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Development of a Nigerian fermented maize food "Akamu" as a functional food

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**DEVELOPMENT OF A NIGERIAN FERMENTED MAIZE
FOOD “AKAMU” AS A FUNCTIONAL FOOD**

By

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partial fulfilment for the degree of

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Development of a Nigerian fermented maize food “Akamu” as a functional food

Abstract

Akamu is a lactic acid bacteria fermented cereal-based food that complements infant diets in most African countries. Uncontrolled fermentation increases the variability in quality and safety of *akamu*. This study was aimed at the controlled fermentation of *akamu* with selected lactic acid bacteria (LAB), investigation of the probiotic potential of the LAB and the effect of variation in production method on the product quality and sensory properties.

PCR-DGGE analysis of traditional *akamu* samples revealed LAB community dominated by *Lactobacillus fermentum*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*. Isolated yeasts were *Candida tropicalis*, *C. albicans*, *Clavispora lusitaniae* and *Saccharomyces paradoxus*.

The isolated *Lactobacillus plantarum* strains (NGL5 and NGL7) fermented irradiated ground maize slurries and produced significant levels of lactic acid ($>73 \text{ mmol L}^{-1}$) and low pH ≤ 3.63 displaying inhibitory activity against *Salmonella enterica* serovar Enteritidis NCTC 5188, *Escherichia coli* 1077 (NCTC 11560), *Bacillus cereus* NCIMB 11925, *Staphylococcus aureus* NCTC 3750 and *Listeria monocytogenes* NCTC 7973 in MRS agar and *E. coli* 1077 in maize slurry fermentation. Viability of both strains of *L. plantarum* at pH 2 after 3 h was reduced from $\geq 8.26 \pm 0.05$ to $\leq 4.94 \pm 0.49 \text{ Log}_{10} \text{ CFU mL}^{-1}$ while incubation in 0.3% bile allowed growth to 5.73 ± 0.13 and $7.93 \pm 0.12 \text{ Log}_{10} \text{ CFU mL}^{-1}$ after 6 h for NGL5 and NGL7 respectively. Auto-aggregation of the *L. plantarum* strains at 37°C (≥ 25 after 5 h) correlated with adhesion to hydrocarbons (<15 , 26, 33 and 64% for Hexane, Hexadecane, Ethyl acetate and Chloroform respectively). The strains failed to exhibit gelatinase or haemolytic activity but adhered to porcine mucin ($\text{OD}_{403 \text{ nm}} \geq 0.63$ with viability $\geq 6.52 \text{ Log}_{10} \text{ CFU mL}^{-1}$) and Caco-2 cells ($\geq 5.13 \text{ Log}_{10} \text{ CFU mL}^{-1}$).

The ash, mineral (Ca, K, Mg, Na, S and Zn), IDF, SDFP and TDF content of the *L. plantarum* fermented ground maize slurries were significantly ($p \leq 0.05$) higher than that of the traditional *akamu* but the peak and final viscosities (139.5 and 68.5 cP respectively) were significantly ($p \leq 0.05$) the least. The aroma, appearance, colour, flavour and texture of the resultant porridges were liked moderately by 75% of the assessors. This study demonstrated that fermentation with the *L. plantarum* strains would contribute towards product safety and the *L. plantarum* strains possessed some probiotic potential that could be beneficial to the consumers particularly in those developing countries where the main staple foods are fermented cereals.

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List of Abbreviations

<u>Abbreviations</u>	<u>Glossary of terms used</u>
ADP	Adenosine diphosphate
AGM	Amyloglucosidase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
BSF	Back Slop Fermentation
CFU	Colony Forming Unit
CVD	Cardiovascular diseases
DF	Dietary Fibre
DGGE	Denaturing gradient gel electrophoresis
DMEM	Dulbecco modified Eagle's minimal essential medium
DNA	Deoxyribonucleic acid
DP	Degree of polymerisation
DRI	Dietary Reference Intake
EPEC	Enteropathogenic <i>E. coli</i>
EPS	Exopolysacharides
FAO	Food and Agricultural Organizations
GIT	Gastro intestinal tract
GRAS	Generally Recognized as Safe
HMWDF	High molecular weight dietary fibre
HPLC	High performance liquid chromatography
IDF	Insoluble dietary fibre
KSW	<i>Koko</i> sour water
LAB	Lactic acid bacteria
LC	Liquid chromatography
LMWDF	Low molecular weight dietary fibre
MEA	Malt Extract agar
MG	Millet–groundnut

MRS	de Man, Rogosa and Sharpe agar
NAD ⁺	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide
NDO	Non digestible oligosaccharides
NGLw	Nigerian LAB isolated from white <i>akamu</i> sample
NGL	Nigerian LAB isolated from yellow <i>akamu</i> sample
NGYw	Nigerian yeasts isolated from white <i>akamu</i> sample
NGY	Nigerian yeasts isolated from yellow <i>akamu</i> sample
OD	Optical density
PBS	phosphate buffered saline
PBST	phosphate buffered saline with 0.05% Tween 20
PCR	Polymerase Chain Reaction
RAPD	Randomly amplified polymorphic DNA
RBCA	Rose Bengal Chloramphenicol Agar
RDS	Rapidly Digestible Starch
RI	Refractive index
RNA	Ribonucleic acid
RNIs	Recommended Nutrient Intakes
rRNA	ribosomal Ribonucleic acid
RS	Resistant starch
SCFA	Short chain fatty acids
SDF	Soluble dietary fibre
SDFP	Soluble dietary fibre precipitated in 78% alcohol
SDFS	Soluble dietary fibre soluble in 78% alcohol
SDS	Slowly digestible starch
T2D	Type 2 diabetes
TBX	Tyrptone Bile X-Glucuronide Agar
TDF	Total dietary fibre
tRNA	Transfer ribonucleic acid
TTA	Titrateable acidity

TTGE	Temporal temperature gel electrophoresis
UNU	United Nations University
WHO	World Health Organization
XLD	Taylor's xylose lysine desoxycholate agar

Dedication

To my beloved Father: **Oha (Chief) Clement Okpasi WEKERE**, and in the loving memory of my late husband: **Prince Obinna Harold ECHEM** (30th December 1966 - 2nd December 2003).

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CHAPTER ONE

LITERATURE REVIEW

1.1 FERMENTATION OF CEREAL-BASED FOODS

1.1.1 Introduction

Fermentation is one of the oldest food preparation methods considered as safe and acceptable for improving the quality and safety of foods. Traditional fermentation technologies were based on natural process whereby wet foodstuff undergo microbial degradation and when the food is edible it was termed fermented were not it was considered as spoilt (Lee, 2009). Over the years, fermentation process has developed such that organic substrate are now being converted into more desirable substances through the action of enzymes or microorganisms under controlled conditions to achieve several specific important functions.

In the human diet fermentation has helped in the enhancement of nutritive and sensory properties (Osundahunsi and Aworh, 2003, Teniola and Odunfa, 2001, Odunfa et al., 2001), decrease of anti-nutritional factors; phytate and polyphenols for nutrient availability (Hellström et al., 2003, Towo et al., 2006), extension of shelf life (Teniola and Odunfa, 2002), inhibition of growth of enteropathogens (Lawal et al., 2009), improvement of digestibility and conferment of health benefits (Elyas et al., 2002, Lei et al., 2006). Lactic acid bacteria (LAB) are involved in the fermentation of many types of food worldwide. Lactic acid fermented cereal-based foods in many African countries forms the

most integral part of the peoples' diet and plays an important role in the nutrition of infants and young children as it is used for the preparation of complementary foods (Adams and Nicolaidis, 1997).

Akamu is a Nigerian traditional lactic acid fermented cereal-based meal, made basically from maize (*Zea mays*), and other cereals; sorghum or millet (Akingbala et al., 1981, Teniola and Odunfa, 2001, Inyang and Idoko, 2006). The traditional process of *akamu* production involves steeping of the grain in excess water for 2 - 3 days, washing, wet milling and wet sieving. The extracted solids are allowed to sediment overnight, during which fermentation by various microorganisms associated with the raw material and utensils take place. The resultant product (*akamu*) varies in colour from white to yellow or dark brown depending on the variety of the cereal used. Addition of an equal part of boiling water to the fermented slurry with vigorous stirring yields a nearly gelatinized lump-less porridge. The porridge which is often eaten with beans cake (*akara*) or beans pudding (*moi-moi*) constitutes an integral part of adult main meals or food for convalescents in many African countries and plays an important role in the nutrition of infants and young children as a complementary food when diluted to thinness of 8 - 10% total solid (Osungbaro, 1990, Teniola and Odunfa, 2001). The fermented slurry when cooked with water produces a stiff gel called *akidi* that serves as convenient food for travellers (Umoh and Fields, 1981).

The traditional technique of *akamu* production that depends on spontaneous fermentation is not without inherent quality, sensory and safety problems. Many studies have utilized an accelerated fermentation (back slopping) method to demonstrate the beneficial effect of fermentation on the shelf life (Ohenhen and

Ikenebomeh, 2007), nutritional and organoleptic quality of cereal-based gruels (Osundahunsi and Aworh, 2003). Inhibition of enteropathogens in fermented cooked and uncooked weaning maize dough was reported by Mensah et al., (1990), Odugbemi et al., (1991), Bakare et al., (1998) and Lawal et al., (2009). Sanni et al., (1999) utilised starter cultures of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* ATCC 10776 in the fermentation of cereal-soybean four paste to establish the ability of starter culture fermentation in the reduction of fermentation time and increased acceptability of product. The study by Kalui et al., (2009) characterised the functional properties of *L. plantarum* and *L. rhamnosus* from *iki*, a Kenyan traditional fermented maize porridge for potentials as probiotics. Studies on the use of starter cultures of lactic acid bacteria in the fermentation of *akamu* for safety and probiotic effects appear to be limited.

This section therefore, reviewed some examples, consumption, key production process and microbiology of African fermented cereal-based foods and the importance of cereal-based fermentation.

1.1.2 Some examples of fermented cereal-based foods

Fermentation is widely used in the processing of cereals for the preparation of a wide variety of dishes in Africa and some other developing countries. Fermented cereal-based food products can be classified on the basis of either the raw cereal ingredients used in their preparation or the texture of the fermented products. The major African cereal-based foods are derived mainly from maize, sorghum, millet, rice, or wheat and texture-wise the products could be in the form of doughs, porridges, beverages or stiff gels. Depending on

locality, various names may be given to the same product or to products that are basically similar but had slight variations in their production processes (Jespersen, 2003). Examples of some of the common non-alcoholic cereal-based foods are shown in Table 1.1.

1.1.3 Preparation of some fermented cereal-based foods

Fermentation is only a step in the processing of fermented cereal products. Generally, pre-fermentation treatments such as drying, washing, steeping, milling, sieving and heating or cooking are some of the processing steps applied in the preparation of these fermented cereal foods (Adeyemi, 1983, Adeyemi and Beckley, 1986, Akingbala et al., 1981, Akinrele, 1970, Osungbaro, 1990). Pre-fermentation treatments of cereals are largely dependent on the type of cereal and on the end product desired. Some of these operations could confer some processing effects in combination with the fermentation.

The traditional method of *akamu* production is summarized in Figure 1.1. Soaking as a pre-fermentation process in *akamu* fermentation increases the moisture content of the grains, thereby softening the grain for ease of milling. The reduced particle sizes from milling operation results in increased surface area for enzyme release and action of microorganisms, while sieving results in very fine smooth slurry.

Milling and sieving pre-fermentation processes have been implicated in nutrient losses (Aminigo and Akingbala, 2004), while soaking has been recorded to influence increase in mineral (Na, K, P, Ca and Mg) levels through significant reduction of anti-nutritional factors: tannin, oxalate, trypsin and phytate (Obasi

and Wogu, 2008). Otitaju, (2009) reported that dry milling after soaking with or without sieving conserves and improves the nutrient levels of fermented maize, with more benefit derived from dry-milling without sieving. Wet milling may not only be implicated in nutrient losses and the production of mainly starch but the introduction of contaminants from the water used in soaking and wet milling of the grains, the milling machine, persons involved in the milling process and perhaps the hygienic condition of the surrounding environment at the time of the *akamu* production.

The processing of fermented maize doughs such as *kenkey* in Ghana is such that the sieving stage is skipped. Fermentation water is simply added to the wet milled mash to produce stiff dough which is then fermented spontaneously and the porridge cooked from the dough (Kpodo et al., 1996). The grains for *obusera* production after sorting are dry milled into flour, before the addition of water and subsequent fermentation for 1 - 2 days. In some cases, the maize flour is first cooked into porridge to which an old stock would be added as starter for fermentation of the porridge before consumption (Muyanja et al., 2003).

To be able to effectively demonstrate the importance of fermentation in the improvement of nutritional quality, ease of edibility and digestibility, shelf life extension, safety, reduction in processing time and cost savings, it would be necessary to improve upon or modify the existing fermentation systems.

Table 1.1: Examples of some fermented non-alcoholic cereal-based foods from Africa, their microbiology and textural characteristics

Product	Countries	Substrate	Fermenting Microorganism	Texture	References
<i>Agidi</i>	Nigeria, Benin	Maize	<i>Lactobacillus</i> spp. (<i>L. fermentum</i> , <i>L. cellobiosus</i>), <i>Pediococcus acidilactici</i> , <i>Aerobacter</i> ; <i>Corynebacterium</i> ; Yeast; Moulds.	Dough	Fields et al., (1981)
<i>Akamu/</i> <i>Ogi</i>	Nigeria, Benin	Maize, sorghum or millet	Lactic acid bacteria: <i>Lactobacillus brevis</i> , <i>L.</i> <i>plantarum</i> , <i>L. fermentum</i> , <i>L. cellobiosus</i> , <i>Pediococcus pentosaceus</i> , <i>P. acidilactici</i> , <i>Bacillus</i> <i>subtilis</i> , <i>Brevibacterium linens</i> , <i>B. oxy</i> , <i>P.</i> <i>pentosaceus</i> Yeast and moulds: <i>Saccharomyces cerevisiae</i> , <i>Rhodotorula graminis</i> , <i>Candida valida</i> , <i>C. krusei</i> , <i>Geotrichum candidum</i> , <i>G. fermentum</i> , <i>Aerobacter</i> ; <i>Corynebacterium</i>	Porridge	Fields et al., (1981), Nago et al., (1998b), Teniola and Odunfa, (2002), Teniola et al., (2005), Omemu, (2011)
<i>Akasa</i> and <i>Koko</i>	Ghana	Millet, maize	<i>Weissella confusa</i> and <i>L. fermentum</i> (Predominant). Others were: <i>L. salivarius</i> , <i>P. pentosaceus</i> , <i>P. acidilactici</i> and <i>L. paraplantarum</i> .	Porridge	Lei and Jakobsen, (2004)
<i>Banku</i>	Ghana	Maize	<i>Lactobacillus</i> spp. Moulds.	Dough	Mensah, (1997)
<i>Borde</i>	Ethiopia	Maize	<i>Weissella confusa</i> , <i>L. brevis</i> , <i>L. viridescens</i> , <i>P.</i> <i>pentosaceus</i> and <i>pentosaceus</i> subsp. (Dominant)	Beverage	Kebede, (2007)

Continues

Table 1.1: *Continued*

Product	Countries	Substrate	Fermenting Microorganism	Texture	References
Gowe	Nigeria, Benin	Sorghum	<i>Lactobacillus fermentum</i> , <i>L. mucosae</i> , <i>Weissella kimchii</i> , <i>W. confuse</i> , <i>Pediococcus acidilactici</i> , <i>P. pentosaceus</i> . Yeast; <i>Kluyveromyces maxianus</i> , <i>Pichia anomala</i> , <i>Candida krusei</i> and <i>C. tropicalis</i>	Beverage	Vieira-Dalodé et al., (2008)
Hussuwa/ Aceda/Ajin	Sudan	Sorghum or millet malt	<i>L. fermentum</i> , <i>P. acidilactici</i> strains (Predominant). <i>P. pentosaceus</i> <i>Enterococcus faecium</i>	Dough/Stiff porridge/ Sourdough	Yousif et al.,(2005), Yousif et al., (2010)
Ikii	Kenya	Maize	<i>L. plantarum</i> , <i>L. rhamnosus</i>	Porridge	Kalui et al., (2009)
Kenkey	Ghana, Botswana	Sorghum, maize, millet	<i>Lactobacillus</i> spp., Yeast: (<i>Candida krusei</i> and <i>Saccharomyces cerevisiae</i>) as predominant organisms	Dough	Jespersen et al., (1994) Halm et al., (1993)
Kisra	Sudan, Ethiopia	Sorghum	<i>L. fermentum</i> , <i>L. amylovorus</i> , <i>L. brevis</i> and <i>Sacch. cerevisiae</i>	Dough	Ali and Mustafa, (2009)
Kunu-zaki	Nigeria	Millet	<i>L. fermentum</i> , <i>L. plantarum</i> , <i>P. pentosaceus</i> <i>Streptococcus</i>	Beverage	Oguntoyinbo and Narbad, (2012)

Continues

Table 1.1: *Continued*

Product	Countries	Substrate	Fermenting Microorganism	Texture	References
<i>Mahewu</i>	Zimbabwe South Africa	Maize, sorghum and millet	<i>Streptococcus lactis</i> , <i>Lactobacillus delbrueckii</i> , <i>L. bulgarius</i>	Beverage	Bvochora et al., (1999)
<i>Masa</i>	Nigeria	Maize	<i>L. plantarum</i> , <i>Pediococcus acidilactici</i> , <i>L.</i> <i>fermentum</i> and <i>Sacch. cerevisiae</i> .	Dough	Oyeyiola, (1990)
<i>Mawe</i>	South Africa Benin	Maize	<i>L. fermentum</i> , <i>L. confuses</i> ; <i>Candida krusei</i> ; <i>Sacch. cerevisae</i>	Porridge	Hounhouigan et al., (1999)
<i>Obusera</i>	Uganda	Millet and sorghum	<i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Lactobacillus</i> (<i>L.</i> <i>plantarum</i> , <i>L. paracasei</i> subsp. <i>paracasei</i> , <i>L.</i> <i>fermentum</i> , <i>L. brevis</i> and <i>L. delbrueckii</i> subsp. <i>delbrueckii</i> , <i>L reuteri</i>), <i>Weissella</i> and <i>Enterococcus</i> spp., <i>Bacillus</i> spp., <i>Streptococcus</i> spp., <i>Fructobacillus</i> spp. Yeast: <i>Sacch. cerevisiae</i> , <i>Clavispora lusitaniae</i> , <i>Cyberlindnera fabianii</i> , <i>Issatchenkia orientalis</i> , <i>Pichia</i> spp.	Porridge/ beverage	Muyanja et al., (2003), Mukisa et al., (2012)
<i>Ting</i>	South Africa	Sorghum, millet or maize	<i>L. fermentum</i> , <i>L. plantarum</i> and <i>L. rhamnosus</i>	Porridge	Madoroba et al., (2009)
<i>Togwa</i>	Tanzania	Maize, sorghum, millet and cassava or their combinations	<i>L. brevis</i> , <i>L. cellobiosus</i> , <i>L. fermentum</i> , <i>L.</i> <i>plantarum</i> and <i>P. pentosaceus</i> . <i>Candida pelliculosa</i> , <i>C. tropicalis</i> , <i>Issatchenkia</i> <i>orientalis</i> and <i>Sacch. cerevisiae</i>	Beverage	Mugula et al., (2003a)

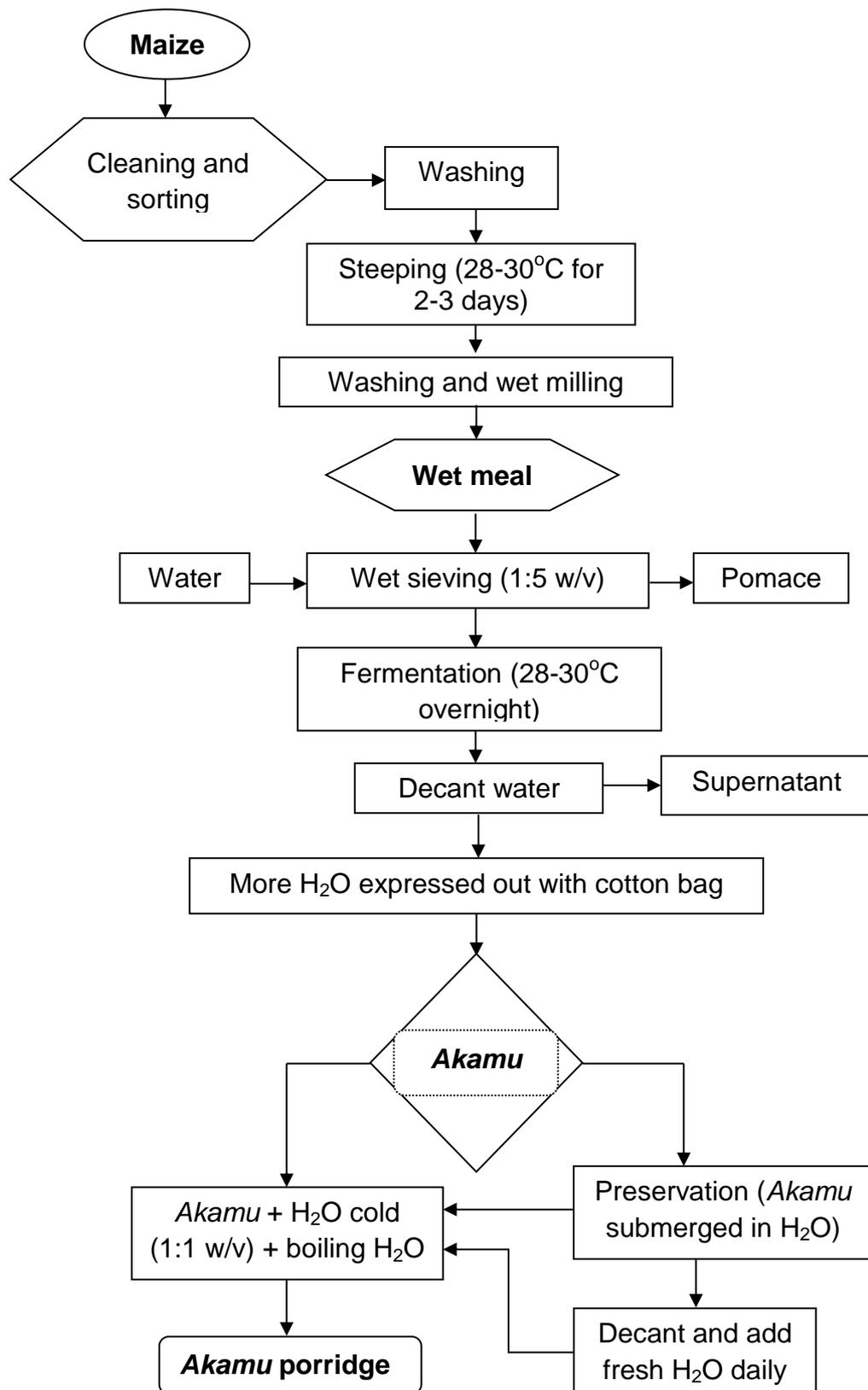


Figure 1.1: The traditional production and preparation process of *akamu* and its porridge in Rivers state, Nigeria

1.1.4 Microbiology of fermented cereal-based foods

The micro-flora associated with the natural fermentation of cereal grains are complex and only the endogenous grain amylase generates fermentable sugars that serve as source of energy for the fermenting organisms. The type and number of microorganisms harboured by the grains depend on many factors such as the soil in which the plants were cultivated, climatic condition under which the grains were produced, weather condition during and after harvesting and the duration and conditions of storage (Fields et al., 1981).

Microbiological evaluation of fermented cereal-based foods had revealed important microorganisms in their manufacture. This includes diverse species of LAB, yeast and moulds, coliforms and pathogenic organisms. Lactic acid bacteria and yeast are the most important groups of microorganisms in the fermentation of cereal-based products with LAB mostly predominant (Table 1.1).

1.1.4.1 Lactic acid bacteria (LAB)

Lactic acid bacteria are a group of bacteria related by certain morphological, metabolic and physiological characteristics (Wright and Axelsson, 2012). They belong to the phylum *Firmicutes*, class *Bacilli* and order *Lactobacillales*. The different families: *Aerococaceae*, *Carnobacterieae*, *Enterococaceae*, *Lactobacillaceae*, *Leuconostoacaceae* and *Streptococcaceae* comprise of a wide range of genera which include *Aerococcus*; *Carnobacterium*; *Enterococcus*, *Tetragenococcus* and *Vagococcus*; *Lactobacillus* and *Pediococcus*; *Leuconostoc*, *Oenococcus* and *Weissella*; *Lactococcus* and *Streptococcus* (Axelsson, 2004, Stiles and Holzapfel, 1997). According to Klein et al., (1998) *Bifidobacterium* are also classified as LAB as they have similar

physiological and biochemical properties and share the same ecological niche (the gastro-intestinal tract) with LAB.

Lactic acid bacteria are generally considered as beneficial microorganisms and some called probiotics have been shown to impart certain health enhancing benefits. Although some of the genera *Streptococcus*, *Lactococcus*, *Enterococcus* and *Carnobacterium* have species or strains that are known to be pathogenic (Wright and Axelsson, 2012). Lactic acid bacteria are typically Gram-positive, catalase-negative rods or cocci that grow under aerophilic to micro-aerophilic and strictly anaerobic conditions, producing lactic acid as a major metabolic products (Reddy et al., 2008). With the exception of *Sporolactobacilli* they are non-sporing and non-motile (Wood and Holzapfel, 1995).

Lactic acid bacteria are heterotrophic and lack many biosynthetic capabilities that generally make them to have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates. They do not possess an electron transport chain and rely mainly on fermentation for energy generation. Due to low energy generation under anaerobic or micro-aerophilic conditions, LAB grow more slowly than aerobic microbes producing smaller colonies of about 2 - 3 mm. The growth of LAB is over a wide range of temperatures from 5 to 45°C with optimum growth pH of 5.5 - 6.5. Some are acid tolerant and most strains are able to grow at pH 4.4 (Reddy et al., 2008).

Depending on the ability to ferment sugars LAB are either classified as homofermentative, or heterofermentative. The homofermentative LAB convert sugars almost exclusively into lactic acid (<85% of lactic acid from glucose) and include the genera *Streptococcus*, *Pediococcus*, *Lactococcus* and some *Lactobacillus*. Heterofermenters include the genera *Leuconostoc* and other *Lactobacillus* that converts sugars into lactic acid (only about 50% of lactic acid from glucose), CO₂, acetic acid, ethanol and some other aromatic compounds. Another group of less well known homofermentative species which produces DL-lactic acid, acetic and CO₂ have been reported (Wood and Holzapfel, 1995, Reddy et al., 2008).

The genus *Lactobacillus* represent the largest group within the family *Lactobacillaceae*, comprising over 100 recognized species and subspecies (Giraffa et al., 2010) and several scientific studies have utilized different methods in their isolation, selection, purification and grouping. The genotypic and phenotypic variations within the genus *Lactobacillus* has been reviewed by Giraffa et al., (2010). Morphologically, *Lactobacillus* species vary in shape from long to short slender rods or short coccobacilli which frequently appear in chains. The lengths are usually between ~1 and 1.5 µm with diameter of ~0.7 to 1 µm (Schär-Zammaretti and Ubbink, 2003).

Lactobacilli are predominantly associated with cereal fermentation where their acidification process serve as preservatives and other fermentation by-product improves the quality of the fermented product. Some *Lactobacillus* that are of intestinal and dairy origin have been associated with some health benefits. The

increasing concern over food and health has led to the characterisation of *Lactobacilli* from other sources for possible health enhancing effects.

1.1.4.2 Yeast

Yeasts are unicellular fungi that are mostly identified on the basis of their morphological and cultural characteristics (Kavanagh, 2005, Harrigan, 1998). Morphologically, yeast vary in shape from ellipsoid to spherical, cylindrical, lemon shaped, pear shaped, curved, ogival, flask shaped or even elongated into pseudo-hyphal, hyphal or dimorphic and reproduction either by budding (asexual) or spore production (sexual). Most young yeast colonies are moist and slimy and could be smooth or rhizoid and of varying colours: pink, white, cream, salmon colour etc and with age some become dry and wrinkled (Fraizer and Westhoff, 1995).

Based on the production of ascospores and ballistospores yeast can be respectively classified as ascosporegenous and sporobolomycetaceae or asporogenous. Ascosporegenous yeast may form pseudomycelium or true mycelium and reproduce by budding, binary fission or arthrospores and include *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, *Kluyveromyces*, *Endomyces* etc. *Sporobolomyces* and *Bullera* (with sickle and ovoid shaped ballistospores respectively) make up the sporobolomycetaceae. While *Candida*, *Rhodotorula*, *Torulopsis* are some examples of asporogenous yeast. Only a few of them form mycelia and reproduce mainly by budding (Harrigan, 1998, Schlegel, 1986). Yeasts are also classified as film or fermentative yeast based on growth pattern in liquid media. Film-yeasts grow as scum or film while fermentative yeasts grow throughout the liquid media.

Unlike bacteria, yeasts are osmophilic and can grow in media of low water activities and acid pH. Yeasts have been reported to be involved in several different types of indigenous fermented foods and beverages (Table 1.1), in coexistence with other microorganisms. *Candida krusei*, *C. tropicalis*, *C. rugosa*, *C. fabianii*, *C. norvegensis*, *Kluyveromyces marxianus* and *Trichosporon asahii* were associated with the fermentation of *fura* a West African spontaneously fermented pearl millet dough with the predominance of *C. krusei* and *K. marxianus* (Pedersen et al., 2012).

1.1.4.3 Methods of microbial identification (LAB and yeast)

Classical food microbiological methods utilised in the identification of LAB and yeast are culture-based in which the microorganisms are grown on microbial media and identified through morphological and biochemical characteristics. These include cultural characteristics of the microbial colonies (form, elevation and margins), Gram and catalase reaction, growth factors and sugar fermentative pattern. This conventional method is simple to perform but time consuming and often lacks discriminatory potency and reproducibility at species level due to the bio-diverse nature of microorganisms (Ehrmann and Vogel, 2005) and cell morphological characteristics can markedly be affected by growth conditions (Stiles and Holzapfel, 1997).

The use of a molecular approach offers a higher level of accuracy, specificity, sensitivity and rapidity (Wakil et al., 2008). Genomic approaches using randomly amplified polymorphic DNA (RAPD) finger printing or sequence analyses of the genes encoding 16S ribosomal RNA (*rrs*) and phenylalanyl tRNA synthase (*pheS*) (Madoroba et al., 2009) gives a better understanding of

the genetic relationships of bacteria, though labour intensive and time consuming. Rapid methods based on direct analysis of DNA in the environment requiring no cell culture and enabling detection of individual species as well as overall profiling of community structural changes with time have evolved. They include single-stranded conformational polymorphism analysis, denaturing gradient gel electrophoresis (DGGE) and temporal temperature gel electrophoresis (TTGE) (Wakil et al., 2008). However, phenotypic characteristics still serves as a benchmark of bacterial classification since they are the principal method available for conventional microbiology laboratory. A polyphasic approach in which both phenotypic and genotypic methods are used would be preferred for explicit and reliable species identifications.

Several studies using the conventional phenotypic approach as well as recent molecular methods have been able to identify different LAB and yeast from fermented cereal products (Table 1.1). Using phenotypic approach: Johansson et al., (1995) identified 121 isolates of LAB from *ogi* and three other Nigeria traditional cereal-based alcoholic beverages; Nago et al., (1998b) reported mixed population of LAB ($9 \text{ Log}_{10} \text{ CFU g}^{-1}$) and yeast ($7 \text{ Log}_{10} \text{ CFU g}^{-1}$), mainly *Lactobacilli* and *Candida* in Beninese *ogi*; Sanni et al. (2002) isolated 106 microbial strains including the genera *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, *Debaryomyces*, *Candida*, *Bacillus*, *Micrococcus*, *Klebsiella*, *Escherichia* and *Aspergillus* from different batches of fermenting maize dough fortified with 20% cowpea and Teniola et al., (2005) identified *Lactococcus raffinolactis*, *Pediococcus* spp., *P. pentosaceus*, *L. plantarum*, *L. suebicus* and *L. brevis* from fermenting *ogi*. Bacteria belonging to the genera *Bacillus* species, *Staphylococcus aureus*, and *Escherichia coli* and yeast such as

Saccharomyces cerevisiae, *Saccharomyces* and *Hansenula* species from spontaneously fermented cereal-legume weaning blends were identified phenotypically while the predominance of *L. plantarum* and *P. acidilactici* were established using genomic methods by Wakil et al., (2008). Pedersen et al., (2012) using both phenotypic and genotypic approach identified different yeast species involved in the fermentation of fermented pearl millet dough.

1.1.4.4 Microbial interaction.

Yeast and lactic acid bacteria (LAB) occur as part of the natural microbial population in spontaneously fermented food and as starter cultures in the food and beverage industry (Shetty and Jespersen, 2006). Predominantly seen in fermented cereals are LAB and yeast, especially *Sacch. cerevisiae* and *C. krusei* (Jespersen, 2003). The acid environment created by LAB in the fermentation medium favours yeast proliferation (Mugula et al., 2003a), while yeasts have been reported to be essential at some stage in stimulating the growth of LAB by providing essential metabolites such as pyruvate, amino acids and vitamins (Akinrele, 1970, Nout et al., 1989b).

According to Aidoo et al., (2006), when yeasts are abundant alone or in stable mixed population with bacteria (usually LAB), their significant impact is on food quality parameter such as taste, texture, odour and nutritive value. The ability of yeast with particular reference to *Sacch. cerevisiae* to produce alcohol, esters and other aroma compounds are implicated in the aroma and flavour of the fermented product (Annan et al., 2003). Excretion of most of the lysine and methionine present in *ogi* has been attributed to yeasts present in the fermentation (Odunfa et al., 2001). The production of CO₂, pyruvate, propionate,

acetate and succinate excreted by *Saccharomyces florentinus* had been implicated in the stimulation of the growth of *Lactobacillus hilgardii* and production of lactic acid in sugary kefir grains (Leroi and Pidoux, 1993). Yeast ability to utilise certain bacterial metabolites as carbon sources and the production of alcohol by yeast may however suppress the LAB growth, thereby lowering the efficacy of organic acid production which is one of the by-product responsible for the characteristic quality of pathogen inhibitions. Moulds such as *Fusarium*, *Aspergillus*, *Penicillium*, *Cephalosporium* spp., *Oospora* spp., and *Cercospora* spp. have also been associated with Nigerian fermented maize (Ohenhen, 2002).

Unwanted spoilage and pathogenic microorganisms from the grains, processing water, utensils, the food handler and the environment may also grow. Their growth occurs slowly at the early stage of fermentation which is associated with the lag phase of microbial growth. Competition for nutrients at this stage may result in a product of varying degree of quality and safety. This phase can be shortened and product of defined characteristic quality and safety obtained through the inoculation of starter cultures.

1.1.5 Starter cultures

Starter cultures are preparation or material containing large numbers of variable microorganisms, which may be added to accelerate a fermentation process (Holzapfel, 1997). Giraffa et al., (2010) specified starter cultures as microorganisms that are intentionally added to raw material to achieve specified end result in the final product and the most common use of starter cultures in

the dairy industry is for the production of lactic acid from milk sugar. Starter cultures facilitate control over the initial phase of a fermentation process suppressing the growth of unwanted microorganisms and generating the desired flavour or textural characteristics as the case may be. Although there could be some growth of endogenous cultures of the substrate, but with large quantities of starter cultures at the start of fermentation would not allow the growth of unwanted microorganisms to levels that would impair the products quality. The use of starter cultures adaptive to the substrate had been suggested to facilitate improved control of a fermentation process and predictability of its product (Holzapfel, 1997).

Modern starter cultures originated from back slop fermentation (BSF): a process that involves the addition of some part of a previous fermentation product as inoculum to start the fermentation of a new product. Nout, (1991) used this method repetitively for 60 fermentation cycles of 24 h each to gradually establish a mixed population of LAB and yeast for a desired product quality in sorghum based infant formula. The main fermentative microorganisms from different fermented products are now been identified, purified and stored in form that can be easily used as pure microbial cultures in controlled fermentation of such or similar products. Starter cultures can come in fresh, frozen or freeze-dried forms. They can be single (one selected strain of particular species of microorganism) or mixed cultures (two or more strains from a species and/or of different families and genera) with definite characteristics that are beneficial in the manufacture of a desired product (Edema and Sanni, 2008).

The choice of starter type depends on the desired product and what is acceptable in a region. African fermented cereal-based foods are usually that of lactic acid fermentation and sometimes in combination with yeast. While in Asian countries it is more of a mould fermentation (Holzapfel, 2002). Fermentation in most cases are usually with mixed-strains as no single strain has the ability to produce all the desired characteristics in a product from sensory to nutritional, safety and health benefits. Fermentation could be affected and the desired product characteristics may be marred if the single-strain culture is degraded by bacteriophages, spontaneous mutation or through the loss of key physiological properties. A mixed culture can result even from the inoculation of a single-strain culture if the raw material is not sterile prior to inoculation and maintained axenic throughout strict control process (Holzapfel, 2002).

The anticipated challenge with the use of starter cultures particularly on a large scale production is the consequence of diminishing the uniqueness of the original product and the loss of characteristics that originally made the product popular (Caplice and Fitzgerald, 1999). According to Holzapfel (1997) the choice of starter culture has to be governed by the ability of the chosen microorganism to:

1. Adapt to the substrate i.e. survive, remain viable and be competitive over unwanted microorganisms in the product. Cultures developed from wild-type strains obtained from the fermented material have been reported to generally adapt better to such food matrix (Holzapfel, 2002)

2. Rapidly produce organic acids or alcohol for fast reduction of pH to levels inhibitory to unwanted microorganisms
3. Be antagonistic against pathogenic and spoilage microorganisms, guaranteeing the safety and increased shelf-life of the product
4. Produce desired primary metabolites of fermentation necessary for the products desired sensory properties
5. Improve the nutritional value of the product by the degradation of anti-nutritional factors
6. Reduce toxicological risks such as the detoxification of biological amines and mycotoxins
7. Confer certain health benefits when consumed

Starter cultures of yeasts such as *Sacch. cerevisiae* have been in use in the bakery and brewing industry. van der Aa Kühle et al., (2005) reported the isolation of a strain of *Sacch. cerevisiae var boulardii* from African indigenous fermented foods with promising probiotic potential. Non-*Saccharomyces* yeasts of the genera *Pichia*, *Torulaspota* and *Candida* were characterised and reported to have promising potentials for use as starter cultures, though in fermentation of brine olives (Silva et al., 2011). A study by Pedersen et al., (2012) investigated the use of yeast species: *Candida krusei*, *Kluyveromyces marxianus*, *Torulaspota asahii*, *C. tropicalis*, *C. norvegensis*, *C. rugosa* and *C. fabianii* isolated from *fura* a West African spontaneously fermented pearl millet product as potential starter cultures.

Although, starter cultures of some LAB and the back slop fermentation have been employed in the fermentation of some African cereal-based food for

various reasons, evidences on health, safety and prebiotic exploration of African cereal-based foods that constitute the peoples' staples need to be researched on with the increasing awareness in health promoting foods.

1.1.6 The importance of fermentation of cereal-based foods

Fermentation is a simple and cheap food processing technique commonly practiced in most African countries. It does not require the use of sophisticated pieces of equipment or any special skill and often the microbial group involved are based on chance. Fermentation like other food processing methods in addition to enhancement of edibility and digestibility of foods, serves several other useful purposes, from the improvement of nutritional and sensory properties to food preservation.

1.1.6.1 Improvement of nutritional quality

Fermented cereal-based porridges are mostly used as weaning foods in most African countries and to obtain porridges with a semi-liquid consistency suitable for infant and young children feeding, the porridges are been diluted with a large quantity of water. This reduces the energy density of the porridges to levels (0.2 - 0.4 kcal g⁻¹) lower than that of breast milk (0.75 kcal g⁻¹) (Svanberg and Lorri, 1997). Reduction of viscosity of starchy porridges has been achieved during fermentation by the addition of germinated cereal grains and amylolytic lactic starter culture (Nguyen et al., 2007); this enabled the preparation of porridges of increased nutrient density that are still sufficiently liquid to be swallowed by infants.

Cereal grains are also major sources of dietary nutrients for all people particularly those in the developing countries where cereals are the major staple. However, the nutritional quality of cereal grains is poor due to deficiencies in certain amino acids, lower protein content, starch and mineral availability (Chavan and Kadams, 1989). Over 60% of cereal grain component are carbohydrates (Haard et al., 1999) and some of the traditional technique involved in their production yields starchy product with very little of other nutrients (Table 1.2).

The application of some legume and oilseed blends such as cowpeas (Osundahunsi and Aworh, 2003), bambara groundnut (Mbata et al., 2009), soybeans (Sanni et al., 1999) and okra seed flour (Otunola et al., 2007) at different ratios to the traditional fermentation of cereal-based staples have exhibited potential improvement of the nutritional quality of the food by elevating the protein content. The availability of lysine in corn meal slurry after undergoing natural lactic acid fermentation at 37°C for 4 days increased from 17.4 to 51.8 mg gN⁻¹ (Fields et al., 1981). Several other studies on *ogi* recorded similar increases in lysine and methionine levels (Odunfa et al., 2001, Teniola and Odunfa, 2001, Osundahunsi and Aworh, 2003). A high lysine-producing mutant of *L. plantarum* (OG 261-5) derived from a wild type strain (OG 261) isolated from fermenting *ogi* was evaluated for nutritional improvement of *ogi* in a modified fermentation process. The result indicated that *ogi* obtained by fermentation with the pure culture of the mutant compared to traditional *ogi* had increased concentration of available lysine from 228.5±12.0 to 525.1±25.8 mg 100g⁻¹, tryptophan from 58.6±8.0 to 114.3± 1.0 mg 100g⁻¹ and tyrosine from 408.5±13.7 to 4636.5±11.3 mg 100g⁻¹. However, the contents of valine, leucine,

isoleucine and phenylalanine were substantially reduced in the modified *ogi* (Adebawo et al., 2000).

Another major contributory factor to the poor nutritional quality of cereal is the presence of some anti-nutritional factors such as phytic acid and phenolic compounds that are bound to minerals such as iron, magnesium, phosphorus, zinc and iron making the minerals unavailable for utilization. The LAB and yeast strains associated with the fermentation of cereal-based foods have the ability to degrade the anti-nutritional factors thereby improving the nutritional values of the fermented foods.

Lopez et al., (1983) and Osundahunsi and Aworh, (2003), reported increased bioavailability of minerals in cereal-based foods from the hydrolysis of phytates by microbial phytase enzymes which released divalent cations such as calcium, magnesium, phosphorus, zinc and iron bound by phytic acids. Reduced tannin levels resulted in increased absorption of iron, except in some high tannin cereals, where little or no improvement in iron availability was observed. The organic acids produced during fermentation has been reported to create an acid matrix that could help in the solubilisation of most iron present in the medium and even contamination iron from mills equipped with iron-containing grindstones consequently, enhancing iron absorption (Greffeuille et al., 2011, Oyarekua, 2011).

Table 1.2: Literature reports on the proximate compositions (%) and energy levels of some Nigerian *ogi* products

Moisture Content	Crude protein	Fat	Ash	Crude fibre	Total Carbohydrate	Energy (KJ g⁻¹)	Reference
10.30±0.21	5.47±0.38	2.56±0.11	0.04±0.24	0.60±0.23	91.3	NA	Aminigo and Akingbala, (2004)
NA	7.5	3.34	0.34	0.78	88.1	NA	Akingbala et al., (2003)
10.9	6.7	1.6	0.4	0.7	79.6	NA	Akingbala et al., (1994)
NA	7.1±0.3	3.5±0.1	0.0±0.0	0.0±0.0	89.4±1.9		Antai and Nzeribe, (1992)
NA	8.8	4.0	1.1	ND	86.2	17.32	Osundahunsi and Aworh, (2003)
6.79	10.5	5.0	1.02	1.40	78.7	14.4	Oluwamukomi et al., (2005)
8.80	11.72	4.22	0.70	NA	83.39	17.51	Egounlety et al., (2002)
6.30±0.14	6.50±0.14	1.66±0.04	0.05±0.01	0.27±0.51	85.22±0.01	NA	Otunola et al., (2007)

NA - Data not available.

ND - Not detected

The lowering of proteinase inhibitors during cereal fermentation increased the proteolytic activity of lactic acid bacteria, providing simple proteins, peptides and amino acids such as lysine, leucine, isoleucine, methionine and tryptophan (Lenaerts, 2004). Sanni et al., (1999) recorded increase in vitamins (riboflavin, niacin, vitamin B12 and ascorbic acid) in fermented cereal blends in comparison with unfermented blends.

Pre-processing stages of milling and sieving during cereal fermentation have however been implicated in reducing the nutritive value of the product through the loss of proteins, minerals and lipids which are components of the germ and aleurone layer usually removed as pomace. Soluble nutrients are wasted in the large volumes of water used during sieving. High levels of organic acid, soluble mono- and disaccharide are lost through the process of water decanting during *akamu* processing (Sefa-Dedeh et al., 2001, Obinna-Echem, 2009). Nago et al., (1998b) recorded a loss of at least 40% of protein and 50% of both macro and micro mineral elements during the production of Beninese *ogi*. It is therefore imperative to research ways of modification of the fermentation system to help in the prevention of nutrient losses.

1.1.6.2 Enhancement of sensory/eating properties

The exclusive sensory properties of fermented foods are attributed to the various end products of microbial metabolism. During cereal fermentation, the metabolic activities of the fermenting LAB result in the production of organic acids; lactic and acetic acid and several aromatic volatiles, such as higher alcohols and aldehydes, ethyl acetate and diacetyl, while the metabolic activity of yeasts produce aromatic compound such as fatty acids, esters, aldehydes

and alcohols . The characteristic distinctive flavours and aromas of these by-products contribute significantly to the unique sensory quality of the fermented products.

Annan et al., in (2003) gave a detailed picture of the aroma compounds in fermented maize as 20 alcohols, 22 carbonyls, 11 esters, seven acids, a furan and three phenolic compounds. Of the total 64 volatile compounds, 51 were ascribed to be contributing to the aroma of the different fermented dough samples. Steeping and fermentation of the grains with cultures of *L. brevis* and *Sacch. cerevisiae* was observed to encourage stronger aroma production in *ogi* by Teniola and Odunfa (2001). Probiotic strains of *L. plantarum* NCIMB 8826 produced high concentrations of volatile compounds when inoculated into cereal-based substrates. The most abundant volatiles detected were oleic acid, linoleic acid, acetic acid, and 5-hydroxymethylfurfural in oat, wheat and barley and malt substrate respectively (Salmeron et al., 2009).

Studies have shown consumer acceptability of various improved sensory qualities of fermented cereal products. The taste, consistency, texture and aroma of fermented porridges from different cereal blends were generally accepted at rating above average by consumers (Sanni et al., 1999). The colour, taste (sourness or sweetness) and aroma of *Gowe* a sorghum-based LAB fermented food was highly appreciated by the taste panel that assessed the product (Vieira-Dalodé et al., 2008). In a test conducted by Teniola and Odunfa, (2001) taste and appearance of flour slurry fermented with *L. brevis* were highly scored. Adequate selection and choice of microorganisms for cereal fermentation can therefore improve upon the known sensory qualities.

1.1.6.3 Safety through pathogen inhibition

Fermentation is generally considered as a safe and acceptable preservation technology for improving the quality and safety of foods. In developing countries, contaminations of cereal-based weaning foods occur through different channels before, during, and after preparation. The raw materials may themselves harbour some contaminants. Enteropathogenic microorganisms from polluted water, dirty utensils, insects, pests, domesticated animals, excreta from the environment could be introduced during and after preparation. Storage at ambient temperature due to economic constraints in turn favours the growth of the pathogens and/or formation of their toxins. Insufficient cooking or reheating of the probably uncovered foods prior to consumption lead to food borne illnesses (Afifi et al., 1998, Lawal et al., 2009). The etiological agents responsible for foodborne diseases are broad and include bacteria, viruses and parasites. Some of the principal pathogens include strains of *Escherichia coli*, *Shigella* spp., *Salmonella*, *Vibrio cholerae* O1, *Campylobacter jejuni*; protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp. and viruses such as Hepatitis A and E, and Rotavirus (Motarjemi, 2002).

Despite the isolation of *B. cereus*, *Clostridium perfringens*, *Aeromonas* spp., and *Staphylococcus aureus* from sorghum-based weaning food by Kunene et al., (1999), fermentation of cereal-based foods have been reported to provide antagonistic environment against pathogenic microorganisms due to the production of organic acids that lower pH to levels inhibitory to some pathogens, overcrowding of the food with desirable lactic acid bacteria making competition difficult for unwanted organisms and the production of antimicrobial agents. The

inhibitory effects of some LAB and/or their antimicrobial agent during the fermentation of various cereal-based foods were shown in Table 1.3.

Yeast such as *Sacch. cerevisiae* and *C. krusei* have been observed to have an inhibitory effect on the growth of mycotoxin producing moulds such as *Penicillium citrinum*, *Aspergillus flavus* and *Aspergillus parasiticus* (Halm and Olsen, 1996). Shetty and Jespersen, (2006) reviewed the capabilities of *Sacch. cerevisiae* strains as prospective mycotoxins decontaminating organisms. The inhibitory effects of the yeasts were shown to be mainly due to substrate competition, but inhibition of spore germination might also occur due to the production of high concentrations of organic acids (Halm et al., 1996).

Studies so far has mainly been with the use of accelerated methods, and has not been able to distinguish clearly between pathogens and the natural fermenting organisms present in the raw materials and those that are been introduced during the inhibitory test. The use of selected starter culture could be of great benefit. Olukoya et al., (1994), developed an improved *ogi* named DogiK with potential use in the prevention and treatment of diarrhoea by using *Lactobacillus* starter cultures with antagonistic activity against diarrhoeagenic bacteria, although there was no *in-vivo* trial in testing the efficiency of the product for diarrhoea prevention or treatment.

Table 1.3: Anti-pathogenic potential of LAB from fermented cereal products

Substrate	Fermenting organism	Pathogens	Level and duration of inhibition	Suspected inhibitory mechanism	References
Maize porridge "Ogi"	<i>Lactobacillus plantarum</i> , <i>L. acidophilus</i>	<i>Vibro cholera</i> , <i>Shigella flexneri</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Inhibition after 36 h	pH and titratable acidity	Lawal et al., (2009)
Tempeh	<i>L. plantarum</i>	<i>Listeria monocytogenes</i>		pH, acidity and inhibitory substances	Ashenafi, (1991)
Ghanaian maize dough	<i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Saccharomyces</i>	<i>S. typhimurium</i> , <i>E. coli</i>	Inhibition after 72 h	Anti-microbial compounds	Sanni et al., (2002)
Maize or high-tannin sorghum sour gruels	Natural lactic acid bacteria in the fermentation	Gram-negative <i>E. coli</i> , <i>Campylobacter jejuni</i> , <i>Sh. flexneri</i> and <i>S. typhimurium</i> Gram-positive <i>B. cereus</i>	Strongly inhibited after 7 h	Low pH, lactic, acetic acids and bacteriocin formation	Svanberg et al., (1992)
		<i>Staphylococcus</i>	Reduction in growth		

Continues

Table 1.3: *continued*

Substrate	Fermenting organism	Pathogens	Level and duration of inhibition	Suspected inhibitory mechanism	References
Mageu sour maize beverage	Natural LAB in the fermentation	<i>Bacillus cereus</i>	Inhibition after 6 h	Decrease in pH	Byaruhanga et al., (1999)
Togwa - cereal gruel	Lactic acid bacteria	<i>C. jejuni</i> and <i>E. coli</i> Cholera toxin	No viable cells after 8 and 24 h respectively No toxin activity	pH ≤4	Kingamkono et al., (1998)
Infant weaning gruel	Natural LAB in the fermentation (Accelerated)	<i>S. typhimurium</i> , <i>Shigella</i> spp., <i>E. coli</i>	Death	Reduced pH	Nout et al., (1989b)
Rice-based gruel	NS	<i>B. cereus</i> Enterotoxin production	Decrease in viable count Inhibited	Enterocin AS-48 from <i>Enterococcus faecalis</i>	Grande et al., (2006)
Millet flour gruel	<i>L. plantarum</i> strain 2.9	<i>B. cereus</i> , <i>S. enterica</i> and <i>E. coli</i> 0157:H7	Viability reduced below detection levels after 8, 24 and 48 h respectively	Bacteriocins	Valenzuela et al., (2008)

NS – Not specified

1.1.6.4 Impact on consumers health

Health benefits from the consumption of fermented cereal-based foods have not been widely studied like dairy and dairy products. However, according to Nout and Motarjemi, (1997) microbial fermentation leads to a decrease in the level of carbohydrates as well as some non-digestible oligosaccharides and polysaccharides. This later reduces side effects such as abdominal distension and flatulence. Some fermentation microorganisms particularly the LAB and few types of yeast that are naturally associated with cereal fermentation are species related to probiotics which have been shown to confer certain health benefits to humans. The health benefits derived by the host is attributed to the gut flora and some endogenous LAB associated with cereal fermentation have been reported to be acid and bile tolerant (Kalui et al., 2010) which could aid in their ability to survive in the gastrointestinal tract (GIT). Thus, they could help to enhance the gut bacterial population, estimated to be at a concentration of 5×10^{11} bacterial cells per gram (Holzapfel and Schillinger, 2002).

Consumption of fermented cereal-based foods in a study in Tanzania was shown to reduce the incidence of diarrhoea in young children who consumed the gruels regularly over a period of nine months, although the mechanism of diarrhoea-lowering was unclear (Lorri and Svanberg, 1994). In another study by Lei et al., (2006), the consumption of fresh *koko* sour water (KSW) a fermented liquid top-layer obtained from the fermentation of millet gruel called *koko* containing live LAB in levels of $8 \text{ Log}_{10} \text{ CFU mL}^{-1}$ with pH of 3.6 ± 0.2 was reported to have alleviated diarrhoea in children below the age of five in Northern Ghana. Further, fermented maize dough is traditionally used to treat shingles by smearing on the body. The pH, carbohydrate, and electrolyte levels

of fermented *kenkey* water were found to be within physiologically acceptable ranges for the treatment and prevention of dehydration in children with diarrhoea (Yartey et al., 1993). *Kenkey* water traditionally is fed to the anorexic malaria patient and recently a cereal based oral rehydration fluid has been formulated from fermented maize (Lei et al., 2006).

Lactic acid bacteria isolated from fermented cereal based gruel *ikii* in Kenya produced varying amounts of exopolysaccharides (EPSs) and some health benefits have been attributed to EPSs as reviewed by Kalui et al., (2010). Among the yeasts, one selected strain that may have application in the prevention and treatment of intestinal infection was reported to be *Sacch. burladii*, a yeast isolated from lychee fruit in Indochina that is thermo-tolerant and grows at the unusually high temperature of 37 °C (Czerucka and Rampal, 2002).

Investigations into the physiological benefits from the consumption of different cereal-based foods obtainable in different regions of Africa and the establishment of their use as vehicles for probiotic microorganisms and prebiotic food ingredients would avail consumers in the developing world such benefits.

