbrueckii MZL2	+13	+9	+10	+9	+9	+9	-
Lb. fermentum MZL1	+8	+9	+9	+17	+14	+10	+9
Lb. fermentum SL2	+9	+17	+8	+13	-	+11	+7
Lb. acidophilus MTL1	+8	+7	+8	+7	+8	+9	+8
Lb. acidophilus SL2	+7	+5	+17	+17	+8	+12	+17
Lb. casei MZL1	-	+17	+7	+9	+8	+13	+11
Lb. casei MZL2	+9	+17	+10	+17	+7	+9	+17

Key: + = Inhibition, – = No inhibition

L = Local variety, MT = Millet, MZ = Maize, S = Sorghum, Lb = *Lactobacillus*

4.2.9 Quality assessment of the different sorghum varieties

The quality assessment of the sorghum varieties showed that the two sorghum varieties used for the malting process: the red sorghum and the white sorghum had good germinative capacities and they are also good varieties for malting in brewing process. The protein content of the white sorghum was 11.37±0.07% while that of the red sorghum was 13.17±0.03%. The germinative energies (GE) of the two sorghum varieties were above 90%, the white sorghum had germinative energy (GE) of 96.33±0.33 while the red sorghum had GE of 95.33±0.67. The germinative capacity of the white sorghum was 98.33±0.33% while that of the red sorghum was 97.67±0.33% (Table 4.7).

4.2.10 LAB treatment and physiological behaviours of the different sorghum varieties before and after malting.

Figure 4.22 shows the measurement of the plumule while Figure 4.23 shows the measurement of the radicle length of the sorghum varieties during malting at the different time intervals. The plumule length and radicle length increased during malting of the sorghum varieties. The red sorghum variety treated with *Lactobacillus plantarum* gave the highest radicle length of 1.30cm and red control gave the least radicle length at day 5. The white sorghum treated with *Lactobacillus casei* had the highest plumule length of 4.00cm while white sorghum control had the least plumule length with value of 1.80cm at day 5.

The weight of the sorghum seeds increased with germination time during the malting process. The white sorghum treated with *Lactobacillus plantarum* gave the highest weight of 4.20g while the red sorghum control gave the lowest weight with value of 2.90g at day 5. Overall, the weight of the challenged sorghum seeds ranged between 1.20-4.20g while that of the control sorghum seeds was between 1.40-3.00g. This is shown in figure 4.24.

Table 4.7: Quality assessment of the sorghum varieties prior to treatment

Parameters	Sorghum Varieties	
	White Sorghum	Red Sorghum
Moisture Content (%)	10.43±0.07	10.60±0.06
Crude Protein (%)	11.37±0.07	13.17±0.03
Total Nitrogen	1.81±0.01	2.10±0.00
Crude Fat (%)	2.36±0.03	3.00±0.00
Ash (%)	1.87±0.03	1.66±0.03
Crude Fibre (%)	1.67±0.03	1.80±0.00
Total Carbohydrate (%)	72.26±0.13	69.70±0.06
Soluble Protein	11.03±0.03	13.00±0.00
Tannins (mg/100g)	23.33±0.33	35.83±0.17
Phytates (mg/100g)	42.33±0.17	37.66±0.44
Protease inhibitors (mg/100g)	1.67±0.16	2.00±0.00
Diastatic Power (IoB Units)	15.67±0.17	14.00±0.29
Germinative Energy (%)	96.33±0.33	95.33±0.67
Germinative Capacity (%)	98.33±0.33	97.67±0.33
Weight of 1000 corns (g)	31.67±0.33	30.67±0.33

*** Values are means of triplicate determinations ± Standard deviation**

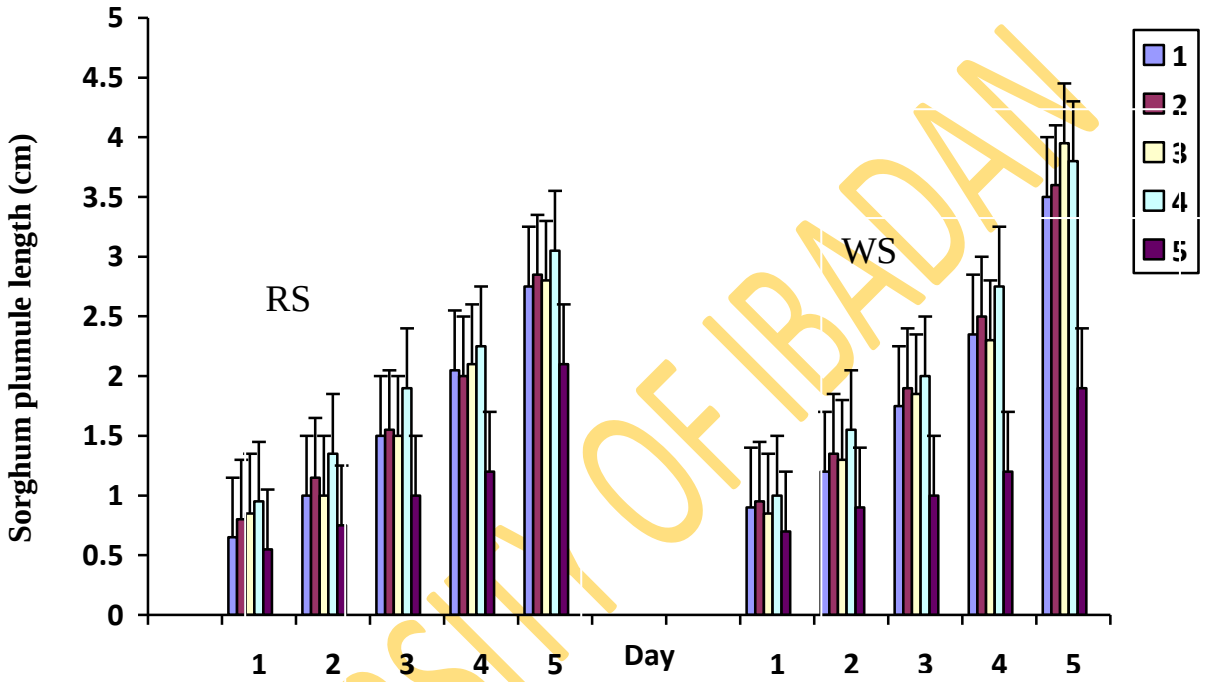

Figure 4.22: Effect of LAB suspension on the plumule length of sorghum seeds during malting at different time intervals.

KEY

1 = *L. plantarum* (LP)
2 = *L. fermentum* (LF)
3 = *L. casei* (LC)
4 = *L. plantarum* + *L. fermentum* (LP+LF)
5=Control

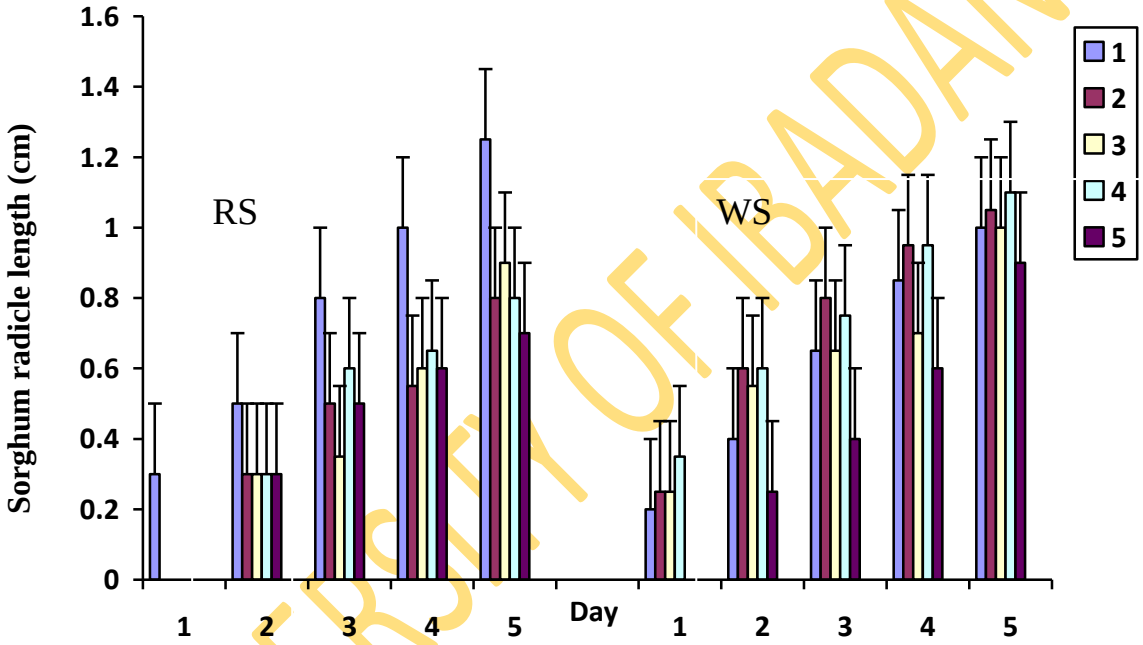

Figure 4.23: Effect of LAB suspension on the radicle length of Sorghum seeds during malting at different time intervals.

KEY

1 = *L. plantarum* (LP)
2 = *L. fermentum* (LF)
3 = *L. casei* (LC)
4 = *L. plantarum* + *L. fermentum* (LP+LF)
5 = Control (LC)

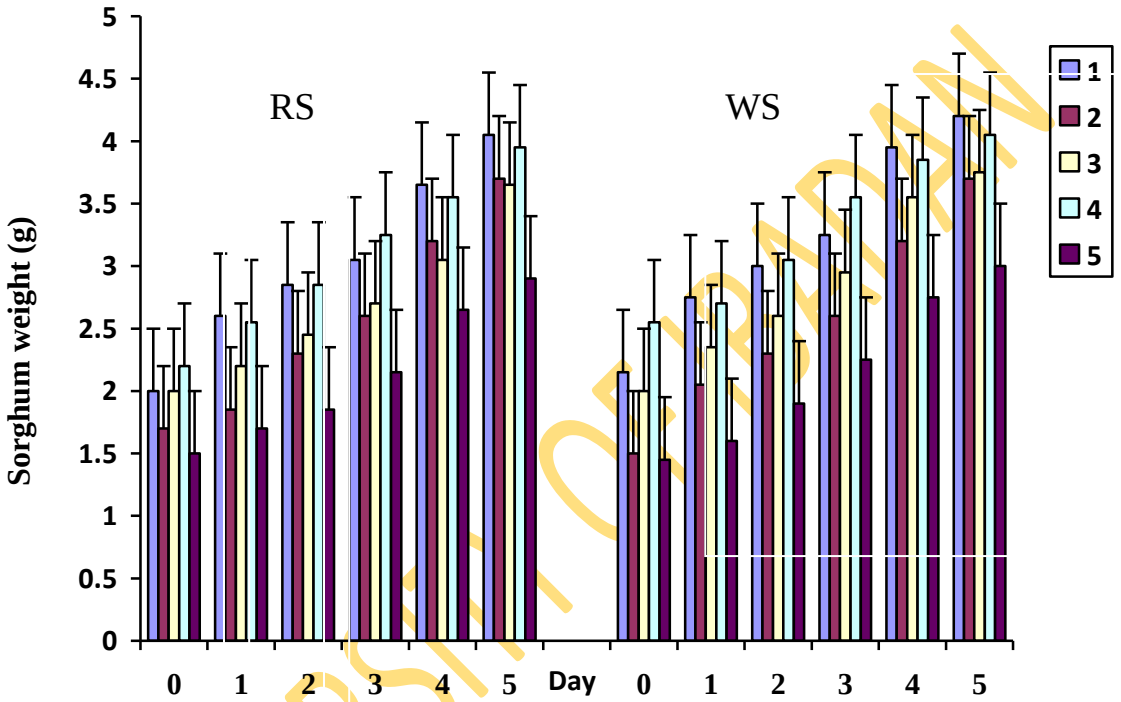

Figure 4.24: Effect of LAB suspension on the weight of Sorghum seeds during malting at different time intervals.

KEY

1 = *L.* plantarum (LP)
2 = *L. fermentum* (LF)
3 = *L. casei* (LC)
4 = *L. plantarum* + *L. fermentum* (LP+LF)
5 = Control

There was also an increase in the amylase activity of the sorghum malt during the germination process from day 1 to day 5. The red sorghum challenged with the combination of *Lactobacillus plantarum* and *Lactobacillus fermentum* had the highest amylase activity with value of 1.98 (Units/ml) at day 5 while the white control sorghum had the least amylase activity value of 0.68 (Units/ml) at day 1 (Figure 4.25)

The protease activity of the challenged sorghum seeds increased steadily during malting. The sorghum varieties treated with *Lactobacillus plantarum*(LP) and with the combination of *Lactobacillus plantarum* and *Lactobacillus fermentum*(LP+LF) had the highest protease activity with values of 1.75 (Units/ml) for red sorghum and 1.72 (Units/ml) for white sorghum. The untreated- sorghum i.e both red and white sorghum varieties gave the least protease activities during malting (Figure 4.26).

Plate 4.1 shows the germination process of the different sorghum varieties at different time intervals while plate 4.2 shows the improvised fermentors for the fermentation of the wort from the different sorghum varieties.

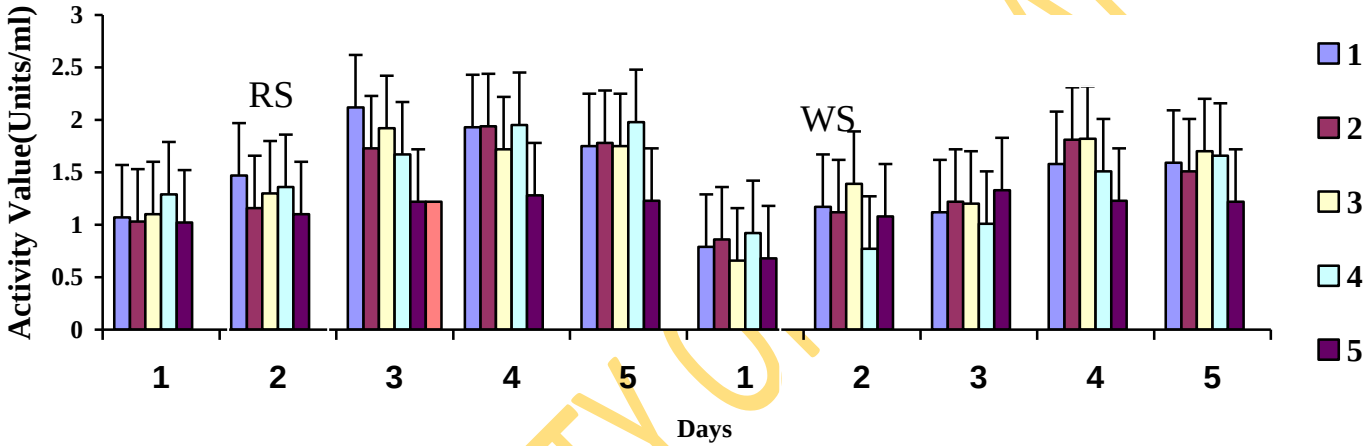

Figure 4.25: Amylase activity of sorghum seeds during malting at different time intervals.

KEY:
1 = *L. plantarum* (LP)
2 = *L. fermentum* (LF)
3=*L. casei* (LC)
4=*L.plantarum +L.fermentum* (LP+LF)
5=Control

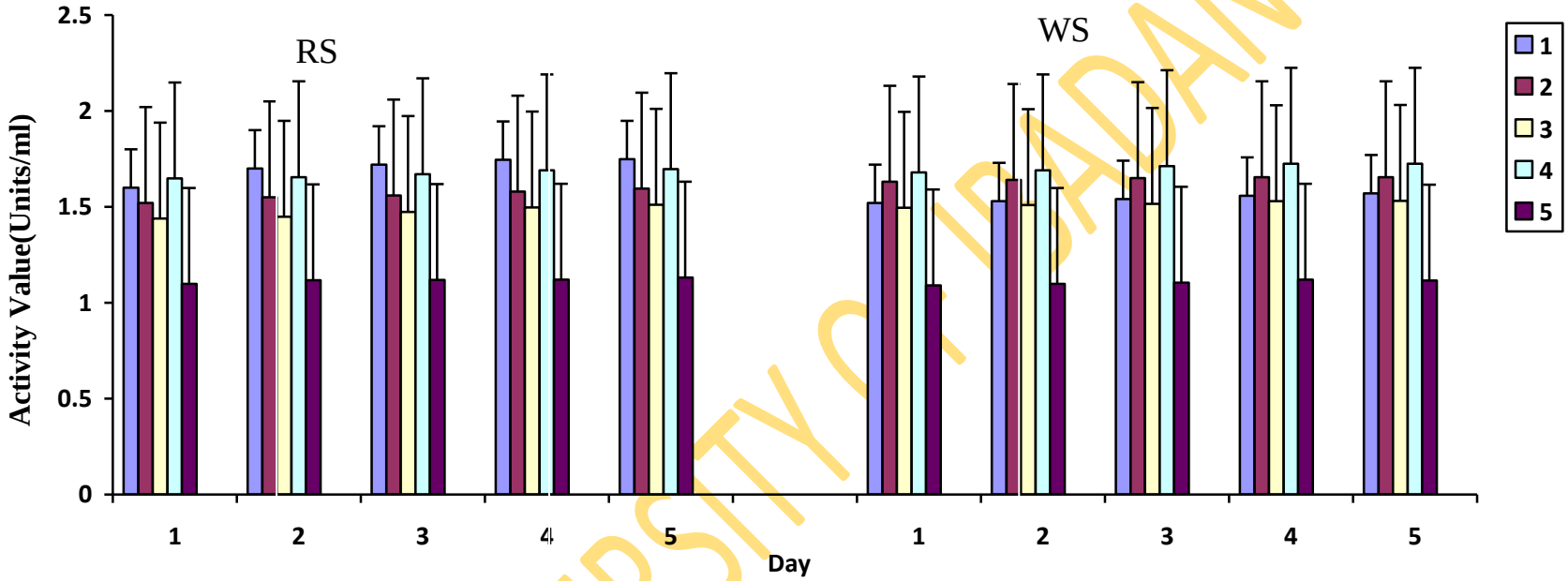

Fig 4.26: Protease activity during malting of the sorghum varieties at different time intervals.