1.1.7 Conclusion

The majority of traditional cereal based foods whether in the form of dough, porridges, beverages or stiff gels consumed in Africa are spontaneously fermented. Diverse multitudes of species of microorganisms depending on

several factors are implicated in the uncontrolled spontaneous fermentation. This does not leave the products without inherent hygienic, nutritional and sensory defects. Lactic acid bacteria and yeast species are the most important groups of the microorganism with LAB mostly predominant. The presence of this mixed culture contribute in conferring some essential quality and safety attributes of the product, though at some stage the presence of yeast suppresses LAB growth which is detrimental to safety. Inhibition of unwanted microorganisms at the early stage of the fermentation and the improvement of general characteristic of the product can be enhanced with the use of appropriate starter cultures. To develop starter cultures from wild type strains endogenous to specific food would require isolation, identification, selection and adequate characterisation of the choice microorganism(s).

1.2 PROBIOTIC LACTIC ACID BACTERIA

1.2.1 Introduction

Lactic acid bacteria (LAB) constitute a diverse group of organisms providing considerable benefits to humankind, some as natural inhabitants of the intestinal tract and others as fermentative lactic acid bacteria used in food industry, imparting flavour, texture and possessing preservative properties (Grajek et al., 2005). In the past century they have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. This has led to the coining of the term probiotics.

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002). By this definition, it implies that the organisms need to be alive and must have been shown in a controlled, human study to exert beneficial physiological effect on the consumer. Thus, cellular components of some microorganisms that have been reported to confer health effects cannot be considered as probiotics as they are only components of the organisms and not the live organisms themselves. Not all natural bacteria or cultures that also stay alive in fermented foods through to consumption are probiotics except they have been isolated, purified, proven not just from *in-vitro* studies but with *in-vivo* studies to have health benefits when administered and adequately documented in literature (Sanders et al., 2007).

Viability however is not necessarily a prerequisite for health benefits but a reasonable measure of probiotic activity, as studies have shown that viability is

not required for probiotic activities such as improved lactose digestion, some immune system modulation activities, anti-allergic and anti-hypertensive effects were therapeutic effects have been linked to non-viable cells, cell components, enzyme activities or fermentation products (Kankaanpää et al., 2003, Nikolic et al., 2012, Kondoh et al., 2009). From the better understanding of viability, many organisms may not be culturable using current methodologies but they still demonstrate viability in different tests. According to Salminen et al., (2010) some organisms may require a sequence of different stimuli to be able to propagate or they may act in a surrounding composing of specific other microbes only. However, consumption of a concentration of 6 Log_{10} viable cells g^{-1} in a product is to be considered functional (Sanders et al., 2007).

Examples of some key genera and species of bacteria used as human probiotics are shown in Table 1.4. The most frequently used bacteria in this group include *Lactobacillus* and *Bifidobacterium* species.

Table 1.4: Key genera and species of microbes studied and used as probiotics

Genus	Species	Genus	Species
<i>Lactobacillus</i>	<i>acidophilus</i>	<i>Bifidobacterium</i>	<i>adolescentis</i>
	<i>brevis</i>		<i>animalis</i>
	<i>delbrueckii</i>		<i>bifidum</i>
	<i>fermentum</i>		<i>breve</i>
	<i>gasseri</i>		<i>infantis</i>
	<i>johnsonii</i>	<i>longum</i>	
	<i>paracasei</i>	<i>Streptococcus</i>	<i>thermophilus</i>
	<i>plantarum</i>		<i>salivarius</i>
	<i>reuteri</i>		<i>faecium</i>
	<i>Escherichia</i>	<i>rhamnosus</i>	<i>Enterococcus</i>
<i>salvarius</i>		<i>clausii</i>	
	<i>coli</i>	<i>Bacillus</i>	<i>cerevisiae</i>
			<i>boulardii</i>

Adopted from Sanders et al., (2007) and modified.

Numerous commercial LAB related products either in the form of liquid (yoghurt) or solid (powder, granules or tablets) are available all over the world (Lin et al., 2006). Most probiotic foods at the markets worldwide are dairy-based and yoghurt is perhaps the most common probiotic-carrying food (Sanders et al., 2007). Dairy products are proven to be well suitable for the growth of probiotics and already have a wide positive image to promote the health of consumers (Helland et al., 2004).

The probiotic market has expanded beyond yoghurt to cheese, fermented and unfermented milk, juices, smoothies, nutrition bars, infant formulas and cereals as food vehicles for delivery of probiotics, only very few attempts however have been made in developing probiotic foods using other fermentation substrates such as cereals for human consumption. This would be of particular importance in the developing world where cereal and cereal products constitute major part of the main daily meals of both adults and children. For LAB endogenous in fermented cereals to be considered probiotic, they need to display certain potentials both from *in-vitro* and *in-vivo* tests. Some of the FAO/WHO, (2002) recommended probiotic characteristics that can be tested using *in-vitro* tests were reviewed in this study.

1.2.2 Characteristics of probiotics

A potentially successful probiotic strain is expected to have several desirable properties in order to be able to exert its beneficial effects to human and animals. Some of these characteristics have been given wide attention in literatures (Saarela et al., 2000, Grajek et al., 2005, Ouwehand et al., 1999,

Sanders et al., 2007). These attributes are in relation to safety, functional and technological properties.

From the safety aspect, probiotic bacteria strain, species and genus should be generally recognized as safe (GRAS) in relation to its origin and characteristics, they should be non-pathogenic or have any history of association with diseases, able to selectively stimulate beneficial bacteria and suppress harmful bacteria, non-toxic, do not conjugate bile salt and are not antibiotic resistant or carry genes that are resistance to antibiotics (Saarela et al., 2000, Sanders et al., 2007, Lin et al., 2006, Mattila-Sandholm et al., 1999). *Lactobacillus* spp. and *Bifidobacteria* spp. isolated from the GIT are generally considered as safe. With the exception of some strains of *Streptococcus* and *Enterococcus*, LAB are rarely pathogenic. They have been widely used for the production of many fermented foods since ancient times without negative effects on humans and are part of the intestinal micro flora (Grajek et al., 2005, Dalié et al., 2010). Although, one of the bio-safety concerns for probiotics is the verification that a potential probiotics does not carry transferable resistance genes, there had been high frequencies of resistance in *Lactobacillus* strains for some antibiotics with few occurrences of less resistant strains (Mathara et al., 2008, Delgado et al., 2005).

Functional properties are often established using *in-vitro* tests and include: viability and persistence of the bacterial in GIT ascertained from acid, bile and gastric juice tolerance and adhesion to the human epithelial surfaces; antagonistic activities against human pathogens through pH reduction and competitive exclusion capability by the production of organic acids and other

antimicrobial substances such as bacteriocins, hydrogen peroxide etc; stimulation of the immune response without inflammatory effects; anti-mutagenic and anti-carcinogenic effects; improvement of bioavailability of nutrients and production of vitamins and enzymes; bowel motility and cholesterol-lowering effects (Grajek et al., 2005, Saarela et al., 2000, Ouwehand et al., 1999, Lin et al., 2011). Safety and functional characteristics tend to overlap as functional requirements established *in-vitro* ought to confer such safety properties when reflected in controlled human studies. It has also been stressed that the results obtained with the *in-vitro* models are not sufficient and require confirmation in double blind, randomized, placebo-controlled human trials (Grajek et al., 2005, FAO/WHO, 2002).

Technological properties are very important in the manufacture and incorporation of the probiotics into food for human benefits. Potential probiotics should have fermentative ability and be of proven stability during processing, storage and shelf-life of the product and compatible with the product format without alteration to the desired sensory characteristics of the product (Grajek et al., 2005). Potential probiotic may not have to fulfil all of these criteria but need to meet the most relevant ones towards the targeted applications. Some of the criteria relevant to this research work were therefore reviewed.

1.2.2.1 pH and organic acid production

Organic acids mainly lactic and acetic acids are end-product of carbohydrate metabolisms by LAB. The production of organic acids results to decrease in pH necessary for preservation by the inhibition or prevention of growth of unwanted spoilage and pathogenic microorganisms. Strong organic acids like lactic acid

has the capacity of creating high extracellular proton concentration, while weak acid like acetic acid increases in their proportion of undissociation. At lower pH, undissociated acid increases and freely diffused through the plasma membrane into the neutrophile cytoplasm, where they dissociate producing protons and thus decreasing the cytoplasm pH. With the acidification of the cytoplasm, the organism shifts its energy production for growth and survival to maintenance of intracellular pH by expulsion of protons (Adams and Moss, 2000). Growth thereby is suppressed. At much lower pH with higher concentration of acid, the rate of diffusion of undissociated acid into the cells becomes greater than the cells ability to expel protons subsequently leading to death of the target organism (Dalié et al., 2010).

1.2.2.2 Acid and bile tolerance

Viability and survival of probiotics through the GIT are vital characteristics if they must exert therapeutic functions (Succi et al., 2005), and viability level of $7 \text{ Log}_{10} \text{ CFU mL}^{-1}$ of product at the time of consumption has been suggested (Ding and Shah, 2007). Although viability at the site of action is presumed to be important, some effects may be mediated by cell components. However, since the viability and activity of probiotics are needed at the lower digestive tract it is expected that they be resistant to acid and bile environment of the upper digestive tract (Ding and Shah, 2007). Acidity of the human GIT depending on the food substrate and the intervals of feeding vary from pH 1.5 to 4.5 (Lin et al., 2006). The LAB is usually in contact with the food substrate in which it is relayed into the gut. The food substance may have some buffering effect that can protect the LAB and thereby, significantly influence their survival in the GIT (Patel et al., 2004).

The ability of LAB to grow in the presence of bile has been reported as important in maintaining preparation and storage of concentrated cultures for use as dietary adjunct. Bile concentration has been assumed to be higher in the upper part of the small intestine (jejunum). Increase in the number of facultative *Lactobacillus* spp. in the upper digestive tract was reported to be caused by the presence of bile tolerant LAB such as *L.acidophilus* (Gilliland et al., 1984).

Acid and bile tolerance of strains of various probiotics including *L. plantarum* isolated from humans have been studied (Ding and Shah, 2007, Lin et al., 2007, Lin et al., 2006). A strain of *L. plantarum* isolated from fermented cereal-based food has been reported to be tolerant to bile and acid (Kalui et al., 2009). All bile depending on concentration are said to be inhibitory to most Gram-positive bacteria including the genera *Lactobacillus*. However, concentration of simulated human gastric system has to be tolerated to a certain extent for viability. Destruction of bacterial cell membrane by bile concentration affects cell permeability, viability and interactions between the membrane and the surrounding environment (Succi et al., 2005).

Although the production of bile salt hydrolase is a common bile resistance mechanism in bacteria (Fang et al., 2009) , microencapsulation has been observed to improve probiotic survival on acid and bile exposures (Ding and Shah, 2007). There have been studies on the effect of cereal and cereal extracts on the improvement of acid and bile tolerance of probiotic microorganisms (Charalampopoulos et al., 2002a, Michida et al., 2006). The viability and stability of *L. plantarum* in simulated gastric condition was reported to be significantly improved by addition of cereal extracts chiefly attributed to the

water-soluble sugar and free amino nitrogen content of the extracts and cell immobilization within the cereal (Michida et al., 2006).

1.2.2.3 Bacterial cell surface hydrophobicity

Bacterial cell surface hydrophobicity is one of the important factors that govern adhesion of bacteria to various surfaces (Zita and Hermansson, 1997, Liu et al., 2004). When two similarly charged bodies approach each other, they are subjected to both attractive and repulsive forces which are additive in effect (Jones et al., 1996). Cell-hydrophobicity represents an attractive force between cells and/or inert surfaces. Hydrophobic attractions have been reported to be principally due to the role of electron-donor/electron-acceptor interactions between two surfaces (Bellon-Fontaine et al., 1996).

Bacterial cell surface hydrophobicity has often been assessed by determining microbial adhesion to solvents or hydrocarbons (Vinderola and Reinheimer, 2003, Mathara et al., 2008, Schillinger et al., 2005, Del Re et al., 2000, Kos et al., 2003). This was based on the comparison between bacterial cell affinity to a monopolar and a polar solvent. The monopolar solvent could be acidic (electron acceptor) or basic (electron donor) but must have similar van der Waals properties (Bellon-Fontaine et al., 1996). Bacteria charges are attributed to their cell wall components (protein and polysaccharides). The amino (-NH₂) and carboxyl (-COOH) group of the regularly arranged protein in the cell walls of *Lactobacillus* spp. are implicated in the positive and negative charges of the cells respectively (Masuda and Kawata, 1985). Bacterial affinity with electron donor would be an indication of more electron acceptor groups on the bacterial cell wall and vice versa. For adequate comparison choices of both acid and

base pairs of solvents (for example, chloroform a strongly acidic solvent and ethyl acetate a strongly basic solvent) are preferred.

Hydrophobic nature of bacteria cell surfaces has been implicated in adhesion to host tissues. High cell surface hydrophobicity have been correlated to increased cell-to-cell adhesion (Del Re et al., 2000, Kos et al., 2003) and suggested to play significant role in interaction with organic mucin layer of the gut (Mathara et al., 2008) and adhesion to epithelial cells (Schillinger et al., 2005). The relationship between the surface hydrophobicity and adhesion ability of probiotic bacteria may give understanding into their ability of proffering certain health benefits as hydrophobicity offers that competitive advantage important for bacterial persistence in the gut (Vinderola and Reinheimer, 2003). Although, it may not obviously be a strong criteria for strong adherence capabilities as some *Lactobacillus* strains with extremely low hydrophobicity were reported to have adherence to epithelial cell models (Schillinger et al., 2005)

1.2.2.4 Aggregation capabilities of probiotics

Achievement of adequate mass through aggregation is very essential for the survival and persistence of probiotics in the GIT and thus a prerequisite for probiotics to manifest beneficial effect (Jankovic et al., 2003). Cell-cell adherence between bacterial cells of the same strain (auto-aggregation) and between cells of genetically divergent strains (co-aggregation) is of importance in several ecological niches, particularly in the human gut (Bao et al., 2010).

Auto-aggregation enables probiotic bacteria to reach adequate mass required for their functionality and it has been suggested to be necessary for adhesion to intestinal mucosal and epithelial cells, while co-aggregation form a barrier that

prevents colonization by pathogenic microorganisms and can inhibit pathogens by production of antimicrobial substances at very close proximity (Kos et al., 2003, Collado et al., 2007a, Collado et al., 2007b, Ouwehand et al., 1999, Tuo et al., 2013). Bacteria cells that aggregate have been suggested to be at advantage of adhesion and aggregation may have positive effects on enhancing LAB tolerance to the GIT system (Jankovic et al., 2003, Xie et al., 2012).

Aggregation phenomenon in many *Lactobacillus* strains are been investigated among others by spectrophotometric assays, in which bacteria aggregation are evaluated by the reduction of light absorbance in the bacterial suspended diluent using a spectrophotometer. Commonly used diluent include phosphate buffered saline (PBS) at different pH (pH 2 - 8) simulating both gastric and intestinal juice (Xie et al., 2012, Bao et al., 2010, Del Re et al., 2000, Zhang et al., 2011, Tuo et al., 2013). Wild *Lactobacillus* strains isolated from fermented foods had not shown high co-aggregation potentials with enteropathogens (Zhang et al., 2011) whereas in the study conducted by Mathara et al., (2008) *Lactobacillus* spp. isolated from fermented milk product and a commercial probiotic strain showed aggregation abilities greater the 50%. Different strains of *Bifidobacterium longum* isolated from human gastric juices had varying aggregation potentials from <20 to \geq 80% while strains from culture collections were unable to auto-aggregate (Del Re et al., 2000). Aggregation capabilities of bacteria were thus reported to be strain specific.

1.2.2.5 Adhesion to intestinal mucus layer and epithelial cells of the human GIT

The epithelial cells of the human digestive tract is overlaid with mucus; a biochemically complex layer containing glycoproteins, antimicrobial peptides, immunoglobulins, lipids and electrolytes (Juge, 2012). The main component being high molecular weight surface active glycoproteins called mucins (Jonsson et al., 2001, Svensson and Arnebrant, 2010). The gastrointestinal mucus is divided into two layers; an outer loose easily removable layer and an inner layer firmly attached to the epithelium. The outer mucus layer harbours glycan-rich domains that provide preferential binding sites and energy for bacterial proliferation while the inner mucus layer protects the underlying intestinal epithelia layer from intestinal contents such as corrosive acid and pepsin of gastric juice and guards the host against infections by pathogens (Uchida et al., 2006, Juge, 2012).

The outer mucus layer is continually being degraded and new components constantly secreted. For prolonged persistence that allows time for exertion of healthful benefits, bacterial adhesion to the mucus layer need to reach the epithelial cells to prevent quick dislodging and washing away by luminal content (Kirjavainen et al., 1998). Adhesion to the epithelial layer thus, ensures persistence of the microorganisms and their colonization of the GIT by preventing immediate elimination by peristalsis (Ouwehand et al., 1999, Kos et al., 2003). Bacterial cells adhered to epithelial cells may be in close contact with immune cells and as such play important role in immunomodulation (Lin et al., 2011).

Attachment of probiotic bacteria to the intestinal mucosal surfaces and epithelial cells and cell lines are among the main selection criteria for probiotic microorganisms (FAO/WHO, 2002). Effective binding has been correlated to some binding receptors such as sugar chains of glycolipids or glycoproteins on the surface of the intestinal mucus (Roos and Jonsson, 2002), specific proteins, peptides, proteinaceous components and carbohydrates on bacterial cell surface (Kos et al., 2003, Girard and Mourez, 2006, Lin et al., 2011) and the ability to recognize and bind to blood group antigens on the intestinal mucus (Uchida et al., 2006, Juge, 2012). Most prominent adhesins include mucus-binding proteins (Mub) of *L. reuteri* 1063 and mannose-specific adhesins of *L. plantarum* Lp6 (Sun et al., 2007). Reports in literature revealed that adhesion of *Lactobacilli* to intestinal mucosal surfaces and epithelia cells are strain specific with adhesive strains having host-residential characteristics (Wang et al., 2008). The production of cell surface proteins with mucus binding capabilities were correlated to bacterial growth media when many strains of *L. reuteri* not of intestinal origin appeared to bind to mucus material in-vitro only when grown in mucin environment (Jonsson et al., 2001).

The assessment of adhesion properties of probiotic bacteria have been achieved through *in-vitro* adherence to mucus and epithelia layers using mucin extracts from human and animals, Caco-2 and HT-29 cells and human intestinal Int-407 (ATCC CCL 6) cells as intestinal models (Baccigalupi et al., 2005, Lin et al., 2011, Wang et al., 2008, Kalui et al., 2009, Monteagudo-Mera et al., 2012). The *in-vitro* epithelial cultures have the morphology and physiology of human enterocyte cells. Caco-2 cells were isolated from a neoplastic tumor of the human large intestine and well developed Caco-2 monolayer is similar to the

small intestine enterocyte-like cell. While HT-29 obtained from the human colon carcinoma cell presents the large intestine and colon goblet-like cells (Grajek et al., 2005)

Just as microbial adhesion to intestinal mucosa and epithelial cells is an important probiotic characteristic; their degradation is considered a potential virulence factor. Microorganisms with extensive mucin-degrading activity would have the potential to invade the host and also facilitate mucosal penetration by potential pathogens. Theoretically, mucinolysis can be defined by changes in the carbohydrate and/or protein content of ethanol-precipitated mucin portions (Zhou et al., 2001). In order to assess potential pathogenicity and local toxicity of some probiotic LAB, Zhou et al., (2001) and Fernández et al., (2005) studied the ability of some *Lactobacillus* spp to degrade mucin and reported the inability of the LAB to utilize the large complex mucin glycoprotein substrate, considering them to be non-toxic and devoid of invasive pathogenicity in the host GIT mucosal defence system.

1.2.2.6 Gelatinase and haemolytic activities.

The ability of probiotics be non-virulent is an important safety criterion. The *in-vitro* ability of bacteria to hydrolyse gelatine into peptide or amino acid units would imply their ability to derange the protective mucus lining of the GIT, while the ability to breakdown red blood cells would cause cessation of the underlying epithelial layer. This would create pathways for infections.

Some studies have demonstrated the absence of gelatinase and haemolytic activity among some probiotic bacteria. Kalui et al., (2009) reported non-

gelatinase and haemolytic activity of *L. plantarum* and *L. rhamnosus* strains isolated from Kenya fermented porridge. In a similar study by Maragkoudakis et al., (2006b) and Ruiz-Moyano et al., (2009) isolates of *Lactobacilli* from dairy products and human/pig faeces respectively were non-haemolytic (γ -haemolysis) except for strains of *L. acidophilus* ACA-DC 295, *L. paracasei* subsp. *paracasei* ACA-DC 126, *L. rhamnosus* ACA-DC 112 and *Lactobacillus* sp. ACA-DC 108 and four different strains of *L. casei* that exhibited partial hydrolysis (α -haemolysis). Alpha haemolysis however, when observed against β -haemolysis is usually interpreted as negative (Maragkoudakis et al., 2006b). According to the report by Salminen et al., (1998) most strains of these LAB reported to have exhibited α -haemolysis have been linked to some clinical cases.

1.2.3 Conclusion

The review had shown that potential probiotics are expected to possess certain safety, functional and technological characteristics. *Lactobacillus* species are generally used in many fermented foods and have been considered as generally safe (GRAS). Viability and persistence through the GIT achieved through tolerance to acidity and gastric components, aggregation, antagonism against pathogens, adhesion to the mucosal and epithelial surfaces are important during the colonization of the gut for a beneficial effect, and for the safety of the food. The potential of probiotic bacteria to ferment a product with the production of adequate metabolic products and remain stable during processing and preservation of the food without any impairment to the products' desired eating qualities are crucial technological properties. It is therefore important to initially ascertain that any isolated potential probiotic possesses

these characteristics using basic *in-vitro* tests. Findings from the in-vitro studies could serve as a baseline data for further studies on the probiotic abilities of such LAB isolates especially if they were not of intestinal origin.

1.3 CEREAL AS ALTERNATIVE FOR FUNCTIONAL FOOD PRODUCTION

1.3.1 Introduction

The food industry is directing new product development towards the area of functional foods and functional food ingredients (Charalampopoulos et al., 2002b). This is due to the tremendous increase in public concern over the quality of life attributed to functional derivatives from what is been consumed and health issues, increasing cost of healthcare and the preference for natural preservation (Charalampopoulos et al., 2002a, Michida et al., 2006).

The concept of functional foods include food or food ingredients that, in addition to basic nutritional functions, exert a beneficial effect on host health or reduce the risk of chronic disease (Huggett and Schliter, 1996). In addition to the normal provision of energy, body building and maintenance, functional foods must demonstrate conferment of health benefits in the amount that is normally consumed in the diet and not to be prescribed as drugs (Carrillo et al., 2013, Grajek et al., 2005). Some of the associated health benefits include modulation of the immune system, alleviation of allergies and lactose intolerance, alleviation of diarrhoea, prevention of initiation, promotion and development of cancer, cardiovascular diseases and osteoporosis, synthesis and enhancement of nutrient bioavailability (Grajek et al., 2005, Saulnier et al., 2009).

The gastrointestinal track is the target for functional foods as it is the interface between diet and metabolic activities (Pang et al., 2012). When properly nourished, the gut can help in maintenance of better health and the influx of probiotic dairy products containing human-derived *Lactobacillus* and

Bifidobacterium species into the market is geared toward healthy gut microbial composition and activities. Prebiotics which are food ingredients that cannot be digested by the human host in the upper gastrointestinal tract and can selectively stimulate the growth of one or a limited number of colonic bacteria (Charalampopoulos et al., 2002b) are as well gaining attention.

This section of the review evaluated the potentials of cereal grain as alternatives to functional food production considering some functional component of cereals and the ability of cereals to sustain growth of probiotic bacteria.

1.3.2 Health implication of some cereal grain components

Over the years, benefits associated with wholesomeness, high fibre content, superior taste, improved satiety and increased energy levels had governed the consumption of foods containing the entire edible portion of grains or 51% of wholegrain ingredient (Kuznesof et al., 2012). Different cereal fractions have been reported to contain many bioactive compounds that act in synergy to influence human metabolism and orchestrates certain health benefits. They include dietary fibre (DF), resistant starch (RS), oligosaccharides, lignans, essential fatty acids, phenolic antioxidant compounds, phytates, vitamins (tocopherols and trienols) and minerals (magnesium and chromium) (Guo and Beta, 2013, Ryan et al., 2011, Dutcosky et al., 2006, Topping, 2007, Slavin, 2003, Gamel and Abdel-Aal, 2012).

The antioxidant components of cereals include phytate, phenolic acids, carotenoids alkylresorcinols, tocopherols and trienols (Poutanen, 2012, Žilić et al., 2011). The phenolic compounds in cereals exist in free soluble conjugate and bound forms. The bound phenolic acid are ester-linked to cell-wall polymers in the outer layer of kernels (Gamel and Abdel-Aal, 2012). They represent the major proportion of phenolic acid in cereals and constitute the main antioxidant in cereals with the greatest potential of scavenging free radicals, inhibiting lipid peroxidation and exhibiting anti-cancer activities (Ragaei et al., 2011, Guo and Beta, 2013, Žilić et al., 2011). Although phenolic compounds bound to mineral elements reduces the bioavailability of the minerals, Đorđević et al., (2010) reported that fermentation of cereals (buckwheat, wheat germ, barley and rye) with *L. rhamnosus*, *Sacch. cerevisiae* enhanced the levels of antioxidant activity and the total phenolic content of the cereals which offers a tool to further increase the bioactive potential of cereal products.

The majority of the carbohydrates and dietary fibre intakes in many countries are obtained from cereal-based foods (Poutanen, 2012). A simple definition of dietary fibre clarifies its constituent make-up and the primary characteristics of been resistant to digestion and absorption in the small intestine (DeVries, 2003). The most dynamic function of dietary fibre has been related to its fermentation, which causes a myriad of biochemical, physiological and microbiological changes in the large intestine (Dutcosky et al., 2006). The short chain fatty acids produced during the fermentation were associated with lowered serum cholesterol and decreased risk of cancer. Undigested carbohydrates increase faecal wet and dry weight and increase intestinal transit (Slavin, 2003). The consumption of whole grain meals and DF may regulate body weight through

the inhibition of food intake by increasing satiety. (Kristensen et al., 2010, Rasoamanana et al., 2013). Other beneficial physiological effects of dietary fibre include lowering plasma lipid (blood cholesterol) concentrations, improvement of glycemic control and decreased hyperinsulinemia (Brown et al., 1999, Chandalia et al., 2000).

There is the abundance of starch in the endosperm of cereal grains. The starch components are classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) or resistant starch (RS). The RDS fraction causes a rapid increase of plasma glucose and insulin levels after ingestion and is associated with high glycemic response. The processing of cereal however results in reduction of RDS with reasonable high amounts of SDS and RS. The SDS is digested slowly in the small intestine and yields moderate glycemic and insulin responses while the RS represents part of starch escaping digestion and not absorbed in the small intestine of healthy humans (Ragaei et al., 2006, Ragaei et al., 2011). The RS are fermented in the large intestine which leads to the production of short chain fatty acids that has been associated with improved cardio-vascular health (Rahman et al., 2007). The different types of RS and their use as functional ingredient including their protective and physiological effects were expatiated by (Fuentes-Zaragoza et al., 2010).

Although, the eating of wholegrain foods has been associated with some health benefits such as reduced risk of cardiovascular diseases, obesity and diabetes (Björck et al., 2012, Weickert et al., 2006), certain barriers to the consumption of wholegrain food such as family taste preference, cooking skill, price and availability of whole grain (Kuznesof et al., 2012) had called for recent

researches into functional cereal foods as an alternative to the consumption of whole grain products. Functional cereal foods are considered as functional foods that are grain based: products from grains such as wheat, maize, rice, oats, barley etc. that are similar to the conventional food but have been modified to provide health benefits over and above basic nutrition (Dean et al., 2007). The components of cereal grains that have gained acceptability in the production of functional cereal foods include: insoluble ingredients such as cereal bran and some cell wall components like cellulose, some hemicellulose and lignin; soluble fibre such as inulin, oligofructose, β -glucans, some hemicellulose and gum acacia (Dutcosky et al., 2006, Dhingra et al., 2012).

There have been extensive studies on the incorporation of cereal components either as wholegrain flours or dietary fibre in wheat and barley products (Kristensen et al., 2010, Ragaei et al., 2011, Robin et al., 2012), it would be important to extend researches into other cereals and cereal product from maize, sorghum and millet that constitute most of the staples in the developing world.

1.3.3 Suitability of cereal as growth support for probiotics

In recent years, cereals have also been investigated regarding their potential use in developing functional foods. Cereals are grown in over 73% of the total world harvested area and contribute over 60% of the world food production (Charalampopoulos et al., 2002b). Cereals contain essential nutrients for fermentation, fibre, carbohydrates, proteins, vitamins, lipids and minerals. These different components are not distributed uniformly but are found in

specific fractions of the grain (Kedia et al., 2008). Fibre is found in the bran fraction while the endosperm mostly contains starch, which is the largest component of the kernel (82% dry basis), thus, starch is the main carbohydrate in cereals.

Amylolytic lactic acid bacteria (ALAB) with their ability to produce α -amylases which are able to hydrolyse raw starch granules could play a major role in cereal fermentation by increasing the availability of easily fermented carbohydrates through partial starch hydrolysis (Sanoja et al., 2000). In a mixed substrate combination of starch with either sucrose or fructose or with both fructose and glucose, *L. fermentum* Ogi E1, displayed the ability to simultaneously use carbohydrate (starch, glucose, fructose and sucrose), produce α -amylase and acetic acid (Calderon et al., 2003). Although, Nguyen et al., (2007) reported that amylolytic strain of *L. plantarum* A6 was incapable of efficient starch fermentation in raw rice/soybean mixture.

Acidification is one of the factors that affect the growth of lactic acid bacteria in natural fermentation. Studies have however, revealed that some probiotic lactic acid bacteria like *L. fermentum* Ogi E1 were particularly acid tolerant and well adapted to the acid conditions that developed during the natural fermentation of cereal dough (Santoyo et al., 2003). Regardless of the acidification during cereal fermentation, cereals have been proven to be suitable substrate for the growth of potential probiotics. Charalampopoulos et al., (2002a) on investigating the overall growth kinetics of four potentially probiotic strains (*L. fermentum*, *L. reuteri*, *L. acidophilus* and *L. plantarum*) cultured in malt, barley and wheat media with pH between 3.40 and 3.77 reported maximum cell population of up

to $10.11 \text{ Log}_{10} \text{ CFU mL}^{-1}$ of all strains. This was attributable to the chemical composition (total fermentable sugars and free amino nitrogen) present in the media particularly the malt media. Malt was substituted with pearl millet-groundnut (MG) slurry in the fermentation of infant gruel in another study by Songré-Ouattara et al., (2009), the substitute supported the growth of *L. plantarum* 6.1 and A6 with MRS count ≥ 8.9 but $\leq 9.1 \text{ Log}_{10} \text{ CFU g}^{-1}$ after 24 h. The presence of some strains of probiotic LAB has been established in fermented cereal foods. Amylolytic strains of probiotic *L. fermentum* were associated with the fermentation of Benin *ogi* and mawe by Agati et al., (1998) and Nigeria *kunu-zakki* by Sanni et al., (2002). Johansson et al., (1995) and Sanni et al., (2002) also isolated amylolytic strains of *L. plantarum* from Nigerian *ogi*.

Microorganisms associated with the fermentation of cereals possess the characteristic properties that are associated with probiotics (Vizoso-Pinto et al., 2006, Olasupo et al., 1999). Although, acid tolerance ability of well documented probiotic species in dairy product and even non-dairy *L. plantarum* has been reported (Mercade et al., 2000). There are indications that LAB isolated from spontaneously fermented cereal based foods were able to withstand the physiological challenges of the GIT and have the potential of surviving and colonizing the GIT. According to Lei and Jakobsen, (2004) and Kalui et al., (2009) LAB isolated from spontaneously fermented cereal based porridges *koko* and *ikii* respectively were tolerant to acid and bile. However, more investigation was required in the area of simulated stomach duodenum passage, hydrophobic characteristic and adhesion to human cell linings.

A strain of *Saccharomyces cerevisiae* var *boulardii* isolated from African indigenous fermented foods has been shown to have promising probiotic potential as the strains were able to tolerate low pH and bile and a few were observed to exhibit pronounced adhesive properties (van der Aa Kühle et al., 2005). *Saccharomyce boursinii* is another strain of yeast that have been considered to have application in the prevention and treatment of intestinal infection (Czerucka and Rampal, 2002). *Saccharomyces boursinii* was considered as a strain of *Sacch. cerevisiae* and *Sacch. cerevisiae* has been reported as one of the predominant yeasts in fermented cereal foods (van der A Kühle et al., 2001).

The high viable counts of diverse strains of LAB in cereals suggest that the incorporation of human-derived probiotic strains in cereal substrate under controlled condition could produce a fermented food with defined and consistent qualities and possible health promoting properties in addition with the prebiotic component of the cereals. Oats amongst other cereals have attracted considerable interest as delivery vehicles for probiotics due to their high content of soluble and insoluble fibres resulting in positive effects on blood cholesterol levels (Angelov et al., 2006). The indigestible components of oat fraction have been reported to support highest growth of *Lactobacillus* strains (Kedia et al., 2008). There is a need to explore other cereals such as maize for the same purpose.

1.3.4 Conclusion

Several studies have presented evidence that cereals form good substrate for the growth of probiotics, some of which are associated with cereal fermentation.

Most natural components of cereal grains display both functional and physiological properties and have been used in the production of health enhancing food. However, some of the important cereal components located in the bran and aleurone cells are often removed during preparation but adequate production techniques with the incorporation of the key physiological ingredients into the cereal products during processing could re-value cereals as an alternative for the production of functional foods.

CHAPTER TWO

RATIONALE, AIMS AND OBJECTIVES OF THE STUDY

2.1 Theoretical background

All over the world, cereals are important staples and the cereals of major importance in Africa are maize (*Zea mays*), sorghum (*Sorghum vulgare*), rice (*Oryza sativa*), and several minor grains such as the millets, especially pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*) (Nout, 2009). Cereals provide important macro and micro nutrients required for human growth and maintenance, and fermentation which is a key production technique for the preparation of various fermented cereal-based foods consumed worldwide has been implicated in the improvement of product quality and safety. The food industries, due to the tremendous increase in public concern over health issues, are however, focusing attention on foods that in addition to the basic nutritional function can exert certain beneficial effects on host health or reduce the risk of physiological disorders (Charalampopoulos et al., 2002b, Michida et al., 2006, Huggett and Schliter, 1996). This has led to the concept of functional foods.

Functional foods are foods with components that have been proven to beneficially affect one or more target physiological functions in the body. These components could be microbial, as in the case of probiotics used in the production of the food, or naturally occurring functional ingredients such as vitamins, minerals, fibre and omega-3 fish oil (Krutulyte et al., 2011). Selected strains of lactic acid bacteria affect health by diverse mechanisms including the colonization of the GIT thereby maintaining gut microbial balance, stimulation of

the immune response, and cholesterol-reducing effects. Cereals have been evaluated to contain the amount of glucose sufficient for microbial metabolism (Marklinder and Lonner, 1994) and a major provider of carbohydrates and dietary fibre in many countries (Poutanen, 2012). Soluble and insoluble cereal carbohydrate components have been implicated in some health enhancing benefits such as lowered risk of CVD through reduction of plasma cholesterol, optimum digesta transit, increased satiety, weight loss and promotion of large bowel function (Björck et al., 2012, Weickert et al., 2006). Cereal grain oligosaccharides also function as prebiotics and increase the level of beneficial bacteria in the large intestine (Topping, 2007). Hence, cereal is seen as offering another alternative to dairy for the production of functional foods.

Akamu and similar other products, such as *uji*, *injera*, *koko*, and *mahewu* in many African countries, are made from cereals such as maize, sorghum and millet. *Akamu* is a popular maize fermented food in Nigeria and the key production process involves steeping, wet milling, wet sieving and fermentation. Its' porridges, which has a smooth texture from the sieving process and sour flavour from the lactic fermentation, are consumed by all ages as complementary food for infants and children, adult main meals, food for the convalescents and when cooked to a stiff gel is a convenient food for travellers (Teniola and Odunfa, 2001, Osungbaro, 1990, Umoh and Fields, 1981).

2.2 Statement of the research problem

Traditionally, *akamu* fermentation has been spontaneous. The microflora involved is complex and the uncontrolled fermentation makes *akamu* a very variable commodity in terms of sensory, nutritional and safety properties.

According to the review by Motarjemi et al., (1993), studies had revealed that African traditional weaning foods are frequently been heavily contaminated with pathogens and thus constitute a major cause of diarrhoea and associated malnutrition in infants and children <5 years. Esrey and Feachem, (1989) reported that 15 - 70% of diarrhoea episode among infants and children <5 years were foodborne and the transmission of the diarrhoea agents were related to specific deficiencies in food hygiene including handling, preparation and storage. The presence of *Escherichia coli* was detected in 80 out of 222 weaning food samples obtained from households in Mashonaland East province of Harare, 15% of which were pathogenic *E. coli* with Enteropathogenic *E. coli* (EPEC) strains as the commonest (Nyatoti et al., 1997). Motarjemi et al., (1993) associated 25% of diarrhoea episode among infants and children with *E. coli*.

Although, many studies have utilized an accelerated fermentation (back slopping) method to demonstrate the beneficial effect of fermentation on quality and safety of cereal-based gruels (Bakare et al., 1998, Lawal et al., 2009, Mensah et al., 1990, Odugbemi et al., 1991, Ohenhen and Ikenebomeh, 2007, Osundahunsi and Aworh, 2003, Otunola et al., 2006, Teniola and Odunfa, 2002), antimicrobial studies with this method have not been able to distinguish clearly between the endogenous microflora present in the raw material or fermentation vessels and those that are been introduced during the study.

Recent trends with the use of pure starter cultures had involved the identification, purification and storage of the main fermentative microorganisms from different fermented products in a form that can be easily used as known microbial cultures in controlled fermentation of such or similar products. *Akamu*

and similar products could be; (1) fermented with selected lactic acid bacteria to produce a safer product that may not necessarily have any other effect on the health of the consumers and (2) used as the vehicle for the introduction of LAB with probiotic characteristics that would be beneficial to the consumer.

2.2 Aims and objectives of the study

2.2.1 General aims of the study

1. To determine whether the safety of *akamu* can be improved by fermentation with selected lactic acid bacteria inoculants.
2. To investigate the potential of probiotics to improve the functional quality of *akamu*.

2.2.2 Specific objectives

The specific objectives and the research approach of this study were as follows:

Stage 1:

1. Characterisation of the lactic acid bacteria population of traditional *akamu* samples using molecular microbiological techniques
2. Isolation and identification of LAB and yeast to be used as microbial inoculant in controlled fermentation of *akamu* using both traditional and molecular microbiological techniques

Stage 2:

3. Identification of one or more LAB and yeast inoculants that will ferment *akamu* and assure its' safety based on predetermined criteria over appropriate time/temperature regimes using routine microbiological

techniques and chemical analysis (high performance liquid chromatography)

4. Assessment of the fermentation abilities of the selected LAB and yeast inoculant in single and mixed culture fermentation of maize, using traditional microbiological and chemical techniques

Stage 3:

5. Investigate if selected LAB can improve the pathogen exclusion properties in *akamu* using traditional microbiological techniques.
6. Investigate the probiotic potential of selected LAB based on some safety, functional and technological characteristics using aggregation, adhesion and other microbiological assays

Stage 4:

7. Assess the effect of variations in the method of production on the products' proximate, mineral, dietary fibre composition, pasting and sensory properties using physical, chemical and sensory tests

2.3 Scope of the study

After a review of literature on cereal fermentation and probiotic potential of LAB in Chapter 1, the schematic summary of the experiments carried out in the 4 key stages were shown in Figure 2.1. Stages 1 and 2 were covered in chapter 3, while stages 3 and 4 were presented in chapter 4 and 5 respectively. Chapter 6 summarises the findings highlighting the strengths and limitations of this research.

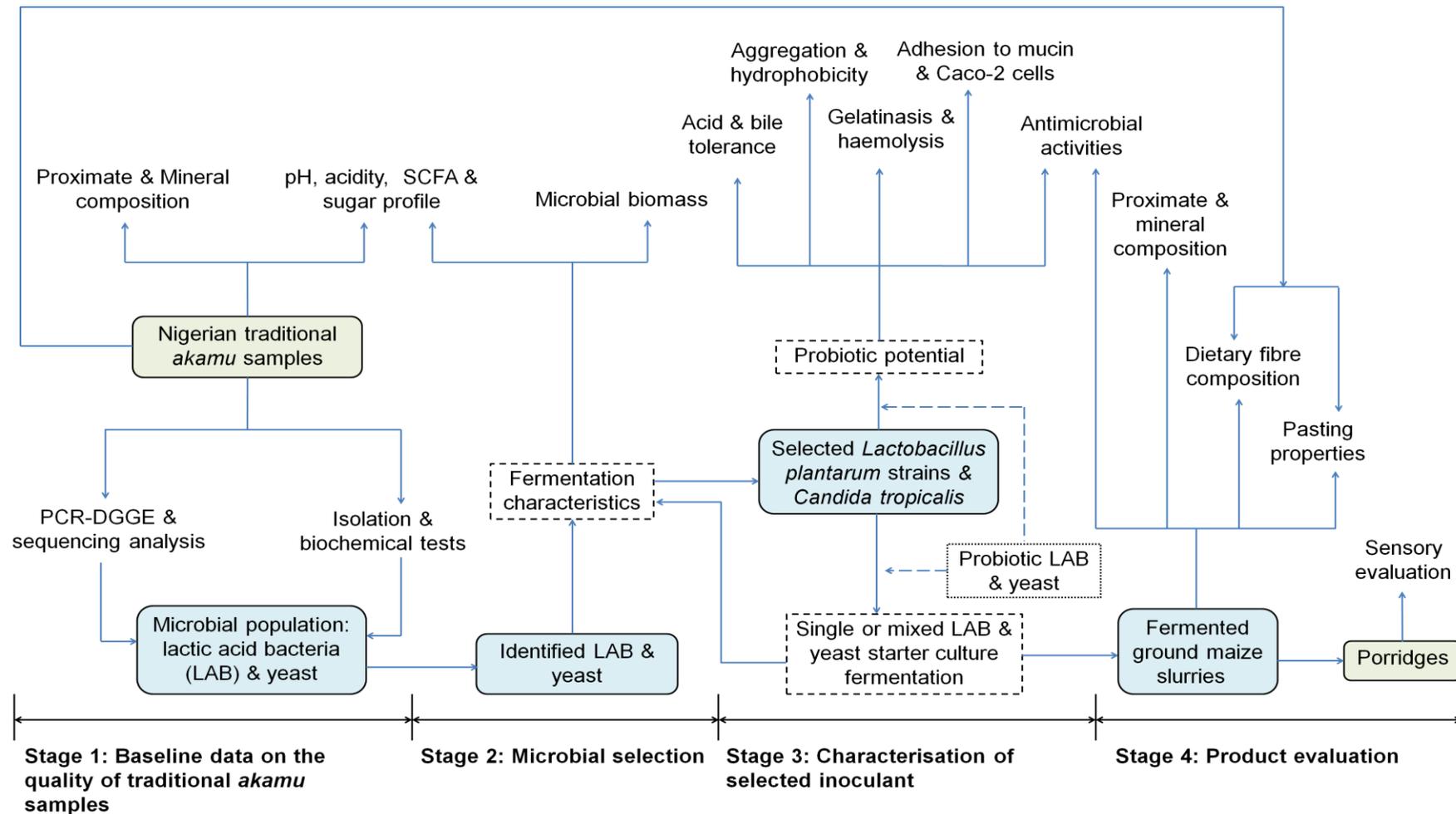


Figure 2.1: A schematic representation of the summary of the experiments carried out
 SCFA - Short chain fatty acids
 PCR-DGGE - Polymerase chain reaction-denaturing gradient gel electrophoresis

CHAPTER THREE

IDENTIFICATION AND FERMENTATION CHARACTERISTICS OF NIGERIAN FERMENTED MAIZE (AKAMU) LACTIC ACID BACTERIA AND YEAST

3.1 Introduction

Akamu is a traditional lactic acid fermented starchy meal, made from cereals such as maize (*Zea mays*), sorghum or millet (Akingbala et al., 1981, Teniola and Odunfa, 2001, Sanni et al., 2002). The porridge forms an integral part of adult main meals in most African countries and plays important role in the nutrition of infants and young children as a complementary food. The fermenting microbial population are mainly lactic acid bacteria: *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Weissella* species and yeasts: *Candida*, *Saccharomyces*, *Geotrichum*, *Kluyveromyces* and *Pichia* species (Nago et al., 1998a, Teniola et al., 2005, Bvochora et al., 1999, Fields et al., 1981, Mugula et al., 2003a, Vieira-Dalodé et al., 2008, Muyanja et al., 2003, Lei and Jakobsen, 2004, Kalui et al., 2009, Kebede, 2007, Oyeyiola, 1990, Wakil et al., 2008, Yousif et al., 2010).

Identification of these microorganisms in the past decades relied on culture-dependent techniques that utilised the phenotypic properties of the microorganisms. Conventional microbiological techniques may be simple to perform but had been reported to be lacking in discriminatory potency and reproducibility at species level. Also, selected microbial growth conditions may influence the cell morphological characteristics and the selection of only small fraction and organisms of interests do not give the true representation of the

complex ecosystem (Ehrmann and Vogel, 2005, Stiles and Holzapfel, 1997, Ampe et al., 1999).

Rapid, sensitive and reliable molecular methods based on direct analysis of DNA in the material require no cell culture and enable detection of individual species as well as the overall profiling of structural changes in microbial community over time. These culture-independent approaches amongst others include polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and PCR-temporal temperature gradient gel electrophoreses (PCR-TTGE) (Muyzer, 1999, Muyzer et al., 1993). The PCR-DGGE analysis involves the extraction of nucleic acids (DNA or RNA), amplification of the genes encoding specific region of rRNA or rDNA using polymerase chain reaction (PCR) and separation of the PCR amplicons in a denaturing gradient gel based on the differential denaturation profile of the different fragments (Ercolini, 2004, Muyzer, 1999). However, selection of nucleic acid and amplification of a particular region of the rDNA aimed at identification of specific bacterial community may sometimes present a limitation to the broad knowledge of the entire population.

For explicit and reliable species identification, the use of a polyphasic approach involving both phenotypic and genotypic methods may be preferred, Wakil et al., (2008) used genotypic methods to demonstrate a major shift in microbial community structure of fermenting cereal-legume weaning food. Several other studies utilizing both phenotypic and genotypic approaches have been able to establish microbial diversity in fermented foods including cereal and legume (Pepe et al., 2004, Ampe et al., 1999, Omar and Ampe, 2000, Mukisa et al.,

2012). Sanni et al., (2002) investigated the microbial ecology of fermenting maize dough fortified with 20% cowpea and reported the predominance of LAB species (63%) of which, 42% were species of *L. plantarum*. Yeasts of the genera: *Saccharomyces*, *Debaryomyces* and *Candida* have also been identified.

Spontaneous fermentation of cereal-based foods are borne out of competitive activities of endogenous or contaminating microorganisms and may take 48 - 96 h for the right microbes to dominate and produce the satisfactory product qualities. The results are products of variable attributes in terms of quality and safety. Although the growth of endogenous cultures of the substrate or those from the ingredients and equipment used in production may be inevitable even with the introduction of starter cultures of the desired microorganism, it is therefore important that selected microbial inocula, to facilitate the process should be adaptive to the substrate from the onset of fermentation. They should possess strong abilities to utilise the components of the substrate with the production of appropriate metabolic end products necessary for the required product stability, safety and without alteration to the appreciated sensory qualities (Holzapfel, 2002).

Lactic acid bacteria in cereal fermentation convert simple sugars to organic acid particularly lactic acid creating the acid environment known to improve product stability and safety. Although yeast have coexisted with LAB in many different types of indigenous fermented foods and beverages, yeast do not produce appreciable amount of organic acids rather their main function had been related to the production of carbon dioxide, alcohol and aroma compound that impact significantly on the food quality parameter such as taste, texture, odour and

nutritive value (Aidoo et al., 2006, Annan et al., 2003, Campbell-Platt, 1987, Sanni and Lonner, 1993). Omemu et al., (2007) reported that the yeast associated with maize fermentation for *ogi* production produced several extracellular enzymes and the amylolytic activities of some of the yeast were implicated in the breaking down of maize starch to simple sugars for other fermenting microorganisms.

This study focused on: (A) Determination of the lactic acid bacteria population in traditional *akamu* samples obtained from Rivers State in Nigeria using culture based microbial technique and by evaluating their DGGE banding patterns and identifying the bacteria by sequencing excised DGGE bands. (B) Confirmation of the identity of conventionally isolated LAB and yeast using direct PCR and sequence analysis. (C) Evaluation of the fermentation characteristics of the identified LAB and yeast isolates based on their growth rate, decrease in pH and increase in titratable acidity and the rate of organic acid production as a basis for the selection of LAB and yeast suitable for the controlled fermentation of ground maize slurries.

3.2 Materials and Methods

3.2.1 Sample and Sample preparation

3.2.1.1 Akamu

Samples used in this study were traditional *akamu* samples obtained from 5 different locations in Rivers State, Nigeria: Mile 3 Diobu (M1, M2 and M3); Emohua (E1, E2 and E3), Rumuokoro (R1 and R2), Aluu (A1) and Worgi (W1). The freshly prepared *akamu* samples (24 h) at the point of sales were sealed in

well labelled polyethylene bags and transferred into plastic containers that were packaged in a cardboard box and posted to the Food and Nutrition unit of Plymouth University, United Kingdom via Worldwide Express Mail Services (EMS).

The samples were received after 3 days and temporarily refrigerated at 4°C overnight. Thereafter, the samples required for microbiological and molecular analysis were taken and the rest of the samples were stored frozen at -80°C until required again for analysis. The samples as ascertained from the salers were all *akamu* prepared following the traditional technique shown in Figure 1.1. The *akamu* samples were made from yellow variety of maize except for sample E1 and W1 which were of the white variety. Although *akamu* is also sold in the market places, Figure 3.1A shows the example of *akamu* at the point of sales by hawker of the product. Some examples of the imported *akamu* samples were shown in Figure 3.1B.

3.2.1.2 Maize flour and fermentation slurry

Organic maize flour (L1530) used for fermentation was obtained from Health Food Shop, Rickard Lanes', Plymouth City Centre in UK. About 50 ± 0.01 g of the flour was weighed into cellophane bags, sealed and irradiated with ^{60}Co at 25.88 ± 0.79 kGy (Becton Dickinson and Company, Plymouth, UK). The slurry for fermentation was thereafter prepared by adding 100 mL of sterile distilled water containing the microbial inocula



Figure 3.1: Examples of Nigerian spontaneously fermented *akamu* samples
(a) Freshly prepared *akamu* at the point of sales by a hawker of the product (b) Some of the imported *akamu* samples

3.2.2 Microbial media and media preparations

The microbial media used in the cultivation, isolation and enumeration of the microorganisms were obtained from Oxoid Ltd (Basingstoke, Hampshire, UK). Generally, the microbial media and dilutents were prepared following the manufacturers instruction by dissolving a known weight of the media powder or tablet as the case may be in the required quantity of distilled water in a beaker. Media that required supplements had the adequate amount of the prepared supplements that would yield the required final concentration added to the media base before or after autoclaving depending on the type of media. The specific weight of media, amount of distilled water used and the respective supplements for the media that required them were shown in Appendix A3.1

3.2.2.1 The preparation of agar plates

The media was dissolved by boiling for 4 min in a microwave and then transferred into 1 L autoclave bottle (Sigma-Aldrich, Gillingham, Dorset, UK) and autoclaved at 121°C for 15 min. The sterile media in the bottles were allowed to cool in a Stuart water bath (Bibby Scientific Ltd, Staffordshire, UK) at 45°C for 30 min and then manually dispensed under the bunsen burner into sterile petri dishes (about 12 - 15 mL per dish). The agar plates were allowed to set at room temperature before storing away in a cold room at 4°C until needed for use.

3.2.2.2 Broth preparation

Broth media were dissolved in warm (60°C) distilled water by stirring on a magnetic stirrer for 2 - 4 min, then dispensed in required amounts into sizeable conical flasks or universal bottles (UB) with or without Durham tubes depending

the test they were required for (overnight growth, serial dilutions or test for fermentation of sugars as the case may be). The flasks were loosely covered with foil papers and the bottles with their caps followed by autoclaving at 121°C for 15 min. The sterile broths when cooled to room temperature ($22\pm 2^\circ\text{C}$) were stored away in a cold room at 4°C until needed for use.

3.2.3 Verification of the sterility of the irradiated maize flour

The sterility of each batch of the irradiated sample was checked as follows:

- (1) Ten grams of the irradiated sample was homogenised in 90 mL of phosphate buffered saline (PBS) ($\text{pH } 7.3\pm 0.2$) and streak plated on Nutrient agar plates. The agar plates were then incubated at 30 and 37°C for 3 - 7 days;
- (2) Slurries were prepared from the irradiated samples without the addition of the microbial inocula and incubated at 30°C. The incubated slurries after 0 to 7 days were checked for microbial growth by streak plating on Nutrient agar plates. The agar plates were incubated at 30 and 37°C for 3 - 7 days.

The sterility of the irradiated samples was confirmed with no microbial growth observed in the incubated plates. Although maize grains for *akamu* production would not ordinarily be irradiated, the essence of irradiation in this study was to ensure that the characteristics being evaluated were that of the inoculated starter cultures.

3.2.4 Microbial community and Direct PCR identification of LAB and yeast in the Nigerian *akamu* samples

The LAB population in the Nigerian *akamu* samples was determined as described by Obinna-Echem et al., (2014) . The DGGE binding pattern of the entire DNA from the *akamu* samples (3.2.1.1) were evaluated and the identity of bacterial DNA on selected bands according to pre-established criteria by sequencing excised DGGE bands was established. In order to obtain viable bacterial cells for further studies, culture-dependent techniques were employed and the identity of the isolates were confirmed using direct PCR and sequencing analysis. The same technique was employed in the isolation and identification of yeast in the selected *akamu* samples M3 and W1.