KEY:
1 = *L. plantarum* (LP)
2 = *L. fermentum* (LF)
3=*L. casei* (LC)
4=*L.plantarum* +*L.fermentum* (LP+LF)
5=Control

Plate 4.1: The germination of the different sorghum varieties at different time intervals.

Plate 4.2: Improvised fermentors for the fermentation of the wort from the different sorghum varieties.

Table 4.8 shows the microbial load during the germination of the sorghum varieties challenged with the LAB isolates. The microbial profiles of the challenged malted sorghum showed a steady decrease in *Bacillus, Staphylococcus* and *Pseudomonas* count from day 1 to day 5 when compared with the unchallenged malted sorghum. The yeasts and moulds count also decreased considerably during malting. The LAB count increased considerably throughout the malting stages in the challenged malt while it reduced in the unchallenged malt. On day 1, *Pseudomonas* and *Staphylococcus* count was 2.0 x 10^3 cfu/ml while at day 5, it reduced to 0 for the sorghum varieties challenged with *Lactobacillus plantarum*.

Table 4.9 shows the moisture content of the malted sorghum subjected to challenge by Lactic acid bacterial isolates for different time intervals. The moisture content reduced from day 0 to day 5 for all the treatments. The red sorghum variety treated with *Lactobacillus casei* (RS) had the highest moisture content with value of 59.37±0.28% at day 0 while the red sorghum treated with the combination of *Lactobacillus plantarum* and *Lactobacillus fermentum* (RS) produced the least moisture content with value of 46.07±0.09% at day 5.

Table 4.10 shows the total carbohydrate content of the malted sorghum subjected to challenge by Lactic acid bacteria isolates for different time intervals. The total carbohydrate content increased from day 0 to day 5. The while sorghum variety treated with *Lactobacillus plantarum* (WS) gave the least value of 32.23±0.15% at day 1 while the red sorghum variety treated with combination of *Lactobacillus plantarum* and *Lactobacillus fermentum* (RS) had the highest carbohydrate with value of 44.67±0.12% at day 5.

Table 4.8: Total Microbial Load during the germination of Sorghum seed varieties

LAB Isolates/ Sorghum Varieties	Day1									
	RS					WS				
	Cell count (cfu/ml) x 10^3					Cell count (cfu/ml) x10^3				
	Bacillus	***Staphylococcus***	***Pseudomonas***	**LAB**	**Yeasts and moulds**	***Bacillus***	***Staphylococcus***	***Pseudomonas***	**LAB**	**Yeasts and moulds**
L. plantarum	-	2.0	2.0	5.0	1.0	2.0	2.0	3.0	7.0	-
L. fermentum	2.0	2.0	1.0	4.0	2.0	2.0	1.0	1.0	5.0	2.0
L. casei	2.0	2.0	1.0	4.0	1.0	1.0	2.0	2.0	3.0	-
L.plantarum+L. fermentum	4.0	5.0	2.0	6.0	2.1	1.0	2.0	1.0	4.0	1.0
Control	10.0	9.0	12.0	3.0	3.0	10.0	7.0	8.0	2.0	2.0

Key: - = No growth RS = Red Sorghum WS = White Sorghum

Table 4.8 Contd: Total Microbial Load during the germination of Sorghum seed varieties

LAB Isolates/ Sorghum Varieties	Day3									
	RS Cell count (cfu/ml) x 10^3					WS Cell count (cfu/ml) x10^3				
	Bacillus	***Staphylococcus***	***Pseudomonas***	**LAB**	**Yeasts and moulds**	***Bacillus***	***Staphylococcus***	***Pseudomonas***	**LAB**	**Yeasts and moulds**
L. plantarum	-	-	-	5.0	-	2.0	1.0	1.0	8.0	-
L. fermentum	1.0	1.0	1.0	4.0	1.0	1.0	-	-	4.0	1.0
L. casei	1.0	-	-	4.0	-	-	-	-	5.0	-
L.plantarum+L. fermentum	-	-	-	6.0	1.0	-	-	-	4.0	1.0
Control	4.0	4.0	3.0	3.0	2.0	5.0	4.0	4.0	3.0	1.0

Key: - = No growth RS =Red Sorghum WS =White Sorghum

Table 4.8 Contd: Total Microbial Load during the germination of Sorghum seed varieties

LAB Isolates/ Sorghum Varieties	Day 5									
	RS Cell count (cfu/ml) x 10^3					WS Cell count (cfu/ml) x10^3				
	Bacillus	***Staphylococcus***	***Pseudomonas***	**LAB**	**Yeasts and moulds**	***Bacillus***	***Staphylococcus***	***Pseudomonas***	**LAB**	**Yeasts and moulds**
L. plantarum	1.0	-	-	8.0	-	-	-	-	6.0	-
L. fermentum	1.0	-	-	5.0	1.0	1.0	-	-	5.0	1.0
L. casei	-	-	-	6.0	-	-	-	-	4.0	-
L.plantarum+L. fermentum	1.0	-	-	7.0	1.0	-	-	-	5.0	-
Control	10.0	8.0	6.0	2.0	4.0	10.0	7.0	5.0	1.0	4.0

Key: - = No growth RS =Red Sorghum WS =White Sorghum

Table 4. 9: Moisture content (%) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB isolates/Sorghum variety	Treatment period (days)/Moisture Content (%)					
	0	**1**	**2**	**3**	**4**	**5**
Lactobacillus plantarum (WS)	59.33±0.008^{a}	54.73+0.03^{g}	50.30±0.06^{g}	49.53±0.03^{c}	47.70±0.10^{ef}	46.33+0.09^{fg}
Lactobacillus plantarum (WS)	58.17±0.08^{ef}	55.73±0.03^{d}	50.53±0.03^{f}	50.13±0.03^{a}	48.20±0.17^{c}	46.83+0.03^{de}
Lactobacillus casei(WS)	58.60±0.35^{d}	56.00±0.03^{c}	51.27±0.06^{d}	49.47±0.08^{cd}	48.93±0.07^{c}	47.63+0.32^{b}
Lactobacillus casei (RS)	59.37±0.28^{a}	55.10±0.05^{f}	51.96±0.03^{b}	49.87±0.08^{b}	49.23±0.03^{b}	48.27+0.07^{a}
Lactobacillus fermentum(WS)	59.20±0.06^{ab}	56.47±0.08^{g}	51.13±0.03^{d}	49.03±0.08^{f}	49.60±0.06^{a}	48.23+0.12^{a}
Lactobacillus fermentum (RS)	58.77±0.03^{cd}	54.60±0.08^{g}	52.97±0.03^{a}	48.63±0.07^{g}	48.80±0.00^{c}	47.17+0.07^{cd}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	57.93±0.03	55.37±0.08^{e}	52.00±0.00^{b}	49.27±0.07^{e}	48.83±0.06^{c}	46.70+0.09^{ef}
Lactobacillus plantarum+ *Lactobacillus fermentum* (RS)	59.03±0.03^{abc}	54.06±0.03^{h}	50.83±0.03^{e}	49.00±0.00^{f}	47.83±0.03^{e}	46.07+0.09^{gh}
Control (WS)	57.23±0.08^{g}	54.10±0.05^{h}	51.97±0.08^{b}	49.30±0.06^{de}	48.97±0.03^{c}	46.63+0.21^{ef}
Control (RS)	58.83±0.08^{bcd}	55.71±0.03^{f}	50.53±0.18^{f}	48.63±0.09^{g}	49.53±0.03^{a}	47.70+0.06^{b}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.10: Total Carbohydrate content (%) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Total carbohydrate content (%)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	32.73±0.07^{e}	32.23±0.15^{h}	36.40±0.16^{h}	40.73±0.15^{fg}	41.90±0.06^{c}	44.53±0.03^{ab}
Lactobacillus plantarum (RS)	33.63±0.09^{cd}	36.77±0.09^{c}	40.70±0.10^{a}	41.43±0.03^{de}	43.03±0.09^{a}	44.07±0.17^{cd}
Lactobacillus casei (WS)	33.50±0.40^{cd}	35.40±0.06^{e}	40.27±0.09^{b}	40.50±0.12^{g}	42.53±0.22^{b}	43.10±0.35^{fg}
Lactobacillus casei (RS)	32.33±0.29^{e}	35.47±0.09^{e}	39.90±0.12^{cd}	41.43±0.18^{de}	41.77±0.03^{c}	42.47±0.12^{h}
Lactobacillus fermentum (WS)	32.83±0.07^{e}	35.93±0.13^{d}	38.97±0.03^{f}	40.87±0.12^{f}	41.47±0.03^{d}	42.73±0.13^{gh}
Lactobacillus fermentum (RS)	32.97±0.12^{de}	34.90±0.11^{f}	39.97±0.09^{cd}	41.90±0.15^{bc}	41.30±0.15^{d}	43.67±0.03^{de}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	33.50±0.06^{cd}	34.20±0.00^{g}	39.17±0.09^{ef}	41.80±0.10^{bcd}	41.33±0.12^{d}	44.17±0.03^{bc}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	34.03±0.09^{bc}	36.07±0.07^{d}	39.37±0.07^{e}	41.70±0.06^{cde}	42.03±0.09^{c}	44.67±0.12^{a}
Control (WS)	33.93±0.03^{bc}	37.97±0.13^{a}	39.80±0.00^{d}	41.33±0.15^{e}	43.07±0.03^{a}	44.17±0.17^{bc}
Control (RS)	34.87±0.13^{a}	37.30±0.06^{b}	39.03±0.09^{f}	41.53±0.03^{cde}	41.77±0.08^{c}	43.00±0.06^{g}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.11 shows the diastatic power of the malted sorghum subjected to challenge by Lactic acid bacteria isolates for different time intervals. The diastatic power of the malted sorghum increased from day 0 to day 5. The white sorghum variety treated with the combination of *Lactobacillus plantarum* and *Lactobacillus fermentum* (WS) gave the highest diastatic power with valve of 25.00±0.29 (IoB Units) at day 5 while the red sorghum variety treated with *Lactobacillus plantarum* (RS) had the least value of 14.50 at day 0. For the control, control (WS) gave value of 22.00±0.00 (IoB Units) on day 5 while control (RS) had a value of 21.17±0.17 (IoB Units) also at day 5.

During the malting of the sorghum varieties, the protease inhibitor of the malted sorghum varieties, the protease inhibitor of the malted sorghum reduced from day 0 to day 5. For all the treatments, excluding the control malts, the protease inhibits of the malted sorghum from day 3 to day reduced to 0.00. The control malt (WS) had the value of 0.65±0.00mg/100g at day 5 and control malt (RS) had a value of 0.80±0.00 also at day 5. The red sorghum variety treated with combination of *Lactobacillus plantarum* and *Lactobacillus fermentum* i.e. (RS) had the least value of 1.83±0.17mg/100g at day 0 (Table 4. 12).

Table 4.11: Diastatic Power (IoBunits) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Diastatic power (IoBUnits)					
	0	**1**	**2**	**3**	**4**	**5**
Lactobacillus plantarum (WS)	16.33±0.17^{bc}	18.17±0.17^{a}	20.67±0.33^{b}	22.00±0.00^{b}	23.00+0.00^{c}	24.17+0.17^{c}
Lactobacillus plantarum (RS)	14.50±0.00^{f}	15.33±0.17^{f}	18.50±0.29^{fg}	19.83±0.17^{efg}	21.67+0.33^{de}	22.67+0.17^{de}
Lactobacillus casei(WS)	16.00±0.00^{c}	17.33±0.17^{b}	19.50±0.00^{cd}	20.17±0.17^{e}	23.00+0.00^{c}	23.83+0.44^{c}
Lactobacillus case (RS)	14.67±0.17^{ef}	16.17±0.17^{e}	17.67±0.17^{h}	19.17±0.17^{hi}	21.00+0.00^{f}	21.67+0.33^{fg}
Lactobacillus fermentum (WS)	16.00±0.00^{c}	17.00±0.29^{bcd}	19.67±0.33^{c}	20.67±0.33^{d}	22.00+0.00^{d}	22.67+0.33^{de}
Lactobacillus fermentum (RS)	14.83±0.17^{def}	17.33±0.44^{b}	18.50±0.29^{fg}	19.67±0.17^{fg}	21.33+0.33^{ef}	22.17+0.17^{def}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	16.83±0.44^{ab}	18.33±0.17^{a}	20.50±0.00^{b}	21.33±0.17^{c}	24.17+0.17^{ab}	25.00+0.29^{ab}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	15.00±0.00^{def}	16.50±0.29^{cde}	18.67±0.17^{ef}	20.00±0.00^{ef}	21.33+0.33^{ef}	22.83+0.17^{de}
Control (WS)	17.33±0.33^{a}	18.33±0.17^{a}	19.33±0.17^{cde}	20.00±0.00^{ef}	21.00+0.00^{f}	22.00+0.00^{ef}
Control (RS)	15.00±0.00^{def}	16.33±0.17^{de}	17.83±0.17^{gh}	19.00±0.00^{i}	20.00+0.00^{g}	21.17+0.17^{g}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.12: Protease Inhibitor (mg/100g) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Protease Inhibitor (mg/100g)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	2.00±0.00^{a}	1.67±0.17^{ab}	1.00±0.00^{c}	0.00	0.00	0.00
Lactobacillus plantarum (RS)	2.00±0.00^{a}	1.50±0.00^{abc}	1.00±0.00^{c}	0.00	0.00	0.00
Lactobacillus casei (WS)	2.00±0.00^{a}	1.33±0.17^{bcd}	1.00±0.00^{c}	0.00	0.00	0.00
Lactobacillus casei (RS)	2.00±0.00^{a}	1.50±0.00^{abc}	1.00±0.00^{c}	0.00	0.00	0.00
Lactobacillus fermentum (WS)	2.00±0.00^{a}	1.33±0.17^{bcd}	1.00±0.00^{c}	0.00	0.00	0.00
Lactobacillus fermentum (RS)	2.00±0.00^{a}	1.50±0.00^{abc}	1.00±0.00^{c}	0.00	0.00	0.00
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	2.00±0.00^{a}	1.00±0.00^{d}	0.50±0.00^{d}	0.00	0.00	0.00
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	1.83±0.17^{ab}	1.00±0.00^{d}	0.50±0.00^{d}	0.00	0.00	0.00
Control (WS)	2.00±0.00^{a}	1.83±0.17^{a}	1.50±0.00^{a}	1.20±0.00^{cd}	1.00±0.17^{d}	0.65±0.00^{de}
Control (RS)	1.83±0.17^{ab}	1.67±0.17^{ab}	1.17±0.17^{b}	1.00±0.17^{c}	0.91±0.00^{d}	0.80±0.00^{def}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

The tannin content of the malted sorghum decreased from day 0 to day 5 during malting. The red sorghum variety used as control i.e control (RS) had the highest tannin content at day 0 with value of 37.83±0.17mg/100g and the white sorghum variety treated with *Lactobacillus casei(*WS) on day 5 had the least tannin content with value of 12.00±0.00mg/100g. The control sorghum varieties i.e control (WS) and control (RS) had the highest tannin at day 5 with values of 20.67±0.00mg/100g and 22.17±0.00mg/100g. There were significant differences ($P<0.05$) between all the values obtained for each day and each variety (Table 4. 13).

Table 4.14 shows the phytate content of the malted sorghum subjected to challenge by lactic acid bacteria isolates for different time intervals. The phytate content of the sorghum decreased during malting i.e from day 0 to day 5. During day 0, white sorghum treated with *Lactobacillus casei* (WS), white sorghum treated with *Lactobacillus fermentum* (WS) and white sorghum treated with combination of *Lactobacillus plantarum* and *Lactobacillus fermentum*(WS) had the highest phytate content with the same value of 38.67±0.33, 38.67±0.44 and 38.67±0.17mg/100g respectively. On day 5, red sorghum treated with *Lactobacillus plantarum* and *Lactobacillus fermentum*(RS) had the least value of 14.33±0.33mg/100g. The control malts i.e control (WS) had 22.83±0.17mg/100g phytate and control (RS) had 21.17±0.17mg/100g at the end of the malting process. There were significant differences between the treatments and the sorghum varieties.

Table 4.13: Tannin content (mg/100g) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Tannin content (mg/100g)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	31.50±0.76^{ef}	30.33±0.67^{ef}	24.50±0.29^{e}	18.00±0.00^{ef}	12.83±0.17^{g}	12.17±0.17^{h}
Lactobacillus plantarum (RS)	35.33±0.33^{d}	33.83±0.17^{cd}	28.33±0.33^{b}	20.67±0.33^{d}	15.33±0.33^{de}	15.00±0.00^{e}
Lactobacillus casei (WS)	32.00±0.00^{e}	30.83±0.17^{e}	23.33±0.33^{fg}	18.33±0.17^{e}	12.50±0.29^{g}	12.00±0.00^{h}
Lactobacillus casei (RS)	35.60±0.00^{d}	33.17±0.17^{d}	27.67±0.17^{b}	22.00±0.00^{bc}	17.33±0.33^{c}	17.00±0.00^{c}
Lactobacillus fermentum (WS)	30.67±0.33^{fg}	29.67±0.33^{fg}	23.67±0.33^{f}	18.33±0.33^{e}	17.00±0.00^{c}	16.00±0.00^{d}
Lactobacillus fermentum (RS)	35.67±0.33^{cd}	33.83±0.17^{cd}	27.83±0.17^{b}	21.67±0.33^{c}	17.00±0.00^{c}	16.33±0.00^{d}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	30.50±0.29^{g}	29.33±0.33^{fg}	22.33±0.17^{h}	17.33±0.17^{fg}	14.00±0.00^{g}	13.33±0.17^{g}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	36.50±0.29^{bc}	34.67±0.33^{bc}	26.33±0.33^{c}	20.17±0.17^{d}	15.83±0.17^{d}	15.16±0.17^{e}
Control (WS)	30.00±0.00^{g}	28.67±0.33^{g}	25.33±0.33^{d}	22.67±0.33^{b}	21.33±0.33^{b}	20.67±0.17^{b}
Control (RS)	37.83±0.17^{a}	35.83±0.17^{a}	30.00±0.00^{a}	25.67±0.33^{a}	23.00±0.00^{a}	22.17±0.17^{a}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.14: Phytate content (mg/100g) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Phytate content (mg/100g)					
	0	**1**	**2**	**3**	**4**	**5**
Lactobacillus plantarum (WS)	38.50±0.29^{ab}	37.33±0.44^{ab}	31.67±0.33^{b}	24.33±0.33^{c}	18.16±0.17^{c}	17.33±0.17^{c}
Lactobacillus plantarum (RS)	37.33±0.17^{c}	36.50±0.29^{abc}	30.33±0.33^{d}	22.67±0.33^{d}	16.33±0.33^{f}	15.67±0.33^{de}
Lactobacillus casei(WS)	38.67±0.33^{ab}	37.33±0.44^{ab}	30.50±0.50^{cd}	24.67±0.17^{c}	17.67±0.33^{d}	17.16±0.44^{c}
Lactobacillus casei(RS)	36.67±0.17^{c}	35.83±0.17^{c}	28.50±0.29^{fgh}	22.67±0.17^{d}	16.17±0.17^{f}	15.67±0.33^{de}
Lactobacillus fermentum(WS)	38.67±0.44^{ab}	37.67±0.17^{a}	29.67±0.33^{de}	24.00±0.00^{c}	17.00±0.00^{e}	16.17±0.17^{d}
Lactobacillus fermentum(RS)	34.33±0.33^{d}	34.33±0.88^{d}	28.33±0.33^{fgh}	21.83±0.17^{e}	16.16±0.17^{f}	15.66±0.33^{de}
Lactobacillus plantarum + *Lactobacillus fermentum*(WS)	38.67±0.17^{ab}	36.50±0.29^{abc}	30.17±0.17^{d}	23.00±0.00^{d}	16.50±0.00^{f}	16.00±0.00^{d}
Lactobacillus plantarum + *Lactobacillus fermentum*(RS)	34.83±0.33^{d}	34.00±0.00^{de}	28.00±0.00^{gh}	21.00±0.00^{f}	15.00±0.00^{g}	14.33±0.33^{f}
Control (WS)	38.33±0.17^{ab}	37.17±0.17^{ab}	33.00±0.00^{a}	29.17±0.17^{a}	23.67±0.33^{a}	22.83±0.17^{a}
Control (RS)	34.50±0.00^{d}	33.50±0.29^{de}	31.67±0.17^{b}	27.00±0.29^{b}	22.00±0.00^{b}	21.17±0.17^{b}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

The α-amylase activity (DU) increased steadily during the malting process from day 0 day 5. The control sorghum i.e control (WS) had the least α-amylase activity with value of 21.50±0.29 (DU) on day 0 while white sorghum treated with *Lactobacillus plantarum* in combination with *Lactobacillus fermentum* had the highest α-amylase activity at day 5 with value of 34.33±0.17 (DU). Overall, the control malts i.e control (WS) and control (RS) had the least α-amylase dextrinizing unit when compared with other sorghum varieties treated with the starter cultures with values of 26.33±0.17 DU and 26.81±0.17 (DU) (Table 4.15) .

The gelatinization temperature of the malted sorghum was increasing from day 0 to day 5. For day 0, white sorghum treated with *Lactobacillus plantarum* (WS) and red sorghum treated with combination of *Lactobacillus plantarum* and *Lactobacillus fermentum* (RS) had the same values of 69.33±0.17 and 69.33±0.44 (^{o}C), there was no significant difference between the treatment given to the two sorghum varieties. For day 5, also *Lactobacillus plantarum* (WS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) gave the highest values of 70.00±0.00 and 70.00±0.29(^{o}C). *Lactobacillus plantarum*(RS), *Lactobacillus casei*(WS), *Lactobacillus casei* (RS), *Lactobacillus fermentum* (WS), *Lactobacillus fermentum* (RS), *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) and control (WS) all had the same values of 69.83±0.17 (^{o}C) at day 5. The gelatinization temperature is shown in Table 4. 16.

Table 4.15: α- Amylase Activity (DU) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/ α- amylase dextrinizing unit (DU)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	22.33±0.17^{e}	24.83±0.33^{bc}	26.83±0.17^{cde}	29.67±0.33^{c}	31.33±0.33^{cd}	31.83±0.17^{de}
Lactobacillus plantarum (RS)	22.67±0.60^{cde}	25.00±0.00^{abc}	25.67±0.17^{f}	28.67±0.17^{d}	29.50±0.00^{fg}	29.67±0.17^{hi}
Lactobacillus casei(WS)	22.50±0.00^{de}	24.67±0.44^{c}	27.00±0.50^{cde}	30.00±0.33^{b}	32.00±0.00^{bc}	32.33±0.17^{cd}
Lactobacillus casei(RS)	23.00±0.00^{bcde}	25.00±0.29^{abc}	26.33±0.17^{ef}	28.50±0.29^{d}	29.00±0.00^{g}	29.17±0.17^{i}
Lactobacillus fermentum (WS)	22.67±0.33^{cde}	24.83±0.33^{bc}	27.33±0.17^{abcd}	31.00±0.29^{b}	32.00±0.00^{bc}	32.33±0.17^{cd}
Lactobacillus fermentum(RS)	23.50±0.29^{abc}	24.83±0.17^{bc}	26.33±0.17^{ef}	28.50±0.00^{d}	29.33±0.17^{fg}	29.83±0.17^{gh}
Lactobacillus plantarum + *Lactobacillus fermentum*(WS)	23.67±0.33^{abc}	25.67±0.44^{ab}	28.17±0.33^{a}	32.00±0.00^{a}	34.00±0.00^{a}	34.33±0.17^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	23.50±0.29^{abc-}	25.50±0.29^{abc}	27.33±0.17^{abcd}	29.50±0.29^{c}	31.00±0.00^{d}	31.67±0.17^{e}
Control (WS)	21.50±0.29^{a}	21.80±0.00^{a}	23.10±0.29^{bcde}	24.16±0.00^{c}	25.50±0.00^{abc}	26.33±0.17^{ef}
Control (RS)	21.62±0.17^{a}	21.90±0.17^{a}	23.00±0.17^{bcde}	24.14±0.00^{c}	25.40±0.17^{abc}	26.81±0.17^{cde}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.16: Gelatinization Temperature (^{o}C) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Gelatinization temperature (^{o}C)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	69.33±0.17^{ab}	69.67±0.17^{a}	69.17±0.17^{a}	69.83±0.17^{a}	70.00±0.00^{a}	70.00±0.00^{a}
Lactobacillus plantarum (RS)	68.50±0.00^{bcd}	69.33±0.17^{ab}	68.83±0.17^{a}	69.50±0.00^{a}	69.67±0.17^{a}	69.83±0.17^{a}
Lactobacillus casei(WS)	69.00±0.00^{abc}	69.33±0.17^{ab}	68.83±0.17^{a}	69.67±0.17^{a}	69.67±0.17^{a}	69.83±0.17^{a}
Lactobacillus casei(RS)	67.83±0.17^{d}	68.67±0.17^{c}	68.83±0.33^{a}	69.67±0.17^{a}	69.67±0.17^{a}	69.83±0.17^{a}
Lactobacillus fermentum (WS)	69.67±0.17^{a}	69.50±0.00^{ab}	68.83±0.17^{a}	69.67±0.17^{a}	69.83±0.17^{a}	69.83±0.17^{a}
Lactobacillus fermentum (RS)	68.67±0.44^{bcd}	69.00±0.00^{bc}	68.83±0.44^{a}	69.50±0.00^{a}	69.67±0.17^{a}	69.8.±0.17^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	69.00±0.29^{abc}	69.33±0.17^{ab}	68.83±0.17^{a}	69.67±0.17^{a}	70.17±0.33^{a}	69.83±0.44^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	69.33±0.44^{ab}	69.33±0.17^{ab}	69.33±0.17^{a}	69.50±0.00^{a}	69.83±0.33^{a}	70.00±0.29^{a}
Control (WS)	68.67±0.33^{bcd}	69.17±0.17^{abc}	68.50±0.29^{a}	69.33±0.33^{a}	69.50±0.29^{a}	69.83±0.17^{a}
Control (RS)	68.67±0.17^{bcd}	69.17±0.33^{abc}	68.83±0.17^{a}	69.50±0.00^{a}	69.50±0.00^{a}	69.50±0.00^{a}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

The pH of the malted sorghum reduced from day 0 to day 5 during the malting process. The pH of red sorghum treated with *Lactobacillus plantarum* i.e *Lactobacillus plantarum*(RS), *Lactobacillus casei*(RS) and *Lactobacillus fermentum*(RS) had the highest pH value and the pH are the same for all the treatments with value of 6.7±0.03 at day 0. The white sorghum variety treated with *Lactobacillus plantarum*(WS) had the least pH with value of 5.4±0.03 at day 5. The control malts also showed reduction in pH with control (WS) that had the value of 7.2±0.03 on day 0 and reduced to 6.2±0.03 on day 5 and control (RS) had pH value of 7.2±0.06 on day 0 and reduced to 6.2±0.06 on day 5. Overall, there was a decrease in the pH value of the sorghum varieties treated with the starter cultures. The pH was between 5.5±0.03 – 6.7±0.03 (Table 4.17).

The crude fibre content of the malted sorghum decreased during malting. Crude fibre of the red sorghum variety treated with *Lactobacillus plantarum* (RS) had the value of 0.90±0.00% at day 0 and it reduced to 0.63±0.03% at day 5. *Lactobacillus casei*(WS), *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) had the same crude fibre content of 0.77±0.03% at day 0 while *Lactobacillus plantarum* (WS), *Lactobacillus fermentum* (RS), *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the same crude fibre content of 0.57±0.03% at day 5. The control malts i.e control (WS) and control (RS) also had their crude fibre content reduced from 0.73±0.03% - 0.67±0.03% and from 0.80±0.00% - 0.60±0.06% respectively (Table 4.18).

The ash content of the malted sorghum increased during the malting process. The red sorghum treated with *Lactobacillus plantarum* and control (RS) had the same ash content of 0.70±0.00 % at day 0. *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) had the highest ash content value of 1.10±0.06% at day 5. The red sorghum sample treated with the *Lactobacillus plantarum* had the least ash content of 0.90±0.06% at day 5. Overall, there was a significant difference ($P<0.05$) between the sorghum treated samples and the control (Table 4.19)

Table 4.17: pH at different time intervals of malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/pH					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	6.7±0.03^{ab}	6.5±0.03^{ab}	6.3±0.03^{ab}	5.8±0.03^{a}	5.6±0.03^{a}	5.5±0.03^{a}
Lactobacillus plantarum (RS)	6.7±0.03^{a}	6.6±0.06^{a}	6.3±0.03^{ab}	5.9±0.06^{a}	5.6±0.07^{a}	5.6±0.03^{a}
Lactobacillus casei(WS)	6.7±0.06^{ab}	6.6±0.07^{ab}	6.4±0.09^{ab}	5.8±0.00^{a}	5.5±0.03^{a}	5.5±0.00^{a}
Lactobacillus casei(RS)	6.7±0.03^{a}	6.5±0.03^{ab}	6.4±0.03^{ab}	5.9±0.06^{a}	5.6±0.03^{a}	5.6±0.03^{a}
Lactobacillus fermentum(WS)	6.6±0.03^{ab}	6.5±0.03^{ab}	6.4±0.06^{a}	5.8±0.03^{a}	5.6±0.06^{a}	5.5±0.03^{a}
Lactobacillus fermentum(RS)	6.7±0.03^{a}	6.6±0.09^{ab}	6.4±0.06^{a}	5.9±0.03^{a}	5.6±0.03	5.5±0.06^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	6.7±0.06^{an}	6.5±0.03^{ab}	6.3±0.03^{ab}	5.8±0.03^{a}	5.6±0.10^{a}	5.5±0.06^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	6.6±0.03^{b}	6.4±0.03^{b}	6.3±0.03^{ab}	5.8±0.07^{a}	5.6±0.06^{a}	5.5±0.06^{a}
Control (WS)	7.2±0.03^{bc}	7.0±0.03^{bc}	6.7±0.03^{ab}	6.6±0.00^{a}	6.4±0.03^{ab}	6.2±0.03^{ab}
Control (RS)	7.2±0.06^{bc}	7.0±0.03^{bc}	6.7±0.05^{ab}	6.6±0.03^{a}	6.5±0.06^{ab}	6.2±0.06^{ab}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.18: Crude fibre content (%) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Crude fibre content (%)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	0.83±0.03^{ab}	0.77±0.03^{ab}	0.70±0.00^{ab}	0.63±0.03^{ab}	0.60±0.00^{ab}	0.57±0.03^{abc}
Lactobacillus plantarum (RS)	0.90±0.00^{a}	0.83±0.03^{a}	0.77±0.03^{a}	0.70±0.06^{a}	0.67±0.03^{a}	0.63±0.03^{ab}
Lactobacillus casei(WS)	0.77±0.03^{bc}	0.77±0.03^{ab}	0.73±0.03^{ab}	0.67±0.03^{ab}	0.63±0.03^{ab}	0.63±0.03^{ab}
Lactobacillus casei(RS)	0.86±0.03^{ab}	0.80±0.06^{ab}	0.73±0.03^{ab}	0.67±0.03^{ab}	0.67±0.03^{a}	0.67±0.03^{a}
Lactobacillus fermentum (WS)	0.77±0.03^{bc}	0.73±0.03^{ab}	0.70±0.06^{ab}	0.63±0.03^{ab}	0.60±0.00^{ab}	0.60±0.00^{abc}
Lactobacillus fermentum (RS)	0.87±0.03^{ab}	0.80±0.00^{ab}	0.67±0.03^{ab}	0.63±0.03^{ab}	0.60±0.06^{ab}	0.57±0.03^{abc}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	0.77±0.03^{bc}	0.70±0.00^{b}	0.67±0.03^{ab}	0.60±0.00^{ab}	0.57±0.03^{ab}	0.57±0.03^{abc}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	0.87±0.03^{ab}	0.76±0.03^{ab}	0.67±0.03^{ab}	0.63±0.03^{ab}	0.60±0.00^{ab}	0.57±0.03^{abc}
Control (WS)	0.73±0.03^{c}	0.77±0.03^{ab}	0.73±0.03^{ab}	0.70±0.00^{a}	0.67±0.03^{a}	0.67±0.03^{a}
Control (RS)	0.80±0.00^{bc}	0.77±0.03^{ab}	0.73±0.03^{ab}	0.70±0.00^{a}	0.63±0.07^{ab}	0.60±0.06^{abc}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.19: Ash content (%) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Ash content (%)					
	0	1	2	3	4	5
Lactobacillus plantarum(WS)	0.83±0.03^{ab}	0.86±0.03^{abcd}	1.00±0.06^{ab}	1.03±0.03^{a}	1.10±0.00^{abc}	1.07±0.03^{ab}
Lactobacillus plantarum(RS)	0.70±0.00^{c}	0.80±0.00^{cd}	0.87±0.03^{cde}	0.87±0.07^{bc}	0.93±0.03^{de}	0.90±0.06^{cd}
Lactobacillus casei(WS)	0.80±0.06^{abc}	0.90±0.06^{abc}	1.00±0.06^{ab}	1.03±0.07^{a}	1.13±0.03^{ab}	1.03±0.03^{ab}
Lactobacillus casei(RS)	0.73±0.03^{bc}	0.80±0.00^{cd}	0.90±0.00^{bcde}	0.87±0.03^{bc}	0.96±0.03^{cde}	0.96±0.03^{bc}
Lactobacillus fermentum(WS)	0.87±0.03^{a}	0.97±0.03^{a}	0.97±0.03^{abc}	1.03±0.03^{a}	1.06±0.07^{abcd}	0.96±0.03^{bc}
Lactobacillus fermentum(RS)	0.77±0.03^{abc}	0.83±0.03^{bcd}	0.83±0.03^{de}	0.83±0.03^{c}	0.87±0.03^{e}	0.83±0.03^{d}
Lactobacillus plantarum+ *Lactobacillus fermentum*(WS)	0.87±0.03^{a}	0.90±0.00^{abc}	0.90±0.00^{bcde}	1.00±0.00^{ab}	1.17±0.03^{a}	1.10±0.06^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	0.77±0.03^{abc}	0.77±0.03^{d}	0.80±0.00^{e}	0.90±0.00^{abc}	1.03±0.03^{abcd}	1.00±0.00^{abc}
Control (WS)	0.80±0.06^{abc}	0.93±0.03^{ab}	0.96±0.03^{abc}	1.00±0.00^{ab}	1.13±0.03^{ab}	1.03±0.03^{ab}
Control (RS)	0.70±0.06^{c}	0.76±0.03^{d}	0.86±0.03^{cde}	0.90±0.06^{abc}	1.00±0.06^{bcd}	0.96±0.03^{bc}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Conversely, the ether extract decreased steadily throughout the period of malting. At day 0, *Lactobacillus fermentum* (RS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) had the highest ether extract of 1.37±0.03% and at day 5, *Lactobacillus fermentum* (RS) and control RS had the highest value of 1.20±0.00%. The white sorghum variety treated with *Lactobacillus fermentum* (WS) had the lowest ether extract with value of 1.06±0.03% at day 5(Table 4. 20).

Table 4.21 shows the protein content of the malted sorghum. The protein content of the malted sorghum increased steadily throughout the period of the malting process from day 0 to day 5. On day 0, *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the highest protein content of 5.40±0.06%. *Lactobacillus plantarum* (WS) had the least protein content of 5.07±0.03%. At day 5, *Lactobacillus fermentum* (RS) had the highest protein content of 6.57±0.07% while *Lactobacillus plantarum* (WS) had the least protein content of 6.37±0.03%. *Lactobacillus plantarum* +*Lactobacillus fermentum* (RS) and control (RS) had the same protein content with values of 6.53±0.03%.