3.2.4.1 Culture based microbial isolation

Ten grams of the *akamu* sample M3 and W1 were homogenised with 90 mL of phosphate buffered saline (PBS) (pH 7.3±0.2) in a stomacher 400 (Seward Ltd, West Sussex, UK) for 1 min and serially diluted (10^{-1} to 10^{-8}) in the same diluent. One hundred microliters (100 µL) of the dilutions were spread-plated on appropriate microbial media and growth conditions for the likely expected microorganisms as shown in Table 3.1. Microbial colonies were selected randomly from plates of highest dilutions based on colony morphology, purified and maintained as described in section 3.2.4.2

Table 3.1: Microbial media and growth conditions for the isolation of the microorganisms in *akamu* sample M3 and W1

Microorganism	Media	Incubation conditions	Incubator
Lactic acid bacteria	de Man, Rogosa and Sharpe (MRS) agar	37°C in 5% CO ₂ for 24 - 48 h	Sanyo MCO-19AIC CO ₂ incubator, Loughborough, Leicestershire, UK
Yeast and mould	Rose bengal chloramphenicol agar (RBCA)	25°C for 72 h	LEEC incubator, LEEC Limited, Colwick Industrial Estate, Nottingham, UK
Enterobacteriaceae <i>Yersinia</i> ,	MacConkey agar Yersinia selective agar	30°C for 24 - 72 h.	Swallow Incubators, LTE Scientific Ltd, Green field, Oldham, UK
<i>Staphylococcus aureus</i> ,	Baird-Parker agar with egg yolk supplement,	37°C for 48 -72 h	LEEC incubator, LEEC Limited, Colwick Industrial Estate, Nottingham, UK
<i>Salmonella</i> and <i>Shigella</i>	Taylor's xylose lysine desoxycholate (XLD) agar,		
<i>Listeria monocytogenes</i> <i>Escherichia coli</i>	Listeria selective agar Tryptone Bile X-Glucuronide (TBX) aga		

3.2.4.2 Maintenance and storage of cultures

Purity of the LAB and yeast isolates were checked by streaking again and sub-culturing on fresh agar plates of the isolation media (Table 3.1), followed by microscopic examinations. The purified LAB colonies were sub-cultured into MRS broth incubated at 37°C for 24 h and the yeast were grown in Malt Extract (ME) broth at 25°C for 48 h. The cells were harvested by centrifugation (Hettich Zentrifugen Rotina 46 S, Tuttlingen, Germany) at 4000 x g for 10 min, maintained in 30% (v/v) glycerol solution of the respective broth and stored either in liquid nitrogen or at -80°C till required for analysis.

3.2.4.3 Phenotypic characterisation

Cell morphology of all isolates was determined using a standard compound microscope (Medilux-12, Kyowa Optical Company Ltd., Sagamihara, Japan). Isolated LAB were Gram-stained and tested for catalase production and other phenotypic properties such as carbon dioxide production from glucose, ammonia from arginine, growth at different temperatures (15 and 45°C) and production of dextran from sucrose as well as the ability to grow in different concentrations (4 and 10%) of sodium chloride in MRS broth, following the methods of Harrigan, (1998) and Schillinger and Lücke, (1987). Sugar (arabinose, cellobiose, esculine, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, mellibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, sucrose, tetrahose and xylose) and starch fermentation by the LAB isolates were determined according to the method of Schillinger and Lücke (1987). Yeast isolates were Gram-stained and identified based on ascospore and ballistospore production, growth in ethanol, resistance to cyclohexamine and sugar fermentation as described by Harrigan (1998). The media for the

fermentation of carbohydrates by the isolated LAB and yeast, hydrolysis of arginine and the yeast ascospore formation were described in Appendix A3.2, Appendix A3.3 and Appendix A3.4 respectively. The test inferences were based on triplicate experiments.

The isolated LAB and yeast were presumptively identified based on their phenotypic characteristics in comparison to known microorganisms using the scheme by Buchanan and Gibbons, (1974) (Bergey's manual of determinative bacteriology), Schillinger and Lücke, (1987) Wood and Holzapfel, (1995) and Harrigan, (1998) for the LAB, then, Barnett and Pankhurst, (1974) and Guillermond, (1920) for the yeast.

3.2.4.4 Genotypic characterization

3.2.4.4.1 DNA extraction

Total DNA were extracted from the *akamu* samples by using DNeasy Mericon Food Kit (Qiagen Ltd, Manchester, UK) according to the manufacturer's protocol. DNA of pure microbial cultures (LAB and yeast isolated from M3 and W1 samples) were extracted from overnight MRS and Malt extract broth cultures of LAB and yeast respectively, by using the Genthra Puregene Yeast/Bacteria Kit (Qiagen Ltd, Manchester, UK) according to the manufacturer's protocol. The quality and quantity of DNA were estimated by using absorbance at 260 nm in a NanoVue plus spectrophotometer (GE Healthcare Ltd, Little Chalfont, UK). The DNA was stored at -20°C until required for use.

3.2.4.4.2 PCR amplification and purification

The conventionally isolated pure bacterial 16S rDNA and yeast 28S rDNA were amplified using primer 27f and 1492r, and NL1 and NL4 respectively. The different bacterial DNAs in the food sample DNA template were amplified with a combination of primers P1 and P2, P3 and P2 as described by Muyzer et al., (1993). The nucleotide sequences of the primers were as shown in Table 3.2.

Table 3.2: Nucleotide sequences of the different primers used for PCR

Primers	Sequence (5'-3')	Reference
P1	CCTACGGGAGGCAGCAG	
P2	ATTACCGCGGCTGCTGG	
P3*	CGCCCGCCGCGCGCGGGCGGGGCGG GGGCACGGGGGGCCTACGGGAGCAGCAG	Muyzer et al., (1993)
NL1	GCATATCAATAAGCGGAGGAAAAG	
NL4	GGTCCGTGTTTCAAGACGG	Siti Hajar et al., (2012)
27f	AGAGTTTGATCMTGGCTCAG	
1492r	TACGGHTACCTTGTTACGACTT	Martin-Laurent et al., (2001)

* Primer 3 contains the same sequence as primer 1 but has at its 5' end an additional 40-nucleotide GC-rich sequence (GC clamp)

PCR amplification reaction mixture was prepared by using MyTaq Mix (Bioline Ltd, London, UK) according to the manufacturer's standard protocol. Amplification was performed in an automated thermocycler (Techne TC-512, Scie-Plas Ltd, Cambridge, UK), with initial denaturation step at 95°C for 1 min, followed by 30 cycles of denaturation for 15 s at 95°C, annealing for 15 s at 55°C and extension for 10 s at 72°C and final annealing at 72°C for 7 min. Aliquot of 2 µL of amplification products were resolved by electrophoresing in 1.2% agarose gel in 1x TAE buffer stained with SYBR safe (Invitrogen, Fisher Scientific, Loughborough, UK) for 20 min. PCR products were purified using SureClean column-free PCR clean-up (Bioline, London, UK) according to the manufacturer instruction. The successfully amplified product of the pure LAB

and yeast DNAs were sent to GATC Biotech Ltd, London, UK, for sequencing. The closest known relatives of the 16S and 28S rDNA nucleotide sequences were determined using the BLAST search programme of the National Centre for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Products of *akamu* DNAs were further analysed by denaturing gradient gel electrophoresis before sending them out for sequencing and the determination of closest relatives from the NCBI search tool.

3.2.4.4.3 DGGE analysis and excision of DNA fragments

Perpendicular electrophoresis was performed with the PCR products (20 µL) at 60°C using 40 to 60% urea-formamide gradient increasing in the direction of electrophoresis at 250 V in 1 × Tris-acetate-EDTA (TAE) buffer for 17 h in a DGGE-2001 apparatus (CBS Scientific Co., San Diego, California, USA). After electrophoresis, the gels were stained with SYBR gold (Qiagen, Manchester, UK) for 20 min and subsequently photographed using a Bio-Rad Imager System equipped with a Gel Doc XR camera and Quantity-One software (Bio-Rad Inc., Hercules, Canada).

DGGE fragments were excised with a sterile pipette and eluted in 20 µL of autoclaved Milli-Q water (18.2 MΩ·cm at 25°C, Millipore Ltd., Watford, UK) overnight at 4°C. One microliter of the eluted DNA was re-amplified by using the same primers (Table 3.2) and PCR condition described earlier in section 3.2.4.4.2.

3.2.5 Fermentation characteristics of the isolated LAB and yeast

3.2.5.1 Microbial inocula: LAB and yeast

The LAB and yeast cultures screened based on fermentation characteristics were isolates from sample M3 as described in section 3.2.4. The LAB strains were: *Lactobacillus helveticus* (NGL1, NGL3 and NGL8), *L. acidophilus* (NGL2 and NGL6), *Lactococcus lactis* subsp. *lactis* (NGL4) and *L. plantarum* (NGL5 and NGL7) while the yeast include *Candida tropicalis* (NGY1) and *C. albicans* (NGY2, NGY3 and NGY4).

Subsequent fermentation studies employed the two *L. plantarum* strains: NGL5 and NGL7 and *C. tropicalis* (NGL1) isolated from *akamu* sample M3 and selected based on their favourable fermentation characteristics. Probiotic *L. plantarum* strain (LpTx) isolated from a probiotic food supplement, obtained from Health Food Shop, Rickard Lanes', Plymouth City Centre in UK and a probiotic yeast: *Saccharomyces boulardii* SB20 (Lallemand Levucell cultures, Biotal Ltd, Pontprennau, UK) were also used in the single and mixed culture fermentations.

3.2.5.2 Inocula preparation

3.2.5.2.1 Cell suspensions from microbial broth cultures

The microbial inocula (section 3.2.5.1) were cultivated by streaking on appropriate agar media with incubation at the organisms' optimum growth conditions (Table 3.1), thereafter; distinct colonies were grown in appropriate broth media at the same growth condition. Cultures were harvested by centrifugation (Hettich Zentrifugen Rotina 46 S, Tuttlingen, Germany) at 4000 x g for 10 min.

Cells of microbial inocula used during the screening of all the isolated LAB and yeast from M3 were washed twice in 0.85% normal saline while in subsequent fermentation experiments, cells were washed in PBS. Dilutions were made such that 1 mL of inoculum produced 8 and 6 Log₁₀ CFU mL⁻¹ for the LAB and the yeast respectively using McFarland standard number 0.5. Double checked at a prefixed absorbance read at 625 nm (Helios Epsilon Unicam Spectrophotometer, Madison, WI, USA) against sterile saline and as viable count on appropriate agar media. The McFarland standard number 0.5 was prepared by adding 0.5 mL of BaCl₂·2H₂O solution (1.175 g of BaCl₂·2H₂O in 100 mL distilled water) to 99.5 mL of the 1% H₂SO₄ (1 mL of H₂SO₄ concentrated in 99 mL of distilled water)

The subsequent single or mixed starter culture fermentation with the selected *L. plantarum* strains (NGL5, NGL7 and LpTx) and yeast: *C. tropicalis* (NGY1) and *Sacch. boulardii* (SB 20) utilized washed cells from an overnight and 48 h broth cultures with microbial concentration of 9 and 7 Log₁₀ CFU mL⁻¹ for the LAB and the yeast respectively.

3.2.5.2.2 Freeze dried *Lactobacillus plantarum* cultures

A 24 h colony of the *L. plantarum* strains from MRS agar plates were inoculated into 50 mL of MRS broth in a 100 mL conical flask and incubated aerobically in a shaking water bath (Haake SWB 20, Thermo Scientific, Hemel Hempstead, UK) at 37°C. After 16 h, the content of the flask was transferred into 500 mL of MRS broth in a 1000 L conical flask and incubated at 37°C overnight in a shaking incubator (Controlled environment incubator shaker G25, New Brunswick Scientific CO. Inc. Edison, New Jersey, USA). Thereafter, the broth

cultures were harvested in batches of 200 mL at 4°C by centrifugation at 6,000 x g for 10 min in an Avanti J-26XP centrifuge (Beckman Coulter, High Wycombe, UK). The harvested cells were pooled together using 50 mL of PBS and washed thrice with the same volume of the diluent.

To minimise any damage from freeze drying, 2 mL of sterile skim milk solution containing 0.2 g of a special bacteriological grade spray-dried skim milk powder (LP 0031, Oxoid, Basingstoke, Hampshire, UK) per millilitre was added to the washed harvested cells. The cell suspensions were flash frozen with liquid nitrogen and freeze dried overnight in Edwards Modulyo bench top freeze dryer (Mecha Tech Systems Ltd, Thornbury, Bristol, UK). The dried cultures were stored away in 30 mL sterile sterilin plastic containers with lid (Fisher Scientific, Loughborough, UK).

3.2.5.3 Fermentation of ground maize slurries

Fermentation studies were set up by thoroughly mixing 50 g of irradiated maize flour with 100 mL of sterile distilled water inoculated with 1 mL of the microbial inocula. The inoculated slurries were distributed in 18 mL quantity into well labelled sterile transparent plastic 50 mL pots with lids and fermented at 30°C for 72 h. With the transparent pots it was possible to visualize some changes like air bubbles from the metabolic activities of some of the fermenting microorganisms in the fermentation.

Samples were aseptically withdrawn after 0, 3, 6, 12, 24, 36, 48, and 72 h of fermentation for pH, titratable acidity, microbial count, and organic acid determinations. The samples for organic acid determination were preserved at -

80°C in Eppendorff tubes until needed. Un-inoculated and a back slop fermentation (with 1 g of traditional *akamu* sample as inocula) served as negative and positive control respectively. Fermentation with the freeze dried *L. plantarum* strain starter cultures had 0.5±0.001 g of the freeze dried starter as inoculum.

3.2.5.4 Physicochemical analysis

3.2.5.4.1 pH and titratable acidity

pH of both the traditional *akamu* samples obtained from Nigeria and the starter culture fermented ground maize slurries were determined with a pH meter (Accumet^R AB10, Fisher Scientific, Loughborough, UK) in 10 mL of mixed slurry (1 mL of slurry and 1 g of *akamu* sample in 10 mL of sterile distilled water respectively). The pH was calibrated against standard buffer solutions (Fisher Scientific, Loughborough, UK) at pH 4.0 and 7.0.

The amount of acid as total titratable acidity (TTA) produced in the fermentation was determined by modifying the method according to Annan et al., (2003). Samples from pH analysis were titrated against 0.1 mol L⁻¹ NaOH with phenolphthalein as indicator. Results were expressed as percentage lactic acid using the formula:

$$\%TTA = \frac{\text{Titre} \times \text{Normality of acid} \times \text{Equivalent weight of acid} \times 100}{\text{Volume of sample} \times 1000} \dots \dots \dots \text{Equation 3. 1}$$

Where equivalent weight of lactic acid is 90.08 g.

3.2.5.4.2 Organic acids and sugar analysis using high performance liquid chromatography (HPLC)

3.2.5.4.2.1 Reagents and standards

All the chemicals used were of analytical or HPLC grade. Concentrated H₂SO₄, ethanol and methanol were purchased from Fisher Scientific, Loughborough, UK. The standard sugars (glucose, fructose, sucrose and maltose) and organic acids (acetic, butyric, lactic and propionic acid) were of Sigma Aldrich, Gillingham Dorset, UK. As described in Appendix A3.5, appropriate concentrations of reagents and standards were obtained by dissolving or making-up appropriate grams or volume in deionised water purified through a Milli-Q system (Millipore Corp., Bedford, MA, USA).

3.2.5.4.2.2 Instrumentation

Organic acid compositions of the fermented ground maize slurries from the isolated LAB and yeast screening process were analysed by the method of Niven et al., (2004) using high performance liquid chromatography (HPLC), Gynkotec (Dionex Corp., Sunnyvale, California, USA) with some modifications in sample preparation. With the breakdown of the Gynkotec HPLC machine, the rest of the chromatographic analysis in this study was carried out in Dionex-Ultimate 3000 UHPLC+Focused (Dionex Softron GmbH, Germering, Germany). The UHPLC was equipped with WPS-3000 autosampler, LPG-3400SD pump, and TCC-3000 column compartment, RI-101 refractive index detector and MWD-3000 Ultra violet detector.

3.2.5.4.2.3 Sample preparation

About 0.5 ± 0.01 g of the Nigerian *akamu* samples was dispersed in 1 mL of Milli-Q water, while 1.5 mL of the frozen fermented ground maize slurry samples in eppendorf tubes were allowed to thaw at room temperature ($22 \pm 2^\circ\text{C}$). The samples were homogenised on a Multi-mixer (Fisher scientific, Loughborough, UK) for 10 s and then centrifuged at 13000 g for 20 min (Sanyo-MSE MSB010.CX2.5 Micro Centaur Centrifuge, Alconbury, UK). The samples that were analysed with the Gynkotek HPLC were acid treated for protein precipitation as follows: 500 μL of the sample supernatant were added to 500 μL of 0.5 mol L^{-1} of HClO_4 and 250 μL of acid treated supernatant were neutralized with equal volume of 1 mol L^{-1} of K_2CO_3 . This sample purification process was to maintain the functionality of the column. Twenty microliter (20 μL) of 7% H_2SO_4 was added to 100 μL of either an acid treated or untreated sample in 400 μL of Milli-Q water. Samples were then filtered through MF-millipore microfiltre (0.20 μm pore size) into vials and sealed with crimp cap (11mm, Ruber/PTFE, Fisher Scientific, Loughborough, UK) and stored at -80°C until needed for analysis. Spiked unfermented ground maize slurries were prepared as described in Appendix A3.6

3.2.5.4.2.4 HPLC conditions, validation and data analysis

The chromatographic separation was achieved with an elution phase of degased 5 mmol L^{-1} of H_2SO_4 in Hi-Plex H Guard column 300 x 7.7 mm (Agilent Technologies, Waghaeusel-Wiesental, Germany). The eluent was pumped at a flow rate of $0.5 \mu\text{L min}^{-1}$ within a pressure limit of 5 - 100 bars. The injected sample volume was 20 μL . Three different column temperatures: 25, 45, and 65°C were used to validate the optimum temperature for better peak

separations and then a temperature of 25°C was upheld for all the analysis. The sugars and organic acids were detected using the Refractive Index (RI) and the Ultra Violet (UV) detectors respectively. The retention times of the sugar and the organic acid standards were noted and used in the identification of the sample peaks.

All data obtained were processed using Chromeleon® 7.1 Chromatography Data System Software (Dionex Softron GmbH, Germering, Germany). A calibration curve for each sugar and the organic acids was obtained from four different concentrations (100, 50, 25 and 12.5 mmol L⁻¹) of the standards' stock solutions. Recoveries were computed by spiking unfermented ground maize slurry with known concentrations of each standard.

3.2.5.5 Enumeration of viable microbial cells

Ten-fold dilutions of 1 mL of the sample (inoculated ground maize slurries) in 9 mL PBS were prepared and plated out using the drop method by Miles and Misra, (1938) on the MRS and RBCA with incubation at 37°C for 24 - 48 h and at 25°C for 48 h for the LAB and the yeast respectively. The number of colony forming unit per milliliter was computed as:

$$\text{CFU mL}^{-1} = \frac{\text{Average number of colonies for a dilution} \times \text{Dilution factor}}{\text{Volume of sample plated}} \dots \dots \dots \text{Equation 3. 2}$$

3.2.6 Statistical analysis

Data obtained were statistically analysed using Minitab (Release 16.0) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences and relationship among variables were evaluated by analysis of variance

(ANOVA) under general linear model and Tukey pairwise comparisons at 95% confidence level.

The rate of growth of the microorganisms were obtained using the microbial model (DMFit version 2.0) by Baranyi and Roberts, (1994). The decimal reduction time or D value (time taken for the surviving microbial population to be reduced by 1 log cycle) and the doubling time (τ) (the average time required for microbial population to double) were computed from the potential maximum growth rate (μ_{max}) in Microsoft Excel 2010 using the formula:

$$D = 1/k \dots\dots\dots\text{Equation 3. 3}$$

$$\tau = 0.693/\mu \dots\dots\dots\text{Equation 3. 4}$$

Where k and μ represent the death and specific growth rates respectively (Adams and Moss, 2000).

3.3 Results

3.3.1 Microbial identification

3.3.1.1 PCR-DGGE analysis

The amplified DNA template from the isolated LAB and the Nigerian *akamu* samples appeared as single bands by the polyacrylamide gel electrophoresis as shown in the example in Figure 3.2. A single band is desirable for successful PCR. Although sample E1 and *Lactobacillus helveticus*, appeared to have double band but at very close proximity.

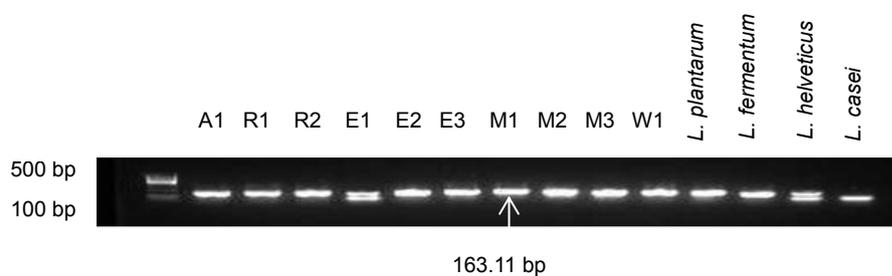


Figure 3.2: PCR amplified product of DNA templates of the *akamu* samples and pure bacteria cultures

The alphanumeric codes (A1, E1, E2, E3, M1, M2, R1, R2 and W1) represent *akamu* samples based on their origin

DGGE analysis of the lactic acid bacteria population in the *akamu* samples produced many distinguishable bands in the separation pattern (Figure 3.3A and Figure 3.3B). Each pattern from the PCR products of the different *akamu* samples produced up to 10 to 20 bands representing different operational taxonomic units. The PCR products obtained for the pure cultures migrated over a narrow region of the DGGE gel in comparison to the *akamu* samples.

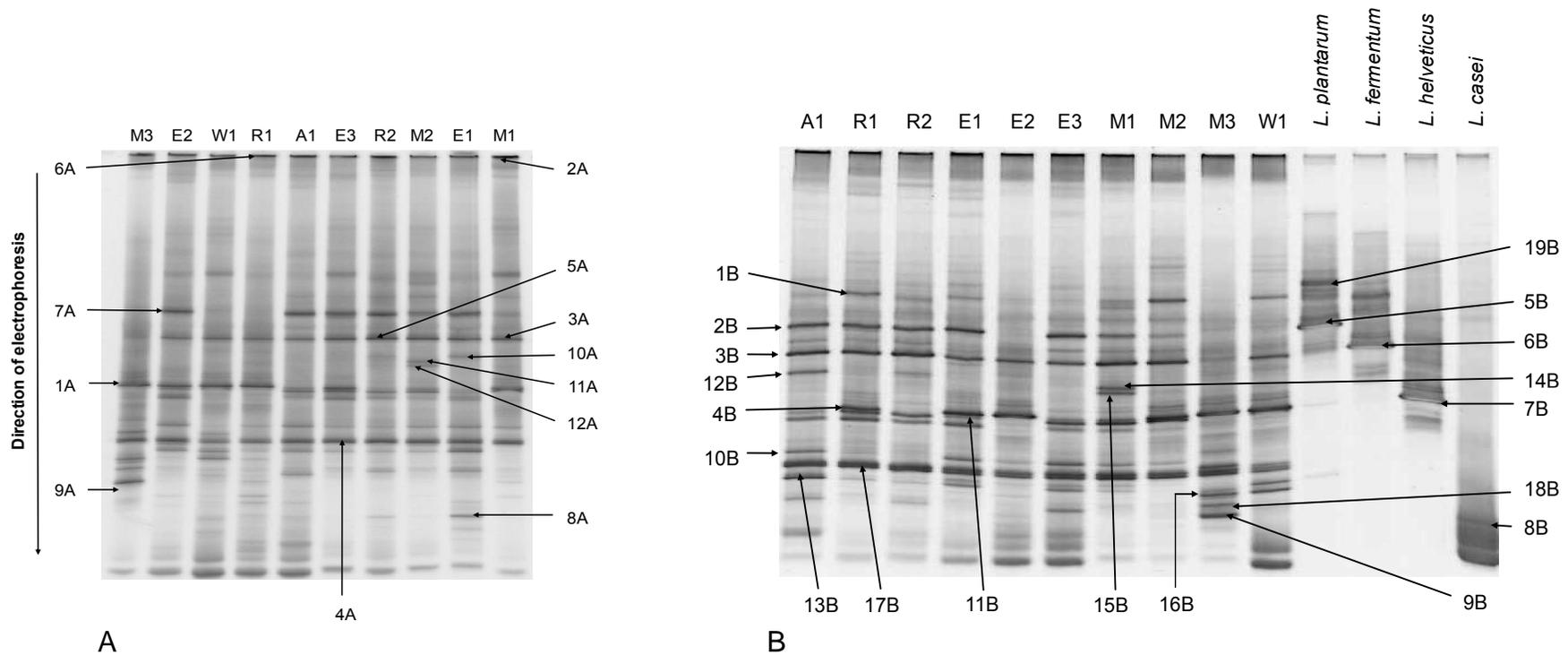


Figure 3.3: DGGE analysis of natural LAB population in 4 - 5 days old traditional *akamu* samples obtained from Nigeria
 (A) First DGGE analysis of the different *akamu* sample PCR products (B) Repeat analysis including PCR product of some pure LAB cultures in the last four wells
 The numeric-alpha codes (1A - 12A) and (1B - 19B) represent bands that were excised for sequencing from the respective Figures.
 The alphanumeric codes (A1, E1, E2, E3, M1, M2, R1, R2 and W1) represent *akamu* samples based on their origin

3.3.1.2 Sequences

Sequencing that returned with an ideal result for pure LAB and yeast DNA's were in the range of 942 - 1105 and 542 - 568 nucleotides respectively. Successful sequences of the *akamu* sample DNAs ranged between 92 and 142 nucleotides. Although, the expected sequence length for the premier pairs: 27f/1492r, NL1/NL4 and P1/P3 were 1500, 680 and 204 base pairs for the LAB, yeast and the *akamu* samples respectively.

Table 3.3: Identities of bands obtained from DGGE analysis (Figure 3.3B) of the *akamu* LAB community

*Excised band	Closest relative	†Maximum identity (%)
2B	<i>Lactobacillus plantarum</i>	96
3B	<i>L. fermentum</i>	100
4B	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> <i>L. helveticus</i>	100
5B	<i>L. plantarum</i>	98
7B	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> <i>L. helveticus</i>	100
8B	<i>L. rhamnosus</i> <i>L. casei</i>	99
9B	<i>L. reuteri</i>	95
15B	<i>L. salivarius</i>	95
16B	<i>Lactococcus lactis</i> subsp. <i>cremoris</i>	100
17B	<i>Zea mays</i> chloroplast	100
18B	<i>L. reuteri</i>	96

*Band excised from DGGE gel shown in Figure 3.3B

†Percentage sequence homology of the nucleotides in the sequence of the DGGE excised fragment and that of the closest relative found in the GenBank
Band fragments with unsuccessful sequencing were not included

3.3.1.3 Identification of the LAB community in akamu samples

In Figure 3.3A, the excised fragments 4A, 7A and 9A corresponding to 17B, 2B and 9B of Figure 3.3B respectively were related to *Zea mays* chloroplast, *L. plantarum* and *L. reuteri* respectively. In Table 3.3, the closest relatives of the excised fragments from the second DGGE gel (Figure 3.3B) were identified as: *L. plantarum*, *L. fermentum*, *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*, *L. rhamnosus* and *L. casei*, and *Lactococcus lactis* subsp. *cremoris*. Although, fragment 6B returned with insufficient sequence length for blasting, it was an already identified *L. fermentum* strain from the pure bacteria culture.

3.3.1.4 Identification of the isolated microorganisms from akamu samples

In this study, there was no growth of Enterobacteriaceae, *Yersinia*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Listeria*, and *Escherichia coli* observed in their respective growth agar plates that were spread plated and incubated as shown in Table 3.1. Lactic acid bacteria and yeast on MRS and RBCA respectively were detected and further identified.

A total of 21 bacterial strains were isolated from the two *akamu* samples: 8 isolates from M3 and 13 from W1. They were presumptively considered as LAB on MRS based on their phenotypic characteristics: Gram and catalase reaction, morphological characteristics of the microbial colonies, growth at different temperature and salt concentrations and sugar fermentative patterns (Table 3.4 and Table 3.5). The bacteria were Gram positive, catalase negative, small short to long rods that were either round, entire, rough, cream or slightly grey and translucent colonies.

Four out of the 13 strains of LAB isolated from W1, were homofermentative rods and using PCR-sequencing analysis, 2 of the isolates were identified as *L. helveticus* and a strain each of *L. rhamnosus* and *L. casei*. Nine strains were heterofermentative rods, able to hydrolyse arginine and some produced gas from glucose, 8 of which were genotypically identified as *L. fermentum* while the sequencing of one strain was not successful.

All the strains isolated from M3 were presumptively considered as homofermentative rods, except for one strain that was coccoid and produced gas from glucose. Genotypically, 3 of the strains were identified as *L. helveticus*, 2 of *L. plantarum* and *L. acidophilus* and 1 of *Lactococcus lactis* subsp. *lactis*.

The phenotypic characteristics of the RBCA isolated yeasts were shown in Table 3.6 and Table 3.7. The yeast isolates were elongated spherical or cylindrical cells. Reproduction was mainly by budding with mycelia production although 2 strains produced ascus with 2 - 4 ascospore. The 3 yeast strains isolated from W1 were identified as *Candida albicans*, *Clavispora lusitaniae* and *Saccharomyces paradoxus*. One out of the 4 yeast strains isolated from M3 was identified genotypically as *Candida tropicalis* (NGY1) while the others were all *C. albicans*.

Table 3.4: Phenotypic characteristics and genomic identity of the LAB isolated from a selected *akamu* sample (W1)

Code	Identity		Growth				Fermentation																				
			NaCl		Temp		Arginine		Glucose																		
	Genotypic	Phenotypic	4%	10%	15°C	45°C	Ammonia	Gas	Acid	Gas	Slime	Arabinose	Cellobiose	Fructose	Galactose	Lactose	Maltose	Mannitol	Mannose	Melezitose	Mellibiose	Raffinose	Ribose	Salicin	Sucrose	Tetrahydrose	Xylose
NGL1w	<i>Lactobacillus fermentum</i>	<i>Lactobacillus acidophilus</i>	+	-	-	+	-	+	+	-	+	-	-	-	-/+	+	+	-	+	n	+	+	-	-	+	+	-
NGL2w	<i>L. fermentum</i>		+	-	-	+	-	+	+	-	-	+	-	+	-	-	-	-	-	n	-	+	-	-	+	-	-
NGL3w	<i>L. helveticus</i>	<i>L. fermentum</i>	n	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	n	-	-	-	-	-	+	-
NGL4w	<i>L. fermentum</i>		+	-	-	+	-	+	+	-	-	+	-	-	-	+	+	-	+	n	+	+	+	-	+	+	-
NGL5w	<i>L. fermentum</i>	<i>L. salivarius spp. salivarius</i>	+	-	-	+	-	+	+	-	-	-	-	+	+	+	+	-	+	n	+	+	+	-	+	+	+
NGL6w	<i>L. fermentum</i>		+	-	-	+	-	+	+	-	-	+	-	+	-	-	-	-	-	n	-	+	-	-	+	-	-
NGL7w	<i>L. fermentum</i>	<i>L. fermentum</i>	+	-	-	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+
NGL8w	<i>L. rhamnosus</i>	<i>L. plantarum</i>	+	-	+	-	-	-	+	-	-	-	+	+	+	-	+	+	+	+	+	-	-	+	+	+	-
NGL9w	<i>L. casei</i>	<i>L. plantarum</i>	+	-	+	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	-
NGL10w	<i>L. fermentum</i>	<i>Pediococcus halophilus</i>	+	+	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	n	-	-	-	n	+	-	+
NGL11w	<i>L. helveticus</i>	<i>L. acidophilus</i>	+	-	-	+	-	+	+	-	-	+	+	+	+	+	+	-	+	n	+	+	+	n	+	+	-
NGL12w	<i>L. fermentum</i>	<i>L. delbrueckii subs delbrueckii</i>	-	-	-	+	-	-	+	-	-	-	+	+	+	-	+	-	-	n	-	-	-	n	+	-	-
NGL13w	SN	<i>L. reuteri</i>	-	-	-	+	+	+	+	+	-	+	-	+	+	-	+	-	-	n	+	+	-	n	+	-	-

All isolate were Gram positive and catalase negative. Esculine, rhamnose, sorbitol, sorbose and starch were negative for NGL1w to NGL6w but not tested for others.

*Weak; n - Not tested. SN – Sequencing was not successful.

NGL2w, NGL4w and NGL6w were difficult to classify phenotypically

Table 3.5: Phenotypic characteristics and genotypic identity of the LAB isolated from a selected *akamu* sample (M3)

Code	Identity		Growth		Fermentation																						
			NaCl		Temp		Arginine		Glucose																		
	Genotypic	Phenotypic	4%	10%	15°C	45°C	Ammonia	Gas	Acid	Gas	Slime	Arabinose	Cellobiose	Fructose	Galactose	Lactose	Maltose	Mannitol	Mannose	Melezitose	Mellibiose	Raffinose	Ribose	Salicin	Sucrose	Tetraose	Xylose
NGL1	<i>Lactobacillus helveticus</i>	<i>Lactobacillus jensenii</i>	-	-	-	+	+	-	+	-	-	-	+	-	-	-	+	-	+	-	-	-	+	+	+	+	-
NGL2	<i>L. acidophilus</i>	<i>L. acidophilus</i>	-	-	-	+	-	-	+	-	-	-	+	+	+	-	+	-	+	-	-	-	-	+	+	+	-
NGL3	<i>L. helveticus</i>	<i>L. helveticus</i>	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	+	+	-
NGL4	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	<i>Leuconostoc mesenteroides</i>	+	-	+	-	-	-	+	+	-	-	+	+	+	+	+	-	+	-	-	-	+	-	-	+	+
NGL5	<i>L. plantarum</i>	<i>L. plantarum</i>	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-
NGL6	<i>L. acidophilus</i>	<i>L. acidophilus</i>	-	-	-	+	-	-	+	-	-	-	+	+	+	-	+	-	+	-	-	-	-	-	+	+	-
NGL7	<i>L. plantarum</i>	<i>L. plantarum</i>	+	-	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
NGL8	<i>L. helveticus</i>	<i>L. helveticus</i>	-	-	-	+	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-

All isolate were Gram positive and catalase negative

*Weak

Table 3.6: Phenotypic characteristics and genotypic identity of the yeast isolated from a selected *akamu* sample (W1)

Code		NGY1w	NGY2w	NGY3w
Identity	Genotypic	<i>Candida albicans</i>	<i>Clavispora lusitaniae</i>	<i>Saccharomyces paradoxus</i>
	Phenotypic	<i>Pichia membranaefaciens</i> ,	<i>Kluyveromyces marxianus</i>	<i>Saccharomyces rouxii</i>
Morphology	Cell	Spherical and elongated, filled with vacuoles.	Cylindrical	Spherical and elongated, in chains
Cultural	RBCA	Round, smooth, white colonies	Pink butryous (rhizoid) colonies	Big pink umbonated colonies.
	Broth	Scum	Scum	Scum
Gram Stain		+	+	+
Ascospores		-	-	+ (deploids)
Ballistospores		-	-	-
Budding		+ No mycelia	+pseudomycelium	+
Resistance to cycloheximide		-	+	-
Ethanol Utilization		+	+	+
Fermentation and gas production	Cellobiose	-	-	-
	Fructose	-	+	-
	Galactose	-	-	-
	Glucose	-	+	+
	Inulin	-	-	-
	Lactose	-	-	-
	Maltose	-	-	-
	Mannose	-	+	+
	Mellibiose	-	-	-
	Raffinose	-	-	-
	Rhamnose	-	-	-
Sucrose	-	+	+	
Tetraose	-	-	-	
Xylose	-	-	-	

Table 3.7: Phenotypic characteristics and genotypic identity of the yeast isolated from *akamu* sample (M3)

Code		NGY1	NGY2	NGY3	NGY4
Identity	Genotypic	<i>Candida tropicalis</i>	<i>C. albicans</i>	<i>C. albicans</i>	<i>C. albicans</i>
	Phenotypic	<i>Sacch. cerevisiae</i>	<i>C. tropicalis</i>	<i>Zygosacch. lactis</i>	<i>C. mecedomiensis</i>
Morphology	Cell	Spherical, ovoid	Cylindrical with rounded ends	Spherical	Elongated
	RBCA	Smooth dull cream domed	Umbonated with two zones of cream and pink colour	Round big pink umbonate colonies	Pink and rhizoid
Cultural	MEA	Smooth shiny cream and convex	Light grey to grey	Flat, smooth, round and greyish white	White butyrous with rough edges
	Broth	Clumped at top corner of conical flask	Sediment	Sediment	Film
Gram Stain		+	+	+	+
Ascospores		Ascus (2-4)	-	Conjugation, ascus (2)	-
Ballistospores		-	-	-	-
Budding		Budding No mycelia	Mycelium with blastospore	-	Multi-lateral budding
Cycloheximide resistance		-	-	-	-
Ethanol Utilization		-	+	+	+
Fermentation and gas production	Cellobiose	-	-	-	-
	Fructose	+	+	+	+
	Galactose	+	-	-	+
	Glucose	+	+	+	+
	Inulin	-	-	-	+*
	Lactose	-	-	-	-
	Maltose	+	+	-	-
	Mannose	+*	+	+	+
	Mellibiose	-	-	-	-
	Raffinose	-	-	-	+*
	Rhamnose	-	-	-	-
	Sucrose	+	+	+	+*
	Tetraose	+	-	-	-
	Xylose	-	-	-	-

*Weak

3.3.2 Fermentation characteristics

3.3.2.1 pH and Titratable acidity

3.3.2.1.1 Akamu samples obtained from Nigeria

The pH, titratable acidity and lactic acid concentrations of the various Nigerian *akamu* samples and the resulting mean for each location are shown in Table 3.8. The samples pH, TTA and lactic acid concentration were in the range of 3.22 - 3.95, 0.60 - 1.59% and 28.47 - 84.29 mmol kg⁻¹. Significant ($p \leq 0.05$) differences were observed among the individual samples.

Table 3.8: pH, titratable acidity and lactic acid levels of the *akamu* samples obtained from Rivers State, Nigeria

Origin	Sample code	pH	TTA (% lactic acid)	*Lactic acid (mmol kg ⁻¹)
Mile 3 Diobu		(3.66±0.15)	(1.11±0.25)	(62.61±8.23)
	M1	3.56±0.03 ^{ef}	0.75±0.05 ^{de}	51.51±1.37 ^{ef}
	M2	3.46±0.01 ^d	0.99±0.00 ^{bc}	78.68±6.29 ^{ab}
	M3	3.95±0.01 ^h	1.59±0.05 ^a	57.65±3.49 ^{de}
Emohua		(3.46±0.04)	(0.79±0.10)	(64±11.90)
	E1	3.42±0.01 ^c	0.90±0.00 ^{bcd}	64.65±2.31 ^{cd}
	E2	3.44±0.01 ^{cd}	0.87±0.05 ^{cd}	84.29±3.02 ^a
	E3	3.53±0.01 ^e	0.60±0.05 ^e	43.10±1.79 ^f
Rumuokoro		(3.29±0.06)	(0.95±0.11)	(69.71±0.15)
	R1	3.22±0.01 ^a	0.84±0.05 ^{cd}	69.56 ±2.64 ^{bc}
	R2	3.35±0.00 ^b	1.05±0.05 ^b	69.85±2.00 ^{bc}
Aluu	A1	3.58±0.00 ^f	0.84±0.10 ^{cd}	61.93±5.63 ^{cd}
Worgi	W1	3.74±0.01 ^g	0.87±0.05 ^{cd}	28.47±0.56 ^g

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). Values for the individual samples were mean of triplicate determinations ± standard deviation. Values in brackets were origin mean ± standard error of mean. Means based on origin did not differ significantly ($p \leq 0.05$).

* Amount of lactic acid obtained using HPLC analysis

3.3.2.1.2 Screening of the LAB and yeast isolates from a selected akamu sample (M3)

The pH and TTA of the ground maize slurries fermented by the different strains of LAB and yeast isolated from *akamu* sample M3 were shown in Figure 3.4 and Figure 3.5 respectively. The rates of increase in pH and TTA of the samples were shown in Appendix A3.7, Appendix A3.8 and Appendix A3.9. The rate of pH decrease in the BSF (0.11) and the samples AL5 and AL7 fermented by the two *L. plantarum* strains: NGL5 and NGL7 (0.10 and 0.12 respectively) within the first 24 h of fermentation were significantly ($p \leq 0.05$) the highest. The pH of these three samples (BSF, AL5 and AL7) was reduced from ≤ 6.18 to ≤ 3.63 after 24 h. Although, other LAB isolates had pH values < 3.35 at the end of the fermentation period, one strain of *L. helveticus* (NGL3) and the fermentation without microbial inoculum had no significant reduction in pH. There was an observed increase in the pH value of the BSF from 3.42 to 3.72 towards the end of fermentation. The observed reduction in pH of the yeast fermentation was from ≥ 6.05 to ≥ 4.89 after 72 h with rate of change of 0.01 and 0.03 for AY2 and AY4 respectively after 24 h.

The AL5, AL7 and the BSF had significantly ($p \leq 0.05$) the highest rate of increase in TTA (0.021 for AL5 and 0.029 for AL7 and BSF) after the first 24 h. The TTA of AL3 fermented by the *L. helveticus* (NGL3) and the control were more or less constant, while significant increases were observed for other LAB isolates towards the end of fermentation. BSF had decreased TTA towards the end of fermentation. Among the yeast isolates, the sample AY1 fermented by *C. tropicalis* (NGY1) had significantly ($p \leq 0.05$) the highest TTA of 0.50% after 24 h although a significant decrease was recorded after 72 h.

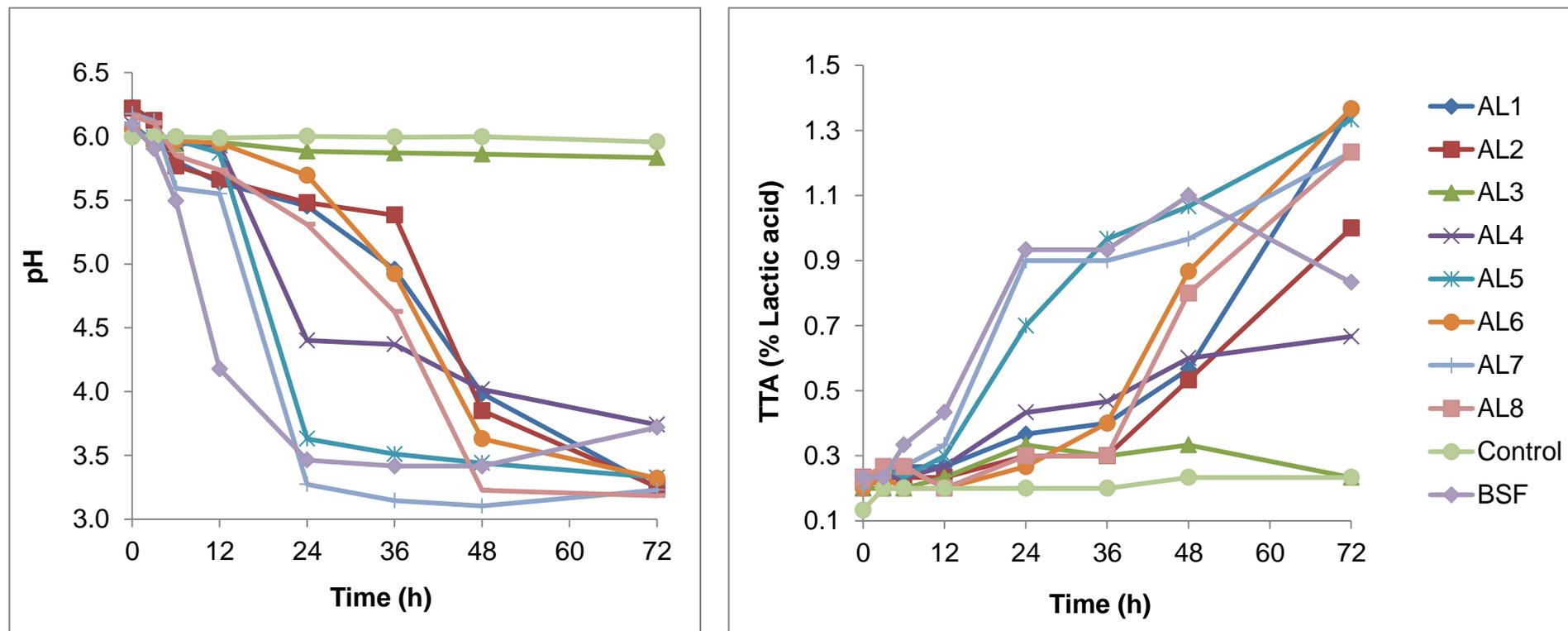


Figure 3.4: pH and total titratable acidity (TTA) of ground maize slurries fermented by the LAB identified from *akamu* sample (M3)
 AL1 – AL8 represent samples fermented by the different Lactic acid bacteria: AL1, AL3 & AL8 for *Lactobacillus helveticus* strains (NGL1, NGL3 & NGL8); AL2 & AL6 for *L. acidophilus* strains (NGL2 & NGL6); AL4 for *Lactococcus lactis* subsp. *lactis* (NGL4); AL5 & AL7 for *L. plantarum* strains (NGL5 & NGL7)
 BSF - Back slop fermentation
 Control - Un-inoculated sample

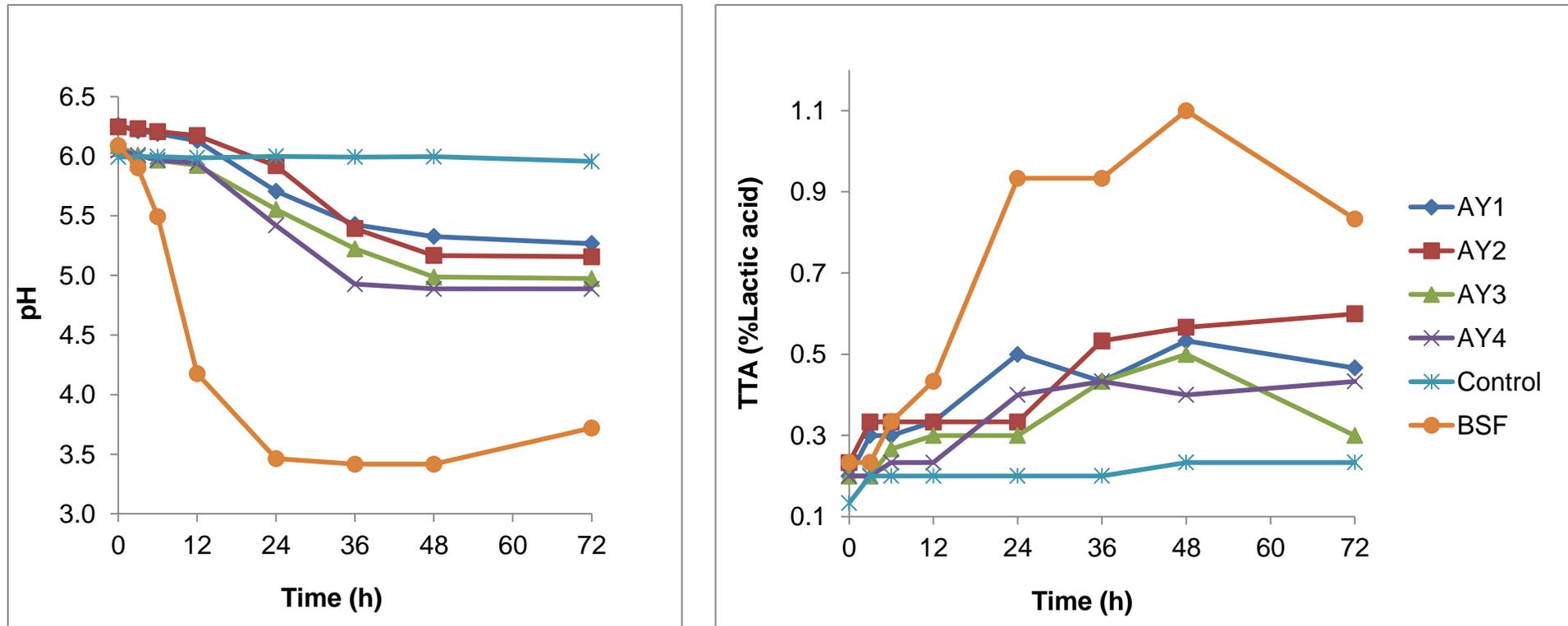


Figure 3.5: pH and total titratable acidity (TTA) of ground maize slurries fermented by the yeasts identified from *akamu* sample (M3)
 AY1 represent sample fermented by *Candida tropicalis* (NGY1)
 AY2 - AY4 represents samples fermented by *C. albicans* (NGY2 - NGY4)
 BSF - Back slop fermentation
 Control - Un-inoculated sample

3.3.2.1.3 Fermentation with the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* SB20

The pH and TTA of the fermentations were shown in Table 3.9 and Table 3.10 respectively. The decrease in pH of the *L. plantarum* strain samples either as single or mixed starter with the yeasts were significantly ($p \leq 0.05$) lower than the yeast single starter fermentation. Fermentation with the *L. plantarum* single and the *L. plantarum* and yeast mixed cultures had pH reduction from ≥ 5.50 to ≤ 3.93 and ≤ 3.52 after 12 and 72 h respectively. While in the yeast single starter fermentation pH decrease was ≥ 4.57 after 72 h. Although the pH (4.31) of the BSF sample after 12 h was significantly ($p \leq 0.05$) higher than the *L. plantarum* starter culture fermentations, it had significantly ($p \leq 0.05$) the least pH (3.37) after 72 h. The TTA of both the *L. plantarum* single ($\geq 1.05\%$) and mixed fermentation with the yeasts ($\leq 0.90\%$) were significantly ($p \leq 0.05$) higher than the TTA of yeast single culture fermentation ($\geq 0.33\%$). The TTA of the yeast single culture fermentation did not differ from that of the control. Back slop fermentation samples after 48 and 72 h had significantly ($p \leq 0.05$) the highest TTA values of 1.17 and 1.41% respectively.

Table 3.9: pH of ground maize slurries fermented by the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* (SB20)

Samples	Time (h)					
	0	6	12	24	48	72
AL5	5.52±0.04	4.64±0.03 ^a	3.80±0.01 ^a	3.51±0.03 ^a	3.42±0.02 ^{ab}	3.43±0.01 ^{abc}
AL5+AY1	5.58±0.05	4.68±0.03 ^a	3.73±0.02 ^a	3.51±0.02 ^a	3.45±0.04 ^{ab}	3.43±0.01 ^{abc}
AL5+ASB20	5.59±0.02	4.78±0.01 ^b	3.75±0.01 ^a	3.56±0.02 ^{ab}	3.47±0.01 ^{bc}	3.45±0.06 ^{bcd}
AL7	5.58±0.04	5.17±0.02	3.93±0.04 ^a	3.55±0.03 ^{ab}	3.43±0.03 ^{ab}	3.40±0.01 ^{ab}
AL7+AY1	5.50±0.07	4.96±0.03 ^c	3.87±0.02 ^a	3.53±0.02 ^a	3.48±0.02 ^{bcd}	3.46±0.03 ^{bcd}
AL7+AY20	5.58±0.02	5.07±0.03 ^d	3.85±0.02 ^a	3.55±0.01 ^{ab}	3.54±0.01 ^d	3.52±0.01 ^e
ALpTx	5.53±0.11	5.08±0.03 ^d	3.69±0.43 ^a	3.56±0.02 ^{ab}	3.42±0.02 ^{ab}	3.43±0.01 ^{abc}
ALpTx +AY1	5.57±0.04	4.97±0.02 ^c	3.90±0.02 ^a	3.53±0.03 ^a	3.47±0.01 ^{bc}	3.48±0.04 ^{bcd}
ALpTx +ASB20	5.61±0.05	4.93±0.02 ^c	3.91±0.01 ^a	3.61±0.01 ^b	3.51±0.01 ^{cd}	3.51±0.02 ^{de}
BSF	5.54±0.06	5.48±0.04 ^f	4.31±0.03 ^b	3.56±0.03 ^{ab}	3.39±0.03 ^a	3.37±0.01 ^a
AY1	5.56±0.04	5.43±0.05 ^f	5.07±0.02 ^c	4.90±0.01	4.83±0.01	4.76±0.01
ASB20	5.59±0.03	5.48±0.02 ^f	4.95±0.03 ^c	4.83±0.03	4.67±0.03	4.57±0.01
Control	5.59±0.01	5.58±0.01	5.52±0.02	5.40±0.03	5.30±0.02	5.23±0.01

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

BSF - Back slop fermentation

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.10: TTA (%Lactic acid) of ground maize slurries fermented by the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* (SB20)

Samples	Time (h)					
	0	6	12	24	48	72
AL5	0.12±0.05	0.21±0.10	0.39±0.05 ^{ab}	0.69±0.05 ^a	0.87±0.05 ^{ab}	1.05±0.05 ^{ab}
AL5+AY1	0.12±0.05	0.21±0.05	0.45±0.09 ^a	0.84±0.29 ^a	0.72±0.09 ^b	0.90±0.09 ^{ab}
AL5+ASB20	0.12±0.05	0.18±0.00	0.45±0.00 ^a	0.66±0.05 ^a	0.81±0.00 ^{ab}	0.84±0.05 ^b
AL7	0.15±0.05	0.15±0.05	0.33±0.05 ^{abcd}	0.66±0.05 ^a	0.96±0.10 ^a	1.11±0.10 ^a
AL7+AY1	0.12±0.05	0.12±0.05	0.36±0.00 ^{abc}	0.60±0.05 ^a	0.78±0.05 ^{ab}	0.87±0.05 ^{ab}
AL7+AY20	0.09±0.00	0.15±0.05	0.36±0.00 ^{abc}	0.63±0.09 ^a	0.78±0.05 ^{ab}	0.87±0.05 ^{ab}
ALpTx	0.15±0.05	0.15±0.05	0.36±0.00 ^{abc}	0.63±0.00 ^a	0.93±0.05 ^a	1.05±0.05 ^{ab}
ALpTx +AY1	0.09±0.00	0.15±0.05	0.39±0.05 ^{ab}	0.63±0.09 ^a	0.81±0.09 ^{ab}	0.84±0.10 ^b
ALpTx +ASB20	0.12±0.05	0.18±0.00	0.39±0.05 ^{ab}	0.63±0.00 ^a	0.84±0.05 ^{ab}	0.84±0.05 ^b
BSF	0.09±0.00	0.15±0.05	0.24±0.10 ^{bcd}	0.75±0.05 ^a	1.17±0.09	1.41±0.14
AY1	0.15±0.05	0.18±0.00	0.24±0.05 ^{bcd}	0.24±0.05 ^b	0.27±0.09 ^c	0.30±0.14 ^c
ASB20	0.09±0.00	0.15±0.05	0.21±0.05 ^{cd}	0.24±0.05 ^b	0.30±0.05 ^c	0.33±0.05 ^c
Control	0.09±0.00	0.15±0.05	0.18±0.00 ^d	0.18±0.00 ^b	0.21±0.05 ^c	0.24±0.05 ^c

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD.

Values after 0 and 6 h did not differ significantly.

BSF - Back slop fermentation

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

3.3.2.1.4 Fermentation with freeze dried cultures of *Lactobacillus plantarum* strains

Figure 3.6 presents the pH and TTA of ground maize slurries fermented by the freeze dried starter cultures of the *L. plantarum* strains. The pH was reduced from 5.96 ± 0.05 to $\leq 3.92 \pm 0.01$ and $\leq 3.43 \pm 0.01$ after 6 and 48 h respectively and with corresponding TTA $\geq 0.51 \pm 0.05$ and $\geq 1.35 \pm 0.09\%$.

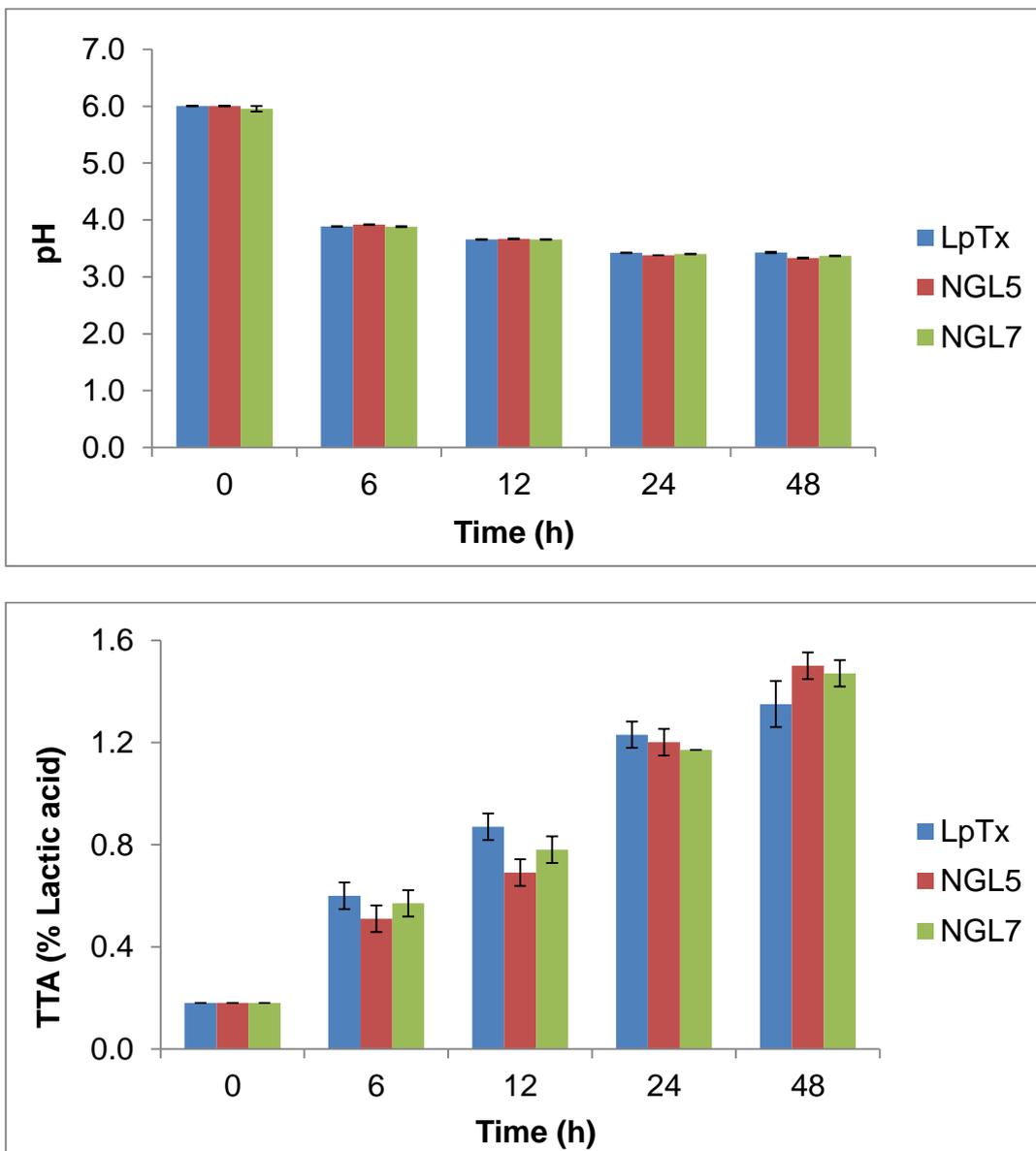


Figure 3.6: pH and total titratable acidity (TTA) of ground maize slurries fermented by freeze dried *Lactobacillus plantarum* strains starter cultures
FLpTx - Samples fermented by commercial probiotic *L. plantarum* strain (LpTx)
FL5 & FL7 - Samples fermented by Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

3.3.2.2 Organic acids and sugar profile

3.3.2.2.1 HPLC Validation

3.3.2.2.1.1 Column oven temperature and identification of sample analyte peaks

The chromatographic separation of the sugars and the organic acids under the 3 different column temperatures were shown in Figure 3.7. At 45 and 65°C, there was poor separation of citric acid and glucose. This co-elution however, was resolved at 25°C, although the total elution time of 40 min at 25°C was longer than 30 min observed at the higher temperatures. In Figure 3.8, the examples of sample analyte peak identification in relation to the standard peaks were shown.

3.3.2.2.1.2 Retention time and recovery of analytes

The retention times, concentrations and the recovery efficiency of the pure standards and the spiked analytes were presented in Table 3.11. The average retention times of the analytes whether as pure or spiked standards were fairly constant. The recovery efficiency of the acid treated standards ranged between 72.73 - 90.70 and 74.80 - 89.34% for the pure and the spiked standards respectively, while the respective percentage recovery efficiency of the non-acid treated standards were 95.71 - 98.45 and 92.33 - 101.29%.

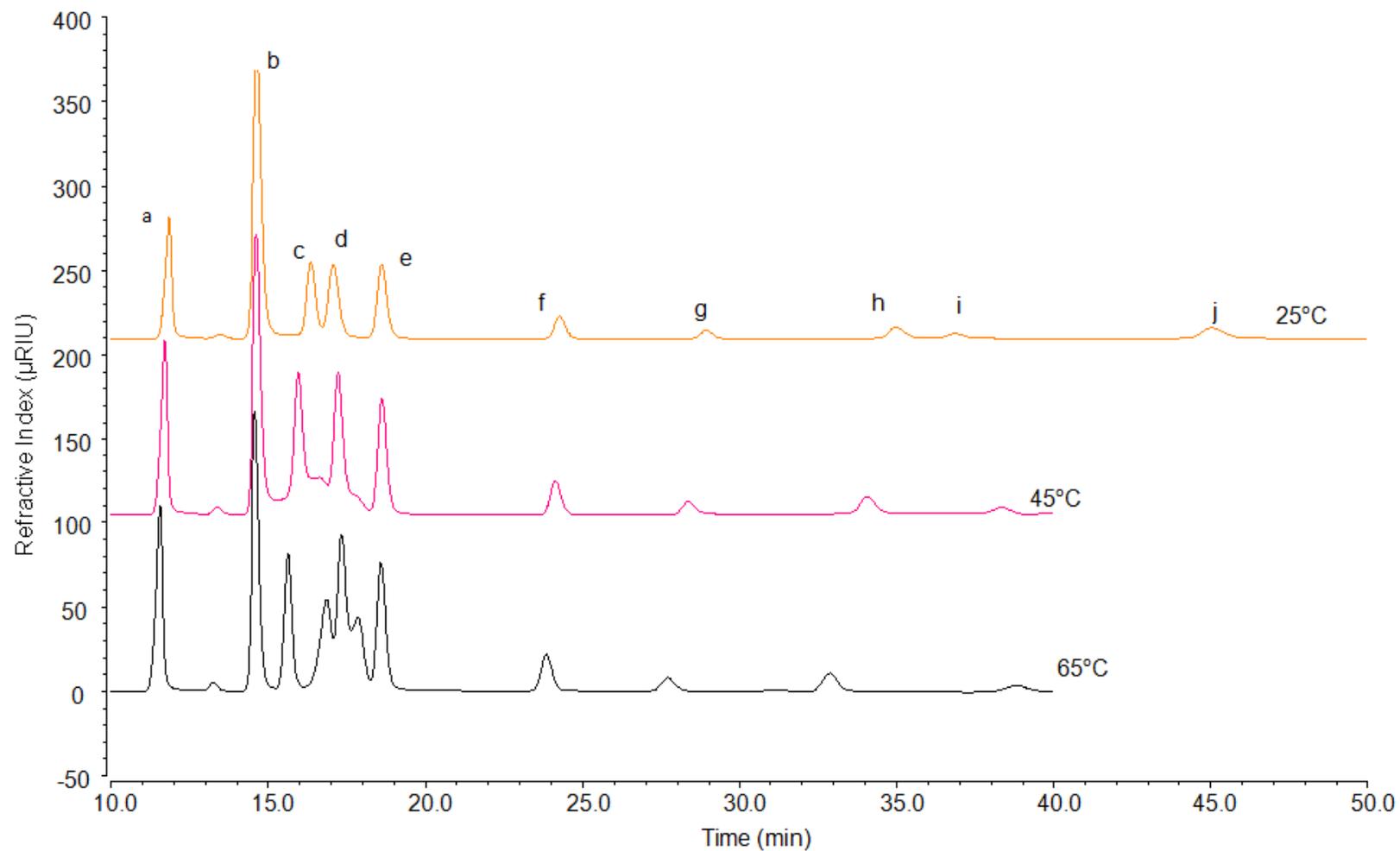


Figure 3.7: HPLC chromatogram showing the separation of standard analyte peaks at different column temperatures. The peak representation were: (a) H_2SO_4 ; (b) maltose; (c) citric acid; (d) glucose; (e) fructose; (f) lactic acid; (g) acetic acid; (h) propionic acid; (i) ethanol and (j) butyric acid

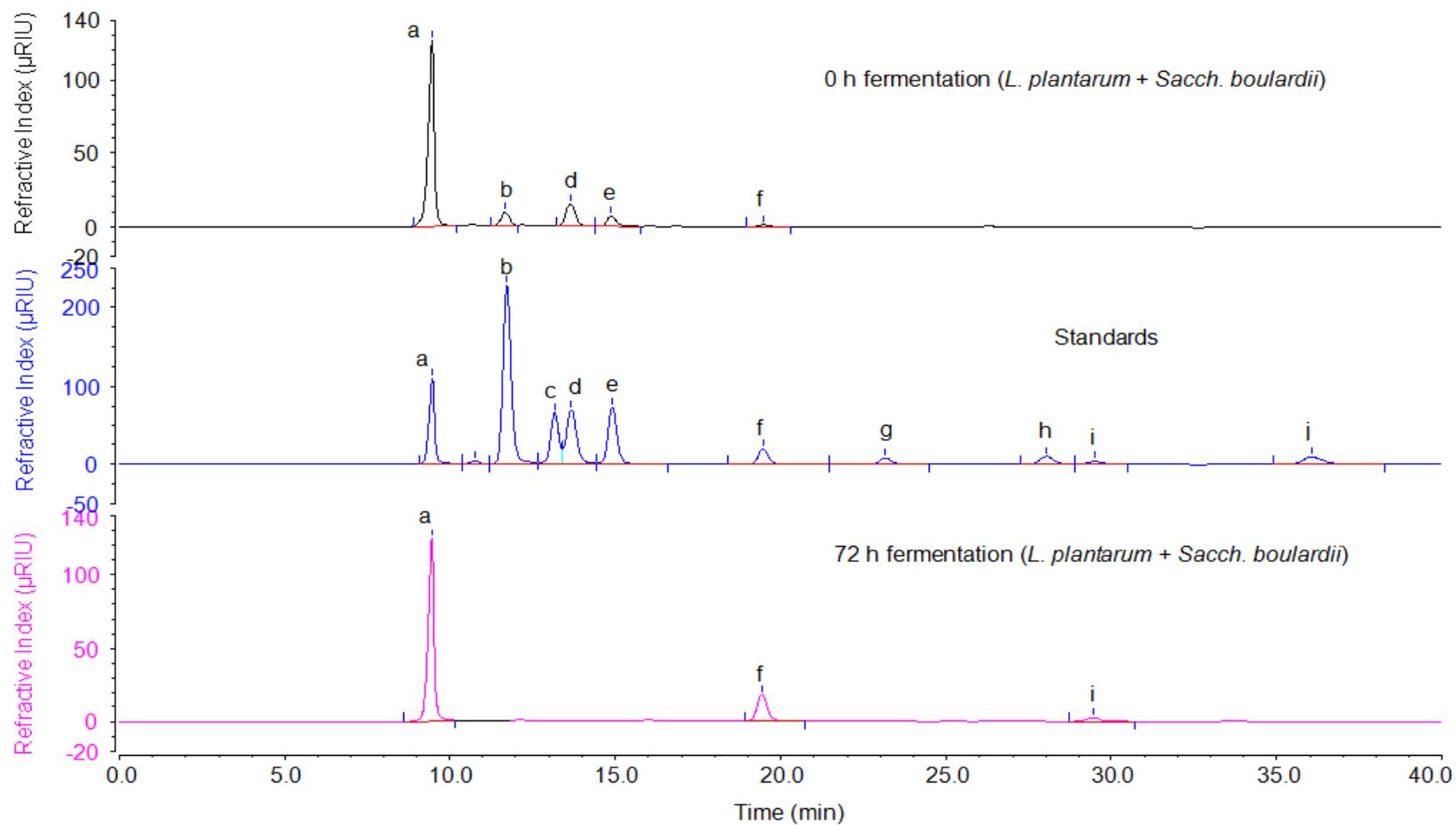


Figure 3.8: The HPLC chromatogram showing the sample analyte and pure standard peaks at 25°C. The peak representation were: (a) H₂SO₄; (b) maltose; (c) citric acid; (d) glucose; (e) fructose; (f) lactic acid; (g) acetic acid; (h) propionic acid; (i) ethanol and (j) butyric acid

Table 3.11: The retention times, concentrations and the recovery efficiency of the pure standards and the spiked analytes

Sample Concentration (mmol L ⁻¹)	Analytes	Average Retention times (Min)	Analyte Recovery (%)	
			Non-acid treated	Acid treated
Standards				
100	Acetic acid	18.53±0.01	97.90	74.39
	Butyric acid	27.03±0.02	96.98	72.88
	Citric acid	10.61±0.00	98.45	72.73
	Lactic acid	15.61±0.00	97.88	76.50
	Propionic acid	21.84±0.00	97.48	73.12
50	Acetic acid	18.53±0.01	96.08	72.94
	Butyric acid	27.04±0.03	95.89	74.79
	Citric acid	10.62±0.00	96.28	74.88
	Lactic acid	15.61±0.01	96.40	90.70
	Propionic acid	21.83±0.01	95.71	74.89
Spiked samples				
50	Acetic acid	18.53±0.00	92.33	74.08
	Butyric acid	27.05±0.04	97.12	76.74
	Citric acid	10.63±0.01	101.29	78.46
	Lactic acid	15.61±0.01	100.53	89.34
	Propionic acid	21.85±0.02	98.39	79.30

3.3.2.2.2 Akamu samples obtained from Nigeria

The lactic acid concentrations ($28.47 \geq LA \geq 78.68$ mmol kg⁻¹) of the various Nigerian *akamu* samples varied significantly ($p \leq 0.05$) from each other (Table 3.8). There were no detectable levels of the other test organic acids except for acetic acid concentrations of 79.44 ± 0.87 and 25.83 ± 9.12 mmol kg⁻¹ that were found in sample M3 and W1 respectively and propionic acid concentration of 13.29 ± 5.22 detected in W1. Thus, they differed from others in that respect. The partial correlation coefficient between the variables pH, TTA and lactic acid concentrations and their respective P-values obtained under 95% confidence interval were as follows: pH and TTA at a constant lactic acid level ($r_{it.l}$) = 0.78 ($p = 0.01$); pH and lactic acid at a constant TTA ($r_{il.t}$) = -0.68 ($p = 0.04$) while TTA and lactic acid with pH been held constant ($r_{tl.i}$) = 0.61 ($p = 0.08$). The probability of 0.08 was an indication of zero correlation at $p \leq 0.05$.

Sugars (glucose and maltose) detected in some of the samples were below the concentration of 5 mmol kg⁻¹. Samples E2, R1 and W1 had glucose levels of 2.24±0.45, 2.48±0.45 and 1.74±0.17 mmol kg⁻¹ respectively while maltose concentrations of 1.26±0.10 and 0.44±0.05 mmol kg⁻¹ was detected only in samples M3 and A1 respectively.

3.3.2.2.3 Screening of the LAB and yeast isolates from a selected akamu sample (M3)

Lactic acid (Table 3.12) and acetic acid were the test organic acids found in some of the samples. Samples AL4, AL5 and AL7 fermented by *Lactococcus lactis* subsp. *lactis* (NGL4), and *L. plantarum* strains (NGL5 and NGL7) respectively and the BSF had lactic acid production rates of 0.33±0.04, 0.84±0.17, 3.34±0.22 and 1.00±0.18 mmol L⁻¹ h⁻¹ respectively.

Acetic acid was not detected in all the samples after the first 12 h. Samples AL1, AL3 and AL8 fermented by *L. helveticus* strains (NGL1, NGL3 and NGL8), AL6 fermented by *L. acidophilus* (NGL6) and AL4 fermented by *Lactococcus lactis* subsp. *lactis* (NGL4) and the un-inoculated sample (control) did not show any level of acetic acid. After 24, 36, 48 and 72 h, the BSF had acetic acid levels of 6.43±4.36, 5.63±3.67, 10.99±1.23 and 33.76±4.92 mmol L⁻¹ respectively while sample AL2 fermented by one of the strains of *L. acidophilus* (NGL2) had acetic acid levels of 3.64±6.30, 3.71±5.45, 6.87±5.70 and 9.32±2.10 mmol L⁻¹ respectively. Samples fermented by the *L. plantarum* strains had acetic acid levels of 3.85±0.58 and 8.96±2.68 mmol L⁻¹ after 48 and 72 h for AL7 and 14.28±4.65 mmol L⁻¹ for AL5 after 72 h. In the yeast fermentation none of the test organic acids were found in the sample.

Table 3.12: Lactic acid (mmol L⁻¹) levels in ground maize slurries fermented by the LAB isolated from a selected *akamu* sample (M3)

Isolates	Sample code	Time (h)			
		24	36	48	72
<i>Lactobacillus helveticus</i>	AL1			13.85±15.04	38.21±9.69
<i>L. acidophilus</i>	AL2			50.60±19.80	138.50±19.80
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	AL4	7.92±1.01	9.57±1.61	5.57±0.12	
<i>L. plantarum</i>	AL5	20.18±4.01	30.03±0.81	38.22±1.40	45.74±3.00
<i>L. acidophilus</i>	AL6		3.80±1.64	24.43±3.86	43.53±4.13
<i>L. plantarum</i>	AL7	80.78±4.42	126.20±75.70	143.92±11.78	179.40±20.40
<i>L. helveticus</i>	AL8		4.15±0.52	20.15±2.95	37.28±0.70
Back Slop Fermentation	BSF	24.13±4.40	27.56±5.81	26.61±3.09	18.36±2.03

*No detectable level of lactic acid was observed after the first 12 h in all the samples except BSF that had lactic acid level of 9.42±1.99 mmol L⁻¹ after 12 h. Sample AL3 fermented by one of the *L. helveticus* strains (NGL3) and the un-inoculated samples (control) were found without lactic acid production. N=3±SD

3.3.2.2.4 Fermentation with the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* SB20

Propionic, butyric and acetic acids were not found in the samples except for acetic acid level of $4.69 \pm 0.07 \text{ mmol L}^{-1}$ observed in BSF after 72 h. Although BSF had significantly ($p \leq 0.05$) the highest levels of lactic acid (Table 3.13) towards the end of fermentation, it was significantly ($p \leq 0.05$) lower than the *L. plantarum* starter culture fermentations after 12 h. The levels of lactic acid in the *L. plantarum* single culture fermentations were significantly ($p \leq 0.05$) greater than the mixed culture fermentation with yeasts. Lactic acid production in yeasts single culture fermentation was $\leq 20.23 \text{ mmol L}^{-1}$. There was no significant ($p \leq 0.05$) change observed in the control.

Ethanol was not discovered in the *L. plantarum* strains single culture fermentation. The incorporation of yeast in the mixed culture fermentation resulted in significant production of ethanol towards the end of fermentation; however, the production of ethanol was significantly ($p \leq 0.05$) higher in yeast single culture fermentation than in the mixed culture fermentation. Ethanol was found in *Sacch. boulardii* (SB20) sample first after 12 h ($42.30 \pm 8.64 \text{ mmol L}^{-1}$) and reached a significant maximum level of $194.00 \pm 24.7 \text{ mmol L}^{-1}$ after 72h. Ethanol levels of 91.37 ± 6.39 and 127.79 ± 26.5 were detected in the *Sacch. boulardii* (SB20) single culture fermentation after 24 and 48 h respectively. *Candida tropicalis* single culture fermentation had an ethanol concentration of 24.40 ± 1.72 , 74.39 ± 3.75 and $77.50 \pm 4.14 \text{ mmol L}^{-1}$ after 24, 48 and 72 h respectively.

Ethanol concentrations in the LAB and yeast mixed culture fermentations varied significantly ($p \leq 0.05$). In the *L. plantarum* and *Sacch. boulardii* SB20 mixed culture fermentation only ALpTx+ASB20 had ethanol level of 9.24 ± 1.18 mmol L⁻¹ after 12 h while the respective ethanol concentrations after 24, 48 and 72 h were 18.31 ± 5.23 , 14.32 ± 2.12 , and 39.10 ± 3.70 mmol L⁻¹ for AL5+ASB20, 9.92 ± 0.52 , 18.83 ± 1.37 and 53.20 ± 10.54 mmol L⁻¹ for AL7+ASB20 and 16.87 ± 2.03 , 10.25 ± 2.54 and 52.90 ± 12.95 mmol L⁻¹ for ALpTx+ASB20. Ethanol was only discovered in ALpTx+AY1 at a concentration of 14.8 and 4.06 mmol L⁻¹ after 24 and 72 h respectively.

In Table 3.14 the concentration of glucose in the BSF and all the LAB starter culture fermentation increased significantly ($p \leq 0.05$) after 6 and 12 h before reduction to levels > 26 mmol L⁻¹ after 72 h. Glucose levels in the yeast single culture fermentation were reduced below 3.20 mmol L⁻¹ after 24 h. Other sugars: maltose and fructose (Table 3.15 and Table 3.16 respectively) were available at an initial concentration of ≤ 4.83 and ≥ 8.12 mmol L⁻¹ respectively but were reduced below 6 mmol L⁻¹ after 12 h. There was continuous significant increase in glucose (16.56 to 87.59 mmol L⁻¹) and fructose (8.91 to 9.46 mmol L⁻¹) with fairly constant level of maltose (about 4 - 5 mmol L⁻¹) observed in the control sample.

3.3.2.2.5 Fermentation with freeze dried cultures of *Lactobacillus plantarum* strains

The effect of fermentation with freeze dried *L. plantarum* strains on the concentration (mmol L⁻¹) of lactic acid and sugars (glucose, fructose and maltose) in ground maize slurries were presented in Table 3.17. The main

organic acid detected in the fermentation was lactic acid and its level increased significantly ($p \leq 0.05$) throughout the fermentation period from 4.20 - 185.32, 4.84 - 182.24, 5.66 - 160.82 mmol L^{-1} for AL5, AL7 and ALpTx respectively. The initial maltose level $< 25 \text{ mmol L}^{-1}$ that was detected in the samples were reduced below 3 mmol L^{-1} after 12 h while the initial fructose levels were below 3 mmol L^{-1} . Glucose found at a concentration $\leq 7.99 \text{ mmol L}^{-1}$ at the start of fermentation in all the samples were observed to have increased significantly ($p \leq 0.05$) to $\geq 23.24 \text{ mmol L}^{-1}$ after 6 h. Although maltose and fructose seemed to be completely utilized in the fermentation, glucose levels $> 7 \text{ mmol L}^{-1}$ were detected at the end of fermentation.

Table 3.13: Lactic acid levels (mmol L⁻¹) of ground maize slurries fermented by the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* SB20

Samples	Time (h)					
	0*	6	12	24	48	72
AL5	5.99±0.13	16.01±0.12 ^a	43.70±0.83 ^a	77.51±2.46 ^{abc}	101.94±1.90 ^a	109.24±1.44 ^{ab}
AL5+AY1	5.99±0.00	14.13±0.49 ^{abc}	44.96±1.18 ^a	77.54±2.25 ^{abc}	90.81±9.35 ^{abc}	100.05±11.96 ^{abc}
AL5+ASB20	6.11±0.06	15.14±0.69 ^{ab}	41.47±14.48 ^a	73.89±5.51 ^{abc}	91.66±3.51 ^{abc}	86.92±3.01 ^c
AL7	6.00±0.25	9.98±0.24 ^{de}	40.52±14.66 ^a	82.93±13.66 ^a	103.50±5.23 ^a	120.09±3.39 ^a
AL7+AY1	6.86±2.76	11.66±0.36 ^{bcd}	41.49±0.03 ^a	72.39±4.02 ^{abc}	82.27±13.26 ^{bc}	97.30±11.18 ^{ab}
AL7+AY20	6.04±0.21	10.65±0.50 ^{cd}	37.21±2.15 ^a	63.88±5.43 ^c	72.74±1.63 ^c	84.57±6.34 ^c
ALpTx	4.69±0.23	11.24±0.42 ^{cd}	36.98±0.43 ^a	73.98±2.05 ^{abc}	100.51±0.50 ^{ab}	110.63±6.12 ^{ab}
ALpTx +AY1	6.10±0.27	10.69±0.63 ^{cd}	37.63±2.42 ^a	75.68±3.62 ^{abc}	90.48±11.61 ^{abc}	94.50±12.82 ^{ab}
ALpTx +ASB20	5.45±0.93	8.42±4.05 ^{def}	37.72±1.81 ^a	66.84±0.25 ^{bc}	80.77±8.55 ^c	89.56±6.79 ^{bc}
BSF	4.45±0.19	5.15±0.21 ^{fg}	17.79±1.29 ^b	79.75±1.09 ^{ab}	123.46±5.88	156.61±9.61
AY1	5.89±0.39	5.29±0.62 ^{fg}	10.69±0.28 ^b	17.16±1.80 ^d	20.23±3.81 ^d	19.21±2.62 ^d
ASB20	5.40±0.14	6.75±0.20 ^{efg}	8.90±0.22 ^b	13.89±0.32 ^d	17.97±1.93 ^d	17.80±3.38 ^d
Control	4.77±0.19	4.75±0.28 ^g	3.88±0.18	2.86±0.63	5.85±0.57	6.53±0.52

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

*Values do not differ significantly

BSF - Back slop fermentation

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.14: Glucose levels (mmol L⁻¹) of ground maize slurries fermented by the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* SB20

Sample	Time (h)					
	0	6	12	24	48	72
AL5	15.52±0.91 ^{efg}	41.50±0.46 ^{bc}	43.14±0.94 ^{ab}	37.28±1.55 ^b	31.81±1.26 ^a	29.63±0.30 ^a
AL5+AY1	19.34±0.00 ^{cde}	43.97±0.32 ^{bc}	24.05±0.96 ^{def}	2.74±1.39 ^c		
AL5+ASB20	27.69±1.20 ^a	67.94±2.67 ^a	14.52±5.93 ^f			
AL7	15.24±0.87 ^{efg}	42.16±1.99 ^{bc}	41.23±8.48 ^{bc}	46.05±8.23 ^a	32.08±2.5 ^a	29.83±1.59 ^a
AL7+AY1	17.62±0.86 ^{cdef}	45.43±2.14 ^b	33.02±0.50 ^{cd}	2.75±1.10 ^c		
AL7+AY20	25.25±1.41 ^{ab}	60.34±2.17 ^a	26.89±1.46 ^{de}			
ALpTx	14.35±0.50 ^{fg}	39.14±1.24 ^{bc}	46.06±1.18 ^{ab}	40.05±1.29 ^{ab}	31.88±1.74 ^a	26.88±2.04 ^a
ALpTx +AY1	21.00±1.96 ^{bc}	40.59±4.20 ^{bc}	33.09±1.18 ^{cd}	3.20±0.54 ^c		
ALpTx +ASB20	20.73±4.15 ^{cd}	31.47±13.87 ^c	22.36±0.94 ^{ef}			
BSF	12.92±0.67 ^g	33.23±3.58 ^{bc}	47.12±4.31 ^{ab}	32.95±1.34 ^b	13.83±0.55	3.08±0.40
AY1	12.26±0.61 ^g	32.41±0.92 ^c	18.95±0.51 ^{ef}			
ASB20	13.69±0.79 ^{fg}	36.72±1.32 ^{bc}	23.39±2.16 ^{def}			
Control	16.56±0.26 ^{defg}	39.57±0.64 ^{bc}	52.48±1.06 ^a	65.75±0.67	80.12±8.86	87.59±9.40

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

BSF - Back slop fermentation

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.15: Fructose levels (mmol L⁻¹) of ground maize slurries fermented by the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* SB20

Sample	Time (h)					
	0	6	12	24	48	72
AL5	9.23±0.30 ^{bcd}	8.04±0.34 ^{ab}	3.54±0.22 ^d			
AL5+AY1	10.72±0.00 ^{ab}	9.93±0.92 ^{ab}				
AL5+ASB20	10.30±0.30 ^{abc}	10.44±0.53 ^a				
AL7	9.32±0.47 ^{bcd}	9.22±0.42 ^{ab}	5.41±2.13 ^{cd}			
AL7+AY1	12.12±1.82 ^a	9.93±0.60 ^{ab}	6.02±0.09 ^{bc}			
AL7+AY20	10.14±0.26 ^{abc}	9.56±0.12 ^{ab}				
ALpTx	8.62±0.26 ^{bcd}	8.29±0.23 ^{ab}	5.85±0.05 ^{bc}			
ALpTx +AY1	10.20±0.54 ^{abc}	9.42±0.73 ^{ab}	5.00±0.31 ^{cd}			
ALpTx +ASB20	9.10±1.85 ^{bcd}	7.20±3.22 ^b	4.17±0.25 ^{cd}			
BSF	7.66±0.46 ^d	7.55±0.73 ^{ab}	7.61±0.83 ^{ab}	4.12±0.35	2.70±0.93	
AY1	8.33±0.54 ^{cd}	7.94±0.61 ^{ab}	3.81±0.63 ^d			
ASB20	8.12±0.30 ^{cd}	8.29±0.60 ^{ab}	4.94±0.04 ^{cd}			
Control	8.91±0.11 ^{bcd}	7.67±0.30 ^{ab}	8.94±0.06 ^a	8.74±0.39	9.03±1.25	9.46±0.73

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

BSF - Back slop fermentation

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.16: Maltose levels (mmol L⁻¹) of ground maize slurries fermented by the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *Candidia tropicalis* (NGY1) and *Saccharomyces boulardii* SB20

Sample	Time (h)					
	0	6	12	24	48	72
AL5	4.00±0.15	2.88±0.13				
AL5+AY1	3.56±0.00	2.42±0.07				
AL5+ASB20	4.03±0.19	2.56±0.18				
AL7	4.15±0.32	3.69±0.13				
AL7+AY1	3.90±0.17	2.92±0.10				
AL7+AY20	4.83±0.47	3.62±1.14				
ALpTx	4.54±0.38	3.71±0.26				
ALpTx +AY1	4.29±0.18	3.13±0.29				
ALpTx +ASB20	4.14±0.80	2.27±1.16				
BSF	3.91±0.31	3.85±0.50	2.96±0.39			
AY1	4.39±0.27	4.29±0.14				
ASB20	4.28±0.31	4.65±0.29	2.12±0.18			
Control	4.78±0.16	4.46±0.26	4.30±0.07	4.06±0.35	3.92±0.36	4.13±0.52

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

BSF - Back slop fermentation

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.17: Levels of lactic acid, glucose, maltose and fructose concentrations (mmol L⁻¹) in ground maize slurries fermented by the freeze dried *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Organic acid /Sugars	Samples	Time (h)				
		0	6	12	24	48
Lactic acid	FL5	4.20±0.15 ^e	63.66±1.32 ^d	110.49±4.69 ^c	162.23±4.33 ^b	185.32±15.75 ^a
	FL7	4.84±0.24 ^e	65.14±1.87 ^d	109.77±0.47 ^c	158.39±13.28 ^b	182.24±8.78 ^a
	FLpTx	5.66±1.19 ^e	63.58±0.86 ^d	105.43±0.09 ^c	154.55±1.06 ^b	160.82±3.39 ^b
Glucose	FL5	5.87±0.92 ^e	24.22±0.87 ^a	23.24±0.62 ^a	14.23±0.58 ^{cd}	7.26±0.66 ^e
	FL7	5.39±0.24 ^e	24.77±0.76 ^a	25.46±0.51 ^a	18.25±2.78 ^b	12.22±0.94 ^d
	FLpTx	7.99±1.78 ^e	25.87±0.87 ^a	23.80±0.22 ^a	17.86±0.17 ^b	15.53±0.54 ^{bc}
Maltose	FL5	22.16±4.02 ^a	10.95±0.19 ^b	3.89±0.14 ^c		
	FL7	24.43±0.54 ^a	10.47±0.38 ^b	3.84±0.35 ^c		
	FLpTx	23.58±0.84 ^a	10.18±0.28 ^b	3.40±0.04 ^c		
Fructose	FL5	1.34±0.25 ^b				
	FL7	1.41±0.14 ^b				
	FLpTx	2.75±1.31 ^a				

Values with the same superscript along the same row and column for the test acid and individual sugars do not differ significantly (p≤0.05). n=3±SD

FLpTx - Samples fermented by commercial probiotic *L. plantarum* strain (LpTx)

FL5 & FL7 - Samples fermented by Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

3.3.2.3 Microbial Analysis

3.3.2.3.1 Screening of LAB and yeasts isolates from a selected akamu sample (M3)

In Table 3.18, variations were observed in the LAB viable counts (Log_{10} CFU mL^{-1}) during the fermentation period. The initial viable LAB count for all the fermentation ranged between 3.72 and 5.64 Log_{10} CFU mL^{-1} for sample AL1 and AL7 respectively, with significant maximum viable count between 8.48 - 9.52 Log_{10} CFU mL^{-1} for AL8 and AL7 respectively towards the end of fermentation except for AL3. AL3 had viable count of 4.22 Log_{10} CFU mL^{-1} after 12 h, with an increase of 2 Log cycle after 24 h. This increase remained stationary throughout the fermentation and the rate of growth of *L. helveticus* NGL3 (0.03 h^{-1}) from the time of detection to end of fermentation (after 72 h) was significantly ($p \leq 0.05$) the least. *L. plantarum* NGL5 in AL5 had significantly ($p \leq 0.05$) the highest growth rate. The BSF sample had significantly ($p \leq 0.05$) the highest initial viable count of 7.52 Log_{10} CFU mL^{-1} and maximum viable count of 9.84 Log_{10} CFU mL^{-1} after 24 h before setting on decline, with growth rate of 0.11 h^{-1} . Summary of the LAB growth model parameters were presented in Table 3.19.

Continuous increase in yeast counts from about 3 - 8 Log_{10} CFU mL^{-1} was observed after 72 h of fermentation (Table 3.20). The yeasts growth model parameters were presented in Table 3.21 with growth rates between 0.12 and 0.19 h^{-1} for NGY4 and NGY2 in samples AY4 and AY2 respectively. Yeast was detected in BSF sample after 48 h. Both LAB and yeast were not found in the control sample.

Table 3.18: Viable LAB count (Log_{10} CFU mL^{-1}) in ground maize slurries fermented by isolates from a selected *akamu* sample (M3)

Isolates	Sample codes	Time (h)						Rate (μ)*
		0	6	12	24	48	72	
<i>Lactobacillus helveticus</i>	AL1	3.72±0.37 ^c	4.27±0.14 ^d	4.99±0.09	6.11±0.15 ^c	8.26±0.51 ^d	8.76±0.14 ^{bc}	0.10±0.01 ^b
<i>L. acidophilus</i>	AL2	4.37±0.23 ^{cd}	4.43±0.11 ^{cd}	5.28±0.06 ^a	5.30±0.15	8.39±0.21 ^{cd}	8.82±0.01 ^b	0.07±0.00 ^{bc}
<i>L. helveticus</i>	AL3 [†]	<3	<3	4.22±0.00	6.16±0.14 ^c	6.19±0.02 ^d	6.12±0.05	0.03±0.00 ^c
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	AL4	5.39±0.08 ^a	5.28±0.15	6.20±0.02	8.18±0.12	9.22±0.09 ^a	9.38±0.05 ^a	0.12±0.05 ^{ab}
<i>L. plantarum</i>	AL5	5.15±0.06 ^{ab}	6.37±0.15	7.22±0.00	9.09±0.05 ^a	9.26±0.07 ^a	9.27±0.05 ^a	0.18±0.00 ^a
<i>L. acidophilus</i>	AL6	4.46±0.25 ^{bc}	4.92±0.10 ^b	5.56±0.01	6.79±0.13 ^b	8.80±0.04 ^{abc}	8.96±0.04 ^b	0.10±0.01 ^b
<i>L. plantarum</i>	AL7	5.64±0.69 ^a	7.48±0.17 ^a	7.45±0.12	8.89±0.04 ^a	9.97±0.07	9.52±0.09 ^a	0.10±0.04 ^b
<i>L. helveticus</i>	AL8	4.48±0.07 ^{bc}	4.70±0.02 ^{bc}	5.30±0.00 ^a	6.98±0.07 ^b	8.56±0.11 ^{bcd}	8.48±0.13 ^c	0.13±0.02 ^{ab}
Back slope fermentation	BSF	7.52±0.10	7.69±0.09 ^a	9.68±0.02	9.84±0.08	9.00±0.00 ^a ^b	8.97±0.22 ^b	0.11±0.00 ^b

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

*Growth rate after 72 h from Baranyi and Roberts, (1994) model.

[†]Data after 12 h when the organism was detected in AL3 samples was utilised in growth rate modelling.

Table 3.19: Growth model parameters of LAB in ground maize slurries fermented by the isolates from a selected *akamu* sample (M3)

LAB	Curve	^a Experimental data		Modelling parameters						
				^b Curvature parameters		^c Primary parameters			^d Statistics	
		yDatMin	yDatMax	mCurv	nCurv	rate	lag	y0	se(fit)	R ²
<i>Lactobacillus helveticus</i>	NGL1	3.72±0.37 ^d	8.76±0.14 ^{de}	10	0	0.10±0.01 ^b		3.73±0.21 ^f	0.18±0.08 ^{bc}	0.99±0.01 ^a
<i>L. acidophilus</i>	NGL2	4.30±0.14 ^d	8.82±0.01 ^{cd}	0	0	0.07±0.00 ^{bc}		4.24±0.07 ^{ef}	0.59±0.05 ^{ab}	0.91±0.01 ^a
<i>L. helveticus</i>	NGL3	4.22±0.00 ^d	6.22±0.05 ^f	0	0	0.03±0.00 ^c	9.76±0.02	4.22±0.00 ^d	0.17±0.11 ^{bc}	0.87±0.08 ^a
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	NGL4	5.23±0.06 ^{bc}	9.38±0.05 ^b	10	0	0.12±0.05 ^{ab}		5.27±0.33 ^d	0.46±0.39 ^{ab}	0.91±0.11 ^a
<i>L. plantarum</i>	NGL5	5.15±0.06 ^{bc}	9.30±0.00 ^b	10	0	0.18±0.00 ^a		5.20±0.06 ^d	0.11±0.05 ^c	1.00±0.00 ^a
<i>L. acidophilus</i>	NGL6	4.46±0.25 ^{cd}	8.96±0.04 ^c	10	0	0.10±0.01 ^b		4.37±0.14 ^e	0.11±0.05 ^c	1.00±0.00 ^a
<i>L. plantarum</i>	NGL7	5.64±0.69 ^b	9.97±0.07 ^a	10	0	0.10±0.04 ^b		6.35±0.47 ^c	0.67±0.41 ^a	0.81±0.19 ^a
<i>L. helveticus</i>	NGL8	4.48±0.07 ^{cd}	8.59±0.05 ^e	10	1	0.13±0.02 ^{ab}	6.69±0.50	4.38±0.15 ^e	0.12±0.14 ^c	0.99±0.01 ^a
Back slop fermentation	BSF	7.52±0.10 ^a	9.84±0.08 ^a	0	0	0.11±0.00 ^b		7.56±0.04 ^b	0.72±0.03 ^a	0.66±0.03 ^b

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable counts ($\text{Log}_{10} \text{CFU mL}^{-1}$)

^b mCurv and nCurv are the curvature parameters at the beginning and end of the linear phase respectively

^c Rate: the potential maximum growth rate of the microorganism (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper asymptote of the sigmoid curve. No value was recorded for yEnd; Lag: The lag phase duration (h)

^d Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

Table 3.20: Viable yeast count (Log_{10} CFU mL^{-1}) in ground maize slurries fermented by the isolates from a selected *akamu* sample (M3)

Isolates	Sample codes	Time (h)						Rate (μ)*
		0	6	12	24	48	72	
<i>Candida tropicalis</i>	AY1	3.72±0.10 ^{bc}	4.65±0.03 ^a	5.75±0.02 ^a	7.65±0.02 ^a	8.10±0.02 ^a	8.24±0.06 ^a	0.17±0.01 ^a
<i>C. albicans</i>	AY2	3.54±0.01 ^c	4.06±0.03	5.19±0.05 ^c	7.55±0.02 ^a	8.34±0.07 ^a	8.36±0.10 ^a	0.19±0.02 ^a
<i>C. albicans</i>	AY3	4.15±0.03 ^a	4.90±0.05	5.63±0.06 ^{ab}	7.52±0.03 ^a	8.02±0.06 ^a	8.11±0.00 ^a	0.14±0.00 ^{ab}
<i>C. albicans</i>	AY4	3.93±0.06 ^{ab}	4.68±0.02 ^a	5.37±0.15 ^{bc}	6.65±0.35	8.01±0.06 ^a	8.16±0.06 ^a	0.12±0.02 ^b
Back Slop Fermentation	BSF [§]	<3	<3	<3	<3	6.53±0.17	7.92±0.25 ^a	0.06±0.01 ^c

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). $n=3 \pm \text{SD}$

*Growth rate after 72 h from Baranyi and Roberts, (1994) model. [§]Data between 48 and 72 h when yeast was detected in BSF sample was utilised in growth rate modelling.

Table 3.21: Growth model parameters of the yeast in maize slurries fermented by the isolates from a selected *akamu* sample (M3)

Isolates	Curve	Modelling parameters							
		^a Experimental data		^b Curvature parameters		^c Primary parameters		^d Statistics	
		yDatMin	yDatMax	mCurv	nCurv	rate	y0	se(fit)	R ²
<i>Candida tropicalis</i>	NGY1	3.72±0.17 ^{bc}	8.24±0.10 ^{ab}	10	0	0.17±0.01 ^a	3.68±0.11 ^{bc}	0.10±0.07 ^{ab}	1.00±0.00 ^a
<i>C. albicans</i>	NGY2	3.54±0.02 ^c	8.41±0.15 ^a	10	0	0.19±0.02 ^a	3.38±0.18 ^c	0.21±0.12 ^a	0.99±0.01 ^a
<i>C. albicans</i>	NGY3	4.15±0.05 ^a	8.12±0.02 ^b	10	0	0.14±0.00 ^{ab}	4.07±0.09 ^a	0.13±0.04 ^{ab}	0.99±0.00 ^a
<i>C. albicans</i>	NGY4	3.93±0.11 ^{ab}	8.16±0.11 ^{ab}	10	0	0.12±0.02 ^b	3.94±0.11 ^{ab}	0.16±0.03 ^{ab}	0.99±0.00 ^a
Back Slop Fermentation	BSF [§]	6.53±0.30	7.92±0.43	0	0	0.06±0.01	6.53±0.30	0	1.00±0.00 ^a

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Estimated initial and maximum viable counts ($\text{Log}_{10} \text{CFU mL}^{-1}$)

^b mCurv and nCurv are the curvature parameters at the beginning and end of the linear phase respectively

^c Rate: the potential maximum growth rate of the microorganism (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper asymptote of the sigmoid curve, had no value; Lag: The lag phase duration (h) had no values

^d Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

[§] Data between 48 and 72 h when yeast was detected in back slop fermentation (BSF) sample was utilised in growth rate modelling.

3.3.2.3.2 Fermentation with the single or mixed starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx), *C. tropicalis* (NGY1) and *Sacch. boulardii* SB20

The viable count and the growth model parameters of the *Lactobacillus plantarum* strains in the single or mixed culture fermentation with *Candida tropicalis* or *Saccharomyces boulardii* SB20 were presented in Table 3.22 and Table 3.23 respectively, while the viable count and growth model parameters of the *C. tropicalis* or *Sacch. boulardii* SB20 in the single or mixed culture fermentation with *L. plantarum* strains were shown in Table 3.24 and Table 3.25 respectively.

There was significant growth of the *L. plantarum* from about 7 - 9 Log₁₀ CFU mL⁻¹. The mixed culture fermentation with yeast did not have any significant influence on the *L. plantarum* growth. Continuous increase in viable count from about 5 - 8 Log₁₀ CFU mL⁻¹ was observed for both yeasts in single culture fermentation while a decline was observed in the mixed *L. plantarum* and yeast culture fermentation after 12 and 24 h for *Sacch. boulardii* (SB20) and *C. tropicalis* (NGY1) respectively. However, yeast growth rate in the mixed fermentation (≥ 0.21 h⁻¹ for NGY1 and ≥ 0.14 h⁻¹ for SB20) did not differ significantly ($p \leq 0.05$) from the single culture fermentation (0.22 and 0.18 h⁻¹ for NGY1 and SB20 respectively) except for *Sacch. boulardii* in mixed fermentation with NGL5 that had significantly lower growth rate of 0.01 h⁻¹. Generally, the growth of NGY1 was significantly ($p \leq 0.05$) higher than SB20 in either the single or mixed culture fermentation.

Table 3.22: Viable *Lactobacillus plantarum* strains count (Log₁₀ CFU mL⁻¹) in ground maize slurries fermented by the single or mixed starter cultures with *Candida tropicalis* or *Saccharomyces boulardii* SB20

Samples	Time (h)						Growth rate (μ)
	0	6	12	24	48	72	
AL5	7.23±0.08 ^a	8.24±0.02 ^a	9.03±0.03 ^a	9.09±0.06 ^{ab}	8.84±0.07 ^{bc}	8.56±0.10 ^{bcde}	0.17±0.01 ^{abc}
AL5+AY1	7.19±0.03 ^{ab}	8.16±0.02 ^{abc}	8.98±0.02 ^a	9.04±0.02 ^{ab}	8.66±0.06 ^c	8.52±0.04 ^{de}	0.17±0.00 ^{abc}
AL5+ASB20	7.17±0.03 ^{ab}	8.18±0.03 ^{ab}	9.00±0.03 ^{ab}	8.94±0.02 ^{bc}	8.70±0.05 ^c	8.43±0.03 ^e	0.17±0.01 ^{abc}
AL7	6.94±0.04 ^{cde}	7.89±0.13 ^{cde}	8.95±0.10 ^{ab}	9.17±0.07 ^a	9.11±0.05 ^a	8.74±0.05 ^{ab}	0.18±0.01 ^{ab}
AL7+AY1	7.06±0.04 ^{bc}	8.03±0.03 ^{abcd}	8.76±0.03 ^c	9.04±0.02 ^{ab}	8.92±0.05 ^{ab}	8.80±0.05 ^a	0.15±0.00 ^{bc}
AL7+ASB20	7.06±0.05 ^{bcd}	7.92±0.05 ^{bcde}	8.83±0.05 ^{bc}	9.04±0.04 ^{ab}	8.91±0.10 ^b	8.72±0.07 ^{abc}	0.16±0.00 ^{bc}
ALpTx	6.82±0.09 ^e	7.76±0.08 ^{de}	8.66±0.02 ^{cd}	8.86±0.04 ^{cd}	8.75±0.03 ^{bc}	8.62±0.08 ^{abcde}	0.16±0.01 ^{bc}
ALpTx+AY1	6.88±0.09 ^e	7.87±0.14 ^{de}	8.56±0.07 ^d	8.84±0.08 ^{cd}	8.74±0.09 ^{bc}	8.69±0.11 ^{abcd}	0.16±0.00 ^{bc}
ALpTx+ASB20	6.89±0.04 ^{de}	7.73±0.20 ^e	8.53±0.05 ^d	8.65±0.08 ^e	8.75±0.05 ^{bc}	8.50±0.02 ^e	0.15±0.01 ^c
BSF	5.72±0.03	6.47±0.05	8.01±0.11	8.71±0.06 ^{de}	8.75±0.09 ^{bc}	8.54±0.01 ^{cde}	0.19±0.01 ^a

Values that share the same superscript in the same column do not differ significantly (p≤0.05). N=3±SD.

BSF - Back slop fermentation

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.23: Growth model parameters of *Lactobacillus plantarum* strains in ground maize slurries fermented by single or mixed starter cultures with *Candida tropicalis* or *Saccharomyces boulardii* SB20

Curve	^a Experimental data		Modelling parameters				
	yDatMin	yDatMax	^b Primary parameters			^d Statistics	
			rate	y0	yEnd	se(fit)	R ²
NG5	7.23±0.08 ^a	9.09±0.05 ^{ab}	0.17±0.01 ^{abc}	7.22±0.08 ^a	8.88±0.05 ^{bc}	0.24±0.05 ^{ab}	0.88±0.06 ^{bc}
NGL5+SB20	7.17±0.03 ^{ab}	9.00±0.03 ^b	0.17±0.01 ^{abc}	7.17±0.03 ^{ab}	8.77±0.02 ^{cde}	0.26±0.01 ^a	0.85±0.01 ^c
NGL5+NGY1	7.19±0.03 ^{ab}	9.04±0.02 ^b	0.17±0.00 ^{abc}	7.18±0.03 ^{ab}	8.80±0.02 ^{bcd}	0.26±0.03 ^a	0.86±0.03 ^c
NG7	6.94±0.04 ^{cde}	9.17±0.07 ^a	0.18±0.01 ^{ab}	6.91±0.07 ^{cde}	9.02±0.05 ^a	0.20±0.06 ^{abcd}	0.95±0.03 ^{ab}
NGL7+SB20	7.06±0.05 ^{bcd}	9.04±0.03 ^b	0.16±0.00 ^{bc}	7.03±0.04 ^{bcd}	8.90±0.01 ^b	0.15±0.02 ^{abcd}	0.96±0.01 ^a
NGL7+NGY1	7.06±0.04 ^{bc}	9.04±0.02 ^b	0.15±0.00 ^c	7.08±0.05 ^{abc}	8.91±0.04 ^{ab}	0.10±0.02 ^{cd}	0.98±0.01 ^a
LpTx	6.82±0.09	8.86±0.04 ^c	0.16±0.01 ^{bc}	6.81±0.10 ^e	8.75±0.03 ^{de}	0.10±0.04 ^{cd}	0.98±0.01 ^a
LpTx+SB20	6.89±0.04	8.75±0.05 ^c	0.15±0.01 ^c	6.87±0.04 ^{de}	8.63±0.04 ^f	0.13±0.05 ^{bcd}	0.97±0.02 ^a
LpTx+NGY1	6.88±0.09	8.86±0.04 ^c	0.16±0.00 ^{bc}	6.89±0.10 ^{de}	8.74±0.06 ^{def}	0.10±0.07 ^{cd}	0.98±0.03 ^a
BSF	5.72±0.03	8.78±0.04 ^c	0.19±0.01 ^a	5.59±0.04	8.67±0.03 ^{ef}	0.22±0.04 ^{abc}	0.97±0.01 ^a

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable count

^b Rate: potential maximum growth rate ($\text{Log}_{10} \text{CFU mL}^{-1} \text{h}^{-1}$); y0: initial point of the sigmoid curve; yEnd: upper or lower asymptote of the sigmoid curve depending on growth trend. There was no Lag time observed.

^c Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

^d The curvature parameters (mCurv and nCurv) at the beginning and end of the linear phase were 10 and 0 respectively except for NGL5+SB20 with mCurv of 0
 NGL5 & NGL7 - Nigerian fermented maize *L. plantarum* strains and LpTx - commercial probiotic *L. plantarum* strain
 NGY1 & SB20 - Nigerian fermented maize *C. tropicalis* and probiotic *Sacch. boulardii* SB20

Table 3.24: Viable count of *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* SB20 (Log_{10} CFU mL^{-1}) in ground maize slurries fermented by the single or mixed starter culture with *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Samples	Time (h)						Growth rate (μ)
	0	6	12	24	48	72	
AY1	5.25±0.04 ^{bc}	6.64±0.11 ^a	7.41±0.05 ^a	7.67±0.03 ^{ab}	7.72±0.05 ^a	7.77±0.09 ^{ab}	0.22±0.04 ^{abc}
AL5+AY1	5.26±0.03 ^{bc}	6.48±0.05 ^a	7.14±0.05 ^b	7.17±0.10 ^{bc}	6.80±0.28 ^b	6.69±0.09 ^c	0.21±0.01 ^{abc}
AL7+AY1	5.21±0.01 ^c	6.64±0.17 ^a	7.09±0.02 ^b	7.09±0.02 ^c	6.67±0.03 ^b	6.40±0.12 ^c	0.26±0.04 ^{ab}
ALpTx+AY1	5.02±0.08 ^d	6.59±0.19 ^a	7.11±0.11 ^b	7.14±0.11 ^c	6.78±0.33 ^b	6.84±0.35 ^c	0.27±0.05 ^a
ASB20	5.43±0.02 ^a	6.40±0.06 ^a	7.58±0.02	7.81±0.02 ^a	8.05±0.04 ^a	8.08±0.06 ^a	0.18±0.00 ^{bc}
AL5+ASB20	5.44±0.04 ^a	6.39±0.06 ^a	7.40±0.05 ^a	6.49±0.03 ^d	6.77±0.08 ^b	7.05±0.55 ^{bc}	0.01±0.01 ^d
AL7+ASB20	5.45±0.03 ^a	6.36±0.00 ^a	7.35±0.04 ^a	6.76±0.06 ^{cd}	6.77±0.16 ^b	6.53±0.07 ^c	0.16±0.00 ^c
ALpTx+ASB20	5.35±0.02 ^{ab}	6.30±0.18 ^a	7.37±0.03 ^a	6.94±0.45 ^{cd}	6.67±0.04 ^b	6.48±0.36 ^c	0.14±0.05 ^c

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). $n=3 \pm \text{SD}$

BSF - Back slop fermentation

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

AY1 - Samples fermented by Nigerian fermented maize *C. tropicalis* (NGY1)

ASB20 - Samples fermented by probiotic *Sacch. boulardii* SB20

Table 3.25: Growth model parameters of *Candida tropicalis* (NGY1) and *Saccharomyces boulardii* (SB20) in ground maize slurries fermented by the single or mixed starter cultures with *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Curve	^a Experimental data		Modelling parameters				
			^b Primary parameters			^c Statistic	
	yDatMin	yDatMax	rate	y0	yEnd	se(fit)	R ²
NGY1	5.25±0.04 ^{bc}	7.79±0.06 ^b	0.22±0.04 ^{abc}	5.28±0.06 ^{ab}	7.69±0.08 ^a	0.12±0.04 ^b	0.98±0.01 ^a
NGL5+NGY1	5.26±0.03 ^{bc}	7.18±0.09 ^{de}	0.21±0.01 ^{abc}	5.26±0.03 ^{ab}	6.95±0.09 ^a	0.27±0.03 ^{ab}	0.86±0.04 ^{ab}
NGL7+NGY1	5.21±0.01 ^c	7.09±0.02 ^e	0.26±0.04 ^{ab}	5.21±0.01 ^{ab}	6.81±0.02 ^a	0.34±0.06 ^a	0.76±0.08 ^{ab}
LpTx+NGY1	5.02±0.08	7.20±0.05 ^{de}	0.27±0.05 ^a	5.01±0.08 ^b	6.97±0.11 ^a	0.27±0.10 ^{ab}	0.88±0.07 ^{ab}
SB20	5.43±0.02 ^a	8.08±0.06 ^a	0.18±0.00 ^{bc}	5.39±0.02 ^a	7.98±0.03 ^a	0.14±0.02 ^b	0.98±0.00 ^a
NGL5+SB20	5.44±0.04 ^a	7.49±0.17 ^c	0.01±0.01	6.26±0.09	0.00±0.00	0.66±0.03	0.83±0.22 ^{ab}
NGL7+SB20	5.45±0.03 ^a	7.35±0.04 ^{cd}	0.16±0.00 ^c	5.44±0.03 ^a	6.85±0.02 ^a	0.36±0.01 ^a	0.77±0.00 ^b
LpTx+SB20	5.35±0.02 ^{ab}	7.41±0.08 ^{cd}	0.14±0.05 ^c	5.47±0.26 ^a	4.55±3.94 ^a	0.43±0.12 ^a	0.70±0.03 ^b

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable count

^b Rate: potential maximum growth rate (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper or lower asymptote of the sigmoid curve depending on growth trend. There was no Lag time observed.