There was also an increase in the free amino nitrogen (FAN) content during the malting of the sorghum varieties challenged with the LAB isolates. At day 0, *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) had the highest FAN content of 13.00±0.58mg/L and the control (WS) also had the same value of 13.00±0.58mg/L. *Lactobacillus plantarum* (RS), *Lactobacillus fermentum* (RS) and control (RS) had the lowest FAN values and the values are 11.33±0.67mg/L. At day 5, LC (WS), *Lactobacillus plantarum*+ *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the highest FAN values of 71.00±1.00mg/L, 71.00±0.58mg/L and 71.67±0.88mg/L respectively. There were no significant differences between the FAN of the samples. The control malts i.e control (WS) and control (RS) had the same FAN content of 68.00±1.00mg/L and 68.00±1.20mg/L (Table 4. 22).

Table 4. 20: Ether Extract (%) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Ether extract (%)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Lactobacillus plantarum(WS)	1.20±0.00^{efg}	1.43±0.03^{e}	1.43±0.03^{abcd}	1.27±0.03^{c}	1.23±0.03^{bcd}	1.13±0.03^{abc}
Lactobacillus plantarum (RS)	1.33±0.03^{abc}	1.60±0.06^{abcd}	1.53±0.03^{a}	1.50±0.00^{a}	1.17±0.03^{cd}	1.10±0.06^{bc}
Lactobacillus casei(WS)	1.17±0.03^{fg}	1.47±0.03^{de}	1.37±0.03^{bcd}	1.33±0.03^{bc}	1.17±0.03^{cd}	1.10±0.06^{bc}
Lactobacillus casei(RS)	1.33±0.03^{abc}	1.70±0.00^{a}	1.50±0.06^{ab}	1.47±0.03^{ab}	1.23±0.03^{bcd}	1.17±0.03^{abc}
Lactobacillus fermentum(WS)	1.17±0.03^{fg}	1.50±0.06^{cde}	1.36±0.07^{bcd}	1.30±0.06^{c}	1.13±0.03^{d}	1.06±0.03^{c}
Lactobacillus fermentum(RS)	1.37±0.03^{ab}	1.67±0.03^{ab}	1.47±0.09^{abc}	1.53±0.03^{a}	1.33±0.03^{ab}	1.20±0.00^{ab}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	1.23±0.03^{def}	1.53±0.03^{bcde}	1.30±0.00^{d}	1.23±0.07^{c}	1.13±0.03^{d}	1.10±0.00^{bc}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	1.37±0.03^{ab}	1.63±0.03^{abc}	1.47±0.03^{abc}	1.50±0.06^{a}	1.33±0.03^{ab}	1.17±0.03^{abc}
Control (WS)	1.23±0.03^{def}	1.50±0.06^{cde}	1.33±0.03^{cd}	1.30±0.06^{c}	1.13±0.03^{d}	1.07±0.03^{c}
Control (RS)	1.30±0.00^{bcd}	1.63±0.03^{abc}	1.57±0.03^{a}	1.50±0.06^{a}	1.33±0.03^{ab}	1.20±0.00^{ab}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.21: Protein Content (%) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Protein content (%)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	5.07±0.03^{e}	5.43±0.03^{b}	5.87±0.03^{def}	6.10±0.06^{de}	6.33±0.03^{abc}	6.37±0.03^{b}
Lactobacillus plantarum (RS)	5.27±0.03^{bc}	5.63±0.03^{a}	6.03±0.03^{abc}	6.30±0.00^{abc}	6.50±0.00^{a}	6.50±0.07^{ab}
Lactobacillus casei(WS)	5.17±0.03^{cde}	5.37±0.03^{b}	5.73±0.03^{f}	6.07±0.03^{e}	6.37±0.12^{abc}	6.50±0.10^{ab}
Lactobacillus casei(RS)	5.37±0.03^{ab}	5.67±0.03^{a}	5.93±0.03^{bcd}	6.26±0.03^{abcd}	6.43±0.03^{abc}	6.47±0.03^{ab}
Lactobacillus fermentum(WS)	5.17±0.03^{cde}	5.43±0.03^{b}	5.87±0.07^{def}	6.10±0.06^{de}	6.30±0.00^{bc}	6.40±0.06^{ab}
Lactobacillus fermentum(RS)	5.27±0.03^{be}	5.60±0.06^{a}	6.06±0.03^{ab}	6.27±0.09^{abcd}	6.47±0.03^{ab}	6.57±0.07^{a}
Lactobacillus plantarum + *Lactobacillus fermentum*(WS)	5.16±0.03^{cde}	5.43±0.03^{b}	5.76±0.03^{ef}	6.20±0.06^{abcd}	6.27±0.03^{bc}	6.40±0.00^{ab}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	5.40±0.06^{a}	5.63±0.03^{a}	6.10±0.00^{a}	6.33±0.09^{ab}	6.47±0.03^{ab}	6.53±0.03^{ab}
Control (WS)	5.13±0.03^{de}	5.40±0.00^{b}	5.97±0.03^{abcd}	6.17±0.03^{bcde}	6.33±0.03^{abc}	6.43±0.03^{ab}
Control (RS)	5.33±0.03^{ab}	5.67±0.03^{a}	6.10±0.06^{a}	6.33±0.07^{ab}	6.43±0.03^{abc}	6.53±0.03^{ab}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.22: Free Amino Nitrogen content (mg/L) at different time intervals of the malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/FAN (mg/L)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	12.33±0.33^{a}	34.33±0.33^{a}	40.67±0.67^{bcd}	53.67±0.88^{ab}	64.67±0.33^{a}	69.00±0.58^{ab}
Lactobacillus plantarum (RS)	11.33±0.67^{a}	31.33±0.67^{e}	37.33±0.33^{f}	49.33±0.88^{c}	61.00±0.58^{bc}	69.00±0.58^{ab}
Lactobacillus casei(WS)	12.67±0.88^{a}	33.33±0.33^{abcd}	40.33±0.88^{cde}	53.33±0.67^{ab}	65.00±0.00^{a}	71.00±1.00^{a}
Lactobacillus casei(RS)	12.00±0.00^{a}	32.33±0.33^{cde}	38.33±0.88^{ef}	50.00±1.16^{c}	62.00±0.58^{bc}	68.00±0.58^{b}
Lactobacillus fermentum(WS)	12.67±0.33^{a}	34.33±0.33^{a}	42.33±0.33^{abc}	53.67±0.33^{ab}	64.67±0.67^{a}	68.67±1.20^{ab}
Lactobacillus fermentum(RS)	11.33±0.33^{a}	32.33±0.33^{cde}	38.67±0.67^{def}	50.00±1.00^{c}	61.67±0.33^{bc}	69.00±1.15^{ab}
Lactobacillus plantarum + *Lactobacillus fermentum*(WS)	13.00±0.58^{a}	34.00±0.58^{ab}	42.67±0.67^{ab}	53.67±0.88^{ab}	64.00±0.00^{a}	71.00±0.58^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	11.67±0.33^{a}	32.00±0.00^{de}	38.67±0.33^{def}	49.33±1.33^{c}	61.67±0.33^{bc}	71.67±0.88^{a}
Control (WS)	13.00±0.58^{a}	33.67±0.00^{abc}	43.00±0.58^{a}	54.33±0.67^{a}	65.33±0.33^{a}	68.00±1.00^{ab}
Control (RS)	11.33±0.88^{a}	33.00±0.00^{abcd}	39.33±1.33^{def}	51.33±0.88^{bc}	62.33±0.33^{b}	68.00±1.20^{ab}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

4.2.11 Characteristic properties of derived wort from LAB- challenged sorghum samples

The yeast cell count (cells/mL) was monitored throughout the fermentation period for the wort. On day 0, there was no yeast cell count i.e it was 0. From days 1 to day 4, the yeast cell count increased from 0.9 x 10^5 – 3.8 x 10^5 (cells/mL) and on the 5th day, there was a decrease in the yeast cell count. On day 4, the control i.e control WS had the highest yeast cell count of 4.3 x 10^5 (cells/mL) while white sorghum challenged with *Lactobacillus fermentum*(WS) had the least yeast cell count of 2.9x10^5 cells/mL(Table 4.23).

The total titratable acidity (TTA) of the wort increased from day 0 to day 5 during the fermentation process. At day 0, control (RS) had the least total titratable acidity of 0.63±0.03% while LF (WS) had the highest value of total titratable acidity of 0.83±0.03%. There was no significant difference($p<0.05$) between *Lactobacillus plantarum* (RS), *Lactobacillus casei*(WS) and *Lactobacillus fermentum* (RS), they had the same TTA with value of 0.73±0.03%. At day 5, *Lactobacillus plantarum* (WS) and *Lactobacillus casei* (WS) had the highest TTA with values of 4.77±0.03% while control (RS) had the least TTA with value of 3.97±0.09%. Overall, the control worts had lowest TTA values when compared with the wort derived from malted sorghum subjected to challenge by Lactic acid bacterial isolates (Table 4. 24**)**.

There was a reduction in the reducing sugar content of the wort from day 0 to day 5. At day 0, *Lactobacillus plantarum* (WS) and *Lactobacillus casei* (WS) had the highest values of 1.80±0.06% and 1.80±0.00% respectively while *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) had the least reducing sugar of 1.37±0.07%. At day 5, *Lactobacillus fermentum* (WS), *Lactobacillus fermentum* (RS), *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) and control (WS) had the highest reducing sugar content of 0.17±0.03% while *Lactobacillus casei*(RS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (WS) had the least reducing sugar content of 0.10±0.06 and 0.10±0.00%. Also on day 5, *Lactobacillus plantarum*(WS), *Lactobacillus plantarum(*RS) and *Lactobacillus casei*(WS) all had the same reducing sugar content of 0.13±0.03%. Overall, control (RS) had the highest reducing sugar content of 0.30±0.06% (Table4.25)

Table 4.23: Yeast cell count (cells/ml) x 10^5 during fermentation of wort

LAB Isolates/ Sorghum Varieties	Treatment Period (Days) / Yeast Cell Count (Cells /ml)											
	0		1		2		3		4		5	
	RS	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS	WS
L. plantarum	-	-	1.0×10^5	1.2×10^5	1.5×10^5	1.8×10^5	2.2×10^5	2.7×10^5	3.4×10^5	3.7×10^5	3.0×10^5	2.8×10^5
L. fermentum	-	-	1.5×10^5	1.1×10^5	1.7×10^5	2.0×10^5	2.5×10^5	2.5×10^5	3.6×10^5	2.9×10^5	2.7×10^5	2.2×10^5
L. casei	-	-	1.3×10^5	1.0×10^5	2.1×10^5	1.9×10^5	3.0×10^5	2.8×10^5	3.3×10^5	3.8×10^5	2.9×10^5	3.4×10^5
L.plantarum+ L. fermentum	-	-	1.2×10^5	0.9×10^5	2.2×10^5	1.5×10^5	3.3×10^5	2.7×10^5	4.0×10^5	3.6×10^5	3.5×10^5	3.2×10^5
Control	-	-	1.0×10^5	1.2×10^5	1.8×10^5	2.1×10^5	2.6×10^5	3.0×10^5	3.4×10^5	4.3×10^5	2.9×10^5	3.3×10^5

Key

- = No growth

Table 4.24: Total Titratable Acidity (TTA) (%) at different time intervals of wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Total titratable acidity (%)					
	0	**1**	**2**	**3**	**4**	**5**
Lactobacillus plantarum (WS)	0.77±0.03^{ab}	1.97±0.03^{def}	2.93±0.03^{de}	4.17±0.03^{ab}	4.57±0.03^{ab}	4.77±0.03^{a}
Lactobacillus plantarum (RS)	0.73±0.03^{abc}	1.80±0.06^{h}	2.77±0.03^{f}	3.87±0.03^{d}	4.43±0.07^{bc}	4.70±0.06^{ab}
Lactobacillus casei(WS)	0.73±0.03^{abc}	2.03±0.03^{cde}	2.93±0.07^{de}	4.10±0.06^{ab}	4.57±0.03^{ab}	4.77±0.03^{a}
Lactobacillus casei(RS)	0.66±0.03^{bc}	1.87±0.03^{fgh}	2.70±0.00^{f}	3.87±0.03^{d}	4.27±0.03^{d}	4.57±0.03^{bcd}
Lactobacillus fermentum(WS)	0.83±0.03^{a}	1.93±0.03^{efg}	2.97±0.07^{d}	4.17±0.07^{ab}	4.50±0.00^{b}	4.67±0.03^{abc}
Lactobacillus fermentum(RS)	0.73±0.03^{abc}	1.83±0.07^{gh}	2.80±0.06^{ef}	3.87±0.03^{d}	4.23±0.03^{d}	4.50±0.06^{cd}
Lactobacillus plantarum + *Lactobacillus fermentum*(WS)	0.77±0.03^{ab}	2.23±0.03^{a}	3.17±0.09^{abc}	4.06±0.12^{bc}	4.47±0.03^{bc}	4.57±0.03^{bcd}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	0.67±0.03^{bc}	2.17±0.03^{ab}	3.03±0.03^{cd}	3.80±0.00^{d}	4.23±0.03^{d}	4.47±0.03^{d}
Control (WS)	0.70±0.06^{bc}	1.47±0.03^{i}	2.33±0.03^{g}	3.43±0.03^{e}	3.67±0.09^{e}	4.03±0.07^{e}
Control (RS)	0.63±0.03^{c}	1.33±0.03^{j}	2.27±0.03^{g}	3.50±0.06^{e}	3.67±0.09^{e}	3.97±0.09^{e}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.25: Reducing sugar content (%) at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Reducing sugar content (%)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	1.80±0.06^{a}	1.17±0.03^{bc}	0.53±0.03^{d}	0.27±0.03^{de}	0.17±0.03^{cd}	0.13±0.03^{b}
Lactobacillus plantarum (RS)	1.60±0.00^{bc}	1.06±0.03^{cd}	0.63±0.03^{cd}	0.37±0.03^{cd}	0.20±0.00^{cd}	0.13±0.03^{b}
Lactobacillus casei(WS)	1.80±0.00^{a}	1.03±0.03^{d}	0.53±0.03^{d}	0.23±0.03^{de}	0.13±0.00^{d}	0.13±0.03^{b}
Lactobacillus casei(RS)	1.47±0.07^{cde}	1.10±0.06^{cd}	0.63±0.07^{cd}	0.30±0.06^{cde}	0.20±0.00^{cd}	0.10±0.06^{b}
Lactobacillus fermentum(WS)	1.73±0.03^{ab}	1.03±0.03^{d}	0.63±0.09^{cd}	0.33±0.03^{cde}	0.23±0.03^{bcd}	0.17±0.03^{b}
Lactobacillus fermentum(RS)	1.43±0.03^{cde}	1.07±0.03^{cd}	0.77±0.03^{bc}	0.37±0.09^{cd}	0.20±0.06^{cd}	0.17±0.03^{b}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	1.53±0.03^{cd}	1.03±0.03^{d}	0.60±0.06^{cd}	0.20±0.00^{e}	0.13±0.03^{d}	0.10±0.00^{b}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	1.37±0.07^{de}	1.00±0.06^{d}	0.73±0.03^{bc}	0.27±0.03^{de}	0.17±0.03^{cd}	0.17±0.03^{b}
Control (WS)	1.50±0.06^{cd}	1.27±0.03^{ab}	0.87±0.03^{ab}	0.53±0.03^{b}	0.33±0.03^{b}	0.17±0.03^{b}
Control (RS)	1.40±0.06^{de}	1.30±0.00^{a}	0.97±0.03^{a}	0.70±0.06^{a}	0.47±0.03^{a}	0.30±0.06^{a}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

The total residual sugar content of the wort samples reduced significantly from day 0 day 5 of the fermentation period. On day 0, *Lactobacillus casei* (WS) had the highest total residual sugar content of 3.63±0.03% while *Lactobacillus casei* (RS) and control (RS) had the least values of 3.27±0.03% and 3.27±0.07%. Also on day 0, *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (WS) had the same total residual sugar content with value of 3.60±0.06%, *Lactobacillus fermentum* (RS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the same total residual sugar content with value of 3.37±0.03%. On day 5, the control worts i.e control (WS) and control (RS) residual had higher total sugar content of 1.23±0.03% while *Lactobacillus plantarum*+ *Lactobacillus fermentum* (WS) had the least with value of 0.93±0.03%. *Lactobacillus plantarum* (WS) and *Lactobacillus casei* (WS) also had the same total residual sugar content with value of 0.97±0.03% at day 5. There were significant ($P<0.05$) differences in the control worts and the wort derived from the malted sorghum challenged with the Lactic acid bacterial isolates (Table 4.26).

Free amino nitrogen content (FAN) of the sorghum wort increased throughout the fermentation period as shown in Table 4.27. On day 0, *Lactobacillus plantarum*(WS) had the highest FAN content of 3.83±0.03mg/L while *Lactobacillus fermentum* (RS) produced the least FAN content of 3.40±0.00mg/L. *Lactobacillus plantarum* (RS), Control WS, and Control (RS) all had the similar values of 3.47±0.03, 3.47±0.07 and 3.47±0.03mg/L respectively. Also at day 0, *Lactobacillus casei* (WS) and *Lactobacillus fermentum* (WS) had the same FAN content of 3.77±0.03mg/L and 3.77±0.07mg/L. *Lactobacillus casei*(RS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) also had the same value of 3.43±0.03mg/L. At day 5, *Lactobacillus plantarum* (WS) had the highest FAN content of 29.97±0.19mg/L while the least FAN was produced by control (RS) with value of 19.37±0.07mg/L. Overall, the treated worts had higher FAN content when compared with the control worts.

The pH of all the worts produced during fermentation were all in the acidic range. The pH reduced considerably from 6.50±0.03 to 2.93±0.03. At day 0, control (RS) had the highest pH value of 6.50±0.03 while LP (WS) had the lowest pH value of 5.16±0.03. At day 5, control (WS) had the highest pH value of 3.80±0.03 while *Lactobacillus plantarum* (WS) had the least pH with value of 2.93+0.03. The pH of the control worts i.e control (WS) and control (RS) had the highest pH value after the fermentation process with values of 3.80±0.03 and 3.75±0.03 respectively when compared with the wort challenged with Lactic acid bacterial isolates (Table 4. 28).