^c Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

^d The curvature parameters (mCurv and nCurv) at the beginning and end of the linear phase were 10 and 0 respectively except for NGL5+SB20 with mCurv of 0
 NGL5 & NGL7 - Nigerian fermented maize *L. plantarum* strains and LpTx - commercial probiotic *L. plantarum* strain
 NGL7 & SB20 - Nigerian fermented maize *C. tropicalis* and probiotic *Sacch. boulardii* SB20

3.3.2.3.3 Fermentation with freeze dried cultures of *Lactobacillus plantarum* strains

In Table 3.26, the freeze dried *L. plantarum* strains were able to grow from an initial count of 8 Log₁₀ CFU mL⁻¹ to a maximum count of 9 Log₁₀ CFU mL⁻¹ after 12 h with growth rate ≥0.066 h⁻¹ before setting on decline. The microbial model parameters were presented in Table 3.27.

Table 3.26: Viable *Lactobacillus plantarum* count in ground maize slurries fermented by the freeze dried starter cultures

Samples	Time					Growth rate	Death rate
	0	6	12	24	48		
FLpTx	8.24±0.00	8.86±0.01	9.04±0.01	8.87±0.01	8.30±0.01	0.07	-0.02
FL5	8.27±0.00	8.96±0.01	9.18±0.01	9.03±0.01	9.03±0.02	0.08	0.00
FL7	8.41±0.00	8.93±0.00	9.24±0.01	9.13±0.01	8.98±0.00	0.07	-0.02

Values differed significantly (p≤0.05). N=3±SD

Table 3.27: Microbial model parameters of *Lactobacillus plantarum* strains in ground maize slurries fermented by freeze dried starter cultures

Curve	^a Experimental data		Modelling parameters			
	yDatMin	yDatMax	^b Primary parameters		^c Statistic	
			rate	y0	se(fit)	R ²
Growth						
NGL5	8.27±0.00	9.18±0.01	0.08±0.00	8.27±0.00	0.09±0.01	0.94±0.01
NGL7	8.41±0.00	9.24±0.01	0.07±0.00	8.45±0.01	0.09±0.00	0.96±0.00
LpTx	8.24±0.00	9.04±0.01	0.07±0.00	8.31±0.00	0.18±0.01	0.82±0.02
Death						
NGL5	9.02±0.02	9.18±0.01	-0.004±0.00	9.14±0.01	0.08±0.00	0.21±0.12
NGL7	8.98±0.00	9.24±0.01	-0.01±0.00	9.23±0.00	0.01±0.01	0.98±0.02
LpTx	8.30±0.01	9.04±0.01	-0.02±0.00	9.07±0.00	0.06±0.01	0.98±0.01

Values that share the same superscript in the same column do not differ significantly (p≤0.05) N=3±SD

^a yDatMin and yDatMax: Estimated initial and maximum viable count

^b Rate: potential maximum growth rate (h⁻¹); y0: initial point of the sigmoid curve; yEnd: upper of lower asymptote of the sigmoid curve depending on growth trend, had no value; There was no Lag time observed.

^c Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

The curvature parameters (mCurv and nCurv) at the beginning and end of the linear phase were 10 and 0 respectively

NGL5 & NGL7 - Nigerian fermented maize *L. plantarum* strains and LpTx - commercial probiotic *L. plantarum* strain

3.4 Discussion

3.4.1 Microbial population of the traditional *akamu* samples

Different lactic acid bacteria (LAB) and yeast were identified from the Nigerian traditional *akamu* samples using both the culture-independent and culture-dependent methods. The absence of the growth of Enterobacteriaceae, *Yersinia*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Listeria*, and *Escherichia coli* in the traditional *akamu* samples may be attributed to the samples acidity (Table 3.8). The product quality and safety may however be influenced by a few other factors such as predominant microorganisms in the grains at the time of preparation of the product in addition to environmental factors and the level of hygienic practices by the food handlers. Contamination may also occur through different channels before, during, and after preparation. Enteropathogenic microorganisms from polluted water, dirty utensils, insects, pests, domesticated animals, excreta from the environment could be introduced during and after preparation. Storage at ambient temperature due to economic constraints may favour the growth of the pathogens and/or formation of their toxins. Insufficient cooking or reheating of the probably uncovered foods prior to consumption may lead to food borne illnesses (Afifi et al., 1998, Lawal et al., 2009).

Although none of the pathogens tested for in the Nigerian *akamu* samples were detected, studies on the improvement of the safety of the *akamu* products would still be important considering the possibilities of contamination at any point from the preparation to the consumption of the product. In a study by Nyatoti et al., (1997) untyped strains of *E. coli* from *Mahewu* and thick maize porridges were isolated. Kunene et al., (1999) identified *Bacillus cereus*, *E. coli*, *Clostridium perfringens*, *Listeria monocytogenes*, *Aeromonas* spp., *Salmonella*

spp., *Staphylococcus aureus*, *Shigella* spp. and *Yersinia* spp. in sorghum powder samples used for the fermentation of sorghum-based weaning food, while only *B. cereus* and *E. coli* were isolated from the corresponding fermented porridge samples with none of the pathogens tested for detected in the cooked fermented porridge samples.

3.4.1.1 The DGGE banding pattern

This study employed PCR-DGGE analysis in the identification of the lactic acid bacteria population present in ten different Nigerian *akamu* samples suspected to be more than 3 days old. The active LAB and yeast isolated from two of the *akamu* samples using conventional microbiological methods were genotypically identified using direct PCR and sequencing analysis. The use of denaturing gradient gel electrophoresis (DGGE) analysis enables the identification of the presence and relative abundance of different species of microorganisms in a microbial population (Muyzer et al., 1993). The mixture of sequences of the various bacteria community in the DNA template of the samples were separated out as bands in the parallel denaturing gel gradient. The relative intensity of each band and its position suggested the common occurrence of particular species of LAB present in the *akamu* samples.

Matching of the relative band positions of previously identified pure LAB cultures with the samples' band pattern gave presumptive idea of what the organisms most likely were. This was against the backdrop that DNA fragment with identical base-pair sequences would have identical melting temperatures and thus stop migrating at a particular position once that temperature was reached (Muyzer et al., 1993). Evidenced in this study was the identification of

the excised DGGE fragments 7A and 2B (Figure 3.3A and Figure 3.3B respectively) as *L. plantarum* and fragment 4B (Figure 3.3B) as *L. fermentum* with their respective band positions matching the band position of the DNA fragment of pure *L. plantarum* and *L. fermentum* cultures respectively. Bands that appeared in all or 90% of the samples were assumed to be linked to the predominant species. Bands common to only one or a few of the lanes with either high or low relative intensities were suspected to be either dominant or minority species peculiar to such samples.

The multiple band patterns of each of the known pure cultures could be attributed to multiple copies of the same strain produced from different cycles of the amplification process. Different species may yield PCR products that co-migrate in DGGE gels which could pose an inherent bias on the identification processes (Marshall et al., 2003).

3.4.1.2 Identity of the DGGE fragments and the isolated LAB

The sequencing analysis revealed that the LAB community in the *akamu* samples were of the genera *Lactobacilli* with a strain of *Lactococcus lactis* subsp. *cremoris* from the genus *Lactococcus*. The identified *Lactobacilli* were: Obligate homofermentative *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*, *L. acidophilus*; facultative homofermenters: *L. plantarum*, *L. rhamnosus* or *L. casei*, *L. salivarius*, and obligate heterofermenters: *L. fermentum* and *L. reuteri*. The commonly occurring bands were identified as *L. fermentum*, *L. plantarum*, and *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*, hence suggesting their predominance in the *akamu* samples. The results of this study were in agreement with reports in literatures on the predominance of *Lactobacillus* spp:

L. fermentum, *L. plantarum* and *L. acidophilus* in fermented cereal foods (Yousif et al., 2010, Vieira-Dalodé et al., 2007, Lei and Jakobsen, 2004, Kalui et al., 2009, Edema and Sanni, 2006). Kalui et al., (2009) and Madoroba et al., (2009) reported the isolation of *L. rhamnosus* from traditional fermented maize and sorghum foods respectively. The dominance of *Lactococcus lactis* subsp. *lactis* was reported in fermented cereal beverages (Kivanç et al., 2011, Muyanja et al., 2003), while the presence of *L. casei* in fermented maize was reported by Dike and Sanni, (2010). The isolation and occurrence of *L. helveticus* in maize fermentation seemed unique. Although a commercial strain of *L. helveticus* ATCC15009 had been employed in the fermentation of sour dough bread (Plessas et al., 2008).

The DGGE profile confirmed the presence of the isolated LABs in the *akamu* samples. However, *L. reuteri*, *L. salivarius* and *L. delbrueckii* subsp. *bulgaricus* were not isolated using culture-dependent techniques a confirmation of the limitations of the culture-dependent methods. (Ampe et al., 1999, Cheriguene et al., 2007). Although, not all the DGGE bands were excised and some fragments were not successfully sequenced which could account for the absence of *L. acidophilus* in the DGGE profile. Fragment 4B had the same homology of 100% for *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*. This suggested the closeness of these 2 strains with similar DNA regions. Omar and Ampe, (2000) encountered similar ambiguity but resolved it with the use of complementary technique such as randomly amplified polymorphic DNA fingerprinting. This study however did not employ these techniques as the main microorganisms with the desired fermentation characteristics were satisfactorily identified to belong to the *L. plantarum* group.

3.4.1.3 Identity of the isolated yeast

The isolated yeast showed variable morphological characteristics and were genomically identified as *Candida albicans*, *C. tropicalis*, *Clavispora lusitaniae* and *Saccharomyces paradoxus*. Nout et al., (1989b) had demonstrated that *C. albicans* can grow very well in fermented porridges with pH \leq 4.0. *Candida albicans* inhabits the mucosal surfaces of the oral and vaginal cavities and the gastro-intestinal tract of humans (Molero et al., 1998), its presence in the *akamu* samples suggested contamination from handlers and this could be worrying as *C. albicans* are potential pathogenic microorganisms.

Candida tropicalis amongst other yeast had been associated with the fermentation of Tanzanian sorghum gruel, maize-cowpea dough, cereal gruel, beverages and doughs (Mugula et al., 2003a, Vieira-Dalodé et al., 2007, Sanni et al., 2002, Pedersen et al., 2012). Mukisa et al., (2012) identified *Clavispora lusitaniae* from fermented sorghum and millet beverages (*obushera*). Reports from literature on the association of *Saccharomyces paradoxus* in maize fermented food seemed scarce, making it an addition to the knowledge of the kinds of yeast that could be found in fermented maize. The presences of yeast in fermentation had been related to the excretion of some essential amino acids and the production of aromatic compounds for improved product nutritional and sensory qualities although the utilization of organic acid and the production of tissue-degrading enzymes involved in food spoilage may also be observed (Jespersen, 2003, Odunfa et al., 2001).

The presumptive identification of the isolated LAB and yeast based on their phenotypic characteristics in comparison to known microorganisms showed

some variations from the genotypic identification. Although, some of the isolates had same identity with both methods, identification based on biochemical test was more related to metabolic functions than genetic closeness.

3.4.2 Fermentation characteristics

3.4.2.1 pH and Titratable acidity

Decrease in pH is been established as an important parameter for assessing how fast a process will reach conditions (pH <4.5) which can inhibit the growth of pathogenic organisms (Nguyen et al., 2007). The range of pH (3.22 - 3.95) of the *akamu* samples obtained from Nigeria was such that would not permit the survival of most unwanted microorganisms. Among the isolated microorganisms that were subjected to the screening process, samples fermented by *L. acidophilus* (NGL2 and NGL6) and *L. helveticus* (NGL1, NGL3 and NGL8) had pH \geq 5.31 after 24 h, a condition that would not favour the inhibition of pathogenic growth. Although with progression in fermentation their pH values were significantly ($p \leq 0.05$) lower than 3.33 except for one of the strains of *L. helveticus* NGL3 with pH of 5.83 after 72 h.

Progression in fermentation of cereal and cereal-legume mixture by endogenous grain microflora, pure or mixed starter cultures has often been linked with increase in microbial numbers concomitant with decrease in pH and increase in titratable acidity (Wakil et al., 2008). This was observed in all the LAB fermented samples except for *L. helveticus* NGL3. This *L. helveticus* strain had poor growth rate with insignificant rate of reduction in pH and acid production. On the contrary, samples AL5 and AL7 fermented by the *L. plantarum* strains: NGL5 and NGL7 had significantly ($p \leq 0.05$) higher microbial

count within the first 24 h of fermentation and thus reached a lower pH ≤ 3.63 with higher %TTA ≥ 0.70 . Similarly, in all other fermentation with *L. plantarum* strains either as single and/or mixed starter cultures with yeast: *Sacch. boulardii* SB20 and *C. tropicalis*, the *L. plantarum* strains were characterised by significantly ($p \leq 0.05$) higher growth rates ($\geq 15 \text{ h}^{-1}$) with maximum viable counts of $9 \text{ Log}_{10} \text{ CFU mL}^{-1}$ after 12 h and concomitant decrease in pH from ≥ 5.52 to ≤ 3.93 . Similar results for *L. plantarum* were reported in the studies by Teniola and Odunfa, (2002) and Mugula et al., (2003a).

The pH and TTA of the control sample that did not change significantly was expected as the maize flour samples were irradiated and sterile distilled water was used for the fermentation. Lactic acid was mostly responsible for the lowering of pH in the fermentations. There was no detectable level of microorganisms in the control for any notable change. The pH and TTA of the yeast single culture fermentation did not show any significant difference from that of the control. This may also be attributed to the little or no lactic acid production by the yeast. Although the pH values for the yeast single culture fermentation were at variance with those reported by Mugula et al., (2003a).

3.4.2.2 Validation of the HPLC performance

The analysis to validate the performance of the HPLC equipment revealed that the acid precipitation process carried out in order to prevent column blockage and thus maintain effective functioning of the column, resulted in lower recovery efficiency. The percentage recovery efficiencies of the acid treated standards were observed to be significantly ($p \leq 0.05$) lower than their untreated counterpart as shown in Table 3.11. The protein concentrations $\leq 7\%$ in the

samples were however not considered as a treat to the column performance hence the acid treatment procedure was skipped for better representation of the amount of analytes present in the samples. The 100% recovery efficiency of lactic acid from the spiked sample was of great importance as lactic acid was the predominant acid produced in the fermentations.

The separation of glucose and citric acid was affected by column temperatures. At higher temperatures glucose interfered with the detection of citric acid. Better separation that enabled adequate quantification of both glucose and citric acid was achieved at 25°C. Citric acid known as one of the predominant acids in fruits (Chinnici et al., 2005), was not detected in the fermented ground maize slurries and its interference with the glucose would not have been of any setback in the sugar quantification for the samples. However, for the analysis of samples suspected to contain both glucose and citric acid, a lower column temperature would be preferable. Interferences in chromatographic analysis can also be resolved by varying the eluent type and molarity as to utilize the differences in the interfering acid strengths (Chinnici et al., 2005).

Although glucose and fructose have similar molecular weight, they were eluted at different times under the column temperature (25°C) used. This was an improvement in elimination of interferences encountered in the use of other chromatographic methods such as the high-performance size exclusion chromatography with refractive index detector (HPSEC-RI) (Giannoccaro et al., 2008). Continuous analysis of large number of samples was achieved through the automatic sample injection system of the Dionex-Ultimate 3000

UHPLC+Focused (Dionex Softron GmbH, Germering, Germany) that required just the refilling of elute at very long intervals.

3.4.2.3 Organic acids, ethanol and sugar concentrations in the samples

Bacteria produce organic acids from the fermentation of carbohydrate and for the *akamu* samples suspected to be more than 3 days old, the sugars may have been converted to mainly lactic acid resulting in high lactic acid contents and undetectable or low sugar levels (<5 mmol kg⁻¹). The amount of sugars detected in the LAB starter culture fermentations suggested the metabolism of these sugars to lactic acid.

The initial increase in glucose level within the first 12 h of fermentation while maltose and fructose where been depleted may imply the breakdown of other complex carbohydrate first to the simple sugar: glucose before its utilization. The consumption of glucose was faster in the mixed culture fermentation with the yeast. Although, glucose levels ≥ 26.88 mmol L⁻¹ were detected in the LAB single culture fermentation after 72 h and was completely depleted after 48 h in the mixed culture fermentation, the lactic acid concentration of the single LAB culture fermentation was significantly ($p \leq 0.05$) greater than the yeast mixed culture fermentation with particular reference to the fermentation with *Sacch. boulardii* SB20. This was an indication of the utilization of the sugars by the yeast in the mixed starter fermentation. Homofermenters such as *L. plantarum* produce more than 85% of lactic acid from glucose; considering that 1 mol of glucose yields 2 mol of lactic acid (Reddy et al., 2008), it would be assumed that other carbohydrates contributed to the high lactic acid levels in addition to the glucose.

In the control, fructose and maltose levels were fairly constant, while the significant increase in glucose level from 16.56 to 87.59 mmol L⁻¹ could be attributable to the hydrolysis of the maize starch to its basic component as glucose by the endogenous grain amylases under the wet condition.

Citric, lactic, acetic pyruvic, succinic and propionic acids have been identified in *bushera* a Ugandan LAB fermented cereal food (Muyanja et al., 2003). In Nigerian *ogi*, the main organic acids were reported to be lactic, butyric, formic and acetic acids by Banigo and Muller (1972). Lactic and acetic acids were reported to be predominant in most cereal fermented food and they act as flavour enhancers (Gobbetti and Corsetti, 1997, Muyanja et al., 2003). As evidenced in this study and the report of other authors' (Songré-Ouattara et al., 2009), lactic acid production is mainly responsible for the lowering of pH and at low pH, the toxicity of acetic acid increases hence both acids also synergistically serve as antimicrobial.

The higher lactic acid production rate by the *L. plantarum* strains: NGL5 and NGL7 is characteristic of homofermentive LAB that produces mainly lactic acid (Sanni et al., 2002). However, the ability of yeast to utilise certain bacterial metabolites as carbon sources (Akinrele, 1970, Leroi and Pidoux, 1993, Silva et al., 2011) and the competition for utilisable sugars could be contributory to the reduction in the lactic acid content in the *L. plantarum* strains mixed culture fermentation with *Sacch. burladii* (SB20) and *C. tropicalis* (NGY1) samples and may explain the increase in pH concomitant with drop in acidity of the BSF sample with the onset of yeast growth. Although, the activities of different fermenting microorganisms and perhaps the predominance of high acid

producing organisms latter in the fermentation stage could account for the significantly highest lactic acid production in the BSF towards the end of the fermentation period.

The production of acetic acid and other organic acids are favoured by heterofermentative pathways. Acetic acid levels of 79.44 ± 0.87 and 25.83 ± 9.12 mmol kg⁻¹ was detected in the traditional *akamu* samples M3 and W1 respectively and the microbial identification revealed the presences of heterofermentative *L. fermentum* and *L. reuteri* in the samples. Microbial succession and the predominance of heterofermenters such as *L. fermentum* towards the end of fermentation have been reported in back slop fermentations of Mexican fermented maize dough *Pozol* (Omar and Ampe, 2000) and Ghanaian fermented millet food *fura* (Owusu-Kwarteng et al., 2012). The production of acetic acid level of 4.69 ± 0.07 mmol L⁻¹ after 72 h in the BSF therefore suggested the onset of the succession of heterofermenters in the mixed microbial population from the old stock. The presence of *L. fermentum* and *L. reuteri* in the stock sample used as inoculum for the BSF has also been established in this study.

Propionic acid was detected in only one of the traditional *akamu* samples W1 at a concentration of 13.29 ± 5.22 mmol kg⁻¹ but none was observed among the LAB fermented samples. Propionic acid in sour dough was reported as an effective antimicrobial inhibitor against *Bacillus* species by Rosenquist and Hansen, (1998) while Niven et al., (2004) associated propionic and butyric acid as spoilage compounds that can alter the organoleptic quality of the fermented

food. Butyric acid was not found in the samples and the detected levels of propionic acid may be unable to cause any negative change organoleptically.

The highest ethanol level was detected in *Sacch. boulardii* SB20 single culture fermentation and higher levels in the mixed culture fermentation with the *L. plantarum* strains. Ethanol production may not be needed in the fermentation for infant and children feeding. The absence and/or very low level of ethanol production in the *L. plantarum* strains and *C. tropicalis* mixed culture fermentation was indicative of the dominant activities of the LAB in the fermentation and as well as a contamination free process. The role of yeast in the flavour of fermented maize products have been reported by Annan et al., (2003), Mugula et al., (2003a) and Omemu et al., (2007). Therefore, the characteristic of *C. tropicalis* to grow in the fermentation with little or no ethanol production could be important in *akamu* production as the product flavour is an important sensory property.

3.4.2.4 Microbial growth analysis

The growth of the LAB during the fermentation of the ground maize slurry followed the known microbial growth pattern. The main phases of growth observed were the log, decelerating and the stationary phases. The lag phase if any was not significant. This implied that the microorganism did not have to undergo any intense synthesis of enzymes or molecules before the utilization of substrate for growth which could be partly due to the phase that they were before inoculation into the substrate. The adaptive nature of the *L. plantarum* strains shown by the quick onset of the log phase is important for early and efficient production of the desired metabolic end product. This may explain why

the rate of organic acid production and the subsequent decrease in pH of the fermentation by the *L. helveticus* strain NGL3 that was observed to have had lag was significantly ($p \leq 0.05$) the least amongst others. The viable counts reached a maximum of $9 \text{ Log}_{10} \text{ CFU mL}^{-1}$ and then fairly constant values were recorded toward the end of fermentation depicting the stationary phase. The cause of change of phase from exponential to stationary may seem unclear as glucose levels $>26 \text{ mmol L}^{-1}$ were still detected after the peak viable count and towards the end of fermentation. Although the *L. plantarum* strains were found to be able to withstand low pH (<3.5), they may not be able to grow exponentially with the accumulation of lactic acid that is their main metabolic by product.

The microbial growth model estimated the growth rate of the individual microorganisms used in the cereal fermentation. The $\text{Se}(\text{fit})$ is the standard error of fitness and it indicated the total deviation of the observed values (viable counts) from the estimated model fit while the R^2 statistics further elucidated how successful the fit was. Some curves were well estimated and others were not. During the screening analysis, the LAB had curve fitness between 91 and 99.8% except for *L. helveticus* of sample AL3 and *L. plantarum* of sample AL7 with 87 and 81% curve fitness. This may be due to the decrease in their viable count after 48 h while others had increases or constant values.

In the BSF samples, differences were observed in the viable count. This may have originated from the effect of frozen storage (as stated in section 3.2.1.1) on the microbial composition of the *akamu* samples used as starters for the different analysis. The use of 1 g of the imported *akamu* sample (M3) that was

refrigerated overnight as starter yielded higher initial microbial count during the screening process as opposed to subsequent fermentations with the use of same *akamu* sample (M3) and quantity of the starter after it had been stored frozen at -80°C for some period of time.

The higher initial microbial load of the BSF during the screening process could have resulted in the highest viable count after 12 h. However, depletion of substrate and production of inhibitory substances by some organisms in the mixed population of the BSF may probably be accountable for decrease in viable count after 48 h and the poor growth rate. Annan et al., (2003) and Wakil et al., (2008) reported such decrease in LAB count in spontaneously fermented cereal and cereal-legume weaning food with the onset of yeast growth.

It could be suggested that the LAB were able to survive the frozen storage of the traditional *akamu* sample used as inoculum in subsequent experiments while the yeasts were unable to. The LAB were able to grow and increased to a fairly constant value towards the end of fermentation as opposed to the initial microbial population in the inoculum used before the frozen storage where reduction by 1 log cycle after 48 h was evidenced when yeast began to grow in the BSF during the early characterisation of the fermentation ability of all the isolated LAB. The fitness of the LAB growth curve in the BSF fermentation during the screening experiment therefore showed significant variation from the observed experimental data with fitness of 66% while in subsequent experiments, the LAB curve for the BSF had fitness >97%.

The growth of *C. tropicalis* and *Sacch. boulardii* SB20 in the fermentation of the ground maize slurry followed a similar growth pattern to that of the LAB except for *Sacch. boulardii* SB20 in the mixed culture fermentation with the *L. plantarum* strains. The fitness of the curve for the yeast single culture fermentation was either 100 or 99%. There was a decrease in viable count after 12 and 48 h for *Sacch. boulardii* SB20 and *C. tropicalis* respectively in the mixed culture fermentation. This also affected the fitness of their growth model curves with *C. tropicalis* having <89% and *Sacch. boulardii* SB20 in mixed culture fermentation with NGL7 and LpTx having 77 and 70% respectively.

Sacch. boulardii SB20 in mixed culture fermentation with NGL5 had very poor growth rate 0.01 h^{-1} when viewed through the entire fermentation period with model curve fitness of 38%. It had an initial exponential growth after 12 h with growth rate of $0.16 \pm 0.01 \text{ h}^{-1}$, thereafter a death phase with 1 log reduction was observed after 24 h followed by another growth phase. This may have resulted in the significant variation in the fitness of the model curve. However, the ability of *Sacch. boulardii* SB20 and *C. tropicalis* (NGY1) to grow in the mixed culture fermentation with the *L. plantarum* strains at a low pH ≤ 3.61 indicated the ability of these yeasts to tolerate the acid environment. The likely mechanism underlining the lactic acid tolerance of a *Candida* spp. at low pH was explained by Halm et al., (2004).

3.4.2.4 Performance of the freeze dried *L. plantarum* strains

Freeze drying is an important means of preserving starter cultures. It was evidenced that the *L. plantarum* strains were able to survive freeze drying from broth cultures and fermented ground maize slurry with pH <3.92 and lactic acid

levels $<63 \text{ mmol L}^{-1}$ after 6 h. This satisfied the requirement of starter cultures to facilitate the fermentation process to levels that would exclude any unwanted microorganism as well as providing a better means of making available the prospective strains for wider usage.

3.5 Conclusion

The PCR-DGGE analysis of the traditional Nigerian *akamu* samples revealed a LAB community dominated by the genera *Lactobacillus* with one strain of *Lactococcus lactis* subsp. *cremoris* from the genus *Lactococcus*. The commonly occurring bands that were identified as *Lactobacillus fermentum*, *L. plantarum*, and *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus* suggested their predominance in the *akamu* samples. The identification of *Saccharomyces paradoxus* seemed an addition to knowledge of the yeast types associated with fermented maize. Although yeasts were conventionally isolated and their identities confirmed with genotypic methods, establishment of the total yeast population will constitute a different study.

The isolated *L. plantarum* strains (NGL5 and NGL7) were observed to have presumably high potential as starter culture due to their significantly ($p \leq 0.05$) higher favourable spectra of fermentation end products. The ability of the *L. plantarum* strains to survive freeze drying would make it easier to relay the starter cultures as freeze dried cultures. Although, the yeast were unable to produce appreciable amount of acid for significant pH decrease, the combination of *C. tropicalis* in fermentation with the LAB may be important for product quality subject to the validation of the production of any likely flavour enhancing compounds by the yeast. The fermentation abilities of the two *L.*

plantarum strains (NGL5 and NGL7) isolated from the Nigerian *akamu* sample M3 and their probiotic counterpart (LpTx) in comparison to the BSF were summarized in Table 3.28. It forms a basis for further studies on their use for product safety and probiotic potential for possible health effects on the consumer.

Table 3.28 Summary of the fermentation characteristics of the two *Lactobacillus plantarum* strains (NGL5 and NGL7) isolated from Nigerian *akamu* and the probiotic strains (LpTx) in comparison to the Back slop fermentation

Sample	pH			TTA (%Lactic acid)			*Lactic acid (mmol L ⁻¹)			*Glucose (mmol L ⁻¹)			Microbial count (Log ₁₀ CFU mL ⁻¹)		
	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h	0 h	24 h	72 h
AL5	5.52 ^e	3.51 ^{bcd}	3.43 ^{abc}	0.12 ^d	0.69 ^c	1.05 ^b	5.99 ^d	77.51 ^c	109.24 ^b	15.52 ^e	37.28 ^{bc}	29.63 ^{cd}	7.23 ^e	9.09 ^a	8.56 ^{cd}
AL7	5.58 ^e	3.55 ^{cd}	3.40 ^{ab}	0.15 ^d	0.66 ^c	1.11 ^b	6.00 ^d	82.93 ^c	120.09 ^b	15.24 ^e	46.05 ^a	29.83 ^{cd}	6.94 ^f	9.17 ^a	8.74 ^{bc}
ALpTx	5.53 ^e	3.56 ^d	3.43 ^{abc}	0.15 ^d	0.63 ^c	1.05 ^b	4.69 ^d	73.98 ^c	110.63 ^b	14.35 ^e	40.05 ^{ab}	26.88 ^d	6.82 ^f	8.86 ^b	8.62 ^{cd}
BSF	5.54 ^e	3.56 ^d	3.37 ^a	0.09 ^d	0.75 ^c	1.41 ^a	4.69 ^d	79.75 ^c	156.61 ^a	12.92 ^e	32.95 ^{bcd}	3.08 ^e	5.72 ^g	8.71 ^{bcd}	8.54 ^d

Values that share the same superscript in the columns for each parameter do not differ significantly (p≤0.05). N=3±SD

BSF - Back slop fermentation

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

* The amount of lactic acid and glucose obtained using HPLC analysis

Values were obtained from Table 3.9, Table 3.10, Table 3.13, Table 3.14 and Table 3.22 for the pH, Total titratable acidity (TTA), lactic acid, glucose and microbial counts respectively.

CHAPTER FOUR

CHARACTERISATION OF *LACTOBACILLUS PLANTARUM* STRAINS ISOLATED FROM 'AKAMU' - A NIGERIAN FERMENTED MAIZE FOOD FOR PROBIOTIC POTENTIAL

4.1 Introduction

Lactobacillus plantarum is a versatile lactic acid bacterium, that is found in various indigenous fermented foods and is commonly associated with the human gastrointestinal tract (GIT) (de Vries et al., 2006, Kalui et al., 2010). *L. plantarum* is among the key species of the genera *Lactobacillus* that is being studied for their ability to confer health benefits. The exertion of health benefits by probiotic *Lactobacillus* strain have been reported to be mediated through early programming of the immune system for better balanced immune response and thus, reducing the risk of development of allergy, provision of essential vitamins, amino acids, organic acids and synthesis of functional enzymes, down-regulation of inflammatory responses, enhancement of commensal microbiota stability and gut mucosal barrier function, inhibition of pathogens and decreased pathogen adhesion to intestinal epithelial (Sanders, 2009).

In order to exert health-enhancing benefits, probiotics must possess certain potentials such as the ability to withstand passage through the GIT, adherence to mucus and/or human epithelial cells and cell lines, reduction of pathogen adhesion to surfaces through aggregation and inhibition of enteric pathogens (FAO/WHO, 2002, Lin et al., 2011). Bacterial resistance to acid and bile is an important criteria that indicate the potential of the bacteria to survive passage

through the GIT and later proliferate to levels adequate for some health benefits (Michida et al., 2006).

The ability of bacteria to aggregate has been proposed to have both food preservation and therapeutic impact on intestinal microbiota (Collado et al., 2007a). Auto-aggregation of probiotic *Lactobacillus* spp. bacterial cells has been suggested to be necessary for adhesion to intestinal epithelial cells (Kos et al., 2003, Del Re et al., 2000), while co-aggregation form barrier that prevent pathogenic colonization of the GIT through the creation of different microenvironment around the pathogens and enhanced antimicrobial activities at close proximity (Collado et al., 2007b, Ouwehand et al., 1999). The adhesion of bacteria to surfaces is governed by cell surface hydrophobicity. High cell surface hydrophobicity have been correlated to increased cell aggregation (Del Re et al., 2000, Kos et al., 2003) and suggested to play significant role in interaction with organic mucus layer of the gut (Mathara et al., 2008) and adhesion to epithelial cells (Schillinger et al., 2005).

Although the inhibition of pathogen adhesion to intestinal surfaces through competitive exclusion by probiotic bacteria has been reported as one of the mechanism of infection prevention, the inhibition of pathogen in the food matrix before consumption would prevent the exposure of the GIT to the unwanted bacteria and thereby minimizing the risk of infection. Antimicrobial activities in fermented cereal-based foods have been mediated by the provision of antagonistic environment against the pathogenic microorganisms. This has been achieved mainly through the production of organic acids with concomitant

reduction of pH to levels (<4.5) inhibitory to most pathogenic organisms (Nguyen et al., 2007).

Potential probiotics themselves are not to possess any factor capable of causing infection as a safety measure (Apostolou et al., 2001, FAO/WHO, 2002). Any ability of potential probiotic to derange the mucus layers and cause cessation of the underlying epithelial cell lines would pose a potential virulence factor.

Some bacterial probiotic potential have been demonstrated using *in-vitro* assays. These potential include tolerance to acid and bile, aggregation, gelatinase and haemolytic capabilities using microbial media and diluents (Zhang et al., 2012, Maragkoudakis et al., 2006b, Xie et al., 2012, Ruiz-Moyano et al., 2009, Kalui et al., 2009, Del Re et al., 2000, Kos et al., 2003); adhesion to mucus and epithelia layers using mucin extracts from human and animals, Caco2 and HT-29 cells and human intestinal Int-407 (ATCC CCL 6) cells as intestinal models (Baccigalupi et al., 2005, Lin et al., 2011, Wang et al., 2008, Monteagudo-Mera et al., 2012).

Although most studies had been with intestinal and dairy isolates, there is increasing interest not just in the development of food products containing beneficial *Lactobacillus* strains but the characterisation of non-intestinal isolates for possible health-enhancing effects. This study was therefore aimed at the characterisation of two strains of *L. plantarum* isolated from Nigerian fermented maize food for possible probiotic potential based on: antimicrobial activities, ability to tolerate acid and bile conditions, hydrophobicity, aggregation potentials,

gelatinase and haemolytic activities, and adherence to mucin and epithelial cell models.

4.2 Materials and Methods

4.2.1 Microbial inocula

4.2.1.1 Lactic acid bacteria and yeast

Studies in this chapter employed the two *Lactobacillus plantarum* strains: NGL5 and NGL7 and the yeast: *Candida tropicalis* (NGY1) isolated from Nigerian spontaneously fermented *akamu* sample (M3) and selected based on their favourable fermentation characteristics as described and discussed in chapter 3. The probiotic *L. plantarum* strain (LpTx) and *Saccharomyces boulardii* SB20 described in section 3.2.5.1 and *L. reuteri* NCIB 11951 obtained from stock culture in the Microbiology laboratory of Plymouth University were used as standards during the characterisation of some of the probiotic potential of the selected LAB and yeast.

4.2.1.2 Pathogens

Antagonistic activities using an agar spot method were assayed against food borne human pathogens: *Bacillus cereus* NCIMB 11925, *Escherichia coli* 1077 (having NCTC number of 11560 but the original strain reference is 1077), *Listeria monocytogenes* NCTC 7973, *Salmonella enterica* serovar Enteritidis NCTC 5188, and *Staphylococcus aureus* NCTC 3750 obtained from stock cultures in the microbiological laboratory of Plymouth University, UK.

Antagonism in fermentation was against *E. coli* 1077. *E. coli* is an indicator organism most commonly chosen in temperate climates and one of the pathogens of concern in African fermented cereal foods (Adams and Moss, 2000, Motarjemi et al., 1993). *Salmonella* Enteritidis 5188 and *E. coli* 1077 were used as control during the experiment on haemolysis.

4.2.2 Microbial media and microbial inocula preparation

The *L. plantarum* strains, the yeast and the pathogens were cultivated on MRS, Malt extract and Nutrient agar respectively with incubation at 30°C for *B. cereus* and 37°C for *L. plantarum* and the others pathogens for a period of 24 h while yeasts were incubated at 25°C for 48 h. MRS, Malt extract and Nutrient broths were used for broth cultures of the *L. plantarum* strains, yeast and the pathogen respectively. All the media were obtained from Oxoid Limited (Basingstoke, Hampshire, UK) and prepared as described in section 3.2.2.

The microbial cell suspensions were prepared as described in section 3.2.5.2.1. Harvested cells were washed twice in PBS (pH7.3±0.2) and suspended with the same diluent such that 1 mL had microbial cell concentration as state under each experiment.

4.2.3 Antimicrobial activities

4.2.3.1 Pathogen inhibition using agar spot method

Antagonistic activity of the *L. plantarum* strains (NGL5, NGL7 and LpTx) and the yeast: *C. tropicalis* (NGY1) and *Sacch. boulardii* SB20 was determined by an agar spot test with some modification to the method described by Majhenić et

al., (2007). Two microlitres of washed *L. plantarum* cell suspensions (c. 9 Log₁₀ CFU mL⁻¹) from an overnight broth culture were spotted on MRS and modified MRS agar plates and incubated both aerobically and anaerobically (using anaerobic gas jackets - 2.5 L AnaeroGen AN0025A, Oxoid Ltd, Basingstoke, England) at 37°C for 24 h.

The modified MRS agars were MRS with 0.2% glucose (MRS.2) and MRS with 0.2% glucose and without Tween 80 (MRS.2-T80). The modification was to check for the cause of the LAB inhibitory activity. The reduced glucose level was to avoid acid production and anaerobic incubation was aimed at preventing H₂O₂ production (Vermeiren et al., 2004), while the removal of Tween 80 was to check the production of antimicrobial agents like bacteriocins (Trias et al., 2008).

For the yeast, 10 µL of washed 48 h cell suspension (c. 7 Log₁₀ CFU mL⁻¹) was spotted on Malt extract agar plates and incubated at 25°C for 48 h. Afterwards, the agar plates were overlaid with 10 mL of molten Nutrient agar (7.5 g L⁻¹) inoculated with 33 µL of cell suspension containing 6 Log₁₀ CFU mL⁻¹ of the following pathogens: *B. cereus* NCIMB 11925, *E. coli* 1077, *S. Enteritidis* NCTC 5188, *Lis. monocytogenes* NCTC 7973, and *Staph. aureus* NCTC 3750. The overlaid plates were incubated at the optimal growth temperature of the respective pathogens: 30°C for *B. cereus* and 37°C for the others. After 24 to 48 h of incubation the plates were observed for inhibition zones and the diameter (mm) of inhibition halos were measured at 3 - 4 different angles using analogue sliding Vernier Calliper 120 mm (Skill Tech 5 in, Frankfurt, Germany) and the average reading was taken.

4.2.3.2 Inhibition of *E. coli* 1077 in ground maize slurries and porridges from 24 h fermentation

The inhibitory ability of the *L. plantarum* strains against *E. coli* 1077 was determined in the fermentation of irradiated ground maize slurries (see section 3.2.1.2 for maize flour source and irradiation) at 22 and 30°C as follows:

4.2.3.2.1 Inhibition in co-inoculation fermentation

One millilitre (1 mL) of washed cell suspensions of each of the *L. plantarum* strains (c. 9 Log₁₀ CFU mL⁻¹) and the *E. coli* 1077 (c. 8 Log₁₀ CFU mL⁻¹) were co-inoculated into 100 mL sterile distilled water that was thoroughly mixed with 50±0.01 g of sterile ground maize. The inoculated slurries were distributed in 18 mL quantity into well labelled sterile transparent plastic 50 mL pots with lids and incubated at 22 and 30°C for 48 h. The use of transparent pots was for easy visualization of changes like bubble production within the fermentation. Every 3 h for the first 9 h of fermentation and at 24 and 48 h, samples were aseptically withdrawn for pH, titratable acidity, and microbial count.

4.2.3.2.2 Inhibition in 24 h *L. plantarum* strains fermented ground maize slurries

The fermentation slurries were prepared as described in section 3.2.5.3 but the distribution of slurries in 9 mL into sterile transparent plastic pots was after 24 h of fermentation with the *L. plantarum* strains at 22 and 30°C. Thereafter, the distributed slurries were inoculated with 1 mL of washed *E. coli* 1077 cell suspension (c. 8 Log₁₀ CFU mL⁻¹). Samples were withdrawn every 10 min for 40 min and at 60, 120 and 180 min for *E. coli* enumeration.

In both the co-inoculation and inoculation of the *E. coli* into 24 h already fermented slurries, slurries without *L. plantarum* inoculum served as control. In a trial run, the same volume of *E. coli* 1077 cell suspension was inoculated into 150 mL of 24 h fermentation.

4.2.3.2.3 Inhibition in porridges from 24 h L. plantarum strains fermented ground maize slurries

Porridges were prepared from 150 mL of 24 h *L. plantarum* fermented slurries by adding equal volume of boiling water and then microwaving for 2 minutes with vigorous stirring after each minute to obtain a lump free porridge. The porridges were distributed in 18 mL amount into well labelled transparent plastic mini pots with lids and allowed to cool in a water bath maintained at 45°C. Thereafter, *E. coli* 1077 was inoculated and enumerated after every 10 min for 40 min.

4.2.3.3 pH and titratable acidity

The pH and titratable acidity (% Lactic acid) of the ground maize slurries' fermentation during the inhibition assay was determined as described in section 3.2.5.4.1.

4.2.3.4 Microbial enumeration

The enumeration of viable *L. plantarum* and *E. coli* cells were as described in section 3.2.5.5. *Lactobacillus plantarum* strains were enumerated on MRS agar supplemented with 0.01% cyclohexamide and *E. coli* 1077 on MacConkey agar incubated at 37°C for 24 - 48h.

4.2.4 Acid and bile tolerance

4.2.4.1 Acid tolerance

The ability of the *L. plantarum* strains to withstand acid condition was investigated using the methods by Lin et al., (2011) and Ding and Shah, (2007). One millilitre of washed *L. plantarum* cell suspension (c. $9 \text{ Log}_{10} \text{ CFU mL}^{-1}$) was inoculated into 9 mL of modified MRS broth (pH 2.0 with 5 M HCl). The pH of 2 has been established as the appropriate pH for screening acid tolerance of probiotic LAB (Kalui et al., 2009, Lin et al., 2006, Lin et al., 2011). The samples were incubated at 37°C for 3 h under anaerobic condition using anaerobic gas jackets (2.5 L AnaeroGen AN0025A, Oxoid, Basingstoke, England). Survival rate was measured by plating out serial dilutions on MRS agar plates at the beginning of the incubation time and every 1 h for 3 h. Acid tolerance at the different time intervals was determined in relation to the initial viable count.

4.2.4.2 Bile tolerance

The method according to Lin et al., (2006) was used in determining the bile tolerance ability of the *L. plantarum* strains. Washed acid stressed cells were re-suspended in 10 mL MRS broth with or without 0.3% ox gall bile (Oxoid Ltd, Basingstoke, England) and incubated anaerobically at 37°C for 24 h. The viable microbial counts were determined on MRS agar after 0, 3, 6 and 24 h of exposure. The viability of the *L. plantarum* strains both in the normal and bile conditions after each experimental time was obtained in relation to the initial viable count before incubation. The effect of the bile salt on the growth of the *L. plantarum* strains was then ascertained by comparing the viability in the MRS broth with those from the bile condition.

4.2.5 Aggregation analysis

4.2.5.1 Bacterial auto-aggregation

Aggregation abilities of the *L. plantarum* strains and the pathogens: *E. coli* 1077 and *S. Enteritidis* NCTC 5188, were evaluated by using the spectrophotometry method described by Del Re et al., (2000) with some modifications. Four millilitre of the bacterial cell suspension ($A_{600\text{ nm}}$ of 0.5) were centrifuged (HARRIER 18/80 Refrigerated, MSE, Lower Sydenham, London, UK) at 6000 x g at 4°C for 10 min and re-suspended in same volume of their culture supernatant diluents and incubated at Room (22 - 24°C) and Body (37°C) temperatures for 5 h. After 2, 4, 5 and 24 h the absorbance (A_{600}) of 1 mL of upper suspension was measured against same volume of PBS as blank. Auto-aggregation percentage was expressed as:

$$\text{Auto-aggregation (\%)} = \left(1 - \frac{A_t}{A_i} \right) \times 100 \dots\dots\dots \text{Equation 4. 1}$$

Where: A_t is the absorbance at the experimental time and A_i is the absorbance before incubation.

4.2.5.2 Bacterial co-aggregation

Bacterial suspensions were prepared as in auto-aggregation. Equal volumes (2 mL) of the *L. plantarum* and the pathogen strains (1:1 v/v) were mixed and incubated at Room (22 - 24°C) and Body (37°C) temperatures without agitation. Absorbance after mixing and after 2, 5 and 24 h of incubation was taken. Co-aggregation was expressed as percentage of reduction in absorbance after 5 h in the mixed suspension compared with individual suspension using the equation by Kos et al., (2003):

$$\text{Co-aggregation (\%)} = \frac{[(A_p + A_i)/2] - A_{(p+i)}}{(A_p + A_i)/2} \times 100 \dots\dots\dots \text{Equation 4. 2}$$

Where: A_p , A_I and $A_{(p+I)}$ represent the absorbance of the pathogen, *L. plantarum* and mixture of pathogen and *L. plantarum* respectively.

4.2.6 Hydrophobicity

Hydrophobic cell surface property based on microbial affinity to mono-polar and apolar solvents was demonstrated using the following pairs of solvents: (a) Chloroform, an acidic solvent and hexadecane and (b) ethyl acetate, a strongly basic solvent and hexane according to the method of Bellon-Fontaine et al., (1996) with some modifications. Equal volume (2 mL) of bacterial suspensions and the individual solvents were mixed by vortexing for 120 s. After 20 min of incubation at room temperature ($22\pm 2^\circ\text{C}$) for complete separation of the two phases, the absorbance of the aqueous phase (1 mL) at 600 nm was measured and the decrease in the absorbance of the aqueous phase was taken as a measure of cell surface hydrophobicity, expressed as follows:

$$\text{Hydrophobicity (\%)} = \left(1 - \frac{A}{A_0}\right) \times 100 \dots\dots\dots \text{Equation 4.3}$$

Where A_0 and A were absorbance values before and after addition of solvents.

4.2.7 Adhesion to mucin and epithelial cells

4.2.7.1 Mucin binding assay

Adhesion to mucin was determined using the method of Roos et al., (2000) and Jonsson et al., (2001) with some modifications. One hundred microlitre of mucin (porcine stomach Type II, Sigma-Aldrich, Gillingham, Dorset, England) solution at a concentration of $100 \mu\text{g mL}^{-1}$ in $50 \text{ mmol L}^{-1} \text{ Na}_2\text{CO}_3$ (prepared as described in Appendix A4.1) in Maxi Sorp surface Nunc-Immuno microtitre wells (Life Technology, Paisely, UK) were incubated at 4°C overnight. The wells were

blocked with phosphate buffered saline supplemented with 0.05% Tween 20 (PBST) at room temperature ($22\pm 2^{\circ}\text{C}$) for 1 h and thereafter washed with the same buffer.

The bacteria were grown in MRS broth at 37°C for 16 h, washed once with PBST and re-suspended in the same buffer to an optical density of 0.5 at 600 nm. In one experiment, 100 μL of the bacterial cell suspensions for each test organism were added into the wells of one of the plates for absorbance measurement and to another set of plates for microbial enumeration. The plates were incubated on an orbital platform shaker (IKA vibrax-VXR S17, Staufen, Germany) at 40 rpm for 1 h at 37°C . Thereafter, the content of the wells were aspirated and the wells were washed thrice with PBST to remove unbound bacterial cells. The plates for absorbance measurement were left to dry in the incubator at 30°C for 30 minutes and the absorbance values ($\text{OD}_{403\text{ nm}}$) were measured in a VersaMax ELISA microplate reader (Molecular Devices, Wokingham, Berkshire, UK). Bound bacterial cells for microscopic examination were estimated under phase contract inverted microscope (Olympus CK30, Yaug-Guan St., Taipei, Taiwan) at a magnification of x 4000.

Each batch of experiment included control wells (mucin coated wells with PBST only) and *L. reuteri* NCIB 11951 was used as standard (Aleljung et al., 1994). Wells that were not coated with mucin but inoculated with bacterial cell suspension were also examined, to ascertain whether the bacteria could bind to the walls of the wells.

Adhesion was expressed as OD of the bacteria in mucin relative to OD of mucin as follows:

$$\text{Adhesion} = (OD_{ml} - OD_m) / OD_m \dots\dots\dots \text{Equation 4. 4}$$

Where OD_{ml} and OD_m represented OD of mucin coated wells inoculated with the LAB and the mucin with only the buffer solution.

To estimate the number of adhered bacterial cells, the bound cells after washing were lifted with 1 mL of buffer into 9 mL of PBST, and enumeration was carried out as in section 3.2.5.5.

4.2.7.2 Adhesion to Caco-2 cells

4.2.7.2.1 Caco-2 cell culture

Caco-2 cells were grown according to the method of Xie et al., (2012). Briefly, Caco-2 cells were grown at 37°C in 5% CO₂-95% air atmosphere (Thermo Scientific Forma Steri-Cult CO₂ incubator, Waltham, Massachusetts, USA) in Dulbecco modified Eagle's minimal essential medium (DMEM) containing L-glutamine (2 mmol L⁻¹), supplemented with 10% (v/v) fetal calf serum, 1% (v/v) non-essential amino acids, 100 U mL⁻¹ penicillin and 0.1 mg mL⁻¹ streptomycin. The Caco-2 monolayers were prepared by seeding in 500 µL of cells (c. 5 Log₁₀ cells mL⁻¹) into six-well tissue culture plates (Fisher Scientific, Loughborough, UK) that were incubated for 21 days, with daily changing of the cultured medium to replenish nutrients and maintain the correct pH.

4.2.7.2.2 Adhesion assay

Adhesion assay was performed following the method of Maragkoudakis et al., (2006a). The growth medium in six-well tissue culture plates of Caco-2

monolayers was aspirated and the cells washed twice with PBS (pH 6.8). Subsequently, 100 μL of bacterial cell suspension $\text{OD}_{600 \text{ nm}}$ (c. $8 \text{ Log}_{10} \text{ CFU mL}^{-1}$) was added to the Caco-2 monolayers. After 1 h of incubation at 37°C in 5% CO_2 , the bacterial cells were aspirated and the monolayers washed 6 times with PBS to release unbound bacteria. Adhered bacterial cells for viable count were lifted with 1 mL of 1% Triton X-100 (BDH, England) into 9 mL of PBS and enumerated as in section 3.2.5.5. Adhered bacterial cells for microscopic examination were fixed with 2 ml of 95% methanol for 10 min. Thereafter, the methanol was aspirated and the well stained with 2 mL of Giemsa stain solution (1:20) (BDH, England) and incubated at room temperature for 30 min. The stain was aspirated and the wells washed with sterile distilled water until no colour was observed in the wash water, dried at 37°C for 1 h and examined microscopically ($\times 4000$) under phase contract inverted microscope (Olympus CK30, Yaug-Guan St., Taipei, Taiwan).

4.2.8 Gelatinase and haemolytic activities

4.2.8.1 Gelatinasis

Gelatinase activity of the *L. plantarum* strains was investigated using the method of Harrigan, (1998). Five microlitre of bacterial cell suspension were spotted on Nutrient agar (Oxiod, Basingstoke, England) supplemented with 0.4% gelatine (Merck, Germany) and incubated at 37°C for 2 - 7 days in anaerobic jars with anaerobic gas jackets (AnaeroGen, Oxide, Basingstoke, England). Thereafter the plates were flooded with 8 mL of saturated NH_3SO_4 and observed for clear zones as positive gelatinase activity.

4.2.8.2 Haemolysis

A colony from 18 h plate culture of the *L. plantarum* strains, *E. coli* 1077 and *S. Enteritidis* NCTC 5188 was streak plated on blood agar composed of Columbia agar base (Oxoid, Basingstoke, England) supplemented with 5% defibrinated sheep blood (TCS Biosciences, Buckingham, UK). The plates were incubated anaerobically at 37°C for 48 h and examined for clear zones (β -haemolysis), green-hued zones (α -haemolysis) and absence of zones (γ -haemolysis) around colonies. *Escherichia coli* 1077 and *S. Enteritidis* NCTC 5188 served as control

4.2.9 Statistical analysis

Statistical differences, relationship among variables and microbial growth rates were analysed as stated in section 3.2.6.

Correlations between 2 or 3 variables such as pH, TTA and lactic acid concentration employed the first-order partial correlation coefficient. Relationship between more than 3 variables such as pH, TTA, *Lactobacillus plantarum* strains and *Escherichia coli* 1077 viable counts utilised multiple regressions and correlations. In which correlation between each pair of variables was determined while holding constant the values of other variables to eliminate any effect of the interaction of the variable held constant on the relationship between the other two variables (Zar, 1999). Interpretations of the correlation results were made using α -level of 0.05, in which p-values less than or equal to 0.05 signified that the correlation was not zero.

4.3 Results

4.3.1 Antimicrobial activity

4.3.1.1 Pathogen inhibition using agar spot method

No inhibition was observed in the modified MRS media under the two test conditions. The inhibitory activity of the *L. plantarum* strains in the MRS agar and that of the yeast against the different pathogens differed significantly as shown in Figure 4.1 and Figure 4.2 respectively. Generally, inhibition under *L. plantarum* anaerobic growth condition (17.2 mm) was significantly ($p \geq 0.05$) higher than the aerobic condition (15.5 mm) (Appendix A4.2)

Among the *L. plantarum* strains, NGL5 exerted the greatest inhibition over all the pathogens in the range of 12.8 ± 0.0 mm for *Lis. monocytogenes* NCTC 7973 to 20.7 ± 0.6 for *S. Enteritidis* NCTC 5188 aerobically; however, anaerobically LpTx exerted maximum inhibition of 23.9 ± 0.1 mm over *S. Enteritidis* NCTC 5188. The most significantly ($p \leq 0.05$) inhibited pathogens by the *L. plantarum* grown in both incubation conditions were the Gram negative enterobacteriaceae: *S. Enteritidis* NCTC 5188 and *E. coli* 1077. *Sacch. bouladii* SB20 was more inhibitory towards *Lis. monocytogenes* NCTC 7973 (8.6 ± 0.3 mm) and *B. cereus* NCIMB 11925 (5.4 ± 0.0 mm) than was *C. tropicalis* (0.9 mm), *Staph. aureus* NCTC 3750 was resistant to both yeasts.

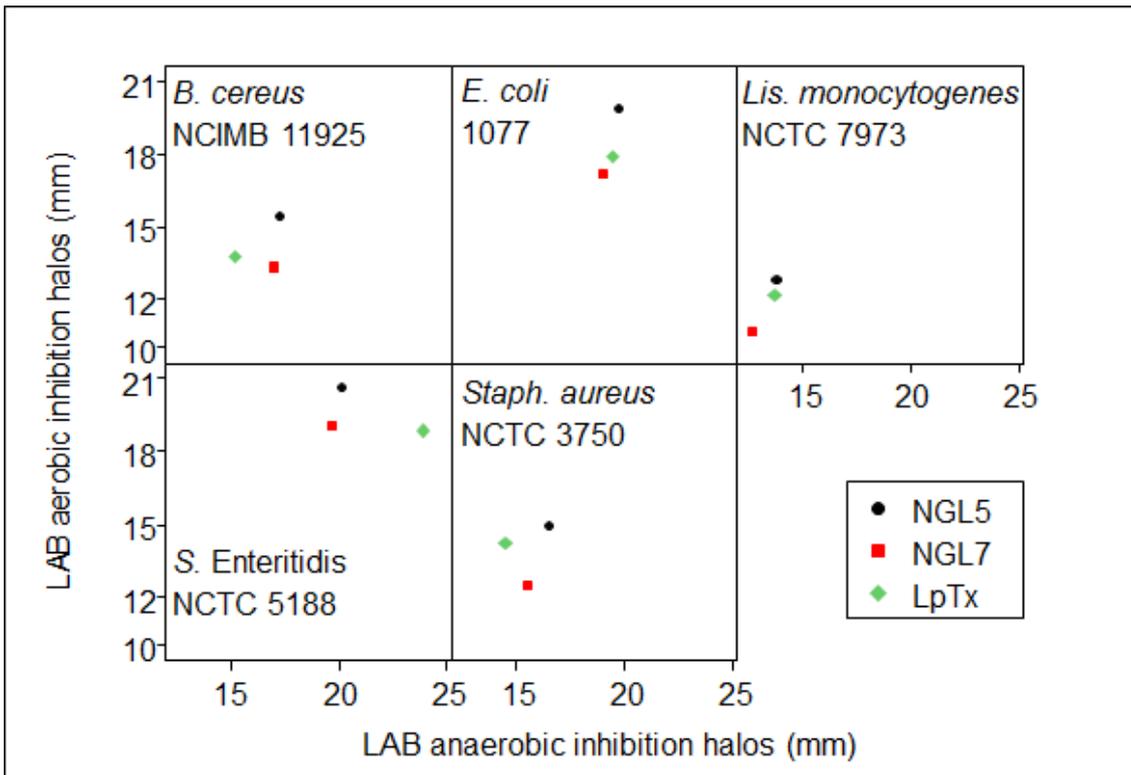


Figure 4.1: Diameter (mm) of the *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) inhibition halos over five foodborne pathogens

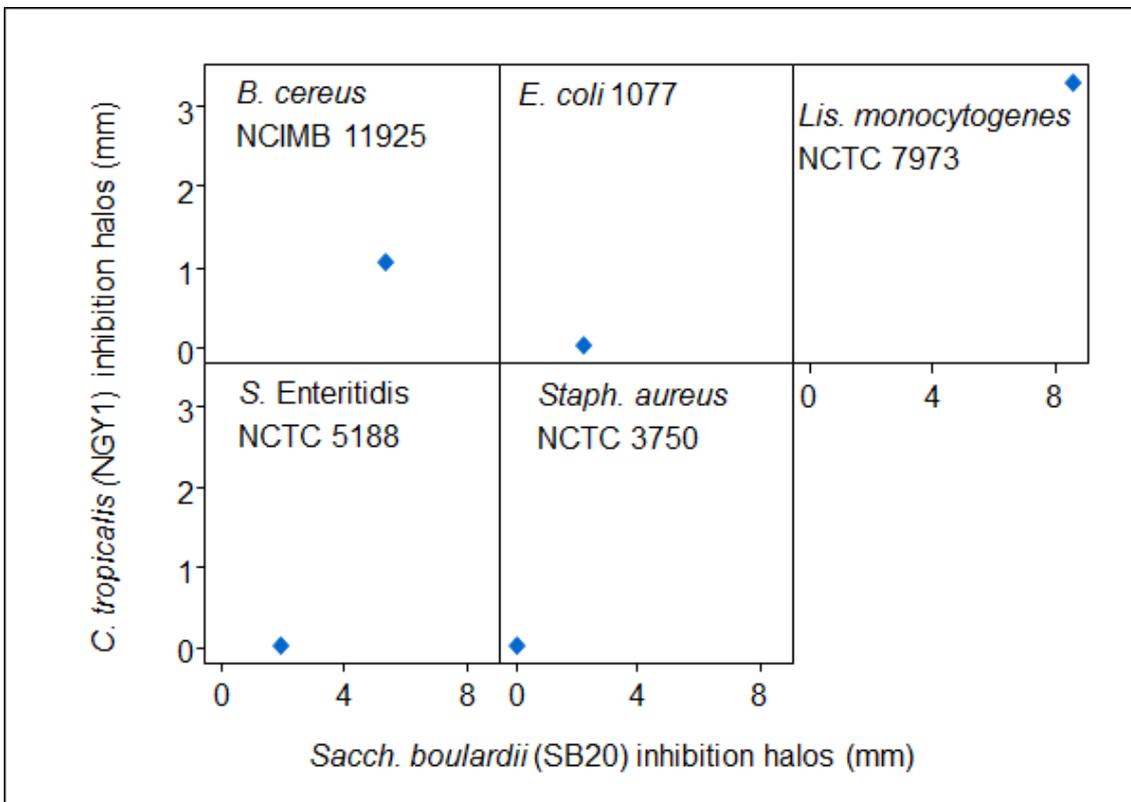


Figure 4.2: Diameter (mm) of *Candida tropicalis* (NGL1) and *Saccharomyces boulardii* (SB20) inhibition halos over five foodborne pathogen

4.3.1.2 Inhibition of *Escherichia coli* 1077 in the fermentation of sterile ground maize slurry

4.3.1.2.1 pH and titratable acidity

4.3.1.2.1.1 Co-inoculation

As shown in Figure 4.3, all the *L. plantarum* strains co-inoculated with *E. coli* 1077 fermented the sterile ground maize slurry with significant reduction in pH $\leq 6.23 \pm 0.08$ - $\leq 3.72 \pm 0.01$ and $\leq 5.50 \pm 0.04$ - $\leq 3.35 \pm 0.01$ after 48 h at 22 and 30°C respectively. Figure 4.4 shows increase in the %TTA from ≥ 0.09 to ≥ 0.72 and ≥ 0.81 after 48 h at 22 and 30°C respectively.

4.3.1.2.1.2 Challenge after 24 h of fermentation with LAB

The pH and TTA of fermentation after 24 h before the inoculation of *E. coli* 1077 at both temperatures were shown in Figure 4.5 and Figure 4.6 respectively. The pH of fermentation after 24 h before inoculation of *E. coli* 1077 was ≤ 3.48 and ≤ 4.17 with TTA value ≥ 0.60 and $\geq 0.51\%$ at 30 and 22°C respectively. The pH and TTA of control sample did not differ with temperature and varied significantly ($p \leq 0.05$ from the *L. plantarum* fermentation. The values of pH and TTA in control sample after 24 h were ≥ 5.22 and $\leq 0.24\%$ respectively.

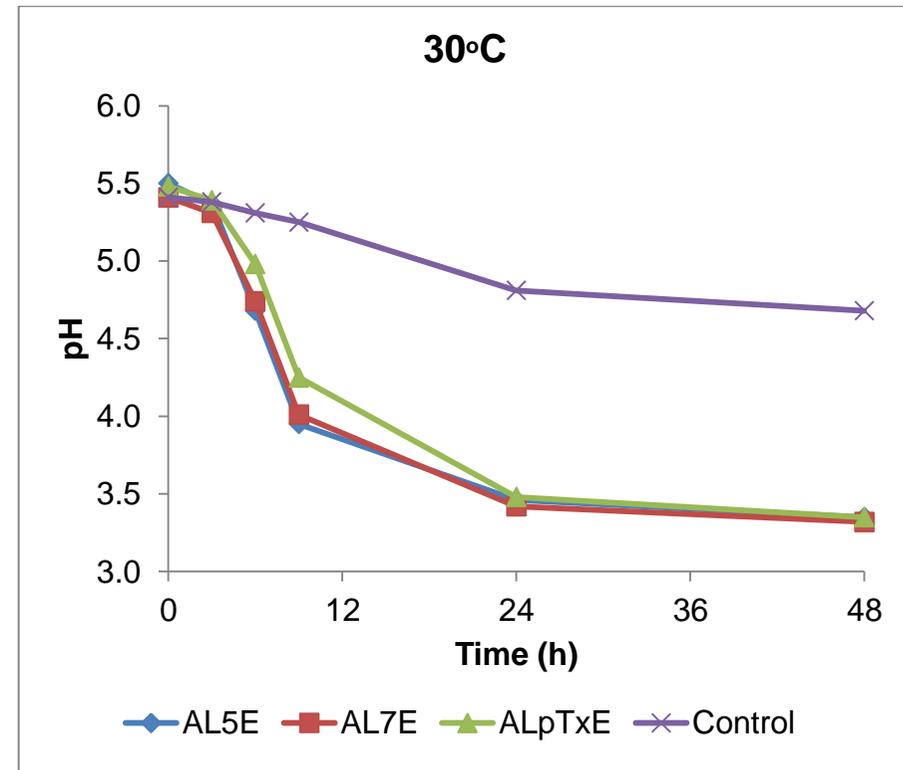
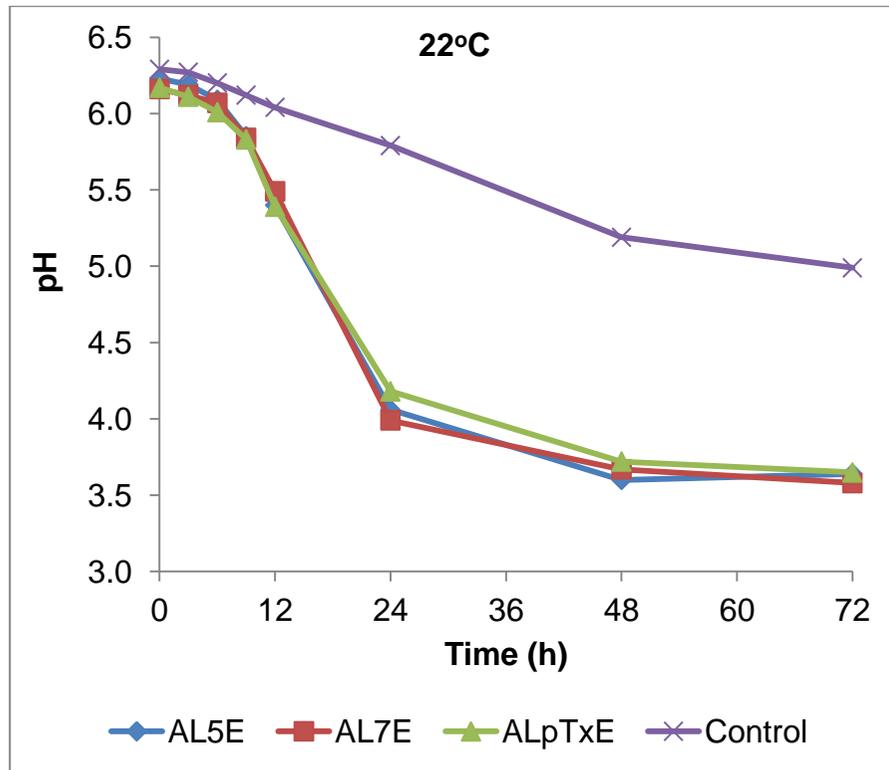


Figure 4.3: pH of sterile ground maize slurries fermented at 22°C and 30°C by the co-inoculation of different strains of *Lactobacillus plantarum* (NGL5, NGL7 and LpTx) and *Escherichia coli* 1077. AL5E, AL7E and ALpTxE represent samples co-inoculated with *E. coli* 1077 and *L. plantarum* strains NGL5, NGL7 and LpTx respectively.

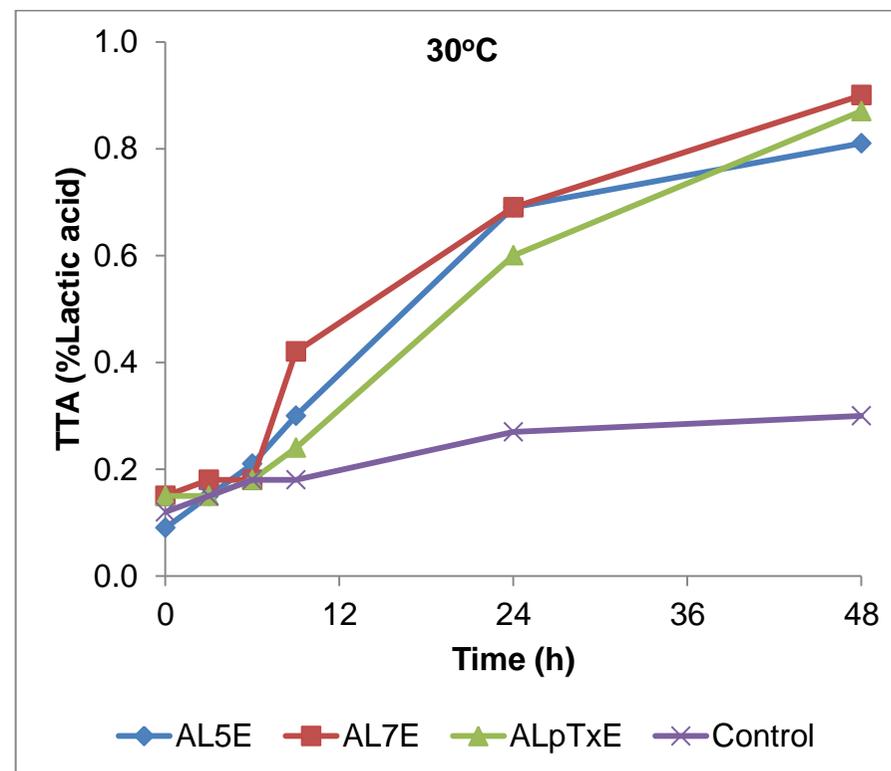
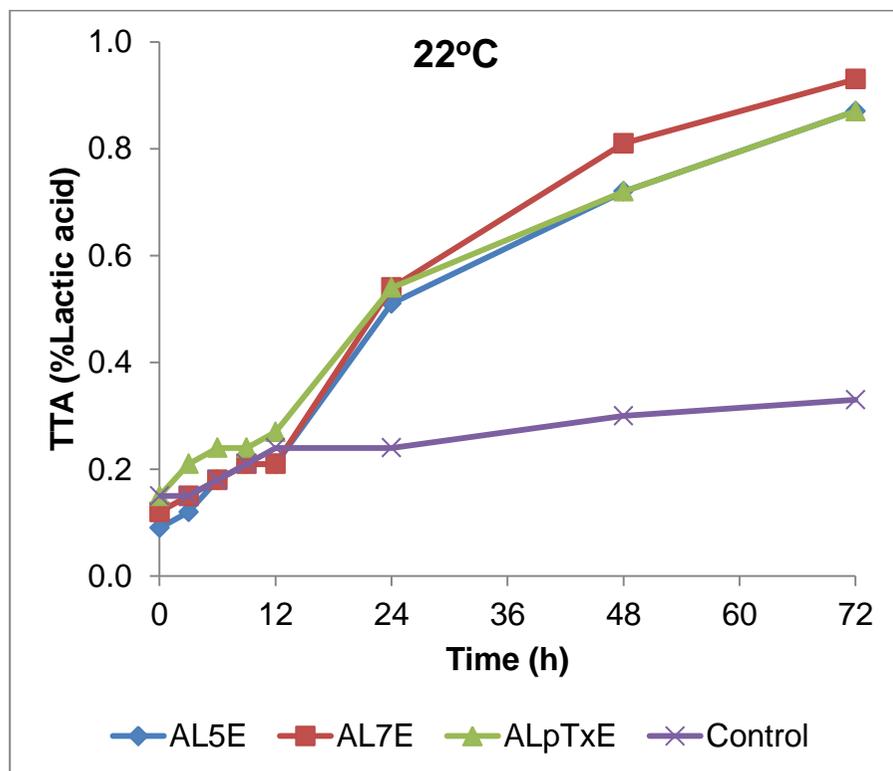


Figure 4.4: Total titratable acidity (TTA) as %Lactic acid of sterile ground maize slurries fermented at 22°C and 30°C by the co-inoculation of different strains of *Lactobacillus plantarum* (NGL5, NGL7 and LpTx) and *Escherichia coli* 1077. AL5E, AL7E and ALpTxE represent samples co-inoculated with *E. coli* 1077 and *L. plantarum* strains NGL5, NGL7 and LpTx respectively.

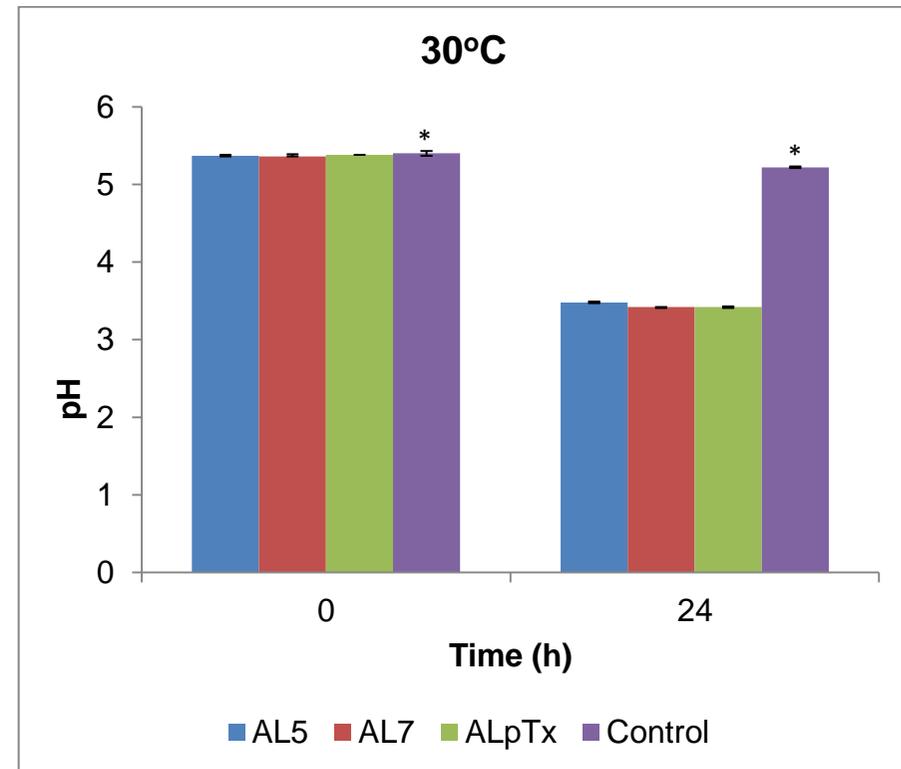
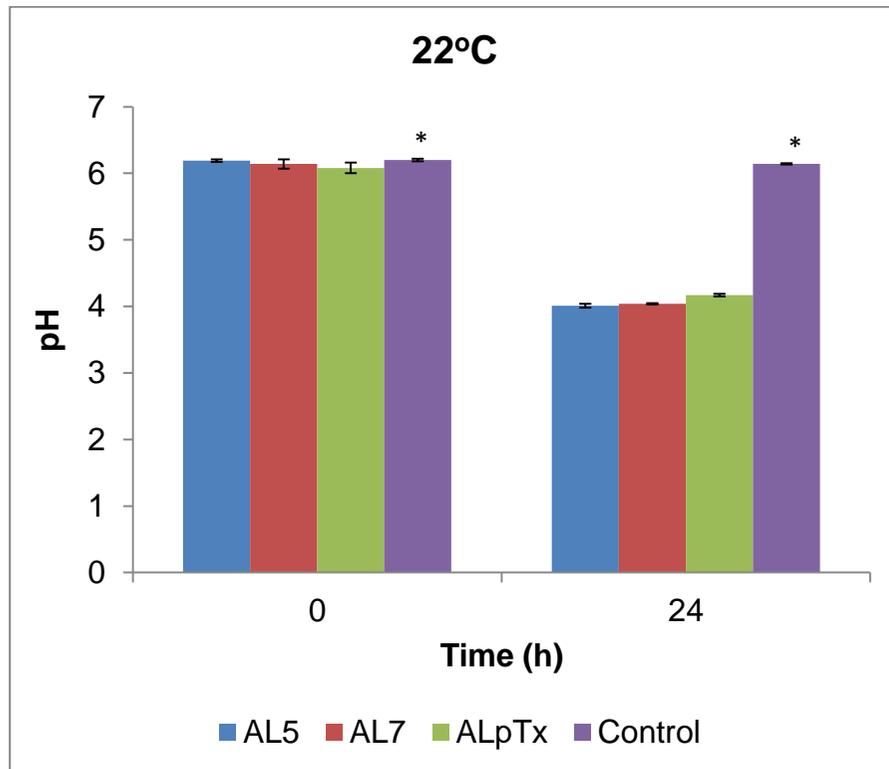


Figure 4.5: pH of sterile ground maize slurries fermented at 22°C and 30°C for 24 h by *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) before the inoculation of *Escherichia coli* 1077
 AL5, AL7 and ALpTx represent samples fermented with *L. plantarum* strains NGL5, NGL7 and LpTx respectively
 *pH of the control samples did not differ significantly ($p \leq 0.05$)

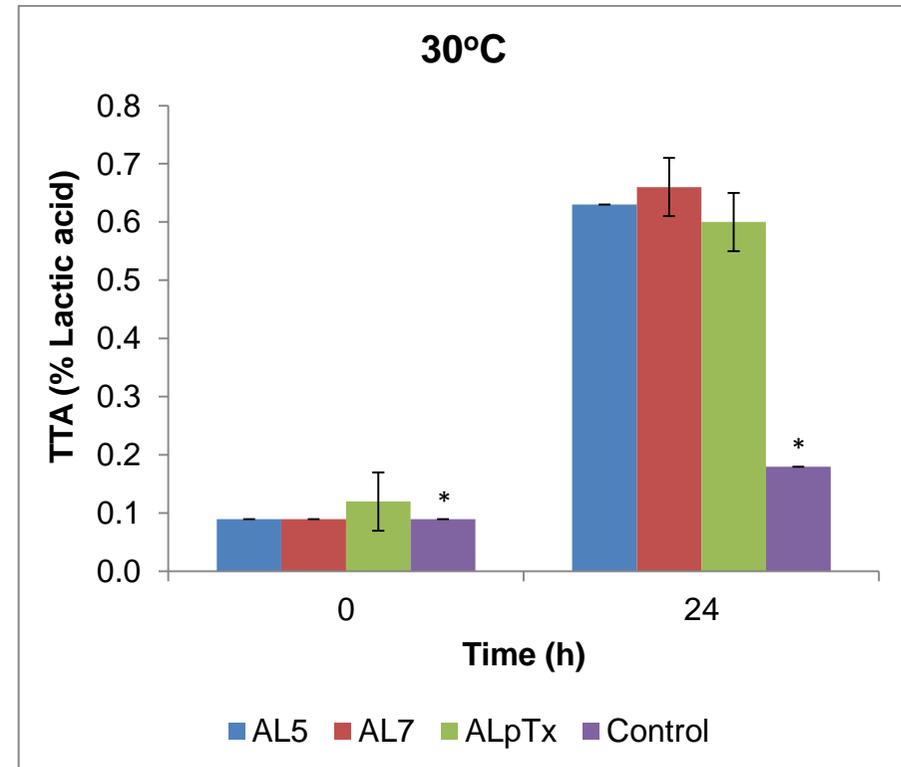
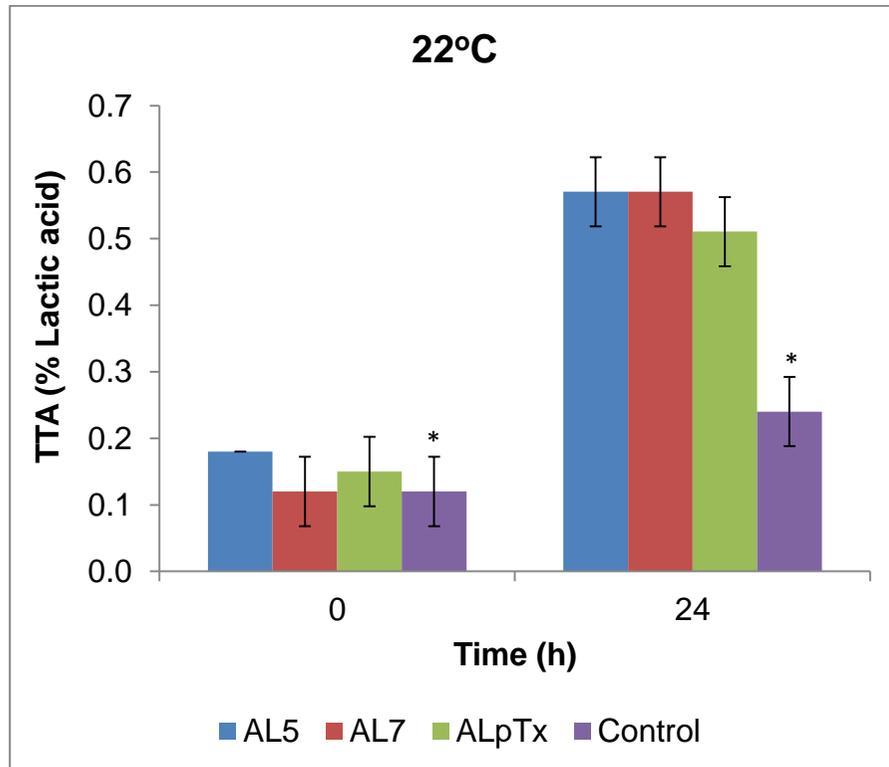


Figure 4.6: Total titratable acidity (TTA) as %Lactic acid of sterile ground maize slurries fermented at 22°C and 30°C for 24 h by *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) before the inoculation of *Escherichia coli* 1077
 AL5, AL7 and ALpTx represent samples fermented with *L. plantarum* strains NGL5, NGL7 and LpTx respectively
 *TTA of the control samples did not differ significantly ($p \leq 0.05$)

4.3.1.2.2 Microbial growth analysis

4.3.1.2.2.1 Co-inoculation

The viable count of the *Lactobacillus plantarum* strains and the *Escherichia coli* 1077 in the co-inoculation fermentation were shown in Figure 4.7 and Figure 4.8 respectively. The growth model parameters of *L. plantarum* strains were presented in Table 4.1. Although, there was a period of lag observed at 22°C, the temperature had no significant ($p \leq 0.05$) influence on the growth rate of the *L. plantarum* strains. Before the decline in the level of *E. coli* 1077, there was an observed increase within the first 6 and 12 h of fermentation at 30 and 22°C respectively. Table 4.2 and Table 4.3 show the model parameters for the growth and death of *E. coli* 1077 respectively.

The growth rate of *E. coli* 1077 at 30°C was significantly ($p \leq 0.05$) lower than at 22°C with values ranging from 0.03 - 0.05 h⁻¹. The doubling time at 30°C (≥ 13.84 h) was therefore significantly ($p \leq 0.05$) higher than at 22°C (≤ 6.09 h) (Table 4.2). The reverse became the case when *E. coli* 1077 was set on decline. The death rate at 30°C (≥ 0.10 h⁻¹) was significantly ($p \leq 0.05$) greater than death rate at 22°C (≤ 0.02 h⁻¹) with decimal reduction time ≥ 41.10 and ≤ 6.00 h at 22 and 30°C respectively (Table 4.3). *E. coli* 1077 was thus below detection limit after 12 h and was completely inhibited after 24 h at 30°C. At 22°C viable counts of over 7.0 Log₁₀ CFU mL⁻¹ were obtained after 24 h but fell below 2 Log₁₀ CFU mL⁻¹ after 48 h for NGL5 and NGL7. Continuous growth (6.72 - 7.59 Log₁₀ CFU mL⁻¹ at 30°C and 6.65 - 8.69 Log₁₀ CFU mL⁻¹ at 22°C) was observed in the control sample with growth rate ≥ 0.09 h⁻¹ and doubling time of ≤ 7.49 h.

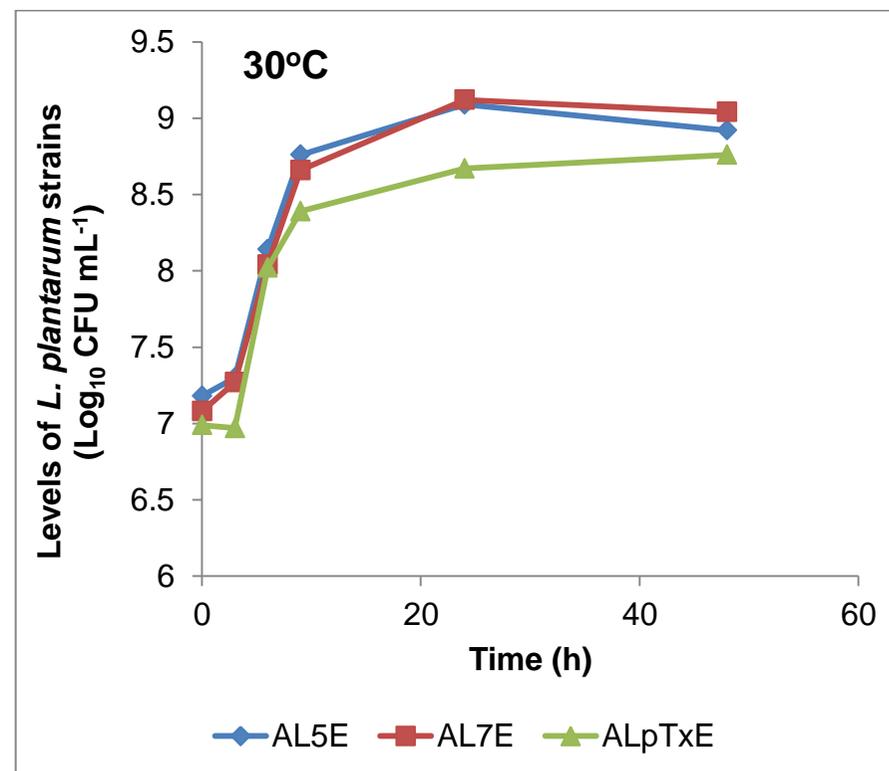
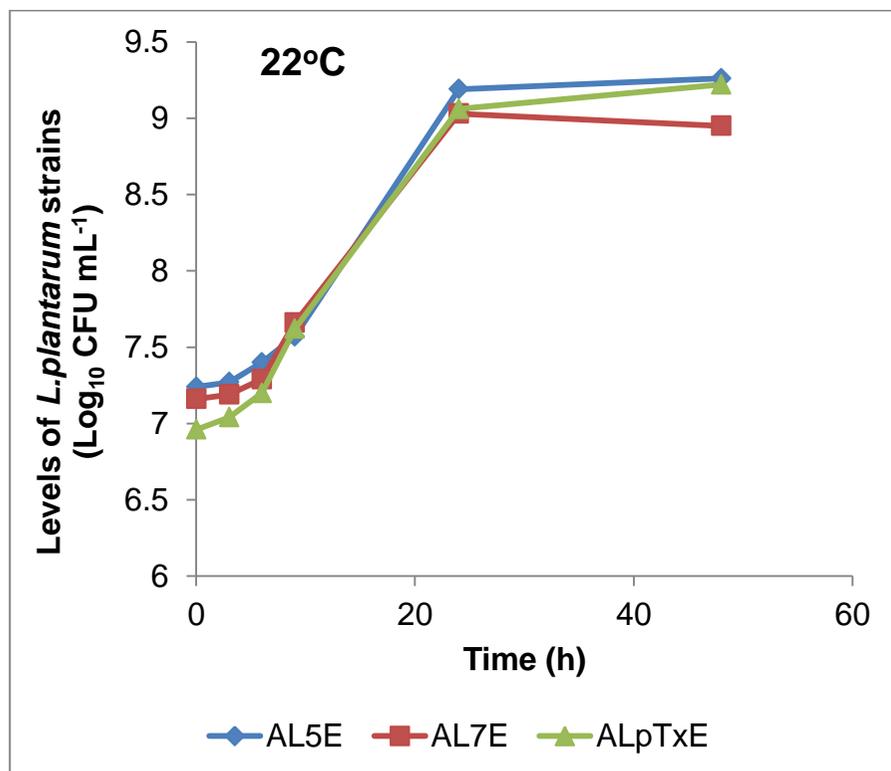


Figure 4.7: Levels of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) in sterile ground maize slurries fermented by co-inoculation of the *L. plantarum* strains and *Escherichia coli* 1077 at 22°C and 30°C
 AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively.

Table 4.1: Modelling parameters for the growth of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) in sterile ground maize slurries co-inoculated with *Escherichia coli* 1077

curve	Temp (°C)	Modelling parameters										T (h)
		^a Experimental data		^b Curvature parameters		^c Primary parameters				^d Statistics		
		yDatMin	yDatMax	mCurv	nCurv	Rate(num)	lag	y0	yEnd	se(fit)	R ²	
AL5E	22	7.20±0.02 ^a	9.28±0.05 ^{ab}	10	1	0.22±0.06 ^a	7.85±1.04 ^a	7.28±0.04 ^a	9.21±0.03 ^a	0.08	0.99	3.04
	30	7.18±0.02 ^a	9.09±0.07 ^c	10	0	0.22±0.05 ^a		7.07±0.11 ^{ab}	9.01±0.00 ^c	0.17	0.95	3.13
AL7E	22	7.15±0.02 ^{ab}	9.31±0.07 ^a	10	0	0.14±0.06 ^a	4.38±3.81 ^b	7.11±0.08 ^{ab}	9.12±0.06 ^{ab}	0.13	0.97	4.71
	30	7.08±0.08 ^{abc}	9.15±0.04 ^{bc}	10	0	0.20±0.03 ^a		6.98±0.07 ^b	9.08±0.05 ^{bc}	0.13	0.97	3.36
ALpTxE	22	6.93±0.08 ^c	9.24±0.05 ^{ab}	10	1	0.14±0.06 ^a	3.38±3.17 ^c	6.91±0.10 ^b	9.19±0.04 ^{ab}	0.09	0.99	4.71
	30	6.96±0.13 ^{bc}	8.78±0.00 ^d	10	0	0.20±0.05 ^a		6.84±0.17 ^b	8.72±0.04 ^d	0.18	0.93	3.44

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable counts (Log_{10} CFU mL^{-1})

^b mCurv and nCurv are the curvature parameters at the beginning and end of the linear phase respectively

^c Rate: the potential maximum growth rate of the microorganism (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper asymptote of the sigmoid curve. Lag: The lag phase duration (h)

^d Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

T: Doubling time ($0.693/\mu$; μ = maximum growth rate)

AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively

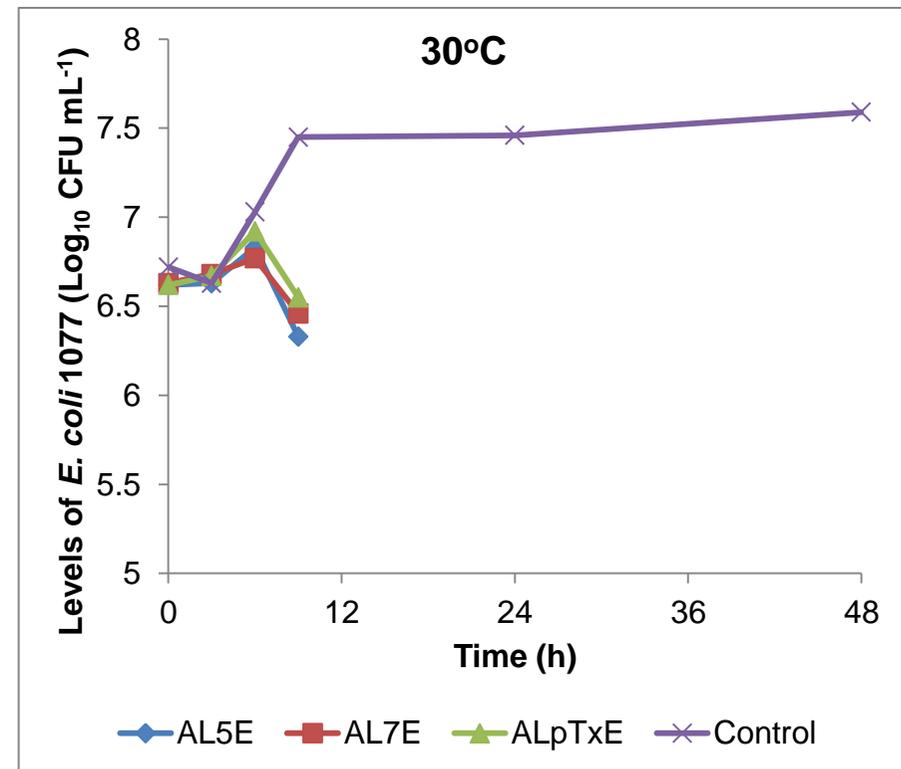
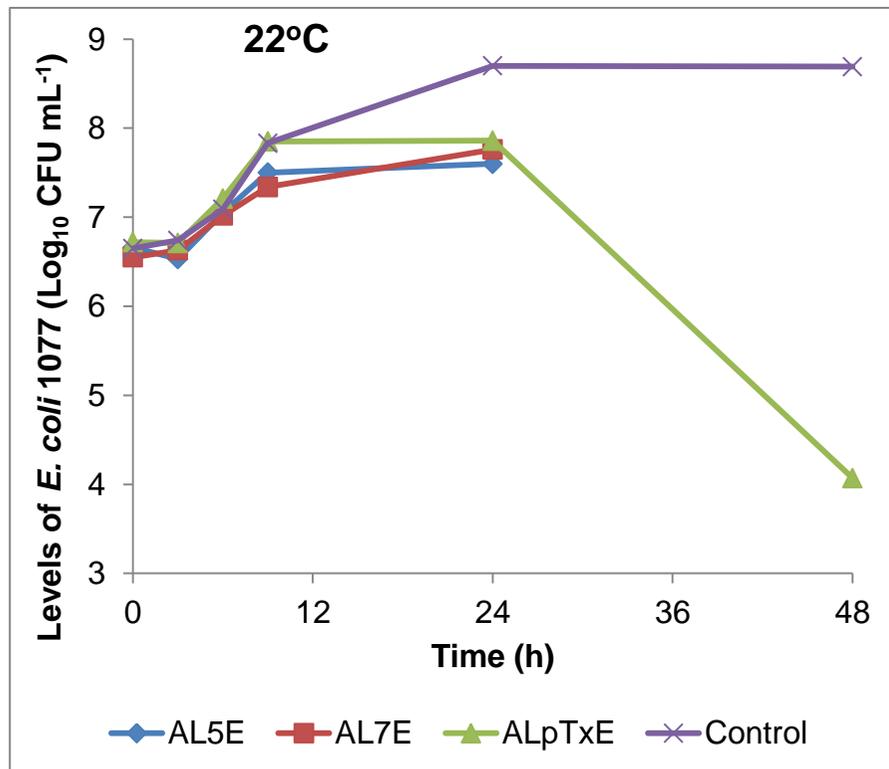


Figure 4.8: Levels of *Escherichia coli* 1077 in sterile ground maize slurries fermented by co-inoculation of the *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) and *E. coli* 1077 at 22°C and 30°C
 AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively.

Table 4.2: Modelling parameters for growth of *Escherichia coli* in sterile ground maize slurries co-inoculated with *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Curve	Temp (°C)	^a Experimental data		Modelling parameters				T (h)
		yDatMin	yDatMax	^b Primary parameters		^c Statistics		
				Rate (num)	y0	se(fit)	R ²	
AL5E	22	6.52±0.02 ^b	7.88±0.17 ^{bc}	0.114±0.00 ^a	6.44±0.04 ^b	0.21	0.86	6.09
	30	6.60±0.06 ^{ab}	6.83±0.06 ^d	0.035±0.01 ^b	6.59±0.06 ^{ab}	0.08	0.76	20.08
AL7E	22	6.51±0.05 ^b	7.98±0.06 ^b	0.131±0.03 ^a	6.44±0.04 ^b	0.15	0.90	5.30
	30	6.60±0.03 ^{ab}	6.77±0.06 ^d	0.028±0.02 ^b	6.60±0.04 ^{ab}	0.01	0.97	25.12
ALpTxE	22	6.71±0.10 ^a	8.01±0.07 ^b	0.124±0.00 ^a	6.56±0.10 ^{ab}	0.20	0.90	5.59
	30	6.58±0.02 ^{ab}	6.92±0.05 ^d	0.050±0.01 ^b	6.59±0.03 ^{ab}	0.10	0.86	13.84
Control	22	6.62±0.06 ^{ab}	8.70±0.02 ^a	0.092±0.00 ^a	6.69±0.07 ^a	0.28	0.88	7.49
	30	6.63±0.09 ^{ab}	7.66±0.22 ^c	0.104±0.02 ^a	6.60±0.07 ^{ab}	0.13	0.90	6.64

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable counts ($\text{Log}_{10} \text{CFU mL}^{-1}$)

^b Rate: the potential maximum growth rate of the microorganism (h^{-1}); y0: initial point of the sigmoid curve; yEnd: upper asymptote of the sigmoid curve had no values; There was no lag duration

^c Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

^d T: Doubling time ($0.693/\mu$; μ = maximum growth rate)

^e mCurv and nCurv are the curvature parameters at the beginning and end of the linear phase were 10 and 0 respectively

^f AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively

Table 4.3: Modelling parameters of the death of *Escherichia coli* 1077 in sterile ground maize slurries co-inoculated with *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Curve	Temp (°C)	^a Experimental data		Modelling parameters				D value (h)
				^b Primary parameters		^c Statistics		
		yDatMin	yDatMax	rate	y0	se(fit)	R ²	
AL5E	22	7.60±0.08 ^a	7.88±0.17	-0.02±0.01 ^a	7.88±0.17	0	1	1.62 ^a
	30	6.33±0.06 ^b	6.83±0.06	-0.17±0.04 ^b	6.83±0.06	0	1	0.78 ^b
AL7E	22	7.76±0.21 ^a	7.98±0.06	-0.02±0.02 ^a	7.98±0.06	0	1	1.75 ^a
	30	6.46±0.07 ^b	6.77±0.06	-0.10±0.01 ^c	6.77±0.06	0	1	0.99 ^b
ALpTxE	22	7.86±0.05 ^a	8.01±0.07	-0.01±0.01 ^a	8.01±0.07	0	1	1.89 ^a
	30	6.55±0.48 ^b	6.92±0.05	-0.12±0.17 ^{bc}	6.92±0.05	0	1	0.91 ^b

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable counts (Log_{10} CFU mL^{-1})

^b Rate: the potential maximum growth rate of the microorganism (h^{-1}); y0: initial point of the sigmoid curve; yEnd: lower asymptote of the sigmoid curve had no values; There was no lag duration

^c Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

D value: Decimal reduction time ($1/\mu$; μ = maximum growth rate)

mCurv and nCurv are the curvature parameters at the beginning and end of the linear phase were 10 and 0 respectively

AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively

4.3.1.2.2.2 Challenge after 24 h of fermentation with *Lactobacillus plantarum* strains

Table 4.4 shows the viable count of the *L. plantarum* strains ($7 - 9 \text{ Log CFU mL}^{-1}$) in the 24 h fermented maize slurry at both temperatures before the inoculation of *E. coli* 1077. The effects of the 24 h fermented samples with $\text{pH} \leq 4.17$ on the viable counts of *E. coli* 1077 were shown in Figure 4.9. The number of *E. coli* 1077 colony forming units were significantly ($p \leq 0.05$) reduced within the first 40 min after inoculation. After 60 min at 30°C *E. coli* 1077 was below detection limit and completely inhibited in LpTx sample after 120 min. Although at 22°C , viable counts of 4.62 ± 0.08 and $5.53 \pm 0.25 \text{ Log}_{10} \text{ CFU mL}^{-1}$ were obtained after 120 min in NGL5 and NGL7 samples respectively, inhibition was observed after 24 h. Fermentation with NGL5 did not show any significant difference in the death rate and the D value of *E. coli* 1077 at both temperatures. At 30°C , the death rate of *E. coli* 1077 in LpTx (-0.10 h^{-1}) and NGL7 (-0.13 h^{-1}) fermented samples were significantly ($p \leq 0.05$) greater than the death rate at 22°C (-0.02 h^{-1}) while the D values from LpTx and NGL7 samples at 30°C were significantly ($p \leq 0.05$) the least ($D_{30} \leq 1.00 \text{ min}$), as shown in Table 4.5. *E. coli* 1077 levels increased from 7.59 to $8.04 \text{ Log}_{10} \text{ CFU mL}^{-1}$ at 30°C but had no significant ($p \leq 0.05$) change at 22°C ($7.79 - 7.77 \text{ Log}_{10} \text{ CFU mL}^{-1}$) in the control.

In the trial run (Table 4.6) where *E. coli* 1077 was inoculated into 150 mL of 24 h *L. plantarum* fermented slurries, *E. coli* 1077 decreased below detection limit after 20 mins. Similar result was obtained when *E. coli* 1077 was inoculated into the porridges from the fermented ground maize slurries as shown in Figure 4.10. The porridges had pH of 3.41 and TTA of 0.72, 0.81 and 0.90 % for samples fermented by NGL5, NGL7 and LpTx respectively.

Table 4.4: Viable count of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) in 24 h fermented sterile ground maize slurries before inoculation of *Escherichia coli* 1077

Temp (°C)	Time (h)	Samples			
		AL5	AL7	ALpTx	Control
22	0	7.44±0.02 ^d	7.11±0.08 ^{ef}	6.97±0.08 ^g	-
	24	9.15±0.03 ^{ab}	9.03±0.09 ^b	9.06±0.05 ^{ab}	-
30	0	7.19±0.05 ^e	7.22±0.05 ^d	6.99±0.03 ^{fg}	-
	24	9.20±0.01 ^a	9.03±0.01 ^b	8.76±0.12 ^c	-

Values with same superscript do not differ significantly ($p \leq 0.05$). N=3±SD

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

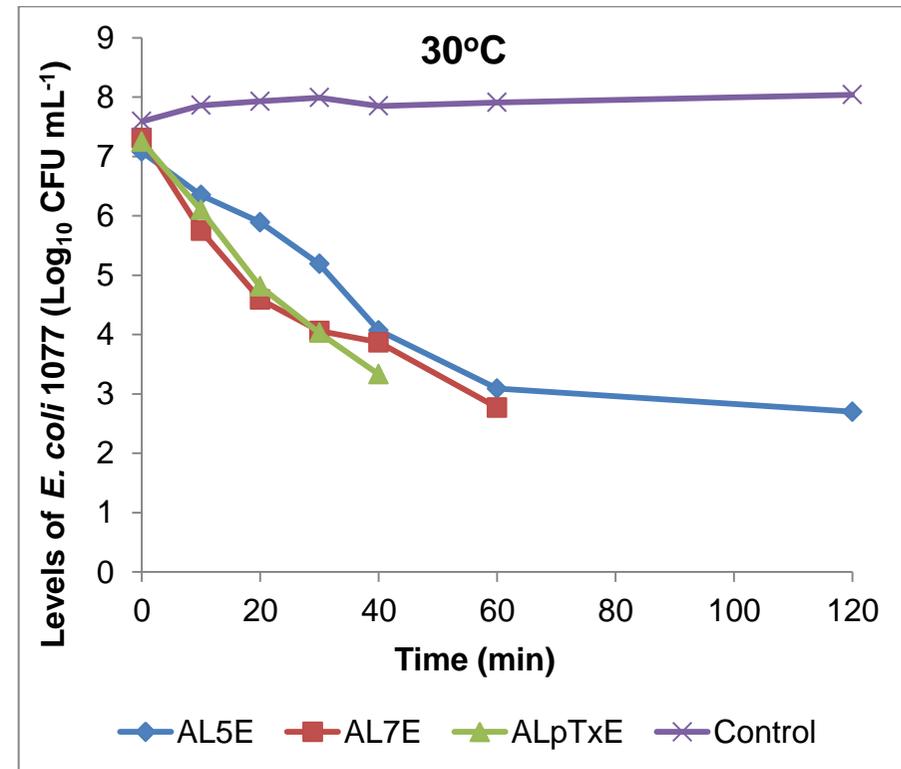
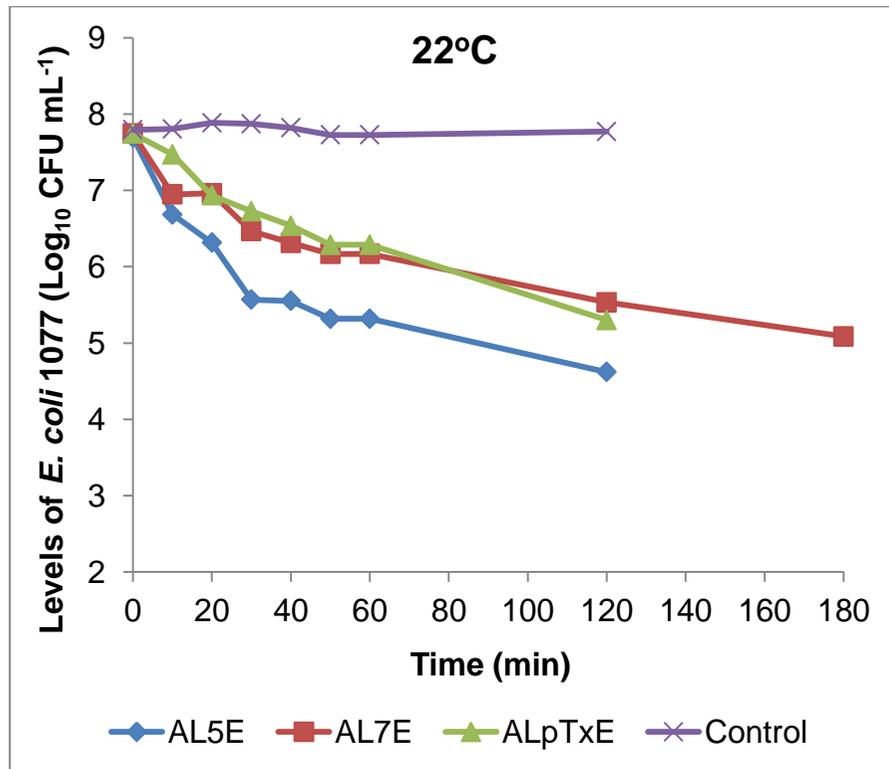


Figure 4.9: Levels of *Escherichia coli* 1077 inoculated into sterile ground maize slurries fermented at 22°C and 30°C for 24 h by *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)
 AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively

Table 4.5: Modelling parameters of the death of *Escherichia coli* 1077 in sterile ground maize slurries fermented at 22 and 30°C for 24 h by *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Curve	Temp (°C)	^a Experimental data		Modelling parameters							D value (min)
				^b Curvature parameters		^c Primary parameters			^d Statistics		
				yDatMin	yDatMax	mCurv	nCurv	rate	y0	yEnd	
AL5E	22	4.62±0.08 ^b	7.70±0.10 ^a	10	0	-0.06±0.02 ^{ab}	7.48±0.17 ^a		0.32	0.89	16.81 ^b
	30	4.07±0.06 ^c	7.08±0.15 ^b	0	0	-0.07±0.00 ^{abc}	7.15±0.10 ^a		0.22	0.96	13.90 ^{bc}
AL7E	22	5.53±0.25 ^a	7.74±0.04 ^a	10	0	-0.02±0.01 ^a	7.39±0.20 ^a		0.24	0.86	41.96 ^a
	30	3.78±0.18 ^{cd}	7.31±0.47 ^{ab}	10	0	-0.13±0.05 ^c	7.17±0.47 ^a	3.98±0.17	0.21	0.97	7.67 ^d
ALpTxE	22	5.30±0.15 ^a	7.74±0.03 ^a	0	0	-0.02±0.00 ^a	7.51±0.21 ^a		0.21	0.92	47.33 ^a
	30	3.33±0.27 ^d	7.25±0.09 ^{ab}	0	0	-0.10±0.01 ^{bc}	7.08±0.10 ^a		0.28	0.96	10.10 ^{cd}

Values that share the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

^a yDatMin and yDatMax: Initial and maximum viable counts ($\text{Log}_{10} \text{CFU mL}^{-1}$)

^b mCurv and nCurv are the curvature parameters at the beginning and end of the linear phase respectively

^c Rate: the potential maximum growth rate of the microorganism (h^{-1}); y0: initial point of the sigmoid curve; yEnd: lower asymptote of the sigmoid curve had no value

^d Se(fit) Standard error of fitting which is the estimated standard deviation of the observed independent values; R²: Adjusted R-square statistics of the fitting

D value: Decimal reduction time ($1/\mu$; μ = maximum growth rate)

AL5E, AL7E and ALpTxE represent samples fermented by *L. plantarum* strains NGL5, NGL7 and LpTx in co-inoculation with *E. coli* 1077 respectively

Table 4.6: The pH, total titratable acidity (TTA) and *Escherichia coli* 1077 levels in 150 mL of ground maize slurries fermented at 30°C for 24 h by the *Lactobacillus plantarum* strain (NGL5, NGL7 and LpTx)

Samples	pH		TTA (% Lactic acid)		<i>E. coli</i> 1077 levels (Log ₁₀ CFU mL ⁻¹) in ground maize slurries		
	Time (h)		Time (h)		Time (min)		
	0	24	0	24	0	10	20
AL5	5.85±0.01	3.37±0.05	0.18±0.00	0.78±0.19	5.36±0.32	2.91±0.09	>2
AL7	5.86±0.01	3.40±0.04	0.17±0.01	0.81±0.09	5.84±0.16	3.21±0.28	>2
ALpTx	5.84±0.02	3.41±0.01	0.12±0.05	0.93±0.05	5.52±0.12	3.74±0.01	>2

N=3±SD

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

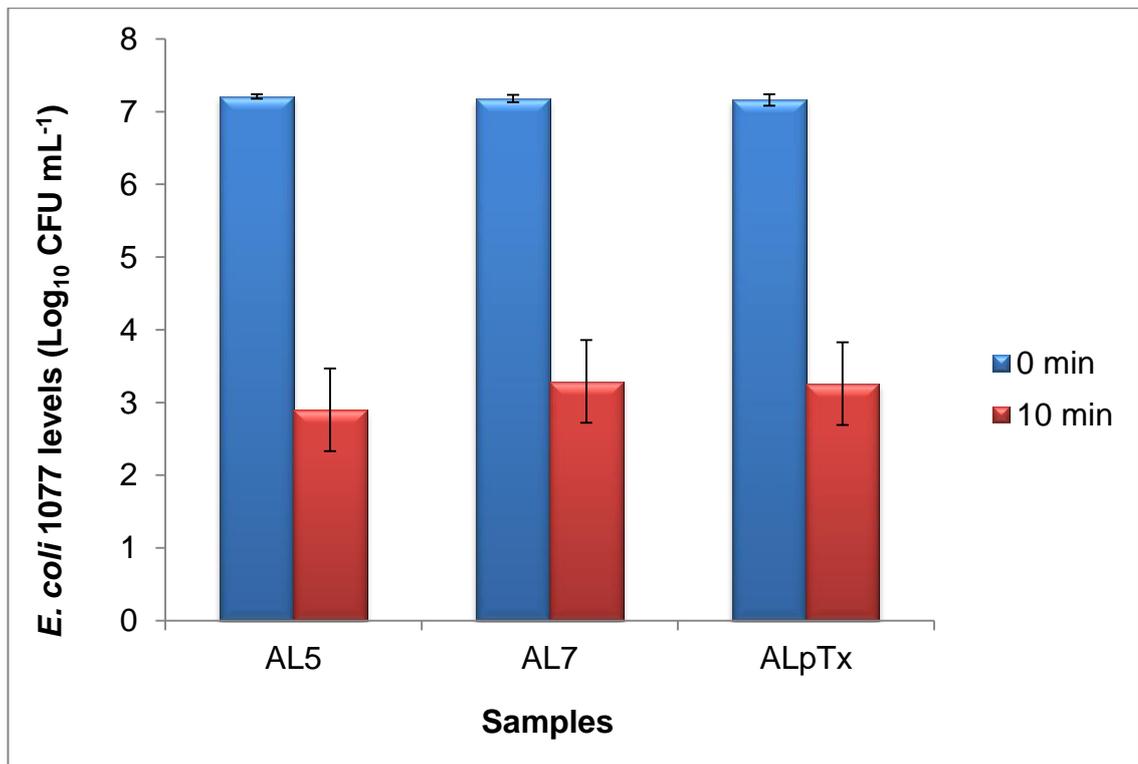


Figure 4.10: Levels of *Escherichia coli* 1077 in *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) fermented ground maize porridges AL5, AL7 and ALpTx represent samples fermented with *L. plantarum* strains NGL5, NGL7 and LpTx respectively.