Table 4.26: Total residual sugar content (%) at different time intervals of wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Residual sugar content (%)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	3.43±0.07^{bcd}	3.07±0.03^{b}	1.93±0.03^{bc}	1.30±0.06^{c}	1.13±0.03^{def}	0.97±0.03^{c}
Lactobacillus plantarum (RS)	3.30±0.06^{d}	3.00±0.00^{bc}	2.06±0.03^{b}	1.53±0.03^{b}	1.20±0.06^{cde}	1.03±0.03^{bc}
Lactobacillus casei(WS)	3.63±0.03^{a}	2.97±0.03^{bc}	1.83±0.03^{c}	1.27±0.03^{cd}	1.10±0.06^{efg}	0.97±0.03^{c}
Lactobacillus casei(RS)	3.27±0.03^{d}	2.93±0.07^{bc}	2.00±0.00^{b}	1.47±0.03^{b}	1.23±0.03^{cd}	1.07±0.03^{bc}
Lactobacillus fermentum(WS)	3.60±0.06^{ab}	2.97±0.03^{bc}	2.03±0.07^{b}	1.57±0.09^{b}	1.30±0.06^{c}	1.13±0.07^{ab}
Lactobacillus fermentum(RS)	3.37±0.03^{cd}	3.03±0.03^{bc}	2.30±0.06^{a}	1.73±0.03^{a}	1.43±0.03^{b}	1.20±0.06^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	3.60±0.06^{ab}	2.93±0.07^{bc}	1.63±0.03^{d}	1.13±0.03^{d}	1.00±0.00^{g}	0.93±0.03^{c}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	3.37±0.03^{cd}	2.97±0.03^{bc}	1.53±0.03^{d}	1.17±0.03^{cd}	1.03±0.03^{fg}	1.00±0.00^{c}
Control (WS)	3.53±0.03^{abc}	3.33±0.03^{a}	2.33±0.03^{a}	1.87±0.07^{a}	1.57±0.03^{a}	1.23±0.03^{a}
Control (RS)	3.27±0.07^{d}	3.37±0.03^{a}	2.23±0.03^{a}	1.77±0.03^{a}	1.43±0.03^{b}	1.23±0.03^{a}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.27: Free Amino Nitrogen (FAN) at different time intervals of sorghum wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/FAN (mg/L)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	3.83±0.03^{a}	11.60±0.06^{cde}	17.33±0.03^{d}	21.73±0.18^{d}	27.40±0.06^{c}	29.97±0.19^{ab}
Lactobacillus plantarum (RS)	3.47±0.03^{c}	11.43±0.03^{efg}	16.60±0.06^{f}	19.90±0.06^{g}	25.30±0.11^{gh}	27.53±0.12^{e}
Lactobacillus casei(WS)	3.77±0.03^{a}	11.70±0.00^{bcd}	17.43±0.09^{d}	21.73±0.03^{d}	27.37±0.07^{c}	29.73±0.09^{b}
Lactobacillus casei(RS)	3.43±0.03^{c}	11.23±0.03^{h}	16.30±0.06^{g}	19.73±0.03^{g}	25.50±0.06^{f}	27.37±0.03^{ef}
Lactobacillus fermentum(WS)	3.77±0.07^{a}	11.73±0.03^{bc}	17.63±0.03^{c}	21.27±0.09^{e}	21.17±0.07^{d}	29.30±0.06^{c}
Lactobacillus fermentum(RS)	3.40±0.00^{c}	11.33±0.07^{gh}	16.33±0.09^{g}	19.83±0.03^{g}	25.27±0.03^{h}	27.20±0.06^{f}
Lactobacillus plantarum+ *Lactobacillus fermentum* (WS)	3.80±0.06^{a}	11.93±0.03^{a}	17.93±0.03^{b}	22.87±0.03^{b}	27.50±0.06^{c}	29.90±0.06^{ab}
Lactobacillus plantarum+ *Lactobacillus fermentum* (RS)	3.43±0.03^{c}	11.53±0.03^{def}	16.77±0.03^{e}	21.33±0.03^{e}	25.60±0.00^{f}	27.56±0.03^{e}
Control (WS)	3.47±0.07^{c}	7.433±0.12^{i}	9.03±0.03^{h}	13.33±0.09^{h}	17.47±0.03^{i}	19.87±0.07^{g}
Control (RS)	3.47±0.03^{c}	6.63±0.12^{j}	8.20±0.06^{i}	11.90±0.06^{i}	17.03±0.03^{j}	19.37±0.07^{h}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.28: pH at different time intervals of wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/pH					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	5.2±0.03^{d}	4.2±0.03^{de}	3.7±0.03^{de}	3.3±0.03^{c}	3.0±0.03^{de}	2.3±0.03^{d}
Lactobacillus plantarum (RS)	5.4±0.03^{bc}	4.3±0.00^{cd}	3.9±0.03^{ab}	3.5±0.03^{ab}	3.2±0.03^{bcd}	3.0±0.03^{bcd}
Lactobacillus casei(WS)	5.37±0.03^{cd}	4.3±0.06^{cd}	3.7±0.03^{ef}	3.2±0.03^{c}	3.1±0.03^{cde}	3.0±0.00^{cd}
Lactobacillus casei(RS)	5.3±0.03^{bc}	4.4±0.03^{bc}	3.8±00^{cd}	3.4±0.03^{b}	3.1±0.06^{bcde}	3.0±0.03^{bcd}
Lactobacillus fermentum(WS)	5.2±0.03^{cd}	4.2±0.03^{de}	3.6±0.03^{efg}	3.3±0.03^{c}	3.1±0.03^{cde}	3.0±0.03^{bcd}
Lactobacillus fermentum(RS)	5.3±0.03^{bc}	4.3±0.03^{cde}	3.9±0.07^{bc}	3.5±0.07^{ab}	3.2±0.06^{abc}	3.1±0.07^{abc}
Lactobacillus plantarum + *Lactobacillus fermentum*(WS)	5.5±0.03^{a}	4.2±0.00^{de}	3.4±0.03^{hi}	3.2±0.00^{c}	2.9±0.03^{e}	2.9±0.07^{d}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	5.5±0.07^{a}	4.2±0.03^{e}	3.6±0.00^{fg}	3.2±0.03^{c}	3.1±0.07^{cde}	3.0±0.03^{bcd}
Control (WS)	6.1±0.07^{de}	6.0±0.03^{de}	5.3±0.03^{cd}	4.6±0.03^{bc}	3.9±0.03^{ab}	3.8±0.03^{cd}
Control (RS)	6.5±0.03^{de}	5.8±0.03^{de}	5.1±0.03^{cd}	4.2±0.03^{e}	3.9±0.03^{bc}	3.8±0.03^{de}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

The ethanol content of the wort increased as the fermentation progressed. There was no ethanol production at day 0, production of ethanol started from day 1 of the fermentation and *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) had the highest ethanol content of 0.63±0.03 (%,w/v) while the lowest ethanol was produced by the control worts i.e control (WS) and control (WS) had the same value of 0.13±0.03 (%,w/v). On day 5 of the fermentation, the highest ethanol content was produced by *Lactobacillus plantarum*+ *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) with values of 4.80±0.06 (%, w/v) and 4.80±0.03 (%, w/v), the control wort i.e control (RS) had the lowest value of 2.10±0.03 (%, w/v). The worts produced from the malted sorghum challenged with the Lactic acid bacterial isolates produced higher ethanol contents than the control worts. There were significant ($P < 0.05$) differences between the values obtained for the ethanol content (Table 4. 29).

Specific gravity of the wort obtained were all in the same range. For day 0, the specific gravity for all the samples were the same and also for days 2, 3 and 4. The treated worts had a specific gravity with a 1.02±0.00 while the control worts had a specific gravity of 1.01±0.00 (Table4. 30).

The colour of the wort reduced considerably as the fermentation progressed. For day 0, *Lactobacillus plantarum* (RS) had the highest colour change with value of 36.60±0.30 while *Lactobacillus plantarum* (WS) had the lowest colour change of 29.17±0.03. For day 5, *Lactobacillus plantarum* (RS) also had the highest colour change of 33.90±0.06 and also *Lactobacillus plantarum* (WS) had the lowest colour with value of 24.87±0.07. The control worts also compared considerably with the challenged sorghum worts (Table 4.31).

Turbidity of the wort increased from day 0 to day 5 during fermentation. For day 0, control (WS) was the most turbid with value of 0.54±0.01 while *Lactobacillus plantarum*(RS), *Lactobacillus casei*(RS) and *Lactobacillus fermentum* (RS) had the least turbidity with the same value of 0.43±0.00. The *Lactobacillus plantarum*(WS) had the highest turbidity with a value of 0.56±0.00 and also *Lactobacillus casei*(WS), *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (WS) also had similar values while *Lactobacillus fermentum* (RS) had the lowest turbidity value of 0.45±0.00 on day 5. Also on day 5, LP(RS), LC(RS), *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) and control (RS) all had the same turbidity with value of 0.46±0.00 (Table 4.32).

The protein content of the wort reduced during the fermentation period (Table 4.33). On day 0, *Lactobacillus casei* (WS) had the highest protein content with value of 1.77±0.03% while *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) had the least value of 1.53±0.03% and the control worts i.e control (WS) had protein contents of 1.63±0.03, control (RS) also had the same value. Also at day 0, *Lactobacillus plantarum*(RS) and *Lactobacillus casei* (RS) had protein content of 1.63±0.03% which was the same as that of the control worts. At day 5, control (WS) had the highest protein content of 1.17±0.03% while *Lactobacillus casei* (RS) had the least protein content with value of 0.93±0.03%. *Lactobacillus plantarum* (RS), *Lactobacillus fermentum* (WS), *Lactobacillus fermentum* (RS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) all had the same protein content with value of 0.97±0.03%. Also, *Lactobacillus plantarum* (WS) and *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) had the same protein content with value of 1.03±0.03% at day 5.

There was a reduction in the total solids of the wort produced from the malted sorghum challenged with the lactic acid bacterial isolates during fermentation. On day 0, LC (WS) had the highest total solids with the value of 13.87±0.03% while *Lactobacillus plantarum* (RS) had the least total solids of 13.03±0.03. Also on day 0, *Lactobacillus casei* (RS) and *Lactobacillus fermentum* (RS) had the same total solids with value of 13.23±0.03%. On day 5, control (WS) had the highest total solid with value of 13.10±0.06% while *Lactobacillus plantarum*+ *Lactobacillus fermentum* (RS) gave the least total solid with value of 12.10±0.00% (Table 4.34).

Table 4. 29: Ethanol Content (%, w/v) at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Ethanol content (%, w/v)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	0.00±0.00^{a}	0.37±0.03^{ef}	0.87±0.03^{abc}	1.57±0.07^{cde}	2.17±0.03^{ab}	4.75±0.03^{efg}
Lactobacillus plantarum (RS)	0.00±0.00^{a}	0.23±0.03^{gh}	0.80±0.00^{cd}	1.43±0.03^{f}	2.17±0.03^{ab}	4.70±0.06^{efg}
Lactobacillus casei(WS)	0.00±0.00^{a}	0.43±0.03^{cde}	0.93±0.03^{a}	1.63±0.03^{bcd}	2.10±0.06^{b}	4.68±0.06^{efg}
Lactobacillus casei(RS)	0.00±0.00^{a}	0.30±0.00^{fg}	0.80±0.00^{cd}	1.43±0.03^{f}	1.97±0.03^{c}	4.60±0.03^{fg}
Lactobacillus fermentum(WS)	0.00±0.00^{a}	0.50±0.06^{cd}	0.93±0.03^{a}	1.67±0.03^{abc}	2.10±0.06^{b}	4.65±0.03^{fg}
Lactobacillus fermentum(RS)	0.00±0.00^{a}	0.33±0.33^{efg}	0.77±0.03^{d}	1.50±0.06^{ef}	1.93±0.03^{c}	4.60±0.06^{fg}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	0.00±0.00^{a}	0.63±0.03^{ab}	0.83±0.03^{bcd}	1.70±0.06^{ab}	2.17±0.03^{ab}	4.80±0.06e^{fg}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	0.00±0.00^{a}	0.43±0.03^{cde}	0.77±0.03^{d}	1.53±0.03^{def}	2.10±0.06^{b}	4.80±0.03^{efg}
Control (WS)	0.00±0.00^{a}	0.13±0.03^{h}	0.37±0.03^{e}	0.87±0.03^{g}	1.66±0.03^{d}	2.20±0.03^{ab}
Control (RS)	0.00±0.00^{a}	0.13±0.03^{h}	0.37±0.03^{e}	0.80±0.00^{g}	1.63±0.03^{d}	2.10±0.03^{b}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.30: Specific Gravity at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Specific gravity					
	0	**1**	**2**	**3**	**4**	**5**
Lactobacillus plantarum (WS)	1.01±0.00^{bc}	1.01±0.00^{d}	1.02±0.00^{bcd}	1.02±0.00^{bc}	1.02±0.00^{abc}	1.02±0.00^{ab}
Lactobacillus plantarum (RS)	1.01±0.00^{abc}	1.01±0.00^{f}	1.02±0.00^{de}	1.02±0.00^{d}	1.02±0.00^{d}	1.02±0.00^{b}
Lactobacillus casei(WS)	1.01±0.00^{c}	1.01±0.00^{cd}	1.01±0.00^{bc}	1.02±0.00^{abc}	1.02±0.00^{a}	1.02±0.00^{ab}
Lactobacillus casei(RS)	1.01±0.00^{abc}	1.01±0.00^{f}	1.01±0.00^{abc}	1.02±0.00^{c}	1.02±0.00^{bcd}	1.02±0.00^{b}
Lactobacillus fermentum(WS)	1.01±0.00^{abc}	1.02±0.00^{cd}	1.02±0.00^{abc}	1.02±0.00^{ab}	1.02±0.00^{a}	1.02±0.00^{ab}
Lactobacillus fermentum(RS)	1.01±0.00^{abc}	1.02±0.00^{ef}	1.02±0.00^{e}	1.02±0.00^{d}	1.02±0.00^{e}	1.02±0.00^{c}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	1.01±0.00^{a}	1.02±0.00^{a}	1.02±0.00^{a}	1.02±0.00^{a}	1.02±0.00^{a}	1.02±0.00^{a}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	1.01±0.00^{a}	1.02±0.00^{cd}	1.02±0.00^{e}	1.02±0.00^{d}	1.02±0.00^{e}	1.02±0.00^{c}
Control (WS)	1.01±0.00^{ab}	1.02±0.00^{g}	1.02±0.00^{f}	1.02±0.00^{e}	1.02±0.00^{f}	1.01±0.00^{d}
Control (RS)	1.01±0.00^{abc}	1.02±0.00^{g}	1.02±0.00^{f}	1.02±0.00^{e}	1.02±0.00^{f}	1.01±0.00^{e}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.31: Colour at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Colour					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	29.17±0.03^{d}	26.77±0.09^{e}	25.33±0.09^{j}	25.27±0.03^{g}	25.03±0.03^{i}	24.87±0.07^{h}
Lactobacillus plantarum (RS)	36.60±0.30^{b}	34.67±0.09^{b}	34.17±0.09^{a}	34.13±0.07^{a}	34.03±0.03^{a}	33.90±0.06^{a}
Lactobacillus casei(WS)	29.43±0.03^{d}	26.77±0.03^{e}	26.30±0.06^{f}	26.30±0.10^{d}	26.17±0.09^{f}	26.00±0.10^{f}
Lactobacillus casei(RS)	37.53±0.68^{a}	34.37±0.03^{cd}	33.87±0.09^{b}	33.87±0.03^{b}	33.57±0.03^{c}	33.27±0.03^{cd}
Lactobacillus fermentum(WS)	29.20±0.00^{d}	26.67±0.09^{e}	25.73±0.07^{h}	25.97±0.03^{e}	25.83±0.03^{g}	25.50±0.06^{g}
Lactobacillus fermentum(RS)	35.83±0.09^{c}	34.53±0.03^{bc}	33.53±0.03^{cd}	33.60±0.06^{c}	33.47±0.03^{c}	33.33±0.03^{c}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	29.30±0.15^{d}	26.33±0.09^{f}	25.50±0.06^{ij}	25.63±0.03^{f}	25.53±0.03^{h}	25.40±0.00^{g}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	36.47±0.03^{bc}	34.30±0.06^{d}	33.43±0.09^{de}	33.53±0.03^{c}	33.27±0.03^{d}	33.13±0.03^{d}
Control (WS)	29.57±0.03^{d}	26.70±0.06^{e}	26.07±0.07^{g}	26.17±0.09^{d}	26.33±0.03^{e}	26.20±0.06^{e}
Control (RS)	36.43±0.03^{bc}	35.07±0.07^{a}	33.77±0.03^{b}	33.87±0.03^{b}	33.73±0.03^{b}	33.57±0.03^{b}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.32: Turbidity at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Lactobacillus plantarum (WS)	0.53±0.00^{a}	0.54±0.00^{b}	0.55±0.00^{a}	0.55±0.00^{a}	0.56±0.00^{a}	0.56±0.00^{ab}
Lactobacillus plantarum (RS)	0.43±0.00^{c}	0.45±0.00^{d}	0.46±0.00^{cde}	0.46±0.00^{e}	0.46±0.00^{c}	0.46±0.00^{e}
Lactobacillus casei(WS)	0.53±0.00^{a}	0.54±0.00^{ab}	0.55±0.00^{a}	0.55±0.00^{a}	0.56±0.00^{a}	0.56±0.00^{c}
Lactobacillus casei(RS)	0.43±0.00^{c}	0.45±0.00^{ef}	0.45±0.00^{def}	0.45±0.00^{d}	0.46±0.00^{d}	0.46±0.00^{g}
Lactobacillus fermentum(WS)	0.53±0.00^{a}	0.55±0.00^{ab}	0.55±0.00^{a}	0.55±0.00^{a}	0.55±0.00^{a}	0.56±0.00^{a}
Lactobacillus fermentum (RS)	0.43±0.00^{c}	0.44±0.00^{ef}	0.45±0.00^{f}	0.45±0.00^{d}	0.45±0.00^{d}	0.45±0.00^{h}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	0.53±0.00^{a}	0.55±0.00^{a}	0.55±0.00^{a}	0.55±0.00^{a}	0.56±0.00^{a}	0.56±0.00^{bc}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	0.44±0.00^{c}	0.45±0.00^{ef}	0.45±0.00^{f}	0.46±0.00^{d}	0.46±0.00^{d}	0.46±0.00^{g}
Control (WS)	0.54±0.01^{a}	0.52±0.00^{c}	0.54±0.00^{b}	0.54±0.00^{b}	0.54±0.00^{b}	0.54±0.00^{d}
Control (RS)	0.46±0.02^{b}	0.45±0.00^{d}	0.46±0.00^{c}	0.46±0.00^{c}	0.46±0.00^{c}	0.46±0.00^{ef}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.33: Protein content (%) at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/protein content (%)					
	0	**1**	**2**	**3**	**4**	**5**
Lactobacillus plantarum (WS)	1.77±0.03^{a}	1.47±0.03^{bc}	1.23±0.03^{abc}	1.13±0.03^{bcd}	1.07±0.03^{bcd}	1.03±0.03^{bcd}
Lactobacillus plantarum (RS)	1.63±0.03^{bcd}	1.27±0.03^{d}	1.10±0.06^{cde}	1.00±0.00^{ef}	0.97±0.03^{ef}	0.97±0.03^{cd}
Lactobacillus casei(WS)	1.73±0.03^{ab}	1.40±0.06^{c}	1.20±0.06^{bcd}	1.13±0.03^{bcd}	1.07±0.03^{bcd}	0.96±0.03^{cd}
Lactobacillus casei (RS)	1.63±0.03^{bcd}	1.17±0.03^{d}	1.03±0.03^{e}	0.97±0.03^{f}	0.93±0.03^{f}	0.93±0.03^{d}
Lactobacillus fermentum(WS)	1.70±0.00^{ab}	1.43±0.09^{bc}	1.27±0.03^{ab}	1.10±0.06^{cde}	1.03±0.03^{cde}	0.97±0.03^{cd}
Lactobacillus fermentum (RS)	1.56±0.03^{cde}	1.17±0.03^{d}	1.06±0.03^{de}	1.03±0.03^{def}	0.97±0.03^{ef}	0.97±0.03^{cd}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	1.73±0.03^{ab}	1.50±0.06^{bc}	1.27±0.03^{ab}	1.17±0.03^{abc}	1.07±0.03^{bcd}	1.03±0.03^{bcd}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	1.53±0.03^{def}	1.20±0.00^{d}	1.10±0.00^{cde}	1.03±0.03^{def}	0.97±0.03^{ef}	0.97±0.03^{cd}
Control (WS)	1.63±0.03^{bcd}	1.70±0.06^{a}	1.37±0.00^{a}	1.26±0.03^{a}	1.20±0.00^{a}	1.17±0.03^{a}
Control (RS)	1.63±0.03^{bcd}	1.47±0.03^{bc}	1.23±0.03^{abc}	1.16±0.03^{abc}	1.10±0.00^{bc}	1.13±0.03^{ab}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