4.3.1.2.2.3 Correlation analysis

The correlation coefficients between pH, TTA, and the levels of the *L. plantarum* strains and *E. coli* 1077 in the co-inoculation fermentation were shown in Appendix A4.3. All the variables were correlated ($p \leq 0.01$) after 24 and 48 h. Viable *L. plantarum* count however, had a negative correlation coefficient with pH after 3 h and as fermentation progressed. *E. coli* 1077 levels had a positive correlation coefficient with pH after 24 h at 22°C and after 6 h at 30°C.

4.3.2 Acid and bile tolerance ability of Nigerian fermented maize food- *L. plantarum* strains

In Figure 4.11, viable counts of both strains of *L. plantarum* at pH 2 after 3 h were reduced from $\geq 8.26 \pm 0.05$ to $\leq 4.94 \pm 0.49$ Log_{10} CFU mL^{-1} . Subsequent incubation of the acid stressed cells in 0.3% ox gall bile media for 6 h resulted in growth enhancement to 5.73 ± 0.13 and 7.93 ± 0.12 Log_{10} CFU mL^{-1} for NGL5 and NGL7. The viability of the acid stressed cells in MRS and MRS with 0.3% ox gall bile for NGL5 and NGL7 were presented in Figure 4.12 and Figure 4.13 respectively. Although, NGL5 cells did not show quick recovery within the first 3 h of exposure to bile, the viability of both strains of *L. plantarum* at each time in normal MRS did not differ significantly ($p \leq 0.05$) from the bile condition.

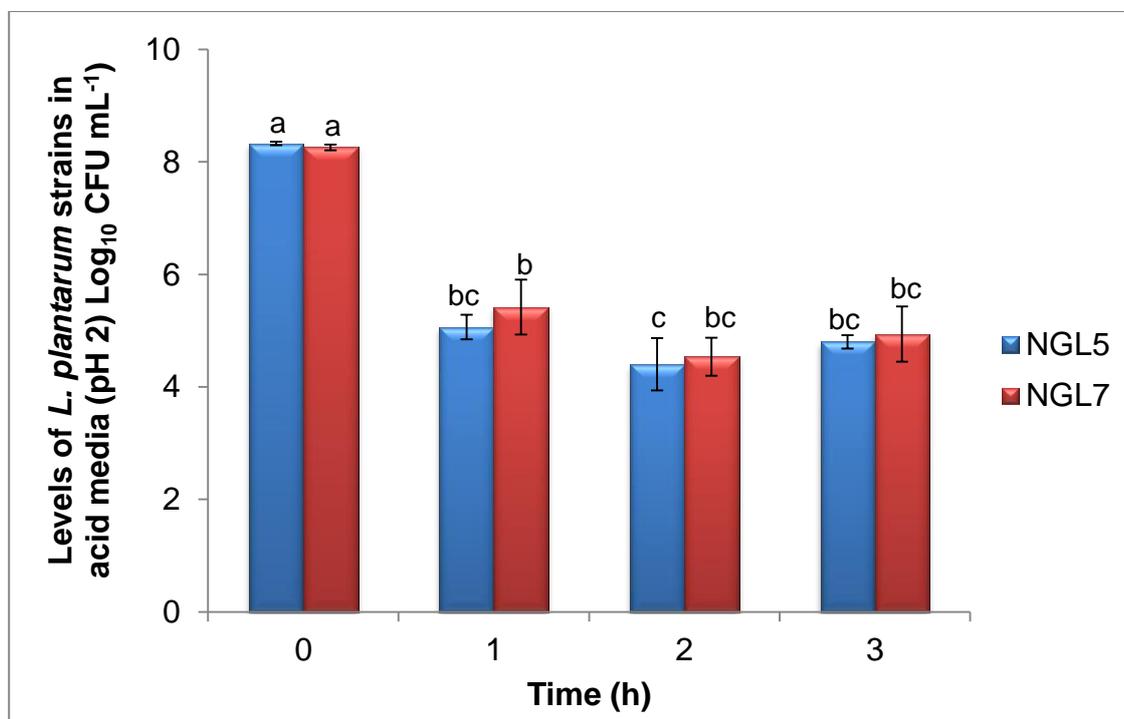


Figure 4.11: Effect of exposure to pH 2.0 on the viable count of two *Lactobacillus plantarum* strains (NGL5 and NGL7) isolated from Nigerian fermented maize food- *akamu*.

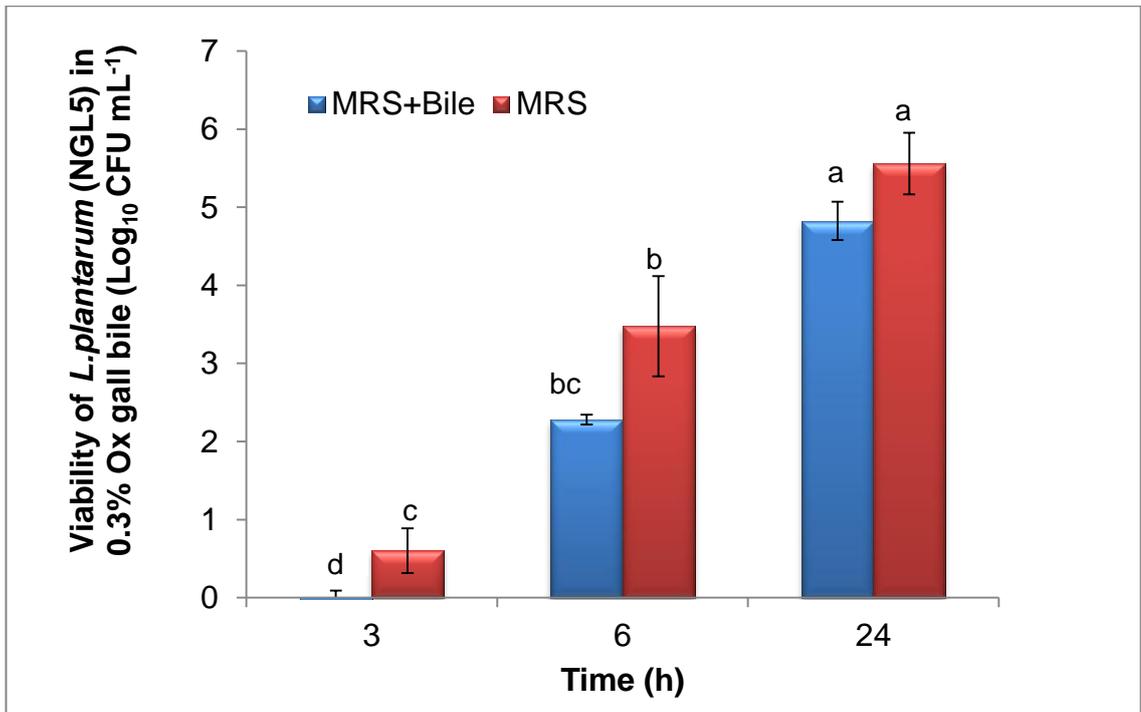


Figure 4.12: Viability of *Lactobacillus plantarum* (NGL5) in MRS broth with and without 0.3% Ox gall bile

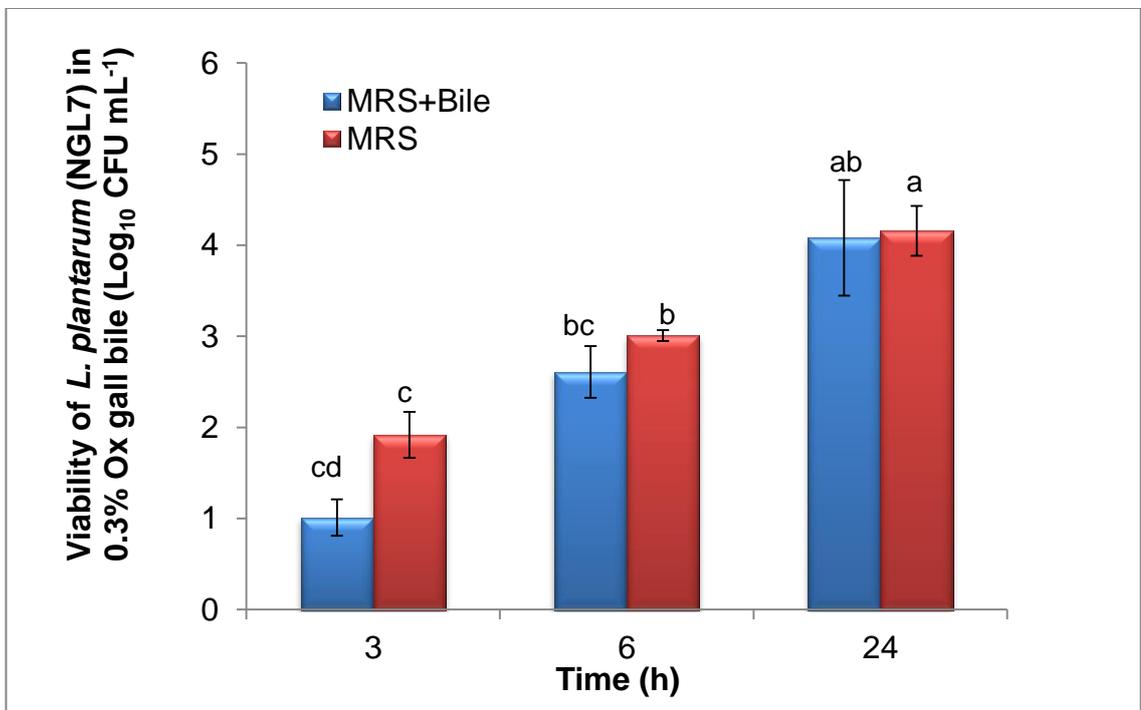


Figure 4.13: Viability of *Lactobacillus plantarum* (NGL7) in MRS broth with and without 0.3% Ox gall bile

4.3.3 Aggregation potential of Nigerian fermented maize food

***Lactobacillus plantarum* strains (NGL5 and NGL7)**

Aggregation ability of the bacterial cells investigated based on their sedimentation characteristics at room (22 - 24°C) and body temperatures (37°C) were shown in Table 4.7. There was significant ($p \leq 0.05$) increase in auto-aggregation of all bacterial cells with time. On the average, auto-aggregation at body temperature was significantly ($p \leq 0.05$) greater than auto-aggregation at room temperature.

After the first 5 h, the auto-aggregation of the *L. plantarum* strains increased from 8 - 17% and 11 - 30% at room and body temperature respectively. While the pathogen had auto-aggregation from <7 to <16% and <3 to <7% at room and body temperature respectively. However, more sedimentation was observed after 24 h with aggregation at room temperature (75 - 90%) significantly ($p \leq 0.05$) greater than at body temperature (61 - 72%). Autoaggregation of the pathogens after 24 h was $\geq 24\%$. The *L. plantarum* strains were unable to co-aggregate with the pathogens (<6%) at both temperatures (Table 4.8).

Table 4.7: Auto-aggregation (%) of the *Lactobacillus plantarum* strains (NGL5, NGL7 & LpTx) and foodborne pathogens at two different temperatures

Bacteria		Temp. (°C)	2 h	4 h	5 h	24
<i>L. plantarum</i> strains	LpTx	37	13.69±0.65 ^b	21.71±1.26 ^{ab}	28.15±1.16 ^a	72.15±0.62 ^d
		Room (22-24)	8.77±0.24 ^d	13.51±0.71 ^c	16.94±0.76 ^c	89.54±1.01 ^a
	NGL5	37	17.25±0.27 ^a	25.23±0.75 ^a	29.61±0.11 ^a	61.59±0.72 ^f
		Room (22-24)	8.52±0.30 ^d	13.18±1.06 ^c	15.25±1.03 ^{cd}	75.48±1.26 ^c
	NGL7	37	11.12±0.45 ^c	19.70±1.00 ^b	24.96±0.36 ^b	67.70±1.28 ^e
		Room (22-24)	8.48±0.89 ^d	13.09±1.20 ^c	16.42±0.50 ^c	79.80±1.30 ^b
Foodborne pathogens	<i>Escherichia coli</i> 1077	37	5.67±0.42 ^e	8.14±2.61 ^{de}	15.56±0.85 ^{cd}	43.74±0.41 ^g
		Room (22-24)	2.91±0.51 ^f	5.31±0.37 ^{ef}	6.36±0.84 ^e	24.23±1.17 ⁱ
	<i>Salmonella</i> Enteritidis NCTC 5188	37	6.21±0.66 ^e	8.90±1.09 ^d	13.63±0.57 ^d	32.98±0.75 ^h
		Room (22-24)	2.58±0.09 ^f	4.32±0.70 ^f	6.50±1.11 ^e	34.23±1.15 ^h

Values with same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

Table 4.8: Co-aggregation (%) of the *Lactobacillus plantarum* strains (NGL5, NGL7 & LpTx) with foodborne pathogens at two different temperatures

<i>L. plantarum</i>	Temp. (°C)	<i>Escherichia coli</i> 1077			<i>Salmonella</i> Enteritidis NCTC 5188		
		2 h	5 h	24 h	2 h	5 h	24 h
LpTx	37	4.58±1.48 ^a	-3.02±0.59 ^d	-27.87±6.36 ^c	6.40±0.76 ^a	5.70±1.11 ^a	-12.28±1.16 ^a
	Room (22-24)	2.29±0.70 ^{ab}	3.35±1.77 ^{ab}	1.94±6.10 ^{ab}	0.01±0.00 ^c	0.01±0.02 ^c	-0.01±0.02 ^b
NGL5	37	-1.20±1.26 ^c	-1.15±0.44 ^{cd}	-10.67±2.23 ^b	3.43±0.23 ^b	3.21±0.29 ^b	-9.27±2.24 ^a
	Room (22-24)	2.47±0.78 ^{ab}	3.89±0.12 ^a	10.50±8.54 ^a	0.01±0.00 ^c	0.01±0.02 ^c	0.00±0.10 ^b
NGL7	37	3.41±0.21 ^{ab}	2.12±1.27 ^{ab}	-11.84±2.55 ^b	2.88±0.66 ^b	1.20±0.32 ^c	-11.23±1.74 ^a
	Room (22-24)	1.50±0.36 ^b	0.89±0.43 ^{bc}	1.00±1.86 ^{ab}	0.01±0.01 ^c	0.01±0.00 ^c	0.08±0.04 ^b

Values with same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

4.3.4 Hydrophobicity of Nigerian fermented maize food *Lactobacillus plantarum* strains

In Figure 4.14, there was significant variation in the ability of the *L. plantarum* strains to adhere to solvent pairs: chloroform/hexadecane and ethyl acetate/hexane. Adhesions to the mono polar solvents were significantly ($p \leq 0.05$) higher than the *n*-alkanes with significant maximal affinity for chloroform an acidic solvent. The general order of display of affinity was chloroform > ethyl acetate > hexadecane > hexane. NGL7 had significantly ($p \leq 0.05$) the highest affinity for all the solvents.

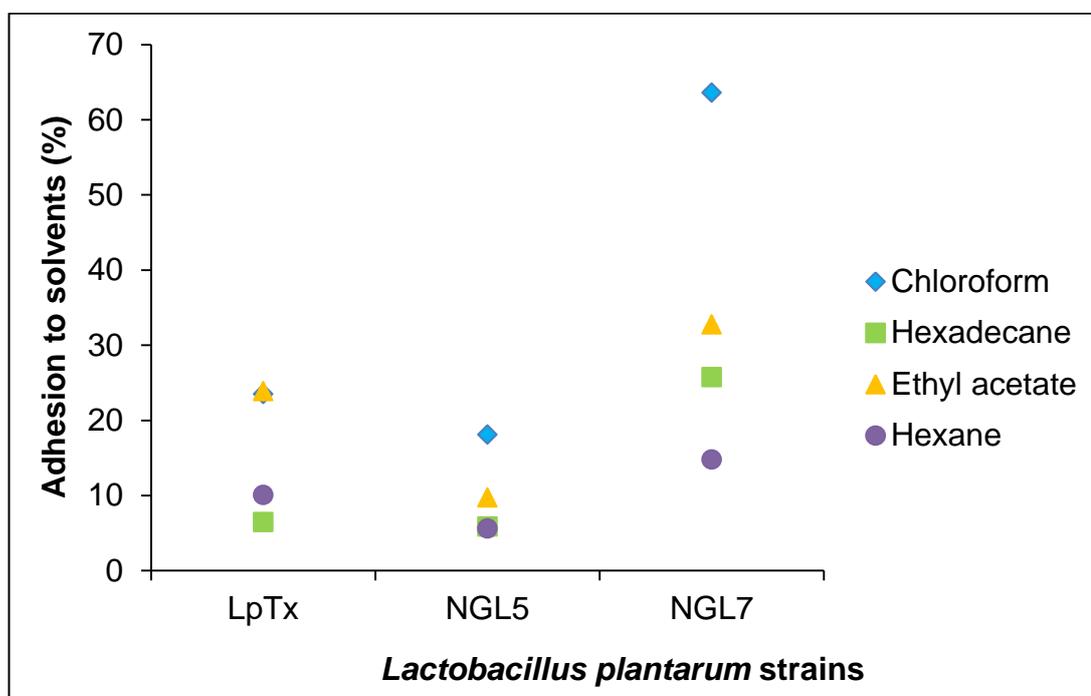


Figure 4.14: Adhesion of *Lactobacillus plantarum* strains (LpTx, NGL5 and NGL7) to solvents

4.3.5 Adhesion to porcine mucin and Caco-2 cells

The ability of the LAB to adhere to porcine mucin was shown in Table 4.9. Adhesion of the *L. plantarum* strains was significantly ($p \leq 0.05$) higher at 37°C (0.43- 1.45) than at 4°C (0.06- 0.32). *L. reuteri* NCIB 11951 had significantly ($p \leq 0.05$) highest adhesion of 1.40 at 4°C. LpTx had significant ($p \leq 0.05$)

reduction and the least adhesion after 20 h at 37°C. The absorbance readings are presented in Appendix A4.4. Adhesion after 2 h at 37°C was taken as a reference condition and the evaluated viable adhered cells ranged between 6.51 - 6.61 Log₁₀ CFU mL⁻¹ for LpTx and *L. reuteri* NCIB 11951 respectively.

More than 80 bacterial cells were microscopically observed to have adhered to the differentiated Caco-2 cells (Figure 4.15). In Table 4.10, the adherent viable counts on the Caco-2 cells ranged between 4.22 and 5.53 Log₁₀ CFU mL⁻¹ for LpTx and NGL5 respectively.

Table 4.9: Adhesion of *Lactobacillus plantarum* strains (LpTx, NGL5 and NGL7) to porcine mucin

LAB	*Adhesion relative to Mucin OD ₄₀₃			Viable cell count at 37°C after 2 h	
	37°C (2 h)	37°C (20 h)	4°C (20 h)	Initial cells	Adhered cells
NGL5	0.72±0.03 ^b	1.07±0.22 ^a	0.11±0.13 ^b	8.43±0.07 ^a	6.52±0.02 ^a
NGL7	0.62±0.04 ^b	0.80±0.07 ^a	0.32±0.27 ^b	8.22±0.02 ^{bc}	6.60±0.11 ^a
LpTx	1.45±0.06 ^a	0.43±0.09 ^b	0.06±0.09 ^b	8.15±0.01 ^c	6.51±0.11 ^a
<i>L. reuteri</i>					
NCIB 11951	0.72±0.04 ^b	0.86±0.04 ^a	1.40±0.39 ^a	8.25±0.02 ^b	6.61±0.10 ^a

Values with same superscript in the same column do not differ significantly (p≤0.05). N=3±SD

*Adhesion = (OD_{mL}-OD_m)/OD_m. N=3 ±SD.

Table 4.10: Viable cell (Log₁₀ CFU mL⁻¹) adherence of *Lactobacillus plantarum* strains (LpTx, NGL5 and NGL7) to Caco-2 cells

<i>L. plantarum</i> strains	Viable cell count	
	Inocula	Adherence
NGL5	8.38±0.02	5.53±0.05
NGL7	8.09±0.04	5.13±0.05
LpTx	8.17±0.10	4.22±0.10

N = 3±SD

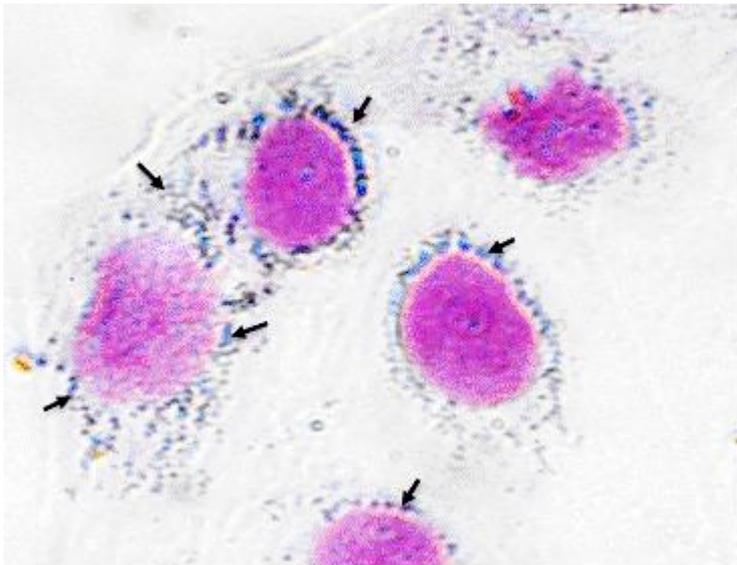


Figure 4.15: Adhered *Lactobacillus plantarum* cells on Caco-2 cell lines
Arrows pointing at some of the *L. plantarum* cells

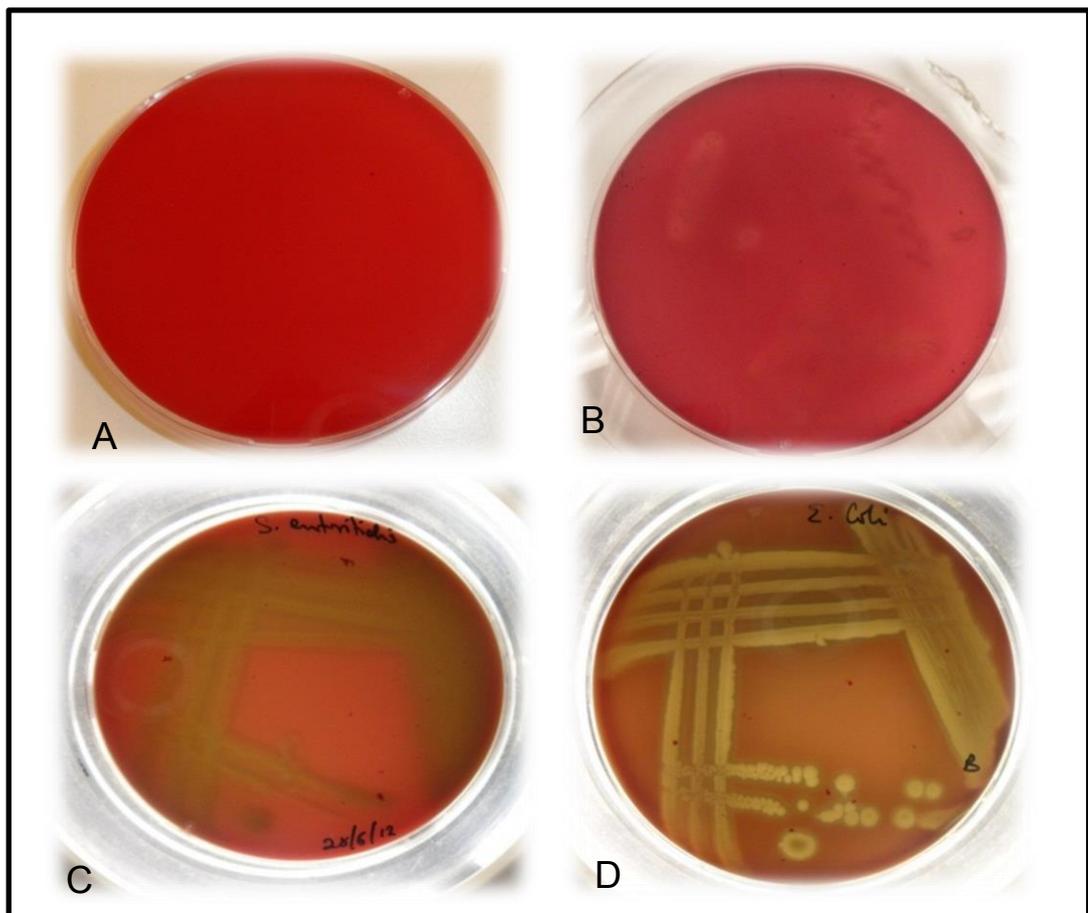


Figure 4.16: Demonstration of haemolytic activity: (A) Fresh blood agar, (B) γ -haemolysis of *Lactobacillus plantarum* strain, (C) α -haemolysis of *Salmonella enteritidis* NCTC 5188 and (D) β -haemolysis of *Escherichia coli* 1077.

4.3.6 Gelatinase and Haemolytic activity

The *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx) were unable to hydrolyse gelatine. The haemolytic activities of the *L. plantarum* strains and the pathogens were shown in Figure 4.16. The strains of *L. plantarum* exhibited no haemolytic activity (γ -haemolysis), while greenish coloration (α -haemolysis) was observed for *S. Enteritidis* NCTC 5188. *E. coli* 1077 had prolific growth with clear zones around its colonies (β -haemolysis).

4.4 Discussion

4.4.1 Antimicrobial activity

Wide spectra of microorganisms are involved in spontaneous fermentation of traditional *akamu*. Given a favourable environment, particular species predominate determining the product quality and safety. The isolated *L. plantarum* strains were found to be among the re-occurring LAB in the traditional *akamu* samples and possessed significant fermentation ability. The isolated *L. plantarum* strains (NGL5 and NGL7) and their probiotic counterpart (LpTx) were able to produce active inhibitory compounds in the MRS agar that was inhibitory against the five relevant foodborne pathogens: *Bacillus cereus* NCIMB 11925, *Escherichia coli* 1077, *Salmonella enteritidis* NCTC 5188, *Listeria monocytogenes* NCTC 7973, and *Staphylococcus aureus* NCTC 3750.

Inhibitory activities of the *L. plantarum* strains at 30°C were independent of the condition (either aerobic or anaerobic) under which they were grown. Inhibition was observed to be mainly due to acidity as there was no inhibition recorded with the modified MRS media. This was in line with the report of Trias et al.,

(2008) that *L. plantarum* strains were without significant activity against *Lis. monocytogenes* and *Staph.aureus* in MRS with 90% reduced glucose. In relation to *L. plantarum* inhibition against *E. coli*, *Salmonella* and *Staphylococcus*, Tejero-Sariñena et al., (2012) had similar results and attributed the inhibitory effect to lactic and acetic acid production from glucose fermentation. The organic acid profile of ground maize slurry fermented by the selected *L. plantarum* strains in section 3.3.2.2.4 and 3.3.2.2.5 of this thesis confirmed the predominance of lactic acid in the fermentation.

Byaruhanga et al., (1999) attributed the inhibition of *B. cereus* in *mage*, a sour dough beverage, to the production of lactic acid and its pH-lowering effect. *Bacillus cereus* under unfavourable environmental conditions such as nutrient depletion and acid pH, form heat resistant endospores (Mensah, 1997). *Bacillus cereus* inhibition was important in preventing spore formation. *Listeria monocytogenes* although the least significantly ($p \leq 0.05$) inhibited by the *L. plantarum* strains, may not constitute a great concern as usually it can be destroyed by heat during food preparation. *Escherichia coli* and *S. Enteritidis* belong to the family Enterobacteriaceae. This family comprises a large group of bacteria whose members characteristically inhabit the human small intestine. As intestinal dwellers they thrive better in an alkaline pH, hence they were considerably inhibited in the acid medium. Kingamkono et al., (1995) attributed inhibition of enteropathogens: *Campylobacter*, *Salmonella*, *Shigella*, *Escherichia*, *Staphylococcus* and *Bacillus* in lactic-fermenting cereal gruel to acidity.

Some microorganisms produce an acid-tolerance response system that protects them against severe acid stress, in which case inhibition is made possible by other by products of fermentation. Inhibition of *B. cereus*, *E. coli* and *Klebsiella aerogenes* in fermenting *Ogi* was associated to bacteriocin production by the dominant LAB: *L. plantarum* and *L. acidophilus* (Lawal et al., 2009).

The evaluation of the inhibitory activity of the *L. plantarum* strains against *E. coli* 1077 in fermenting ground maize slurry revealed that the growth and metabolism of the *L. plantarum* strains was not limited by the fermentation temperatures (22 and 30°C) regardless of the observed short period of adaptation of the *L. plantarum* strains at 22°C. This may be attributed to the availability of carbohydrate for utilization after adjustment at low temperature. However, the period of lag may have influenced the rate of decrease in pH at the same temperature. The initial variation in sample pH may probably be due to differences in batches of the purchased maize samples and storage period of the irradiated ground maize. One batch of the sample (fermentation at 30°C) had lasted over a year in storage after irradiation while the other batch (sample fermented at 22°C) were freshly purchased and irradiated.

Growth of *E. coli* 1077 in the control sample and the change in pH and acidity observed at both temperatures with the production of gas bubbles after 24 h confirmed the ability of *E. coli* to utilize some carbohydrate present in the slurry with gas production. The utilization of glucose by *E. coli* had been reported by (Fooks and Gibson, 2002), however not much of the acid was probably produced for significant reduction in pH. The ability of *E. coli* 1077 to carry out its metabolic activities at near neutral pH in co-inoculation with the *L. plantarum*

strains must have accounted for the observed growth at the early stages of the fermentation.

Suppression in the growth of *E. coli* 1077 when decline in viable count had no relationship with pH or acidity may be an attribute of competition for nutrient. In the co-inoculation samples, decline in viability after 9 h at 30°C to undetectable levels and inhibition with progression in fermentation was due mostly to acidity. Complete inhibition of *E. coli* 1077 after 24 h at 30°C may also suggest the role of fermentation temperature. At 30°C, higher rate of decrease in pH to levels lower than the strength of the predominant lactic acid (pK_a 3.86) (Adams and Moss, 2000) most have resulted in greater concentration and higher diffusion of the undissociated acids into the cytoplasm. Sudden cytoplasmic acidification at higher temperature compared to the effect at 22°C would have necessitated the complete inhibition after 24 h at 30°C.

Similar inhibition of enteropathogens in fermented cereal food had been reported in literature (Kingamkono et al., 1994, Svanberg et al., 1992, Valenzuela et al., 2008). Nout et al.,(1989b) reported *E. coli* levels below 2.70 Log_{10} CFU g^{-1} after 24 h at 30°C in weaning porridges fermented using inoculum recycling. Porridges prepared from fermentation with $\text{pH} \leq 4.0$, would probably have the same range of pH and microbial contaminants may not survive coupled with the heat treatment during porridge preparation. This was evidenced in the porridges with $\text{pH} \leq 3.4$ prepared from the *L. plantarum* strains fermented ground maize slurries that were maintained at 45°C. *E. coli* 1077 was below detection level after 20 min of inoculation.

Candida tropicalis (NGY1) in the agar spot assay was observed to be without antimicrobial activity against the tested pathogens while *Sacch. boulardii* SB20 showed some level of inhibition. Pathogen inhibition on agar plates by yeast in this present study seemed unique as literature report on similar studies appeared limited. Comparison with related results was therefore difficult and the inhibitory mechanisms of the *Sacch. boulardii* SB20 although unclear, could be attributed to acidity and ethanol production. In section 3.3.2.1.3 and 3.3.2.2.4, fermentation with *C. tropicalis* (NGY1) produced neither significant decrease in pH nor amount of ethanol for the anticipation of antimicrobial activity. Fermentation with *Sacch. boulardii* SB20 was however accompanied with lactic acid concentration of 20.23 mmol L⁻¹ after 48 h and ethanol levels in the range of 42 to 194 mmol L⁻¹ after 12 and 72 h of fermentation.

Edema and Sanni (2008) observed antimicrobial activities in maize meal fermented by *Sacch. cerevisiae*, but attributed their finding to the presence and growth of endogenous LAB in the unsterilized substrate. In another study by Halm and Olsen, (1996) the inhibitory effects of yeast in food matrices were shown to be due to substrate competition while Shetty and Jespersen (2006), attributed the antagonistic property of *Sacch. cerevisiae* to the polysaccharide part of the cell wall that is involved in surface binding of pathogenic toxins.

4.5.2 Acid and bile tolerance

Most strains of *L. plantarum* have been reported to be highly tolerant of acid (Parente et al., 2010). The *L. plantarum* strains: NGL5, NGL7 and LpTx in this study survived the acid environment and the growth of the acid stressed cells was enhanced to 6, 8 and 9 Log₁₀ CFU mL⁻¹ after 3, 6 and 24 h respectively for

NGL7 in the bile condition. Survival of approximately $5 \text{ Log}_{10} \text{ CFU mL}^{-1}$ at the end of the 3 h of exposure to pH 2 was one log cycle less than $6 \text{ Log}_{10} \text{ CFU mL}^{-1}$ reported by Ouwehand and Salminen (1998) as the requirement for conferment of health benefit in the host. This however, could be dependent on the initial microbial inocula and the buffering effect of the substrates in the media. The food substance in which the LAB is relayed into the GIT may influence the intensity of the adverse effect that the acid and gastric juice may have on the LAB and thereby increasing the survival and viability of the organism (Patel et al., 2004).

Bile in the human GIT is an important factor that affects bacteria cell viability and interaction with the environment as it is known to destroy the lipids and fatty acid components of bacteria cell membrane (Succi et al., 2005). The increase in the levels of the acid stressed *L. plantarum* cells in MRS was not significantly ($p \leq 0.05$) different from the 0.3% ox gall bile condition. This was an indication that the *L. plantarum* strains tested in this study showed significant tolerance to 0.3% ox gall bile even after been exposed to acid (pH 2.0). The ability of LAB to tolerate bile salt in vitro could imply their capability to survive in the human small intestine. Although the result indicated that some cells of the NGL5 were not able to recover within the first 3 h of exposure to bile. Similar viable cell counts for *L. plantarum* strains isolated from *ikki* were reported by Kalui et al., (2009). Mathara et al., (2008) reported high tolerance of *L. plantarum* strains to 0.5% ox gall after acid stress. The resistance to bile have been related to the activity of bile salt hydrolase which can hydrolyse combined bile salt and hence reduce toxicity of the bile salt (Bao et al., 2010).

4.4.3 Aggregation potential

Auto-aggregation properties of *Lactobacillus* species had been reported to be strain dependent, varying from strain to strain as well as co-aggregation to enteric bacterial pathogens (Bao et al., 2010). The *L. plantarum* strains investigated were able to auto-aggregate at both temperatures, although aggregation was significantly ($p \leq 0.05$) greater at 37°C than at room temperature. The auto-aggregation values obtained in this study were higher than the values reported for some strains of *L. fermentum* (Bao et al., 2010) but lower than that of *Bifidobacterium longum* (Del Re et al., 2000). The auto-aggregation potential of the *L. plantarum* strains would be an advantage in achieving greater mass that is necessary for exerting certain health benefits and may aid the prevention of pathogen colonization.

Co-aggregation of *Lactobacillus* spp. with pathogens enhances the prevention of pathogen colonization (Bao et al., 2010), however, the *L. plantarum* strains were unable to co-aggregate with the pathogens irrespective of the experimental conditions. This was comparable with the literature report that co-aggregation of enteropathogenic *E. coli* 3014 and *S. Typhimurium* with *L. plantarum* L4 was less than 5% (Kos et al., 2003). Although, in this study, there was no co-aggregation of the *L. plantarum* strains with *E. coli* 1077 and *S. Enteritidis* NCTC 5188, acid production by the *L. plantarum* strains during fermentation had proven to be capable of significantly inhibiting the pathogen in the food sample which is of relevance in the consumption of safe product.

4.4.4 Adhesion to porcine mucin and Caco-2 cells

The *L. plantarum* strains were able to adhere to porcine mucin with adhesion values ≥ 0.62 and adhered viable count between $6.51 - 6.61 \text{ Log}_{10} \text{ CFU mL}^{-1}$ for LpTx and *L. reuteri* NCIB 11951 respectively (Table 4.9). Some studies have revealed that effective binding of *L. plantarum* strains are mediated by the secretion of extracellular protein (Hevia et al., 2013, Sánchez et al., 2011). It could be suggested that the *L. plantarum* strains in this present study may possess proteins with adherence capabilities in addition to the binding receptors of the mucin.

For prolonged persistence that allows time for exertion of healthful benefits, bacterial adhesion to the mucus layer need to reach the epithelial cells to prevent quick dislodging and washing away by luminal content (Kirjavainen et al., 1998). The isolated *L. plantarum* strains were characterised with the ability to also adhere to Caco-2 cell linings. The adhesion of the *L. plantarum* strains to the epithelial cell culture (Caco-2) based on the enumeration of the bacterial colonies in this study indicated that $4 - 6 \text{ Log}_{10} \text{ CFU mL}^{-1}$ were adherent to the Caco-2 cells. This was higher than values reported in similar analysis for *L. plantarum* ACA-DC 112 (Maragkoudakis et al., 2006a) and some other *Lactobacillus* spp. (Monteagudo-Mera et al., 2012). The adhesion of the isolated endogenous *L. plantarum* strains (NGL5 and NGL7) to Caco-2 cells was significantly ($p \leq 0.05$) higher than the probiotic strain (LpTx). The type of strain and not its origin may be an influencing factor to adherent properties. Considering the adhesion potential of the isolated Nigerian *akamu L. plantarum* strains (NGL5 and NGL7) in comparison with the commercial probiotic strain (LpTx), it could be suggested that the *L. plantarum* strains (NGL5 and NGL7)

would be able to utilize the binding sites and possibly proliferate and prevent colonization by pathogens in the GIT.

4.5.5 Hydrophobicity

Hydrophobic cell surface properties of the *L. plantarum* strains based on microbial affinity to mono-polar and a polar solvent pairs: Chloroform/hexadecane and ethyl acetate/hexane were observed to be that of favourable acid-base interaction with increased affinity to the mono-polar solvents. The cells could be assumed to have more electron donating properties attributable to the presence of carboxylic groups on microbial cell surfaces (Bellon-Fontaine et al., 1996). Differences in the hydrophobicity of the strains further suggested variation in the structure and surface composition of each strain (Pan et al., 2006).

In this study, the observed correlation ($p \leq 0.01$) between hydrophobicity as determined by adhesion to hydrocarbons and bacterial adhesion to mucin was that of ethyl acetate and hexane, while there was no correlation with adhesion to Caco-2 cells. Although hydrophobicity has been reported as an indicator for adherence ability (Kos et al., 2003, Del Re et al., 2000, Liu et al., 2004, Pan et al., 2006), it may not be a basic criterion for strong adhesion in comparison to mucin and epithelial cells models. The auto-aggregation of the *L. plantarum* strains at 37°C correlated with hydrophobicity and adhesion to mucin. There was no correlation observed in the Caco-2 adhesion and the other parameters except for aggregation at 37°C after 24 h. This suggested that high percentage auto-aggregation would be an advantage in adhesion of bacterial to epithelial

cells. The strong relationship between hydrophobicity and auto-aggregation confirmed the observation made by Del Re et al., (2000).

4.4.6 Gelatinase and Haemolytic activity

In line with the FAO/WHO, (2002) recommended safety attributes, the *L. plantarum* strains: NGL5, NGL7 and LpTx did not exhibit gelatinase and haemolytic activity. This was in agreement with the literature report on non-haemolytic and non-gelatinase activities of strains of *L. plantarum* isolated from Kenyan fermented maize porridges (Kalui et al., 2009) and the non-haemolytic activity of other *Lactobacillus* species reported by Maragkoudakis et al., (2006b) and Zhang et al., (2012). Gelatinase activity would derange the important mucus protective lining of the GIT and create pathway for infections while haemolysis would cause cessation of the underlying epithelial layer (Kalui et al., 2009). It would be good to investigate other safety attributes such as resistance to antibiotics and the possession of transferable resistant genes.

4.5 Conclusion

The summary of the probiotic potential of the *L. plantarum* strains were presented in Table 4.11. The *L. plantarum* strains: NGL5 and NGL7 were able to exert antimicrobial activities against five foodborne pathogens mainly due to acidity. In the co-inoculation and 24 h fermented maize slurry, *E. coli* 1077 was absent after 24 h and 180 min respectively. This implies that the use of the *L. plantarum* strains as starter cultures in *akamu* fermentation can improve the safety of the product. *Sacch. boulardii* SB20 showed inhibition against all the pathogens except for *Staph. aureus*. Although the mechanism of inhibition by

Sacch. boulardii SB20 is unclear and may require investigation. The *L. plantarum* strains were able to tolerate acid and bile condition, exhibited good auto-aggregation potentials and adhesion to hydrocarbons, porcine mucin and Caco-2 cells. With respect to safety issues, there was no gelatinase and haemolytic activity observed. This suggested that the organisms would be able to survive passage through the GIT, aggregate and adhere to the intestinal mucosa and epithelial cells for beneficial health effects without posing any risk.

Table 4.11: Summary of the probiotic potential of the two *L. plantarum* strains (NGL5 and NGL7) isolated from Nigerian *akamu* and their probiotic counterpart (LpTx)

Probiotic potential	Experimental conditions	<i>Lactobacillus plantarum</i> strains		
		NGL5	NGL7	LpTx
Pathogen Inhibition using Agar spot Assay	37°C			
<i>Bacillus cereus</i> NCIMB 11925	Aerobic	++	+	+
	Anaerobic	++	++	++
<i>Escherichia coli</i> 1077	Aerobic	+++	++	++
	Anaerobic	+++	++	++
<i>Listeria monocytogenes</i> NCTC 7973	Aerobic	+	+	+
	Anaerobic	+	+	+
<i>Salmonella</i> Enteritidis NCTC 5188	Aerobic	+++	+	+
	Anaerobic	+++	+++	+++
<i>Staphylococcus aureus</i> NCTC 3750	Aerobic	++	+	+
	Anaerobic	++	++	+
<i>E. coli</i> 1077 inhibition in ground maize slurries				
Co-inoculation fermentation	22°C, 24 h (pH≤4.18)	7.60	7.76	7.86
	30°C, 24 h (pH≤3.48)	Inhibition	Inhibition	Inhibition
24 h <i>L. plantarum</i> fermentation	22°C, 120 min (pH≤4.17)	4.62	5.53	5.30
	30°C, 120 min (pH≤3.48)	<3	<3	Inhibition
Porridges from 24 h fermentation	30°C, 20 min (pH 3.41)	<3	<3	<3
Acid tolerance (pH 2)	37°C 3 h	4.80	4.94	NT
Bile tolerance (Survival relative to initial viable count after 6 h)	MRS+0.3% bile	2.28	2.61	NT
	MRS (control)	3.01	3.48	NT
Hydrophobicity	22±2°C			
Chloroform		++	+++	+++
Ethyl acetate		+	+++	+++
Hexadecane		-	+++	-
Hexane		-	+	+
Aggregation				
Auto-aggregation after 4 h	22±2°C	+	+	+
	37°C	+++	+++	+++
Co-aggregation	22±2°C	-	-	-
	37°C	-	-	-
Adhesion				
Porcine mucin	37°C, 2 h	6.52	6.60	6.51
Caco-2 cells		5.53	5.13	4.22
Gelatinasis and haemolysis		-	-	-

+ Values from 10 to 14; ++ Values from 15 to 19; +++ Values ≥20; NT Not tested, - Negative
*Units: Diameter of inhibition halos (mm); Microbial viable counts (Log₁₀ CFU mL⁻¹); Aggregation and hydrophobicity (%)

CHAPTER FIVE

PROXIMATE, MINERAL AND DIETARY FIBRE COMPOSITION, PASTING CHARACTERISTICS AND SENSORY ANALYSIS OF TRADITIONAL NIGERIAN *AKAMU* AND STARTER CULTURE FERMENTED GROUND MAIZE SLURRY.

5.1 Introduction

All over the world, cereals constitute one of the most important sources of dietary protein, carbohydrates, minerals, vitamins and fibre (Blandino et al., 2003). However, cereals are poor in their nutritional quality compared to dairy and dairy products due to the deficiency of certain essential amino acids, lower protein, starch and mineral availability and the presences of anti-nutritional factors such as phenolic compounds, tannin and phytates (Chavan and Kadams, 1989).

Fermentation has been reported to be of great importance in the improvement of the nutritional quality and safety of cereal foods through the production of organic acids that provides optimum pH condition for the enzymatic degradation of anti-nutritional phytates that are bound to the minerals and for the lowering of proteinase inhibitors. This had led to the synthesis of certain amino acids (Adebawo et al., 2000, Odunfa et al., 2001, Teniola and Odunfa, 2001, Osundahunsi and Aworh, 2003), increase in vitamins (riboflavin, niacin, vitamin B12 and ascorbic acid) and mineral availability (calcium, magnesium, phosphorus, zinc and iron) (Oyarekua, 2011, Sanni et al., 1999, Greffeuille et al., 2011). Several other techniques, also employed to improve the nutritional

quality of cereal based foods, include supplementation legumes and pulses, and different processing methods such as soaking, milling and germination (Aremu, 1993, Mbata et al., 2009, Osundahunsi and Aworh, 2003, Aremu et al., 2011). On the other hand, some of these techniques such as milling and wet sieving as in the case of *akamu* production have been implicated in the losses of nutrients and organic acid components (Sefa-Dedeh et al., 2001, Obinna-Echem, 2009, Nago et al., 1998a). It is therefore imperative to study the fermentation process to promote changes which would reduce nutrient losses.

Dietary fibres are primarily considered to be of plant origin and in cereal grains they are concentrated in the bran, representing 18.1 - 86.7% of the grain weight for oat and maize respectively with only about 13.1 - 19.6% in whole maize grain (Vitaglione et al., 2008). Depending on the solubility of dietary fibre (DF) in water, it is classified either as water-insoluble dietary fibre (IDF) or water-soluble dietary fibre (SDF) (Ragaei et al., 2011, Vitaglione et al., 2008). Although, the solubility of DF is demonstrated under conditions which may not exist in the human GIT (Topping, 2007), the SDF comprises of DF that are precipitated in a solution of one part water and four part ethanol (SDFP) and dietary fibre that remains soluble (SDFS) in 78% aqueous ethanol (McCleary et al., 2011).

In cereals, the bran is rich in IDF while SDF are concentrated near the endosperm (Vitaglione et al., 2008). Cereal grains are usually processed before consumption and the process of refining cereals removes proportionally more of the insoluble dietary fibre that is concentrated in the bran than of the soluble dietary fibre. A comparison of the dietary fibre contents of various whole grains

showed that oats, rye and barley contain about one-third soluble fibre and the rest is insoluble fibre (Slavin, 2003). Soluble fibre has been associated with cholesterol lowering and improved glucose response, while insoluble fibre was associated with improved bowel emptying (Slavin, 2003). A recent study by Roberts et al., (2013) revealed that SDF can also promote intestinal health and prevent infective diarrhoea by inhibiting epithelial adhesion and translocation by pathogenic bacteria. There are limited studies on the total dietary fibre composition of whole grain maize and its products while cereal such as barley, millet, rye, sorghum, wheat, rice and oat and their products have received wider attention (Ragae et al., 2011, Kristensen et al., 2010, Brennan and Cleary, 2005, Sullivan et al., 2013, Andersson et al., 2013, Shahidi and Chandrasekara, 2013, Guo and Beta, 2013, Hollmann et al., 2013).

Starch is a major polysaccharide in cereals, which plays important role in food viscosity. It is a semi crystalline granule composed of two main glucan: amylose and amylopectin (Frazier, 2009). The normal maize starch consists of 75% branched amylopectin and 25% of linear amylose and this varies significantly depending on the maize variety (Sandhu et al., 2004). When heated in excess water, starch granules imbibe water and swell to several times its initial size, ruptures and simultaneously releases amylose into the medium, which causes increase in viscosity. According to Tester and Morrison, (1990) the amylopectin component of starch is mainly responsible for starch swelling while amylose restrict swelling maintaining the integrity of the starch granule. Modification of starch structure may occur during food process which may have influence on the pasting properties of the food.

Fermentation had been reported to change the chemical composition and amorphous region of starch granule composed mainly of amylose and short chain amylopectin and hence affect the physical and rheological properties of the food (Lu et al., 2005, Yuan et al., 2008). Steeping and wet milling of grains have also been reported to influence the physical and chemical properties of starch (Zheng et al., 1998). During traditional *akamu* (spontaneously fermented maize starch slurry) production, the grains are steeped in water for efficient wet milling and starch yield before fermentation and the *akamu* slurries are prepared with boiling water to obtain gelatinized gruel for consumption. It would be important therefore, to investigate the effect that different production method could have on the pasting properties *akamu* samples.

Various microbial metabolites (lactic, acetic, oleic and linoleic acids, esters, higher alcohols and aldehydes, ethyl acetate and diacetyl and 5-hydroxymethylfurfural) are produced during microbial fermentation of cereal and have been implicated in the enhancement of the shelf-life and sensory characteristics of such products (Charalampopoulos et al., 2002b, Salmeron et al., 2009). Fermented cereal foods are known and appreciated for certain specific eating qualities, the sensory attributes (appearance, colour, flavour, texture, sweetness or sourness) are therefore crucial in their acceptability. Fermentation of *Gowe* a sorghum-based gruel with selected starter of *L. fermentum* alone or in combination with *K. marxianus* was found to have sensory characteristics (colour, taste and aroma) that were acceptable to consumer as the traditional spontaneous product (Vieira-Dalodé et al., 2008). Stronger and acceptable *ogi* aroma was observed in fermentation with starter cultures of *L. brevis* and *Sacch. cerevisiae* (Teniola and Odunfa, 2001).

In this study, the process of *akamu* production was modified by fermenting ground whole maize grain slurries with starter cultures of *L. plantarum* strains. The aim was to investigate the effect of the production method on the proximate, mineral and dietary fibre composition and the pasting property of the product in comparison to traditional *akamu* samples and the consumer sensory acceptability of the new product.

5.2 Materials and Methods

5.2.1 Samples

5.2.1.1 Proximate and mineral analysis

Proximate and mineral analysis was performed on triplicate samples of all of the Nigerian traditionally fermented *akamu* samples (section 3.2.1.1) except for sample W1. Unfermented irradiated and un-irradiated ground whole maize (IGM and GM respectively), and ground maize slurries that were fermented at 30°C for 0, 24 and 72 h by the isolated *L. plantarum* strains (NGL5 and NGL7) and their probiotic counterpart (LpTx) were also analysed. The fermentations were set up as described in section 3.2.5.3.

5.2.1.2 Dietary fibre and pasting properties

A selected traditional *akamu* sample (M3), irradiated ground whole maize slurries fermented by *L. plantarum* strain NGL5 and the unfermented irradiated and un-irradiated ground whole maize (IGM and GM respectively) were the samples analysed for dietary fibre and pasting properties.

5.2.1.3 Sensory evaluation

Sensory evaluation was carried out on porridges prepared from ground maize slurries fermented by the three *L. plantarum* strains (NGL5, NGL7 and LpTx). Porridges from unfermented (UUF) and artificially acidified unfermented (ACD) maize slurries served as controls.

5.2.2 Proximate analysis

Proximate analysis was carried out using the AOAC official method 923.03 (AOAC, 1995). Experiments were performed in triplicate except for ash and energy that were carried out in duplicates.