Table 4.34: Total Solids (%) at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Total solids (%)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	13.77±0.03^{a}	13.20±0.06^{cd}	13.03±0.03^{ef}	12.90±0.00^{d}	12.83±0.03^{de}	12.67±0.03^{cd}
Lactobacillus plantarum (RS)	13.03±0.03^{d}	12.67±0.03^{e}	12.53±0.03^{g}	12.40±0.06^{ef}	12.30±0.06^{f}	12.13±0.03^{e}
Lactobacillus casei(WS)	13.87±0.03^{a}	13.10±0.00^{d}	12.90±0.06^{f}	12.80±0.06^{d}	12.70±0.06^{e}	12.63±0.03^{d}
Lactobacillus casei(RS)	13.23±0.03^{c}	12.80±0.06^{e}	12.47±0.03^{gh}	12.40±0.06^{ef}	12.30±0.06^{f}	12.23±0.03^{e}
Lactobacillus fermentum(WS)	13.80±0.06^{a}	13.47±0.03^{b}	13.20±0.00^{cd}	13.07±0.03^{c}	12.93±0.03^{cd}	12.77±0.03^{bcd}
Lactobacillus fermentum(RS)	13.23±0.03^{c}	12.73±0.07^{e}	12.53±0.03^{g}	12.47±0.03^{e}	12.30±0.06^{f}	12.20±0.06^{e}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	13.60±0.06^{b}	13.23±0.03^{cd}	13.07±0.03^{de}	13.03±0.03^{c}	12.93±0.03^{cd}	12.80±0.06^{bc}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	13.17±0.03^{cd}	12.47±0.03^{f}	12.30±0.06^{i}	12.20±0.06^{g}	12.13±0.03^{g}	12.10±0.00^{e}
Control (WS)	13.53±0.03^{b}	13.70±0.06^{a}	13.57±0.03^{a}	13.40±0.00^{a}	13.23±0.03^{a}	13.10±0.06^{a}
Control (RS)	13.57±0.09^{b}	13.27±0.09^{c}	13.13±0.07^{de}	13.07±0.00^{c}	13.00±0.00^{bc}	12.83±0.03^{b}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

The total soluble solids of the wort increased steadily during the fermentation period. On day 0, *Lactobacillus fermentum* (WS) had the highest total soluble solids with value of 7.77±0.03 (^{o}Brix) while *Lactobacillus plantarum*+*Lactobacillus fermentum* (RS) and control (WS) gave the least total soluble solids with value of 7.13±0.03 (^{o}Brix), *Lactobacillus plantarum*(WS) and *Lactobacillus plantarum*+*Lactobacillus fermentum*(WS) also had the same total soluble solids with value of 7.63±0.03 (^{o}Brix) and 7.63±0.07 (^{o}Brix) respectively. On day 5, *Lactobacillus fermentum* (WS) had the highest total soluble solids with value of 9.10±0.06 (^{o}Brix) while *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) and control (RS) had the least value of 8.77±0.03 (^{o}Brix). *Lactobacillus plantarum* (WS) and *Lactobacillus fermentum* (WS) had the same total soluble solids with value of 8.97±0.03 (^{o}Brix) and also, *Lactobacillus plantarum* (RS) and *Lactobacillus casei* (RS) also had the same value of 8.93±0.03 (^{o}Brix) at day 5 (Table 4.35).

Figure 4.27 shows the total fermentable sugar of the wort. The total fermentable sugars increased from day 1 to day 4 during fermentation, but on the 5th day the fermentable sugars reduced drastically. On day 1, the *Lactobacillus fermentum* (RS) treated wort had the highest total fermentable sugar with value of 1.72 ± 0.10g/100g; while on day 5, *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the highest total fermentable sugar with value of 2.68 ± 0.08g/100g.

Table 4.35: Total Soluble Solids (^{o}Brix) at different time intervals of the wort derived from malted sorghum subjected to challenge by Lactic acid bacteria isolates.

LAB Isolates/Sorghum Variety	Treatment period (days)/Total Soluble Solids (^{o}Brix)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	7.63±0.03^{abc}	8.27±0.03^{b}	8.53±0.03^{g}	8.77±0.03^{de}	8.90±0.00^{efg}	8.97±0.03^{bcd}
Lactobacillus plantarum (RS)	7.43±0.03^{de}	8.13±0.03^{bcd}	8.57±0.03^{fg}	8.73±0.03^{e}	8.80±0.00^{g}	8.93±0.03^{cde}
Lactobacillus casei(WS)	7.73±0.03^{a}	8.50±0.00^{a}	8.83±0.03^{abcd}	8.97±0.03^{bc}	9.13±0.07^{bc}	9.07±0.03^{abc}
Lactobacillus casei(RS)	7.53±0.03^{cd}	8.27±0.03^{b}	8.73±0.03^{cde}	8.90±0.00^{c}	9.00±0.06^{cdef}	8.93±0.07^{cde}
Lactobacillus fermentum(WS)	7.77±0.03^{a}	8.50±0.06^{a}	8.83±0.03^{abcd}	9.03±0.03^{ab}	9.17±0.03^{ab}	9.10±0.06^{ab}
Lactobacillus fermentum (RS)	7.57±0.03^{bcd}	8.17±0.07^{bc}	8.73±0.03^{cde}	8.87±0.03^{cd}	8.97±0.03^{def}	8.90±0.06^{def}
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	7.63±0.07^{abc}	8.53±0.03^{a}	8.86±0.03^{abc}	8.97±0.03^{bc}	9.03±0.03^{bcde}	8.97±0.03^{bcd}
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	7.13±0.03^{g}	8.10±0.00^{cd}	8.60±0.06^{efg}	8.73±0.03^{e}	8.87±0.03^{fg}	8.77±0.03^{f}
Control (WS)	7.13±0.03^{g}	8.13±0.08^{bcd}	8.60±0.06^{efg}	8.77±0.07^{de}	8.90±0.06^{efg}	8.80±0.06^{ef}
Control (RS)	7.23±0.12^{fg}	8.00±0.00^{d}	8.57±0.03^{fg}	8.70±0.00^{e}	8.80±0.00^{g}	8.77±0.03^{f}

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

4.2.12 Sensory properties of products of LAB-challenged sorghum fermentation

Results of the sensory evaluation carried out on the fermented sorghum samples were presented in Table 4.36. The sorghum samples challenged with the LAB isolates were rated better when compared with the unchallenged sorghum samples. Samples *Lactobacillus fermentum* (WS), *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the highest same value of 7.80^{a} in terms of appearance. In taste rating, *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) had the highest rating value of 8.10^{a}. The flavour/aroma rating indicated that *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) scored significantly higher than all other samples with value of 8.10^{a} while in terms of colour rating, samples *Lactobacillus plantarum* (WS), *Lactobacillus plantarum* + *Lactobacillus fermentum* (WS) and *Lactobacillus plantarum* + *Lactobacillus fermentum* (RS) had the highest colour rating with value of 7.60^{a}. The overall acceptability assessment showed that the samples challenged with the LAB isolates were more acceptable than the samples not challenged with the LAB isolates.

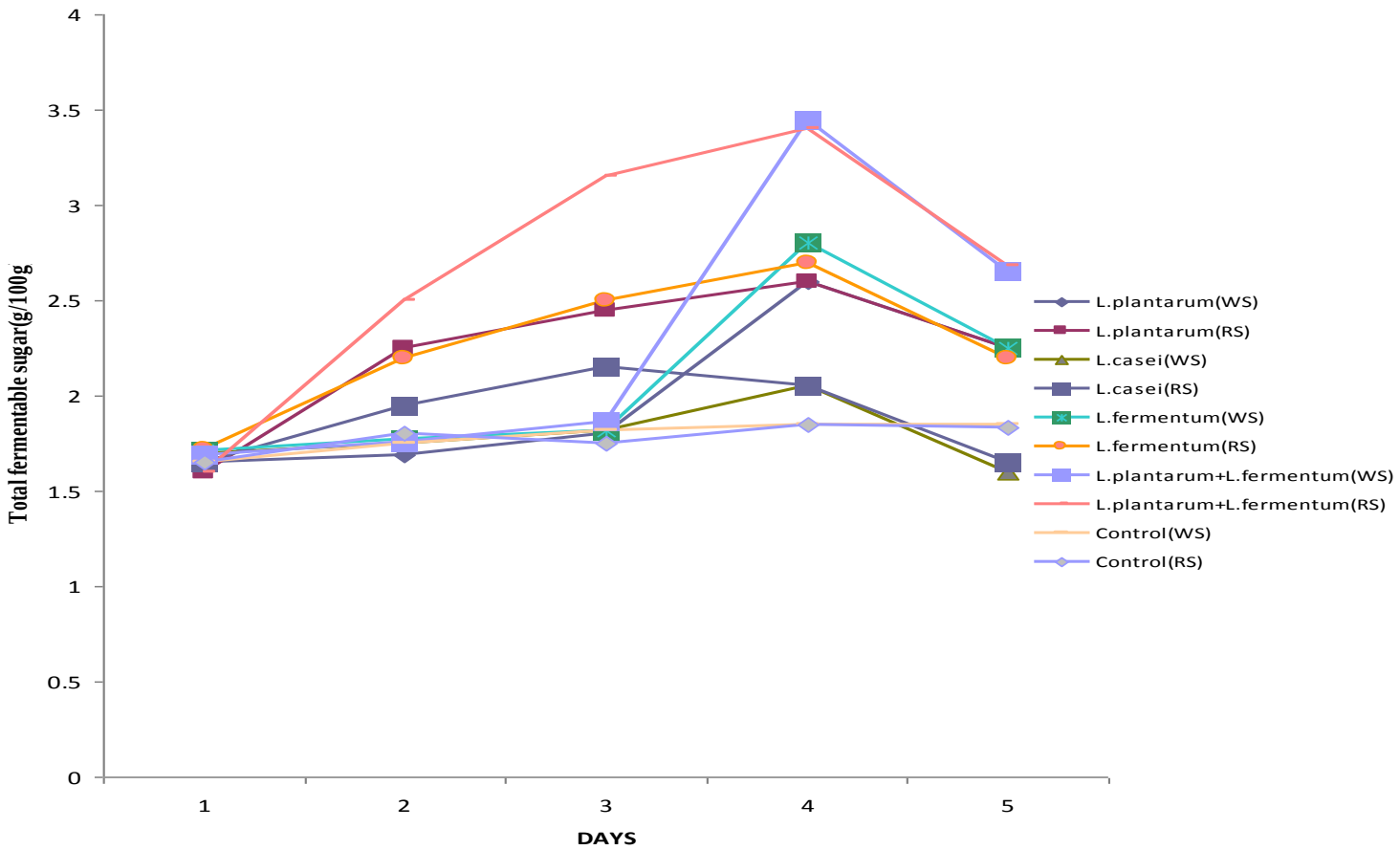

Figure 4.27: Total fermentable sugar at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates.

Table 4.36: **Sensory Evaluation Results of the Produced Fermented Sorghum Drink Samples**

Samples	Sensory Parameters			
	Appearance	**Taste**	**Flavour/Aroma**	**Colour**
Lactobacillus plantarum (WS)	7.60[a]	7.90[a]	8.00[a]	7.60[a]
Lactobacillus plantarum (RS)	7.60[a]	8.00[a]	7.80[a]	7.50[a]
Lactobacillus casei (WS)	7.40[a]	7.30[a]	7.70[a]	6.80[a]
Lactobacillus casei (RS)	7.30[a]	7.70[a]	7.40[a]	6.60[a]
Lactobacillus fermentum(WS)	7.80[a]	7.40[a]	7.30[a]	7.20[a]
Lactobacillus fermentum (RS)	7.20[a]	7.00[a]	7.00[a]	6.90[a]
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	7.80[a]	8.10[a]	7.50[a]	7.60[a]
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	7.80[a]	7.80[a]	8.10[a]	7.60[a]
Control (WS)	2.10[b]	2.80[a]	2.70[b]	2.30[b]
Control (RS)	2.10[b]	2.30[a]	2.20[b]	2.00[b]

***Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.**

KEY

WS-White sorghum variety

RS-Red sorghum variety

CHAPTER FIVE

5.0 DISCUSSION

One hundred and twenty- seven strains of lactic acid bacteria (LAB) were isolated and characterized from fermented cereal gruels. All the lactic acid bacteria isolated were Gram positive, catalase negative and oxidase negative. The cultural and biochemical properties of the LAB isolates was determined with reference to Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986). The lactic acid bacteria isolated were identified as *Lactobacillus plantarum* (32), *Lactobacillus acidophilus* (19), *Lactobacillus brevis* (31), *Lactobacillus casei* (12), *Lactobacillus delbrueckii* (8) and *Lactobacillus fermentum* (25). The heterofermentative lactobacilli were represented by *Lactobacillus brevis* and *Lactobacillus fermentum*, while others were homofermentative. These organisms have been reported by Halm *et al.* (1993) to be responsible for various fermentation processes involved in food production. Dike and Sanni (2010) also isolated lactic acid bacteria from various fermented cereal gruels. Onilude *et al.* (2005) also reported the involvement of various types of lactic acid bacteria in fermented cereal gruels (maize, sorghum and millet). The dominance of lactic acid bacteria in spontaneous fermentation of cereals gruels has also been reported by some researchers (Odunfa and Adeyele, 1985; Olukoya *et al.* 1993a; Ogunbanwo *et al.* 2003; Wakil and Daodu, 2011).

Among all the lactic acid bacteria isolated, three of them were selected for use as starter culture for the malting of sorghum. They were selected based on their ability to produce antimicrobials (lactic acid, hydrogen peroxide, diacetyl and bacteriocin), enzyme production and high antagonistic activity against pathogenic organisms. The organisms selected as starter cultures were *Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus fermentum.* They were used singly and also in combination.

The lactic acid bacteria isolates were screened for the rate of production of antimicrobial compound since lactic acid bacteria are reported to be important in the biopreservation of food and feed (Messens and De Vuyst, 2002). In this study, the isolated lactic acid bacteria, *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus acidophilus* and *Lactobacillus delbrueckii* were observed to produce more of lactic acid, hydrogen peroxide and diacetyl an observation in line with the findings of Borch and Molin

(1988) which reported an increase in the production of lactic acid with time due to low pH. Low pH (4.0-6.0) permits the growth of lactic acid bacteria. The organisms were also able to inhibit pathogenic organisms selected as indicator organisms when compared with other LAB isolates. The rate of production of these antimicrobials and inhibitory activity formed an important factor for the selection of the LAB strains for further screening by subjecting them to various physiological tests in the search for suitable starter cultures for malting of sorghum. Furthermore, the results observed in this study were in accordance with the work of Ogunbanwo *et al.* (2003) who reported that production of the primary metabolite, lactic acid and the resulting pH decrease is the main preserving factor in food fermentation. The metabolites of selected LAB have antagonistic activities against all the indicator organisms used in this work. The isolated LAB produced antimicrobial compounds to varying degree. The increase in the production of lactic acid with time have been attributed to lowered pH which permit the growth of LAB. The antimicrobial effect of lactic acid is due to undissociated form of acid which penetrates the cell membrane and liberates hydrogen ion in the neutral cytoplasm thus leading to inhibition of vital cell functions. The inhibitory effect of hydrogen peroxide produced by LAB has also been reported (Ogunbanwo *et al.,* 2003). The proliferation of spoilage organisms and food-borne pathogens can be prevented by low pH and high concentrations of organic acids (Adam and Hall; 1988 and Cintas *et al.,* 2001).

All the LAB isolates were able to grow very well when they were subjected to various physiological tests in terms of their behaviour to the different carbon source , the pH of the medium, nitrogen sources , cation tested on the isolates and subjection to varying temperature. Curing of their plasmids, however, brought a different behaviour of the LAB isolates. Plasmid DNA are small circular DNA molecules carrying some other genes and they are capable of autonomous replication. Plasmids are relatively much more unstable molecules than the bacterial chromosome (Klaenhammer *et al.,* 2002). The most important biosynthetic and catabolic pathways in lactic acid bacteria are chromosomally encoded. However, other properties such as carbohydrate metabolism and proteolytic activities may involve both plasmid-linked and chromosomal genes. The plasmid-linked functions include fermentation of lactose, sucrose, galactose, mannose, xylose, glucose, proteinase activity, phage resistance, amino acid metabolism, bacteriocin production, antibiotic resistance, citrate utilization, exopolysaccharide production, DNA restriction and modification

(Ward *et al.*, 2004). The number of plasmids and molecular sizes obtained in this study was also in accordance with the range obtained by Wang and Lee (1997). The reason in the behaviour of the cured LAB isolate could be that the isolates were plasmid-linked (Gonzalez and Kunka, 1986). From this study, it is relevant that the economically important characters of lactic acid bacteria are plasmid encoded. The use of these plasmids in genetic engineering techniques in the brewing industry will go a long way towards constructing a strain with all desirable characters. A report of the work of Lee and Moon (2003) shows that isolation and characterization of these plasmids have provided a good opportunity to improve the performance and stability of starter cultures.

All the test isolates best utilized glucose as carbon source for the production of lactic acid used in this work. LAB convert glucose to lactic acid with 100% yield. This agrees with the work of Mossel *et al.* (1995) who reported that all LAB ferment glucose to produce lactic acid. Also, glucose is the only one of many sugars found in nature; more complex pentose sugars such as arabinose, xylose and ribose under normal conditions are not degraded by lactic acid bacteria (Adesokan *et al.*, 2009). The work of Leam and Gashe (1994), described glucose as an important component of microbial nutrition which is used for energy and metabolite production. As a result, these various carbon sources have different effect on growth and amylase production of the LAB isolates.

The effect of different cation concentrations used i.e Magnesium sulphate on the growth of the LAB isolates shows that there was an increase in growth of the, different LAB isolates used as the concentrations increased, even though, the LAB isolates responded to the cation in different manners. This is in accordance with the work of Pelczar *et al.* (1993) in which it was stated that during the growth of LAB isolates in the presence of different inorganic salt, the inorganic salt tend to form complexes with the lactic acid that is produced by the organisms. Enzymes require free metals for their activity such as $MgS0_4$ that was used in this work. It is therefore necessary to ensure that the metals are in sufficient amount in the medium of growth of the isolates (Keith, 1992). Also, the effect of various concentrations of cation tested on enzymes i.e amylase, invertase and protease shows that the LAB isolates performed very well using the different concentration of $MgSO_4$ and the enzyme that was produced best was amylase (Keith, 1992).

With different pH, the LAB isolates were able to grow within pH range of 5.5 – 9.2 although pH 5.5 was optimal for growth and enzyme production. High activity of the organisms at this pH was responsible for high enzyme production especially amylase enzyme. Price, (1992) stated that many enzymes are only active within a fairly narrow range of pH and exposure to pH values outside this range can lead to irreversible loss of activities. A pH that does not favour growth whether high or low will not also favour enzyme production. The activity of the enzyme will thus be reduced. High activity of the organism at this point (pH 5.5) is also responsible for higher amylase production (Leam and Gashe, 1994). Keith (1992) also stated that the activity of enzymes are profoundly affected by the buffer used to adjust the medium to a particular pH and the ionic strength of the assay mixture.

The effect of temperature on the growth of the LAB isolates shows that optimum temperature for growth of LAB was 30^{o}C. This agrees with the work of Giraud *et al.* (1991) who reported that the optimum temperature for the growth of lactic acid bacteria is 30^{o}C which incidentally also favours enzyme production by the LAB isolates. A decrease in growth was observed at 40-50^{o}C, although there are some LAB that can grow at 45^{o}C which is one of the test for differentiating the various species of *Lactobacillus.* It was observed that the LAB did not grow very well at temperature above 60^{o}C and this is in accordance with the work of Ilori *et al* (1995) who observed that at high temperature above 60^{o}C, LAB will not grow. At very low temperature, enzyme production reduces and at a very high temperature, enzyme production reduced considerably the more (Styer, 1981). Giraud *et al.* also opined that the best result for enzyme production were obtained when incubation temperature is the same as temperature for growth. The activity of enzyme here is linked with specificity of enzyme to temperature as reported by Styer (1981). The author reported that enzymes have specific temperature at which they act best. At a temperature below or above this, the activity of the enzymes decreases. This may be due to denaturation of the enzymes at higher temperature because they are protein whose bonds can be easily broken down at high temperature (Lealem and Gashe, 1994). The growth of LAB is inhibited at temperature greater than 40^{o}C (Ahmed *et al.,* 2006) because the high temperature stress can cause thermal damage leading to disruption of hydrogen bonding and hydrophobic interactions. Cell viability has also been reported to decline when temperature increases beyond growth-optimal levels (Ahmed *et al.,*

2006). Of the three enzymes- invertase, amylase and protease, results show amylase to be produced best by the LAB isolates at temperature of 30^{o}C.

Using different concentrations of peptone as nitrogen source, it was discovered that peptone favoured growth and metabolism of the LAB isolates. Peptone also favoured amylase production by the LAB isolates. The rate of production of enzymes by the LAB isolates shows that amylase was the most prominent enzyme produced by the isolates. All the test isolates used for this work used peptone for lactic acid production. Lactic acid bacteria require complex nitrogen sources which is very important in lactic acid production and as growth factors (Suma *et al.,* 1999). However, proteolytic activity is first required before nitrogen can be consumed. Because of this slow catabolism, a state of nitrogen limitation is created when using urea which resulted in the suppression of possible metabolic regulatory mechanisms such as the repression of catabolic enzymes and amino acid transport (Aharonowitz, 1980). A slow metabolic rate is also related to a low specific growth rate, which could lead to reduction in lactic acid production. Also, rich media such as peptone contain adequate amounts of minerals needed for the growth of LAB.

The antimicrobial activities of the LAB strains were investigated against indicator organisms earlier reported to be associated with sorghum malting as pathogenic and spoilage organisms apart from moulds (Jay, 2000a). Of all the LAB isolates used, *Lactobacillus plantarum* had the highest effect on *Bacillus subtilis* followed by *Lactobacillus casei* and *Lactobacillus fermentum*. This implies that there was hope of successfully combating spoilage organisms like bacteria with the LAB strains (Jay, 2000b). This result is in accordance with the work of Thanh *et al.* (2010) who used LAB metabolites to inhibit growth of *Escherichia coli, Listeria monocytogenes* and *Salmonella typhimurium*.

Generally, the two sorghum varieties used for this work had good germinative properties as reflected in their high viabilities, with no tendency to dormancy (Palmer, 1989). Their initial moisture contents were within the range (10-11.5%) for malting sorghum grains (Aisien, 1988). The moisture content obtained in this work for the two unmalted sorghum varieties were 10.43 $\pm$ 0.07 for white sorghum variety and 10.60 $\pm$ 0.06 for the red sorghum variety. Ogu *et al.* (2006) also reported moisture content of unmalted grains to be in the range 8.5-10.5%, a range of choice for the unmalted sorghum used for this work. Their total nitrogen contents were equally within the acceptable values (1.6-2.03%) for unmalted grains. In spite of the ideal range stated

above, it has been argued that Total Nitrogen (TN) values greater than 1.9% will usually give run-off problems during filtration and render the beer less stable, due to the formation of chill haze (O'Rourke, 2002). On the other hand, levels must be high enough to impart body to the beer, good head formation as well as healthy fermentation (O'Rourke, 2002). The protein content obtained for the two sorghum varieties were 11.37 ± 0.07 for white sorghum and 13.17 ± 0.03 for red sorghum, these two values obtained corresponds with the works of Ogu *et al.* (2006). The fat content was 2.36 ± 0.03 for white sorghum and 3.00 ± 0.00 for red sorghum and this was in the range (1.93 ± 3.85%) for unmalted sorghum samples. The ash contents was also in the range obtained for unmalted sorghum (1.43 -2.18%) in the work of Stephen (2008).

The Germinative Energy (GE) and the Germinative Capacity (GC) of the two cultivars or varieties were high enough (>90%), compared with the recommended (i.e not less than 90) (O'Rourke, 2004) for malting. This means that all the varieties were suitable for malting. The GE and GC also fall within the ranges (>95%) prescribed as acceptable for malting grains (Briggs *et al.*, 1981). These two properties have direct bearing on suitability of cereals for malting. The two sorghum varieties had high 1000 corn weight above 30g which also shows that they were suitable for malting. The higher the corn weight, the higher the expected brewers extract. The thousand corn weight was between the values (25 to 35g) reported by Serna-Saldivar and Rooney (1995). Similar results were reported by Ogu *et al.*, (2006). These slight differences in germination energies and germination capacities could be attributed to differences in the sorghum varieties, storage periods and conditions as well as germination temperature (Novellie, 1962). The two sorghum varieties used had good germinative properties as revealed in their high viabilities with no tendency of dormancy. Data obtained on the properties of the two sorghum varieties are within the range for malting sorghum grains and are consistent with other reports (Aisien, 1988; Palmer, 1989).

The moisture content of the malted sorghum during malting reduced for the two sorghum varieties when lactic acid bacteria was used as starter culture for the steeping process. High moisture content encourages microbial growth and allows increase in metabolic rate, which depletes the extract content. According to Okon and Uwaifo (1985), the problem of high moisture content could be reduced by kilning the sorghum after malting. It has also been reported that increased germination moisture

improved sorghum, pearl millet and barley malt quality in terms of Diastatic power, Free Amino Nitrogen (FAN), hot water extract and malting loss (Palmer, 1989). Germinating sorghum grains have the tendency to rapidly loose water taken up during steeping and therefore it has been found necessary to spray germinating grains at intervals during the germination phase because the higher the level of moisture content, the higher the resulting malt quality (Morall *et al.*, 1986; Palmer, 1989 and Dewar *et al.*, 1997a). The moisture content is an important indication of sorghum malt quality for brewing purposes. A useful way of improving the quality of malt is to enhance the moisture content of the germinating grain during malting (Dewar *et al.*, 1997b).

The total carbohydrate of the malted sorghum increased during malting which shows that starch was present in the sorghum. Starch is the major storage form of carbohydrate in sorghum and millets. The increase in the total carbohydrate content of the malt was due to the high gelatinization temperature of the sorghum (Okafor and Aniche, 1998). The increase in total carbohydrates could also be attributed to particularly starch and soluble sugars which are the principal substances for fermenting microorganisms; therefore, degradation and a subsequent increase in starch contents are expected to occur (Ejigui *et al.*, 2005).

The ash content of the treated malted sorghum also increased during the malting process. There were not much significant differences between the control malt and the treated sorghum malt. The increase could be due to metabolic action of added LAB as starter for the fermentation process. Obasi *et al.* (2009) also observed an increase in mineral content of pearl millet with increased soaking time and germination time.

There was also a significant reduction in the fat content i.e the ether extract as germination progressed in the treated cereal. This was as a result of the action of hydrolytic enzymes existing in the sorghum grains, coupled with the mobilization of soluble materials into roots and shoots for germination, thus reducing the level of these nutrients in the seeds.

The malting process reduced the fat content in the grains. However, the level of fat obtained in the sorghum malts used in this work were slightly higher than what was reported by Serna-Saldivar *et al.* (2004) probably due to the addition of LAB as starters. Serna-Saldivar *et al.* (2004) reported that the maximum fat content as set by the brewing industry is less than 1.0%. The reduction in the fat content could also be

due to the utilization of oxidized lipids to generate energy for growth and cellular activities. On the other hand, since germination generally reduce the fat content, the germination period for the sorghum could be extended in order to further reduce the fat content. Lower fat contents are also desirable to reduce oxidation of off-flavours in the final beer.

The gelatinization temperature of the malted sorghum increased during germination for both the treated malt and the control malts. The gelatinization temperature obtained in this work is in accordance with the report of Beta and Corke (2001) who also reported sorghum starch gelatinization temperature as being between 67-73^{o}C. At these temperatures, the starch in the grains will be converted to sugars by hydrolytic enzymes.

The Free Amino Nitrogen (FAN) content of the malted sorghum subjected to challenge by lactic acid bacteria isolates increased during germination. There was an increase in FAN for both the treated malt and the control malt, but a high increase was observed in the sorghum malt that was treated with the combination of starter cultures. The increase in FAN levels is an indication that using different mashing systems, the FAN level could support yeast function during fermentation (Agu, 2006). According to the work of Lowe and Arendt (2004), one of the beneficial effects of the application of LAB starter cultures during malting is the increase in FAN level of the sorghum malt which was also observed in this study. FAN is produced during malting by the action of endogenous proteinases and peptidase enzymes on the protein reserves of the grain. The subsequent breakdown products are collectively referred to as FAN. For all the sorghum varieties tested, malting improved malt FAN, which increased with increasing germination time over the 5 days of malting. These findings support the work of Nout and Davies (1982), Dewar *et al.*, (1997a), who reported that the proteolytic activity of sorghum malt increased with germination time. Similarly, Morrall *et al* (1986) also reported an increase in malt FAN up to 6 days of germination. The FAN level potentially would meet the minimum requirements for yeast growth.

Malting also improved the quality of the sorghum protein, which increased with increasing malting time (Donaldson, 1999). The same trend was observed in this work. The high protein content shown by the lactic acid bacteria -treated sorghum sample may be due to the action of proteinases of the starters. The increase in protein supports the idea that the increase in protein digestibility is probably due to structural

changes and the enzymic hydrolysis of proteins into more digestible forms such as amino acids and small peptides. The evidence also supports the suggestion that the simple technology of malting offers a means of improving the quality and digestibility of sorghum protein. Odunfa (1985) reported an increase in amino acid content of fermented legumes and cereals. There was no significant difference between the sorghum treated malt and the control malt. The increase in protein content of the malt could also result from the mobilization of storage nitrogen of the sorghum to produce the nutritionally high quality proteins needed by the young plant for its development. This is important to the nutrition of infants and children in developing nations who depend largely on gruels of cereals to meet their energy and protein needs. The increase in protein content of malt obtained in this work was also in agreement with the work of Ariahu *et al* (1999) who also reported an increase in protein content of African breadfruit.

The Diastatic power (DP), which is a measure of extent of enzyme development of the malt increased during the malting process. DP measures the overall amylase activity, and was taken as the parameter to determine the sorghum malt quality based on the standard of the Southern African sorghum malt industry in which sorghum malt quality was defined in terms of its overall DP (Raschke and Taylor, 1995). The DP of malts was higher because of the temperature and time they were subjected to and also because of the addition of LAB as starter cultures. The DP of the malts increased with increasing germination time in agreement with what has been reported by other researchers; Morrall *et al*., (1986), Dewar *et al*., (1997a). The results obtained in this work is also in agreement with the work of Briggs *et al.*, (1981) who reported that diastase (amylases and other relevant starch-degrading enzymes) increases in quantities when grains are germinated. It has also been reported that such parameters as DP, β-amylase activity, FAN all increase with increasing time of germination (Pelember *et al.*, 2002).

The protease inhibitor of the sorghum -treated malt reduced drastically during the germination process for all the sorghum treated with the LAB. This was due to the addition of LAB during malting and malting process also cause a reduction in anti-nutritional factors (Malleshi and Klopfenstein, 1996). The tannin content and phytate also reduced considerably during malting. Fermentation reduces anti-nutritional factors as reported by many researchers including Chavan and Kadam (1989). Cereals and legumes contain significant amounts of anti-nutrients, which

further lower their nutritional quality. The reduction in the anti-nutrients might have been due to hydrolysis during fermentation, an observation similar to those of Chavan and Kadam (1989).

It was observed that the pH of the malted sorghum treated with the LAB starter culture reduced considerably when compared with the pH of control malt. The final pH of the malted sorghum was within acidic range and this could be as a result of the *Lactobacillus* used as starter culture. The LAB converted the sugar into acid during the malting process and this lowered the pH of the malted sorghum (Haikara and Laitila, 1995). Lactic acid bacteria could only grow very well in the acidic range as reported by Oliver-Daumen (1988). The low pH observed with the LAB cultures was due to the production of lactic acid by the cultures and this was probably responsible for the inhibitor of the coliforms. Low pH inhibits coliforms and other food- borne contaminants by dissociating their cell membranes (Stiles, 1996). Also, according to Elyaas *et al.* (2002), the increased acidity and low pH as a result of fermentation enhances the keeping quality of fermented foods, by inhibiting microbial growth and also contributing to the flavour of the processed food.

There was a decrease in the crude fibre content of the LAB- treated malted sorghum. The crude fibre content decreased with fermentation time. The crude fibre content of the LAB -treated malted sorghum reduced when compared with the control malt. The expected decrease in the fibre content could be attributed to the partial solubilization of cellulose and hemicellulose components of the grains by microbial enzymes. A previous study has also reported a significant decrease of fat and fibre contents of cereals during fermentation (Ejigui *et al.*, 2005).

The plumule and the radicle length increased during germination for the LAB-treated sorghum when compared with the untreated sorghum. There were significant differences between the LAB- treated sorghum and the untreated. The greater change in the rootlets and shootlets lengths was observed between the second and third day of germination. This may be due to the fact that the activities in the endosperm occur mostly during this period. Palmer and Bathgate (1976) also reported that enzyme activity is highest during the early stages of germination since the first 2-3 days coincide with the movement of the growth hormone (Gibberellins). The rootlets and shootlets of the LAB- treated sorghum malt increased when compared to the control malts. This could occur due to the addition of LAB as starter culture. In general, the radicle length was larger than the plumule length. According to Briggs *et al.* (1981),

high nitrogen sorghum was observed to respire and grow vigorously. Lengths of plumule and radicle indicate growth and probably the hydrolysis of high molecular structures within the sorghum. It may also indicate the rate of loss of dry matter during germination period. It may also be pointed out that the behaviour of plumule and radicle lengths during germination could be affected by the pericarp thickness. The reports of Adeola (1991) also indicated that the thickness of the pericarp influences the growth of the root of sorghum kernels, and hence, the germination behaviour and it also yielded good quality wort.

The weights of the sorghum seeds also increased with germination time for both the control and the treated malts, although, the starter cultures had little or no effect on the weight of the sorghum seeds. There has been no information on the effect of starter cultures on the weight of seeds during germination. The weight of the sorghum grains reduced after malting when compared with the weight of the grains before malting. Malting loss is the loss in weight of grains after malting. In sorghum malt, high malting loss is linked to good diastatic power (Owuama, 1999a).

Amylase activity increased during the malting process for the LAB-treated sorghum when compared with the untreated sorghum. It was observed that amylase activity increased steadily from the first day to the fourth day of germination. All the sorghum samples had their highest amylase activity towards the fourth day. It could, according to Novellie (1962) be concluded that the enzymes were synthesized during the germination stage. One of the beneficial effects with the application of LAB starter cultures during malting is increase in amylase activity (Lowe and Arendt, 2004). Important physiological processes associated with the germination phase are synthesis of amylase, proteases and other endogenous hydrolytic enzymes (Palmer, 1989). Amylase is synthesized in the starch endosperm during the development of the sorghum grain. Lauriere *et al.* (1985) also reported that during the course of germination, the bound enzyme in the sorghum grain is apparently released by endospermal proteinases resulting in an increase in the free and active forms of amylase. The result obtained in this work also agrees with the result of Elkhalifa and Bernhandt (2010) that in germinated sorghum, amylase activity reached a maximum on the 3rd day and decreased steadily thereafter. Amylase activity in grains have been found to break down starch to maltose and with germination its activity increases, thereby lowering the viscousity of the food (Correia *et al.*, 2008). It is also interesting to note that amylase activities could be correlated with food processing of sorghum.

For industrial beer preparations, high α-amylase and β-amylase activities are desired (Dicko *et al.*, 2006). Amylase activity was also reported to increase with increase in moisture content during malting according to Novellie (1962). The α-amylase activity (DU) of the malted sorghum increased for the treated LAB treated malt while for control malt it was lower. The application of LAB starter cultures during malting increase the α- amylase activity (DU) of malt as reported by Lowe and Arendt (2004).

The protease activity also increased steadily during the malting process for the LAB-treated sorghum compared with the untreated sorghum. This could be as a result of the addition of LAB as starter cultures because the protease activity of the control malt was lower than the started treated malt. The increase in protease activity can be explained by the beginning of proteolysis initiated by a slight temperature rise within the grain bed. According to Taylor and Boyd (1986), proteolysis occurs optimally at 43^{o}C to 50^{o}C in sorghum malts. Alternatively, the increase may be due to the presence of exo-peptidases. According to Lewis and Young (1995), exo-peptidases tolerate the heat of kilning because they are heat stable and persist in the endosperm after kilning.

Monitoring of the microbial profiles during germination shows that the use of LAB during steeping inhibited the growth of spoilage pathogens especially coliforms and moulds. The treated sorghum showed a steady decrease in *Bacillus* species, *Staphylococcus* species, *Moulds* and *Pseudomonas* count whereby the untreated (control) malts showed steady increase in microbial counts. The inhibition of pathogenic organisms observed could be attributed to the production of lactic acid by the Lactic acid bacteria (LAB) (Vaughan *et al.*, 2005) and to extracellular antimicrobial compounds including bacteriocins, hydrogen peroxide etc, by strains of *Lactobacillus*. *Lactobacillus plantarum* is known to produce antifungal proteins. The number of coliforms decreased during germination for the treated sorghum malt. Similar results have been reported for other fermented cereal foods (Nout, 1991). The disappearance of coliforms may be attributed to the acid production by the dominating lactic acid bacteria. Steinkraus (1996), indicated that most coliforms are acid-intolerant and are inhibited as low pH is achieved. The numbers of LAB increased in the treated sorghum malt when compared with the control malts, the reason being the presence of the inoculated LAB in the treated malt. On the other hand, pathogenic organisms were able to grow well in the control malts. Boivin and Malanda (1997) also demonstrated how the application of starter cultures could inhibit the growth of

undesirable moulds, production of mycotoxins and reduction of spoilage pathogens when applied to the steeping water during malting.

The composition of wort is critical for healthy yeast fermentation. Deficiencies in any of the critical components can be responsible for a sluggish fermentation (Declan and Arendt, 2003). The yeast cells count peaked on days 1-4 and decreased thereafter. After day 4, the yeast cell count dropped considerably. Yeast viability remained high which indicated that the physiological condition of the yeast is not impaired. The sorghum wort was nutritionally rich enough and contains ample FAN to satisfy the yeast requirements. At the same time far less residual unassimilated nitrogen remains in beer (Macfadden and Clayton, 1989). The yeast was viable throughout the fermentation period (Declan and Arendt, 2003).

Total titratable acidity (TTA) of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria increased from day 0 to day 5 during the fermentation of the wort. The increased in the TTA was pronounced in the starter culture treated wort i.e it had high effect on the wort derived from malted sorghum treated with the LAB isolates than the control worts. The increase in TTA is one of the common features in the fermentation of fermented food and beverages (Abegaz *et al.,* 2002). During fermentation, the metabolic activities of lactic acid bacteria and yeasts lead to production of lactic acid and ethanol from the break down of hexoses and pentoses (Adams and Moss, 2008) and this explains the increase in titratable acidity of the wort. Similar observations have also been made by Hounhouigan *et al.* (1999) and Mugula *et al.* (2003) who observed increase in titratable acidity of 'mawe' and 'togwa' respectively while using LAB as starter culture in the preparation of these products. The accelerated acidification observed constituted an asset that can contribute to the improvement of the final product quality as observed in other studies (Holzapfel, 1997; Ross *et al.,* 2002). The growth of pathogens can also be avoided with the rapid acidification obtained (Michodjehoun-Mestres *et al.,* 2005).

It was also observed that the reducing sugar and total residual sugar of the wort reduced during fermentation. The decrease of the reducing sugars and total residual sugar with prolonged fermentation was attributed to utilization by the fermenting yeast i.e *Saccharomyces uvarum*. The sugars are being utilized to produce ethanol, carbon dioxide and other metabolites according to the work of Mensah (1997). Reducing sugars and residual sugar are prime component of fermentation, after consumption of which alcohol is produced. Reducing sugar and the total

residual sugar decreased with the increase in fermentation days. Michodjehoun-Mestres *et al.* (2005) also reported a decrease in the reducing sugar of 'gowe'. He observed that reducing sugar content decreases with the fermentation period due to the fact that these sugars were progressively being used as substrates by the fermenting microorganisms for production of ethanol and other metabolites production over time. A reduction in total sugars was conversely correlated with the total titratable acidity of wort. The amount of reducing sugar and total residual sugar in beer influences the organoleptic properties of beer and indicates the completion of fermentation.

The Free Amino Nitrogen (FAN) of the wort derived from malted sorghum challenged with lactic acid bacteria increased considerably when compared with the control worts. The free amino nitrogen gives estimate of the amount of amino acids, ammonia, and in addition, the terminal α- amino nitrogen groups of peptides and proteins in the wort. The work by Taylor *et al.* (1985a) provided evidence to support the ninhydrin assay as a good indicator of yeast fermentation performance. Evaluation of FAN content in wort indicates how well yeast can grow and reproduce. Owuama (1999b) reported that a high level of FAN in wort is necessary to support rapid and proper fermentation. FAN is important because it is an essential component of yeast nutrition in brewing as it promotes proper yeast growth and fermentation efficiency (Lekkas *et al.*, 2007). It also plays a role in the maintenance of foam stability of beer. The FAN obtained in this work was slightly lower than the FAN obtained by other workers.

Beer fermentation is a natural acidification process. The pH of all the worts including the control reduced considerably during fermentation. The pH started dropping after 24 hrs. A rapid drop in pH can be correlated to the yeast viability and yeast growth. Wort amino acid stimulates yeast growth, which in turn promotes pH decline. A low pH is desirable to inhibit gram-negative wort spoilage bacteria and also for the final flavour of the beer (Fix, 2000). The reason why the pH of the wort was within range could also be as a result of the *Lactobacillus* used as the starter culture. The starter culture converted the sugar into acid during the malting process and this lowered the pH of the wort produced from the malted sorghum. The *Lactobacillus* species could only grow and survive in the acidic range. Also, at this pH, enzyme activity is optimized while beer flavour is improved. No great

differences in pH were observed between the fermentation of the starter treated wort and the control.

There was a progressive increase in the alcohol content of beer produced from both treated and control sorghum with increase in the period of fermentation. The wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates had the highest ethanol content. The ethanol produced was at its peak on the 5^{th} day. The alcoholic content of beer is usually regarded as a measure of its strength (Hough *et al.*, 1971). The ethanol production rates also reflected the cell growth pattern. The level of alcohol produced during the course of fermentation in this work shows the fermentative performance of the yeast used. The higher alcohol content produced by the starter treated wort showed that the components of the wort i.e sugars, peptides, amino acids were easily fermentable by the yeast probably because of the addition of starters compared with the control worts with low ethanol content. The higher ethanol content could also be attributed to high specific gravity of the wort. The ethanol content obtained in this work was also in the range of that obtained by Seema (2005) during fermentation of sorghum wort. Okafor and Aniche (1987) also reported an increase in ethanol content of sorghum wort from day one to day five of fermentation in their work and their final beer gave 3.09 (%, w/v). A good quality beer depends on the amount of alcohol present in the beer and this result in a long shelf life (Okafor and Aniche, 1987).

It was observed in this study that the specific gravity of the wort derived from malted sorghum subject to challenge by lactic acid bacteria isolates was 1.02 $\pm$ 0.00 while that of the untreated sorghum also had the same value. There were no significant differences between the LAB-treated wort and the untreated wort. The specific gravity obtained in this wort was in the same range with the specific gravity of beer (Demuyakor and Ohta, 1993). Urias-Lugo and Serna-Saldivar (2005) also reported beer to have a specific gravity of 1.03. The decrease in specific gravity of the samples shows that some of the sugars have been converted into alcohol which is less dense than water. A similar observation was also made by Egemba and Etuk (2007). The gravity fall also corresponded with an increase in the yeast population. This is because yeast cells multiply when the nutrients were available in the medium. An increase in yeast population indicated that more yeast cells utilized the sugar in the wort more readily, thereby bringing down the specific gravity of the wort (Malomo *et al.*, 2011).

Overall, the colour of both worts from the LAB-treated and untreated sorghum reduced considerably throughout the fermentation period. Colour formation can be attributed to the malting process. The colour could also be attributed to the higher content of reducing sugars and alpha amino nitrogen that favoured heat treatments used for mashing (Urias-Lugo, 2001). In general, the LAB added during the malting process had very little impact on the resultant wort colours. Only a marginal colour difference was observed between the control wort and the starter treated wort. However, the colour of the wort obtained in this study was slightly higher than the EBC (1998) requirement. Colour and flavours that are imparted to beer are as a result of Maillard reactions (reactions between free amino nitrogen and reducing sugars of the malt) basically developed on kilning and then further modified on mashing according to the work of Fix (1999).

The turbidity of the worts increased during the fermentation period. The highest turbidity was recorded by the 5th day. The control worts also gave high turbidity value like the treated worts but with no significant difference. The multiplication of the yeasts cells led to an increase in the turbidity of the wort during the fermentation period. A clear bright laboratory wort is usually preferred, although in most cases malts that give turbid worts in the laboratory usually give bright wort in the brewery due to the thicker layer of spent grains that occurs when a lauter turn is used (Etok' Akpan, 2004).

The protein content of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates reduced significantly during fermentation compared with the control. Although this was contrary to the report of Elyaas *et al.* (2002) who reported that increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles. The reduction in protein could also result from nutrient depletion by microorganisms during fermentation.

The total solids reduced during fermentation while the total soluble solids increased. Such decrease in the total solids was attributed to the alcoholic fermentation carried out by yeast (Abegaz *et al.,* 2002). During fermentation, the metabolic activities of yeasts lead to the production of lactic acid and ethanol from the breakdown of hexoses and pentoses. This explains the decrease in the total solids during fermentation.

The total fermentable sugars reduced at the end of fermentation when inoculated with *Saccharomyces uvarum* with an increase in fermentation period. This may signify the effect of higher utilization of fermentable sugars by the yeast. According to the work of Kouadio-Florent *et al.* (2008), it was reported that sugars were utilized as carbon and energy sources by yeasts during fermentation. There were no significant differences between the treated wort and the untreated wort. The amount of fermentable sugars reduced at the end of fermentation process. This is because the yeast cells utilized these sugars which were converted to ethyl alcohol and carbondioxide (Brauhaase, 2000).

The result of sensory evaluation indicated that the sorghum beer treated with starter cultures were acceptable when compared with the beer that was not treated, that is the control beer. The starter culture treated beer had a good taste, appearance, aroma and also the colour was good when compared with the control beer. When all the attributes were compared for the treated worts and control worts, the treated beer had the highest rating by the consumers that evaluated the fermented sorghum drink. The overall acceptability assessment showed that the samples challenged with the LAB isolates were more acceptable than the samples not challenged with the LAB isolates. The results of acceptability assessment obtained in this study was in accordance with the work of Holzapfel (2002) who also reported that the use of LAB as starter culture for the fermentation of foods and beverages contribute to aroma or flavour development.

CONCLUSION

This study was carried out to isolate and identify lactic acid bacteria from spontaneous fermented cereal gruels and to use the lactic acid bacteria as biocontrol agents for the malting process. This has become necessary because of the problem of contamination during malting by fungi and coliforms which are of particular concern to maltsters and brewers. Contamination of sorghum during malting by fungi and coliforms are of particular concern to both maltsters and brewers.

In this study, lactic acid bacteria (LAB) have been shown to be effective at inhibiting unwanted microorganisms in sorghum malting. The inhibition of *Bacillus, Staphylococcus, Pseudomonas* and *Moulds* by LAB is attributed mainly to the low pH, resulting from the production of lactic acid and acetic acids (Vaughan *et al.*, 2005). Synthesis of bacteriocins by the LAB also play a role in inhibiting pathogenic

organisms during malting. Of significance with respect to sorghum malting is that many anti-microbial producing lactic acid bacteria have been isolated from samples of raw sorghum. LAB also improved the safety and quality of malt,

LAB starter cultures are applied in the brewing industry for their ability to improve mash and wort characteristics while ultimately resulting in a better beer. LAB as starter cultures is a more effective and natural option. The utilization of LAB starter cultures in malting reduces the fungal contamination during malting, lowers the aerobic bacterial flora and leads to a higher quality regardless of the natural variation. The use of LAB improve taste and flavour stability of starter beers. The use of LAB as starter cultures also reduced the growth of spoilage pathogens, reduced antinutritional factors of sorghum during malting and also improved the end products.

The results obtained in this study showed that the LAB starter treated worts was better than the control wort and also their flavour stability was better. The results of proximate analysis of the treated malted sorghum and the treated wort were with the acceptable limits expected for a fermented sorghum drink.

In conclusion, this study demonstrated the use of biological control methods, involving inoculation with *Lactobacillus* starter cultures, which have shown the most promise for the control of spoilage pathogens, reduced antinutritional factors of sorghum during malting and also improved the end products. The two sorghum varieties used for this work were suitable for malting and brewing processes. The characterization and confirmation of the identities of the LAB were carried out. Physiological and biochemical studies were also carried out on the LAB isolates in other to obtain the appropriate starter for the work. The identified *Lactobacillus* strains have the potentials of being used as starter cultures because of their reported use in literature. The ability of the LAB isolates to produce antimicrobial compounds in appreciable amounts was also used to combat the problem of high microbial load and the presence of mycotoxins in sorghum malt which improved the quality of the end product. The result obtained from the physiological and nutritional studies on the different sorghum varieties treated with the lactic acid bacteria cultures performed very well compared with the untreated sorghum. Overall, the sensory results were favourable towards the LAB-treated beer. The beneficial values of LAB can be harnessed and enhanced for the improvement of alcoholic beverages.

RECOMMENDATIONS

The use of chemicals for the treatment of sorghum should be discarded because it is dangerous to human health. There should be large scale production of *Lactobacillus* species that could be given to maltsters and brewers both at the traditional level and commercial scale that can be used for fermentation of sorghum in the brewing industry so as to improve the quality of the finished products. Sorghum grains must be stored in a dry, cool place to prevent mould growth during malting. Good brewing practices must be maintained as this will also enhance the microbiological stability of the finished product. The addition of LAB starters to steep water results in sorghum malt that is microbiologically safe with the absence of or very low levels of coliforms and fungi.

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APPENDICES

MRS (De Man Rogosa and Sharpe) Medium

Peptone (Oxoid)	5.0 g
Lab-Lemco powder (oxoid)	4.6 g
Yeast extract (oxoid)	2.0 g
Dextrose	10.0 g
Tween 80	0.5 ml
Dipotassium hydrogen phosphate	1.0 g
Sodium acetate	2.5 g
Tri-ammonium citrate	1.0 g
Magnesium sulphate	0.025 g
Agar (oxoid)	7.5 g
Distilled water	500 ml
pH	5.5

Bernfeld Reagent (1951)

3,5-Dinitrosalicyclic acid	1 g
2N NaOH	20 ml
Potassium sodium tartarate	20 g
Distilled water	100 ml