5.2.2.1 Moisture and Total solid content

The moisture content of 10.000±0.001 g of the samples was calculated after drying at 105°C to constant weight in an air oven (UT 6200 Thermo Fisher Scientific, Loughborough, UK) using Equation 5.1.

$$\text{Moisture (\%)} = \frac{\text{wet weight (g)} - \text{Dry weight (g)}}{\text{Sample weight}} \times 100 \dots \text{Equation 5. 1}$$

$$\text{Total dry matter (\%)} = 100 - \text{Moisture (\%)} \dots \text{Equation 5. 2}$$

5.2.2.2 Ash content

Sample were weighed (500±1 mg) in duplicates into pre-weighed porcelain crucibles and incinerated in a muffle furnace (AAF-11/18 Carbolite, Hope, UK) at 550°C for 12 h. Thereafter the samples were cooled to room temperature (22±2°C) in a dehumidification chamber and re-weighed. Ash was calculated using equation 5.3.

$$\text{Ash (\%)} = \frac{(\text{Weight of crucible + residue}) - \text{Weight of crucible (g)}}{\text{Sample weight}} \times 100 \dots \text{Equation 5. 3}$$

5.2.2.3 Lipid content

Fat was estimated by exhaustive extraction of 1.000 ± 0.002 g of dried samples with petroleum ether using rapid soxhlet extraction apparatus (Soxtherm SE-416, Gerhardt, Bonn, Germany). Briefly, 1.000 ± 0.002 g of the samples were weighed into cellulose thimbles and lightly plugged with cotton wool. The thimbles were placed into a wire support and inserted into a pre-weighed extraction beaker containing 2 - 3 anti-bump stones (Acros Organics, New Jersey, USA). Using a bottle-top dispenser (Eppendorf Varispenser, Eppendorf UK Ltd, Stevenage, UK), 140 mL of petroleum ether was added into the beaker that was properly mounted on the heating plate of the Soxtherm unit. The extraction process was carried out following the instruction from the the Multistat unit. At the end of the extraction, the beakers were removed from the unit into a fume cupboard. The thimbles and their holders were removed from the beaker and under full fume extraction traces of solvent were allowed to evaporate before re-weighing the beakers. The extracted lipids were quantified using equation 5.4.

$$\text{Lipids (\%)} = \frac{\text{Final weight of beaker} - \text{Initial weight of beaker (g)}}{\text{Sample weight}} \times 100 \dots \text{Equation 5.4}$$

5.2.2.4 Protein content

The determination of protein was by Kjeldahl method which measures protein from the total nitrogen content of the sample. About 100 - 150 mg of the dried samples were weighed into weighing boats and transferred into micro Kjeldahl boiling tubes along side with a catalyst tablet containing 3.5 g Potassium sulphate, 0.105 Copper sulphate and 0.105 Titanium dioxide (Kjeltabs TCT AA21, Thompson and Capper Ltd, Cheshire, UK). Using a bottle-top dispenser, 10 mL of 98% sulphuric acid was dispensed into the Kjeldahl boiling tubes. Two

tubes containing the reagents only served as blank while four more tubes: two with acetanilide and two with casein served as standard for the correction of nitrogen efficiency and for the standard reference respectively. The samples were digested in a Kjeldatherm digestion unit (Gerhardt, Bonn, Germany) under the following conditions: initial heating at 105°C for 15 min followed by a raised temperature of 255°C for 60 min and 380°C for 45 min or more till the samples had changed colour to emerald green before cooling to 60°C.

After the digestion, nitrogen was assayed in a Vapodest distillation unit with the aid of Vapodest manager software (Gerhardt, Bonn, Germany). The process involved the addition of excess sodium hydroxide to the acid digestion mixture which converted the ammonium ion in the sample digest to ammonia gas. The amount of ammonia released and collected in a receiving solution containing boric acid was quantified by back titration with Sulphuric acid.

The total nitrogen content of the samples was calculated using equation 5.5. Acetanilide nitrogen was computed by replacing the sample titre with acetanilide titre in equation 5.5. The nitrogen values were corrected to 100% efficiency with acetanilide values using equation 5.6 and 5.7, and then converted to protein using equation 5.8.

$$\text{Sample Nitrogen (\%)} = \frac{(St - Bt) \times M \times MW \text{ (mg)}}{\text{Sample weight (mg)}} \times 100 \dots \dots \dots \text{Equation 5.5}$$

Where *St* and *Bt* represent the sample and blank titre respectively, *M* is the molarity of acid and *MW* is the molecular weight of Nitrogen.

$$\text{Efficiency of Nitrogen recovery (\%)} = (ASN/10.36) \times 100 \dots \dots \dots \text{Equation 5.6}$$

Where *ASN* is the acetanilide sample Nitrogen value.

Corrected Nitrogen (%) = (% Nitrogen / % Efficiency) X 100.....Equation 5.7

Protein (%) = % corrected Nitrogen X 6.25.....Equation 5.8

5.2.2.5 Carbohydrate content

The carbohydrate content was determined by difference.

Carbohydrate (%) = 100 – % (Protein + Ash + Fat)Equation 5.9

5.2.2.6 Energy content using Bomb Calorimetry

Although energy can be calculated from carbohydrate, fat and protein using the conversion factors of 4, 9 and 4 kcal g⁻¹ respectively, a bomb Calorimeter (Mod. 1356, Parr Instrument Company, Moline, IL, USA) was used for the determination of the gross energy (MJ kg⁻¹) of the samples (section 5.2.1.1) in this study. One gram of the dried sample was prepared into a pellet using a pellet press and weighed into a crucible that was placed inside a stainless steel container (the Bomb) filled with 435 psi of oxygen. The sample was ignited through a wired thread inside the bomb and combusted. During the combustion, the core temperature and pressure in the crucible was raised to 1000°C and 2900 psi respectively. Under this condition, all the organic matter was oxidized, the resultant heat was transferred into the surrounding water jacket where it was detected and the internal microprocessor of the Bomb calorimeter converted the information into the energy value that was expressed in MJ Kg⁻¹ of sample.

5.2.3 Mineral analysis

The calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulphur and zinc content of the samples was evaluated according to the standard methods of AOAC (2005) using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES Varian 725-ES, Melbourne, Australia).

Briefly, the mineral elements in about 0.1 ± 0.05 g of air oven dried samples were transferred into solution by acid (10 mL of 70% Nitric acid) digestion in a Kjeldatherm Digestion System with Scrubber Unit, using the following temperature regime: 60°C for 1 h, 90°C for 1 h, 110°C for 30 mins and 135°C for 4 h. The digest when cool were made up to 50 mL with distilled water in a volumetric flask and transferred into 50 mL centrifuge tubes and sealed. Thereafter, the mineral composition were analysed in an ICP-OES (Varian 725-ES, Melbourne, Australia) under the following conditions: plasma, auxiliary and nebulizer flow rate of 15, 1.50, 0.68 L min⁻¹ respectively at a viewing height of 8 mm, replication read time and instrument delay time of 4 and 10 s respectively.

The measured spectra analyte lines in nanometre (nm) were: Ca: 317.933; Cu: 327.395; Fe: 238.204; K: 766.491; Mg: 285.213; Mn: 257.610; Na: 589.592; P: 213.618; S: 181.972 and Zn: 213.857. Validation of the analytes concentration was performed using standard stock solution made up of 1, 4, 10 and 20 mg L⁻¹ of the macro elements: Ca, Mg, P, Na, K and S and 0.01, 0.04, 0.1 and 0.2 mg L⁻¹ of the micro elements: Cu, Zn, Mn, and Fe respectively. Calibration curves with linearity of 0.998 for each of the macro and micro elements were obtained from the four different concentrations of the standards stock solutions. Residues

of any likely chemical reagent in the samples were corrected with the blank. Results were expressed in mg kg^{-1} of dry sample.

5.2.4 Dietary fibre analysis

The samples dietary fibre fractions IDF, SDFP and SDFS were determined using an integrated total dietary fibre assay kit (K-INTDF 06/12 Megazyme International, Co. Wicklow, Bray, Ireland) according to the integrated enzymatic-gravimetric and chromatographic procedure of AOAC Method 2011.25 (Megazyme, 2012). The method was based on the solubilisation and hydrolysis of non-resistant starch to D-glucose, maltose and resistant maltodextrins by the combined action of two enzymes: pancreatic α -amylase and Amyloglucosidase. Blanks without any sample were analysed simultaneously with the samples and served as controls.

The key stages in the total dietary fibre determination were summarised in Figure 5.1. Briefly, after the vacuum filtration of the digest, the protein content of the dried residue was determined by Kjeldahl method and ash was determined gravimetrically following the AOAC, (1995) standard methods. Values were used in correcting the IDF content.

Deionization was performed using 20 mL syringes loaded with thoroughly mixed 4 g each of freshly prepared Amberlite FPA 53 (OH-) and Ambersep 200 (H+) (Megazyme International, Co. Wicklow, Bray, Ireland) that were mounted on the column of vacuubrand vacuum pump (GMBH+Co, Wertheim, Germany) and eluted at the rate of 1.0 mL min^{-1} into 50 mL Duran bottle.

Chromatographic determination of the SDFS was performed using high performance liquid chromatography (HPLC), Dionex-Ultimate 3000 UHPLC+Focused (Germany). The UHPLC was equipped with WPS-3000 autosampler, LPG-3400SD pump, and TCC-3000 column compartment, RI-101 refractive index detector and MWD-3000 Ultra violet detector. The chromatographic separation of 50 μL injected sample volume was achieved with an elution phase of Milli-Q water containing $\text{Na}_2\text{Ca-EDTA}$ (50 mg L^{-1}) in a PL Hi-Plex H Guard column $50 \times 7.7 \text{ mm}$ (Agilent Technologies, Germany). The eluent was pumped at a flow rate of 0.5 mL min^{-1} within a pressure limit of 5 - 100 bars at a column oven temperature of 75°C . The sugars were detected using the Refractive Index and the retention times of the standards were noted and used in the identification of the sample peaks.

The data obtained were processed using Chromeleon[®] 7.1 Chromatography Data System Software (Thermo Scientific, Germany). Calculations were made using Microsoft Excel 2010 package following the formulas provided in the Megazyme instructional manual.

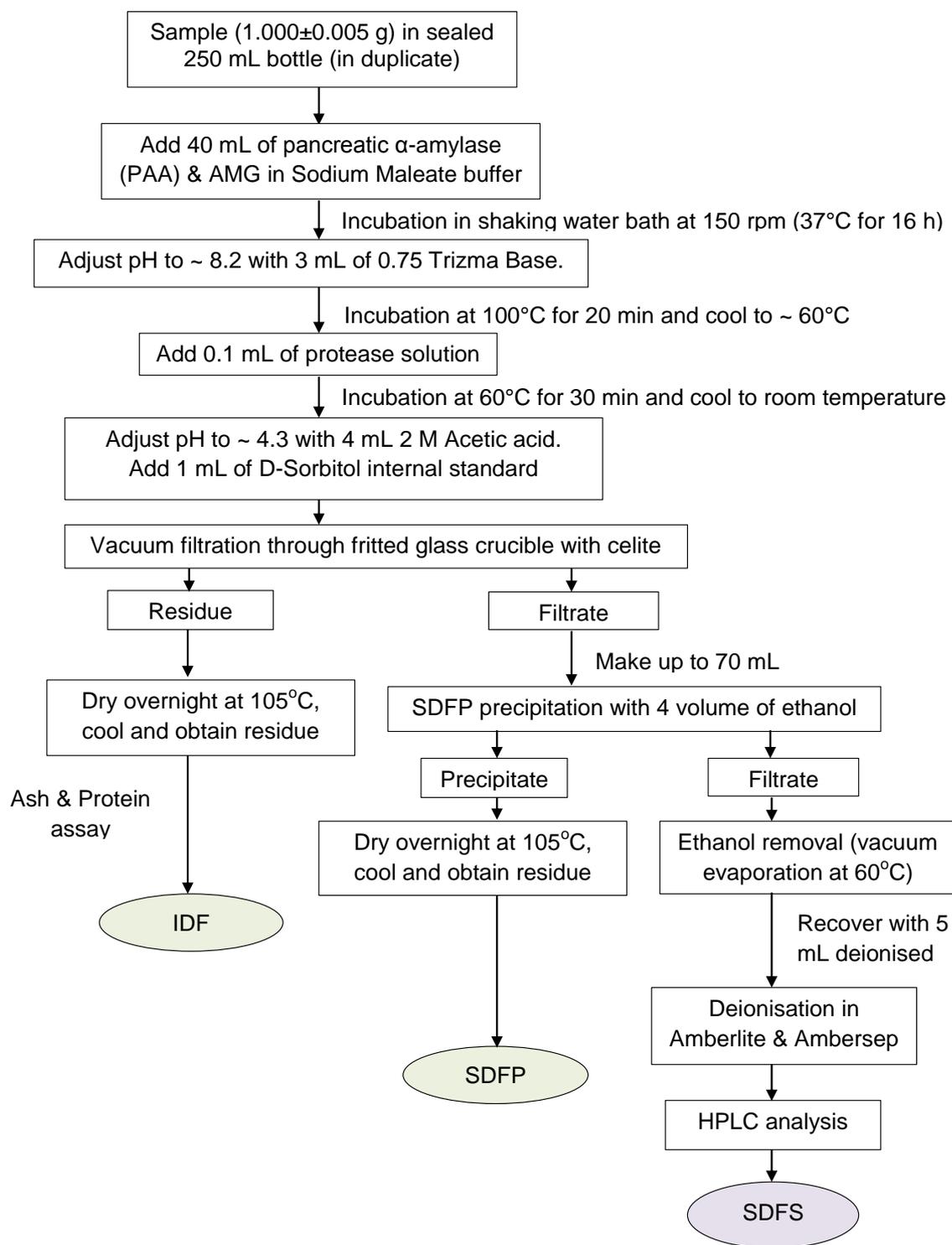


Figure 5.1: Scheme of the AOAC 2011.25 integrated total dietary fibre assay Showing the key stages in the quantification of IDF, SDFP and SDFS

*Modified from Megazyme, (2012)

5.2.5 Pasting characteristics

5.2.5.1 Sample preparation

The pasting characteristics was carried out on a selected traditional *akamu* sample (M3), irradiated ground maize slurries fermented with starter cultures of *L. plantarum* strain (NGL7), un-fermented irradiated and un-irradiated ground maize. The slurries were freeze dried in Edwards Modulyo bench top freeze dryer (Mecha Tech Systems Ltd, Thornbury, Bristol, UK) and ground to flour using an 80 mm unglazed laboratory mortar and pestle (Sigma Aldrich, Gillingham Dorset, UK). The resultant flour and the irradiated and un-irradiated ground maize samples were sieved using 500 µm sieve size in a motorized sieve shaker (Retsch AS 200 basic, Haan, Germany) at amplitude of 80 mm for 5 to 10 s.

5.2.5.1 Test procedure

Pasting properties of samples suspended in distilled water were determined using a Rapid Visco Analyser (RVA-4 series, Newport Scientific, Warriewood, Australia) following a standard method described in the instructional manual. Briefly, 25 mL of distilled water was added into a clean canister. The dry samples were weighed out in 2.93 g quantity and added to the water in the canister. A paddle was placed into the canister and jogged for 6 - 10 times to disperse the sample. Thereafter, the canister was mounted on the RVA and analysis carried out using standard 1 profile. The profile involved an idle temperature of 50°C, initial paddle rotation at 960 rpm for 10 s and then at 160 rpm for the rest of the test period. Samples were heated to 95°C at a rate of 12°C min⁻¹, held at 95°C for 3.7 min and then cooled at the same rate to 50°C.

The RVA pasting curve was automatically plotted and values of the pasting parameters were determined using ThermoLine for Windows version 2.2 software (Newport Scientific, Warriewood, Australia). The measured parameters were the pasting temperature, peak time, peak final viscosity, breakdown, holding strength and setback. The viscosities, temperature and time were expressed in Centipoise (cP), degree Celsius ($^{\circ}\text{C}$) and minutes respectively.

5.2.6 Sensory analysis

In line with the Plymouth University policy, the sensory evaluation protocol received the approval of the Human Ethical Committee of the Faculty of Science and Technology (Approval shown in Appendix A5.1).

5.2.6.1 Porridge preparation

Sensory evaluation was carried out on porridges prepared from ground maize slurry fermented by the *L. plantarum* strains (NGL5, NGL7 and LpTx). Artificially acidified unfermented and unfermented maize slurries served as controls. Acidification was achieved using food grade monohydrate citric acid (Fisher Scientific, UK) to a concentration of 77 mmol L^{-1} (4.05 g per 250 mL of slurry) that corresponded to the average concentration of acid in the fermented samples. The frozen slurries were allowed to completely thaw at 25°C for 3 h. To 250 mL of the sample slurry was added equal volume of boiling water and then microwaved for 2 minutes with vigorous stirring after each minute to obtain a lump free porridge. The porridges were distributed in 15 mL samples into labelled transparent plastic mini pots with lids and maintained at 45°C .

5.2.6.2 Recruitment of panelists and Hedonic test

Thirty panellists were recruited from within the University of Plymouth staff, postgraduate and undergraduate students via e-mail invitation (Appendix A5.2) and verbal communication. Although not all the panellists were persons that were accustomed to the type of fermented maize porridges, they were consumers of various other types of fermented products like youghurts. In the morning (10.00 am to 12.00 noon) of the day of the sensory evaluation, after briefing and signing consent (Appendix A5.3 and Appendix A5.4 respectively) at a reception area, the panellists were invited to sit at sensory evaluation booths where porridge samples were presented in random order with a ballot sheet for each sample. Instructions on the ballot sheet (Appendix A5.5) directed the panellists to evaluate a range of sensory attributes (appearance, colour, aroma, Sourness (acidity), flavour, texture (smoothness) and overall acceptability). Samples were evaluated and scored on a 9-point hedonic scale, with the degree of likeness of the product attribute expressed as follows: 1- dislike extremely, 2 - dislike very much, 3 - dislike moderately, 4 - dislike slightly, 5 - neither like nor dislike, 6 - like slightly, 7 - like moderately, 8 - like very much and 9 - like extremely.

5.2.7 Statistical analysis

Statistical differences and relationship among variables were analysed as stated in section 3.2.6. Correlation between the proximate composition variables: protein, ash, fat and energy utilised multiple regressions and correlations as stated in section 4.2.9.

The non-parametric Friedman test and 2-sample t-test were employed in determining the statistical differences among the product sensory attributes.

5.3 Results

5.3.1 Proximate composition

5.3.1.1 Akamu samples

The proximate composition of the traditional *akamu* samples are shown in Table 5.1. Carbohydrate content was $\geq 90\%$ of which $< 1\%$ were sugars. The samples did not differ significantly ($p \leq 0.05$) based on their origin. However, some variation was observed among the individual samples. Sample E1 had significantly ($p \leq 0.05$) the least protein content ($3.19 \pm 0.01\%$). All samples had low lipid levels ($\leq 3.68\%$) with ash content in trace amounts $\leq 0.4\%$ or absent. Correlation coefficient between carbohydrate and the other nutrient variables: protein, lipids and ash with their corresponding p-values were 0.984 ($p = 0.016$), -0.994 ($p = 0.006$) and -1 ($p = 0.000$) respectively. Correlation coefficient between energy and each of the nutrient variables: protein, carbohydrate and lipids while holding the others constant was 1.

5.3.1.2 *L. plantarum* starter culture fermented ground maize slurries

The proximate composition of the ground maize slurries (AL5, AL7 and ALpTx) fermented by the *L. plantarum* strains (NGL5, NGL7 and LpTx) and the original ground irradiated (IGM) and unirradiated (GM) maize was presented in Table 5.2. The moisture content of the ground maize was $\leq 12.16\%$ while that of the fermented slurry were $\geq 67.49\%$. There were no significant differences observed in the ash (0.89 - 1.12%) and CHO (88.13 - 91.00%) contents of the samples.

The lipid and energy content of the samples varied from 1.47 - 4.07% and 16.27 - 18.14 KJ g⁻¹ respectively. Fermentation resulted in significant reduction in the energy and lipid levels of the samples after 72 h. The initial protein content (7.45%) of the *L. plantarum* NGL5 sample (AL5) was greater than the ground maize (6.54%).

Generally, for the starter culture fermented ground maize slurries, carbohydrates had a negative correlation coefficient with the lipids (0.960; p=0.000) and proteins (0.694; p=0.018). The correlation coefficient between energy and each of the nutrient variables: carbohydrates, proteins and lipids while holding the others constant was -0.633 (p=0.037), -5.97 (p=0.052) and -0.606 (p=0.048) respectively. The comparison of the samples protein and energy values expressed as percentages of the daily requirements were presented in Appendix A5.6

Table 5.1: Proximate composition and energy values of spontaneously fermented *akamu* samples obtained from different parts of Rivers State in Nigeria

Origin	Sample codes	Moisture (g 100 ⁻¹ g)	Total Solid (g 100 ⁻¹ g)	Protein (g 100 ⁻¹ g DM)	Lipids (g 100 ⁻¹ g DM)	Ash (g 100 ⁻¹ g DM)	Carbohydrate (g 100 ⁻¹ g DM)	*Energy (KJ g ⁻¹ DM)
Mile 3 Diobu		(47.43±1.01)	(52.57±1.01)	(5.52±0.42)	(2.79±0.46)	(0.33±0.07)	91.36±0.79	(17.80±0.07)
	M1	47.30±0.35 ^c	52.70±0.35 ^c	4.73±0.24 ^d	2.57±0.20 ^{de}	0.40±0.00 ^a	92.30	17.91±0.04 ^{abc}
	M2	49.24±0.26 ^b	50.76±0.26 ^d	5.70±0.07 ^c	2.13±0.00 ^f	0.20±0.01 ^b	91.98	17.66±0.02 ^{bc}
	M3	45.75±0.12 ^e	54.25±0.12 ^a	6.13±0.12 ^{bc}	3.68±0.08 ^a	0.39±0.01 ^a	89.80	17.82±0.24 ^{abc}
Emohua		(49.36±1.10)	(50.64±1.10)	(5.65±1.27)	(2.62±0.10)	(0.13±0.00)	(91.60±1.41)	(17.94±0.24)
	E1	47.60±0.13 ^c	52.40±0.13 ^c	3.19±0.01 ^e	2.44±0.15 ^{ef}	0.00	94.37	17.54±0.07 ^{bc}
	E2	49.08±0.08 ^b	50.93±0.08 ^d	6.33±0.03 ^b	2.80±0.12 ^{cd}	0.20±0.00 ^b	90.67	18.37±0.08 ^a
	E3	51.39±0.20 ^a	48.61±0.20 ^e	7.43±0.14 ^a	2.62±0.08 ^{de}	0.20±0.00 ^b	89.75	17.92±0.05 ^{abc}
Rumuokoro		(48.77±2.18)	(51.23±2.18)	(5.34±0.98)	(2.40±0.62)	(0.10±0.00)	(92.17±0.45)	(17.51±0.22)
	R1	46.58±0.19 ^d	53.42±0.19 ^b	6.31±0.03 ^b	1.77±0.08 ^g	0.20±0.00 ^b	91.71	17.29±0.29 ^c
	R2	50.95±0.40 ^a	49.05±0.40 ^e	4.36±0.33 ^d	3.02±0.08 ^{bc}	0.00	92.62	17.72±0.05 ^{bc}
Aluu	A1	47.81±0.08 ^c	52.19±0.07 ^c	6.37±0.03 ^b	3.15±0.08 ^b	0.40±0.00 ^a	90.08	17.97±0.28 ^{ab}

Values with same superscript in the same column do not differ significantly ($p \leq 0.05$). $N=3 \pm SD$

Values for the individual samples were mean of triplicate determinations \pm standard deviation.

Values in brackets were location mean \pm standard error of mean.

Means based on location did not differ significantly ($p \leq 0.05$).

DM = Dry matter

*Energy values obtained using Bomb calorimeter

The alphanumeric sample codes: A1, E1, E2, E3, M1, M2, R1, R2 and W1 represent *akamu* samples based on their origin

Table 5.2: Proximate composition and Energy values of ground maize slurries fermented with starter cultures of *Lactobacillus plantarum* strains (LpTx, NGL5 and NGL7)

Samples	Time (h)	Moisture (g 100 ⁻¹ g)	Total Solid (g 100 ⁻¹ g)	Protein (g 100 ⁻¹ g DM)	Lipids (g 100 ⁻¹ g DM)	Ash (g 100 ⁻¹ g DM)	Carbohydrate (g 100 ⁻¹ g DM)	*Energy (KJ g ⁻¹ DM)
GM		12.16±0.06 ^e	87.84±0.06 ^a	6.54±0.10 ^{bc}	4.00±0.00 ^a	1.09±0.03	88.36	18.06±0.31 ^a
IGM		11.86±0.06 ^e	88.14±0.06 ^a	6.45±0.09 ^{bc}	3.20±0.00 ^c	1.03±0.04	89.32	18.14±0.01 ^a
ALpTx	0	69.13±0.55 ^{ab}	30.87±0.55 ^{de}	6.79±0.04 ^{bc}	4.07±0.12 ^a	1.01±0.06	88.13	17.49±0.06 ^{abc}
	24	68.75±0.06 ^{ab}	31.26±0.06 ^{de}	6.50±0.17 ^{bc}	1.47±0.11 ^e	1.04±0.01	91.00	17.28±0.78 ^{abcd}
	72	69.12±0.22 ^{ab}	30.88±0.22 ^{de}	6.64±0.21 ^{bc}	1.80±0.00 ^{de}	0.97±0.02	90.59	16.63±0.06 ^{cd}
AL5	0	68.50±0.13 ^{bc}	31.50±0.13 ^{cd}	7.45±0.72 ^a	3.74±0.12 ^{ab}	1.12±0.05	87.69	17.77±0.11 ^{ab}
	24	68.04±0.14 ^{cd}	31.96±0.14 ^{bc}	6.40±0.04 ^{bc}	3.33±0.11 ^{bc}	0.89±0.03	89.38	16.27±0.13 ^d
	72	67.49±0.14 ^d	32.51±0.14 ^b	6.32±0.11 ^c	2.13±0.12 ^d	0.97±0.03	90.58	16.90±0.25 ^{bcd}
AL7	0	69.12±0.23 ^{ab}	30.88±0.23 ^{de}	7.00±0.01 ^{ab}	3.70±0.10 ^{ab}	0.97±0.18	88.32	17.95±0.21 ^a
	24	68.91±0.22 ^{ab}	31.09±0.22 ^{de}	6.96±0.24 ^{abc}	3.40±0.40 ^{bc}	0.93±0.02	88.70	17.62±0.04 ^{abc}
	72	69.33±0.42 ^a	30.67±0.42 ^e	6.58±0.07 ^{bc}	2.20±0.20 ^d	0.95±0.04	90.26	16.67±0.03 ^{cd}

Values with same superscript in the same column do not differ significantly (p≤0.05). N=3±SD

IGM and GM represented the irradiated and un-irradiated ground maize.

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

Ash content of the sample did not differ significantly.

DM = Dry matter

*Energy values obtained using Bomb calorimeter

5.3.2 Mineral composition

The mineral composition of the different *akamu* samples obtained from Nigeria and the unfermented irradiated (IGM) and un-irradiated (GM) ground maize and their *L. plantarum* strain fermented counterpart are presented in Table 5.3 and Table 5.4 respectively. There were no significant variations observed for Ca, Cu, Fe, Mg, Mn, Na and S among the traditional *akamu* samples based on sample origin, samples from Emohua however were significantly ($p \leq 0.05$) lower in Mg, K and P (101.26, 275 and 795.3 mg kg⁻¹ respectively) while lower Zn concentration (5.54 mg kg⁻¹) was observed in Rumuokoro samples. Except for Ca, Na and Zn, the mineral concentration of the individual samples varied significantly ($p \leq 0.05$) even between locations. Comparison of the unfermented and the starter culture fermented ground maize showed no significant variation in the concentration of Ca, Cu and S. Fermentation led to significant increases in the concentration of Fe and Na but the unfermented and un-irradiated ground maize had significantly ($p \leq 0.05$) higher concentrations of K, Mg, Mn, P and Zn than its irradiated and fermented counterpart. Concentrations of Fe in the starter fermentation increased significantly with progression in fermentation while K concentration was on the decline. Comparison of the samples mineral levels expressed as percentages of the daily requirements were presented in Appendix A5.7

Table 5.3: The mineral composition (mg kg⁻¹ DM) of *akamu* samples obtained from different parts of Rivers State in Nigeria

Origin	Sample Codes	Calcium (Ca)*	Copper (Cu)	Iron (Fe)	Potassium (K)	Magnesium (Mg)	Manganese (Mn)	Sodium (Na)*	Phosphorus (P)	Sulphur (S)	Zinc (Zn)
Aluu Mile 3 Diobu	A1	46.37±12.12	1.53±0.47 ^{ab}	57.90±6.33 ^a	493.84±13.17 ^c	162.23±3.83 ^{au}	3.08±0.06	214.30±22.40	995.08±14.39 ^{abu}	621.40±22.40 ^c	9.92±1.40
		(57.84±6.66)	(1.74±0.20)	(40.22±3.96)	(595.80±56.40 ^u)	(167.90±16.40 [†])	(3.00±0.30)	(213.40±3.84)	(915.30±34.20)	(616.65±7.21)	(8.06±0.59)
	M1	70.30±18.60	2.39±0.55 ^a	41.49±0.63 ^{bcd}	371.20±4.36 ^d	107.85±5.26 ^d	2.24±0.07 ^e	215.30±22.00	789.06±13.46 ^e	609.10±29.30 ^{cd}	6.82±0.99
	M2	65.40±18.30	1.64±0.17 ^{ab}	50.89±13.33 ^{bc}	711.60±28.80 ^a	219.32±14.04 ^a	2.58±0.14 ^d	212.94±4.46	942.90±46.70 ^c	610.30±23.10 ^{cd}	9.50±1.14
	M3	37.88±1.31	1.18±0.18 ^b	28.27±0.53 ^d	704.53±11.83 ^a	176.62±1.66 ^b	4.19±0.14 ^{bc}	211.98±4.55	1014.00±10.80 ^b	630.51±7.22 ^{bc}	7.86±2.13
Emohua		(57.13±4.48)	(1.87±0.13)	(52.4±12.4)	(275.73±22.2)	(101.26±4.94)	(3.37±0.48)	(224.78±5.02)	(795.26±28.20)	(630.94±19.30)	(8.92±1.9 ^u)
	E1	46.92±6.97	1.52±0.28 ^{ab}	101.44±9.13 ^a	337.90±16.83 ^d	114.95±2.80 ^d	4.68±0.22 ^a	222.70±21.90	857.60±36.30 ^d	658.60±21.20 ^{ab}	9.41±1.95
	E2	64.21±4.11	1.95±0.49 ^{ab}	27.47±2.52 ^d	297.97±7.59 ^e	106.3±4.25 ^d	1.51±0.18 [†]	235.91±3.96	842.43±9.91 ^{de}	676.84±15.44 ^{bc}	8.51±0.57
	E3	60.30±20.30	2.12±0.20 ^b	28.40±0.80 ^d	191.33±10.59 ^f	82.53±2.60 ^e	3.88±0.02 ^c	215.71±9.65	685.76±10.82 ^f	557.30±18.80 ^d	8.83±3.28
Rumuokoro		(48.66±3.26)	(1.25±0.09)	(30.80±3.07)	(403.40±60.00)	(109.70±13.90)	(3.49±0.44)	(220.19±7.28)	(854.00±122.00)	(676.90±21.10)	(5.54±0.65 ^u)
	R1	43.79±7.73	1.35±0.22 ^b	31.28±11.74 ^{cd}	269.33±2.08 ^e	78.87±1.25 ^e	2.52±0.09 ^e	210.08±13.14	580.50±17.90 ^g	630.71±5.47 ^{bc}	5.50±2.51
	R2	53.53±5.53	1.16±0.19 ^b	30.32±1.78 ^d	537.46±7.00 ^b	140.52±5.40 ^c	4.46±0.26 ^{ab}	230.30±17.80	1127.70±11.80 ^a	723.01±15.23 ^a	5.58±0.34

Values with same superscript in the same column do not differ significantly (p≤0.05). N=3±SD

Values for the individual samples were mean of triplicate determinations ± standard deviation

Values in brackets were location mean ± standard error of mean.

*No significant different between sample means.

^uLocation means that are significantly (p≤0.05) different from others in the same column.

DM = Dry matter

Table 5.4: The mineral composition (mg kg⁻¹ DM) of ground maize and ground maize slurries fermented by starter cultures of *Lactobacillus plantarum* strains (NGL5, NGL7 and LpTx)

Samples	Time (h)	Calcium (Ca)	Copper (Cu)	Iron (Fe)	Potassium (K)	Magnesium (Mg)	Manganese (Mn)	Sodium (Na)	Phosphorus (P)	Sulphur (S)	Zinc (Zn)
GM		68.71±5.92	1.36±0.41	17.56±1.62	2458.90±38.20 ^b	863.00±19.90 ^a	3.08±0.11	210.30±20.90 ^a	2227.00±47.10 ^a	780.80±48.20	20.64±3.44
IGM		77.25±5.72	1.66±0.43	17.40±0.93	2317.90±35.80	786.09±4.31	2.83±0.17	209.68±9.23 ^a	2015.30±16.60 ^b	760.31±9.71	19.22±2.32
ALpTx	0	79.04±8.28	1.11±0.27	17.98±0.61	2163.90±22.10	759.25±8.36	2.81±0.15	289.04±14.26	1817.60±18.20	781.90±30.90	17.42±0.90
	24	80.59±7.68	1.27±0.11	20.06±0.54	2239.60±40.50	761.59±4.96	2.97±0.09	291.68±11.70	1868.30±23.60	798.41±12.78	17.89±1.57
	72	73.08±5.37	1.65±0.32	23.86±1.84 ^a	2096.60±102.10	771.70±34.30	2.97±0.17	292.90±34.60	1787.60±61.70	770.30±24.90	16.54±1.66
AL5	0	81.00±27.50	1.24±0.41	17.16±2.07	2664.20±108.10 ^a	799.69±16.28	2.71±0.07	308.30±34.10	1818.00±54.60	789.20±114.20	18.08±2.99
	24	76.11±10.39	1.81±0.36	17.28±0.40	2394.20±63.70	773.20±26.30	2.77±0.13	289.30±19.90	1791.00±34.50	796.70±85.00	19.66±1.78
	72	94.62±9.55	1.62±0.44	18.66±0.48	2189.90±54.40	732.40±48.20	2.77±0.20	288.90±35.60	1776.30±98.60	781.10±41.70	21.57±1.38
AL7	0	73.27±7.25	1.62±0.35	18.90±1.27	2213.90±62.30	773.00±25.70	2.80±0.14	291.10±22.60	1755.60±63.70	773.50±35.00	17.40±2.24
	24	76.47±9.28	1.41±0.45	19.40±0.60	2069.60±124.20 ^c	727.30±23.50 ^b	2.70±0.16	300.98±16.58	1716.00±38.60	769.80±20.80	16.81±2.65
	72	78.93±5.50	2.01±0.35	19.25±1.02	2044.20±17.60 ^c	764.50±33.20	2.95±0.16	284.34±5.59	1817.00±76.20	765.60±38.10	16.71±0.90

Values with superscript in the same column were significantly ($p \leq 0.05$) different from others. N=3±SD

DM = Dry matter

ALpTx - Samples fermented by the commercial probiotic *L. plantarum* strain (LpTx)

AL5 & AL7 - Samples fermented by the Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

GM - un-irradiated and unfermented ground maize

IGM - irradiated unfermented ground maize

5.3.3 Dietary Fibre composition

Figure 5.2 shows an example of the chromatogram of the standard and the 72 h *L. plantarum* strain (NGL5) fermented sample extract showing the peaks of the non-digestible oligosaccharide (NDO) also known as malto-oligosaccharides, maltose, glucose and other components.

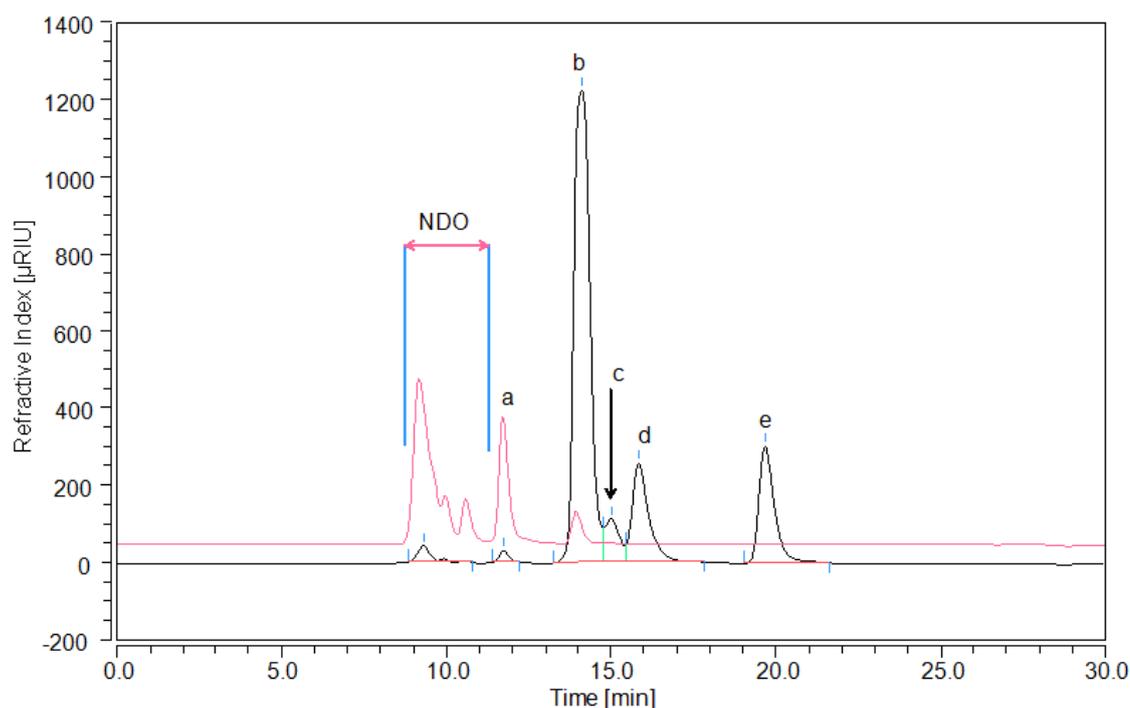


Figure 5.2: Chromatogram of 72 h *Lactobacillus plantarum* strain (NGL5) fermented sample extract (black) overlaid with the LC RT standard (pink). a= Maltose; b = Glucose; c = Xylose; d = Sorbitol (IS) and e = Glycerol

In Table 5.5, the range of values for the IDF, SDF and TDF content of the samples were 35.10 - 82.05, 26.97 - 50.80 and 85.90 - 126.94 mg g⁻¹ respectively. The IDF and TDF of the 72 h *L. plantarum* fermented sample was the highest. The selected traditional *akamu* sample (M3) had the lowest IDF and the highest SDF (50.80 mg g⁻¹) with particular reference to its SDFS (35.40 mg g⁻¹) content. The unfermented sample had the lowest SDFS values of 13.50 mg g⁻¹.

In Table 5.6, the sugar components of the hydrolysed polysaccharides as determined using the HPLC include xylose, glucose and maltose.

Table 5.5: Dietary fibre composition (mg g⁻¹ DM) of a selected traditional *akamu* sample (M3) and ground maize slurries fermented by *Lactobacillus plantarum* strain (NGL5)

Sample	Time (h)	IDF	SDFP	SDFS*	SDF	TDF
IGM		65.75	24.00	13.50±0.00 ^e	37.50	103.25
AL5	0	60.50	10.68	16.28±0.02 ^d	26.97	87.47
	24	62.16	14.57	17.94±0.05 ^c	32.51	94.66
	72	82.05	23.16	21.73±0.10 ^b	44.89	126.94
<i>Akamu</i> (M3)		35.10	15.40	35.40±0.04 ^a	50.80	85.90

*The HPLC analysis was performed in duplicate and all the values differed significantly (P≤0.05)

AL5 - Sample fermented by the *L. plantarum* strain (NGL5)

IGM - Irradiated unfermented ground maize

M3 - Nigerian *akamu* sample

Table 5.6: The sugar content (mg mL⁻¹) of the sample extracts

Sample	Time (h)	Xylose	Glucose	Maltose
IGMF		4.39±0.09 ^a	47.25±0.22 ^b	0.93±0.00 ^a
AL5	0	4.57±0.04 ^a	46.38±0.23 ^c	0.89±0.00 ^b
	24	3.37±0.02 ^b	42.75±0.29 ^d	0.42±0.00 ^d
	72	2.99±0.03 ^b	43.16±0.02 ^d	0.51±0.01 ^c
<i>Akamu</i> (M3)		2.97±0.22 ^b	54.20±0.16 ^a	0.38±0.00 ^e

Values with the same superscript in the same column do not differ significantly (p≤0.05)

AL5 - Sample fermented by the *L. plantarum* strain (NGL5)

IGM - Irradiated unfermented ground maize

M3 - Nigerian *akamu* sample

5.3.4 Pasting characteristics

Figure 5.3 presented an example of the RVA pasting curve showing the peak viscosity, holding strength and final viscosity of the traditional *akamu* sample (M3), un-irradiated and unfermented ground maize and the fermented irradiated ground maize sample. The pasting parameters were shown in Table 5.7. The irradiated ground maize and its *L. plantarum* strain fermented samples had an undetected pasting temperature with very low peak and final viscosities.

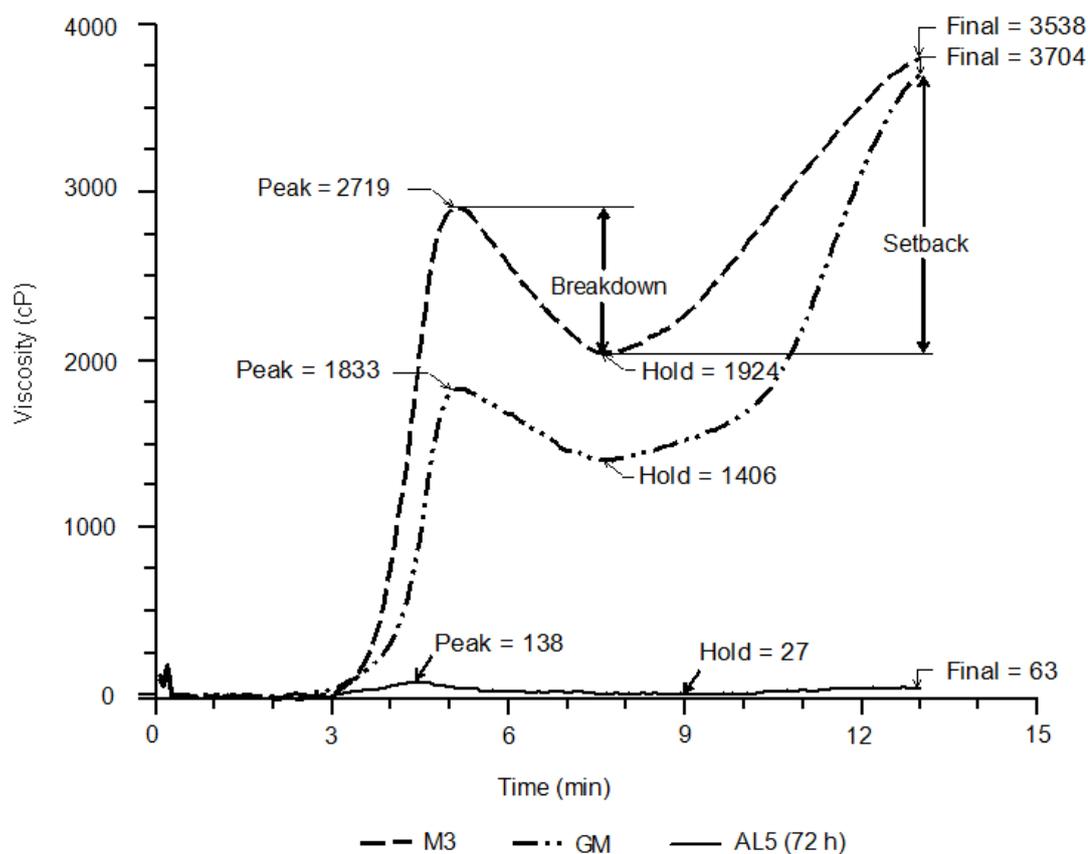


Figure 5.3: RVA pasting curve showing the peak viscosity, holding strength and final viscosity of the traditional *akamu* sample (M3), unfermented and un-irradiated ground maize (GM) and the irradiated ground maize slurries fermented by *Lactobacillus plantarum* strain. AL5 (72) - Irradiated ground maize slurry fermented by the *L. plantarum* strain (NGL5) at 30°C for 72 h

Table 5.7: The pasting properties of Nigerian *akamu* sample (M3), irradiated ground maize slurries fermented by *Lactobacillus plantarum* strain (NGL7) and unfermented, irradiated and un-irradiated ground maize

Samples	*Time (h)	Peak viscosity	Holding strength	Breakdown	Final viscosity	Setback	Peak Time (Min)	Pasting Temp (°C)
AL5	0	128.70±22.00 ^c	33.67±10.97 ^c	95.00±12.29 ^c	72.67±14.36	39.00±6.08 ^c	4.53±0.07 ^b	
	72	139.50±2.12 ^c	33.50±9.19 ^c	106.00±7.07 ^c	68.50±7.78	35.00±1.41 ^c	4.63±0.05 ^b	
GM		1920.50±123.70 ^b	1415.00±12.70 ^b	505.50±111.00 ^b	3819.00±162.00 ^a	2404.00±149.00 ^a	5.10±0.05 ^a	76.67±3.43
IGM		135.33±9.71 ^c	58.67±10.26 ^c	76.67±0.58 ^c	108.33±10.21	49.67±1.53 ^c	4.47±0.00 ^b	
M3		2641.00±54.30 ^a	1880.50±49.60 ^a	760.50±33.90 ^a	3378.80±110.60 ^b	1498.30±83.00 ^b	5.32±0.13 ^a	76.86±0.80

Values with the same superscript do not differ significantly (p≤0.05). N=3±SD

*Fermentation time

AL5 - Sample fermented by the *L. plantarum* strain (NGL5)

GM - Un-irradiated and unfermented ground maize

IGM - Irradiated unfermented ground maize

M3 - Nigerian *akamu* sample

5.3.5 Sensory evaluation

Sensory evaluation data were presented in Figure 5.4 and Figure 5.5. The aroma, appearance, colour and texture of all the treatment showed normal distribution ($p \leq 0.05$) using Anderson-Darling Test (Appendix A5.8) except for the artificially acidified (AUU) sample and PLpTx flavour, PLpTx and PL5 sourness and the overall acceptability of PL7 and control (UUA) samples. There were no significant differences between treatments in the appearance, colour and flavour. The assessors rating the sensory attributes of all the treatments had mean scores above 5 (which is neither like nor dislike) except for the flavour and sourness for *L. plantarum* strain fermented samples and aroma for PLpTx were rated below 5.00. Sourness of the acidified sample (score above 3 - moderately dislike) was significantly ($p \leq 0.05$) the least acceptable having first and third quartile rating of 1.75 (extreme dislike) and 4.25 (slightly dislike) respectively. In Figure 5.6, the third quartile (75%) of all the assessors rating for the treatments' aroma, appearance, colour, texture and the overall acceptability was that of moderate likeness except for the overall acceptability of the artificially acidified samples.

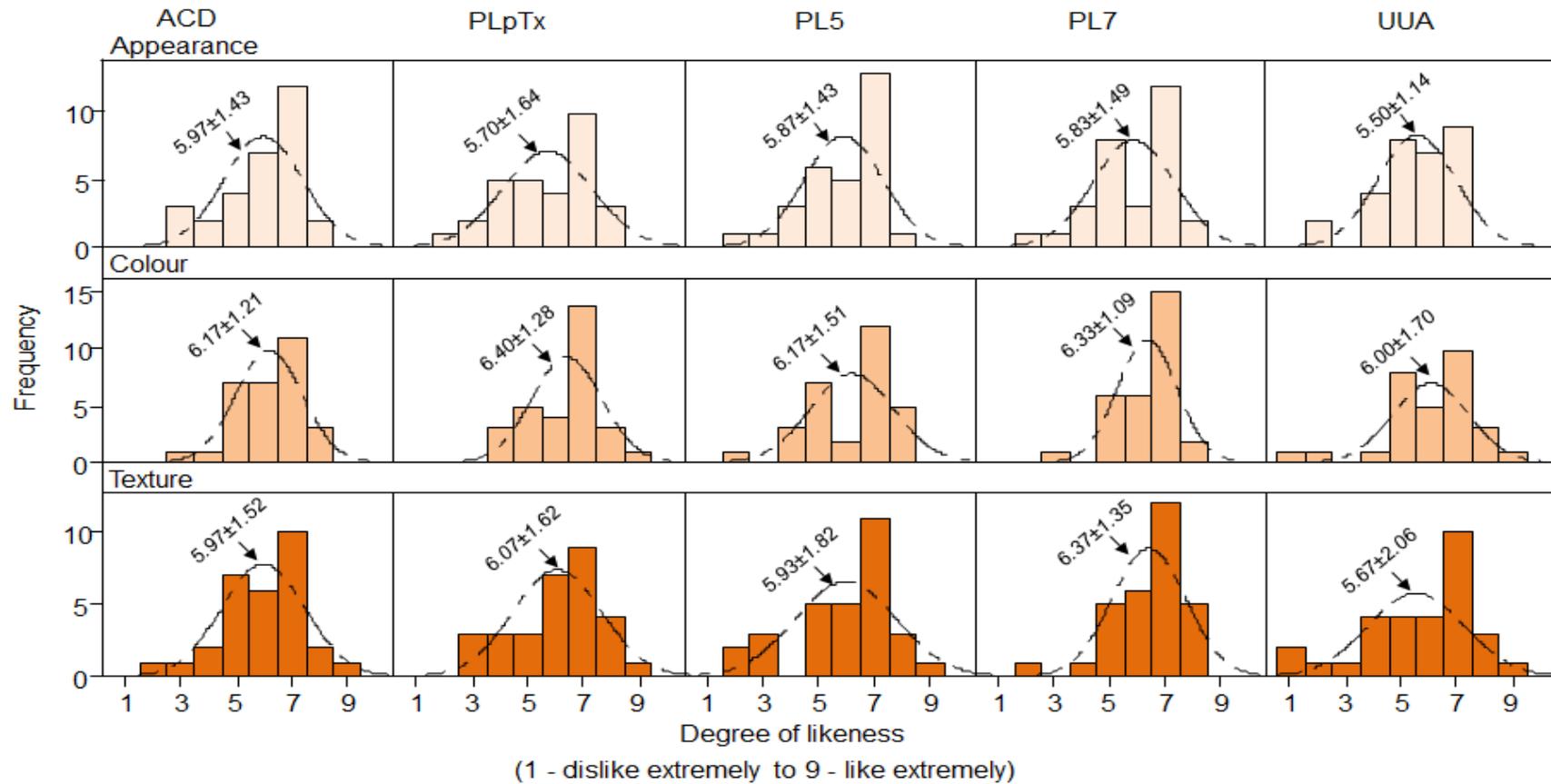


Figure 5.4: Histogram and distribution fit of the sensory attributes: appearance, colour and texture rating of potential probiotic maize porridges
 Mean \pm standard deviation, n=30
 ACD - Artificially acidified; UUA - Unfermented un-acidified; PL5 and PL7 - Porridges of ground maize slurries fermented by *L. plantarum* strains isolated from *akamu* sample (M3).
 PLpTx - Porridges of ground maize slurries fermented by commercial probiotic *L. plantarum* strain

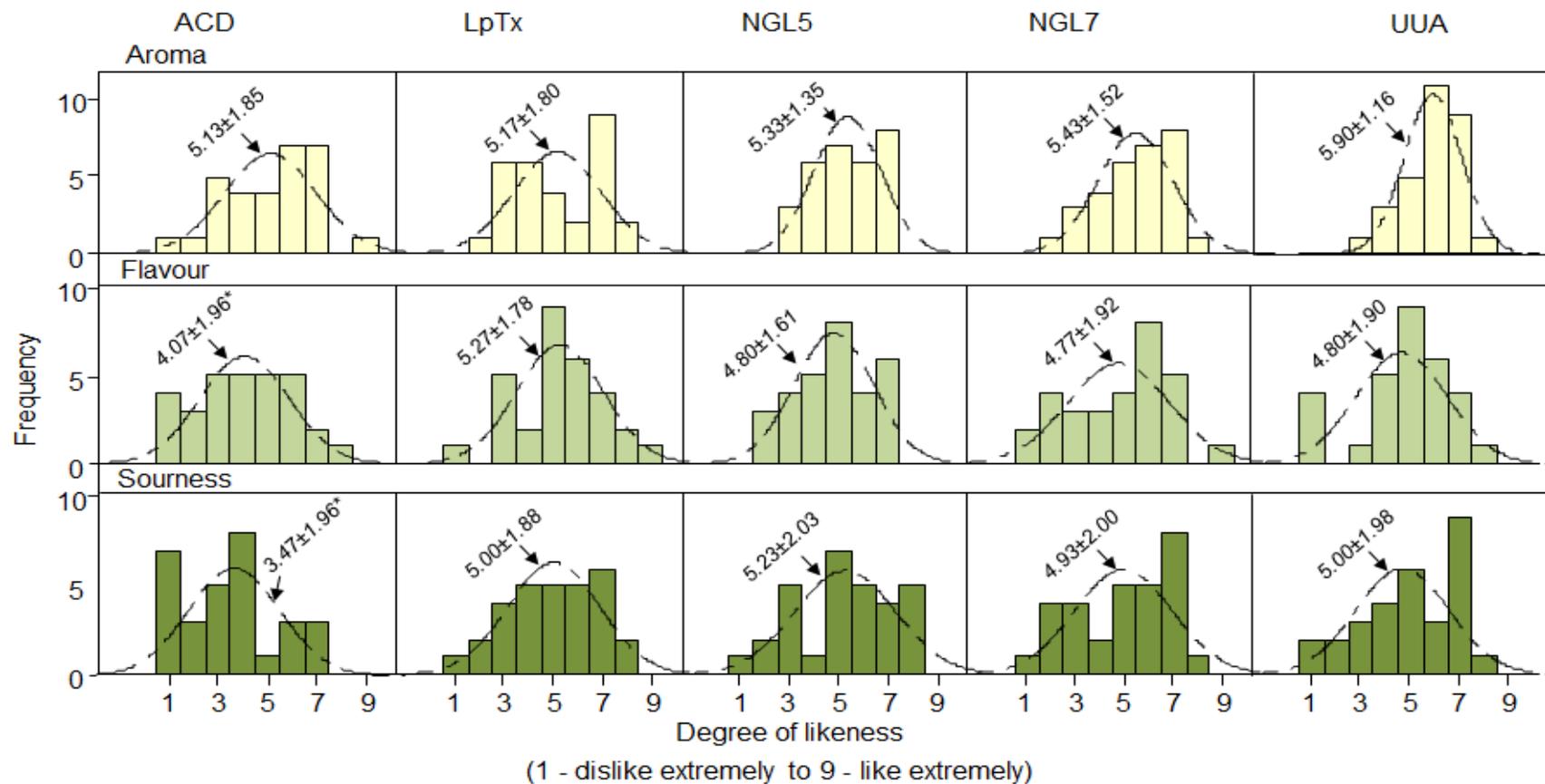


Figure 5.5: Histogram and distribution fit of the sensory attributes: Aroma, flavour and sourness rating of potential probiotic maize porridges

*Value were significantly ($p \leq 0.05$) different from others. (Mean \pm standard deviation, $n=30$)

ACD - Artificially acidified; UUA - Unfermented un-acidified; PL5 and PL7 - Porridges of ground maize slurries fermented by *L. plantarum* strains isolated from *akamu* sample (M3)

PLpTx - Porridges of ground maize slurries fermented by commercial probiotic *L. plantarum* strain

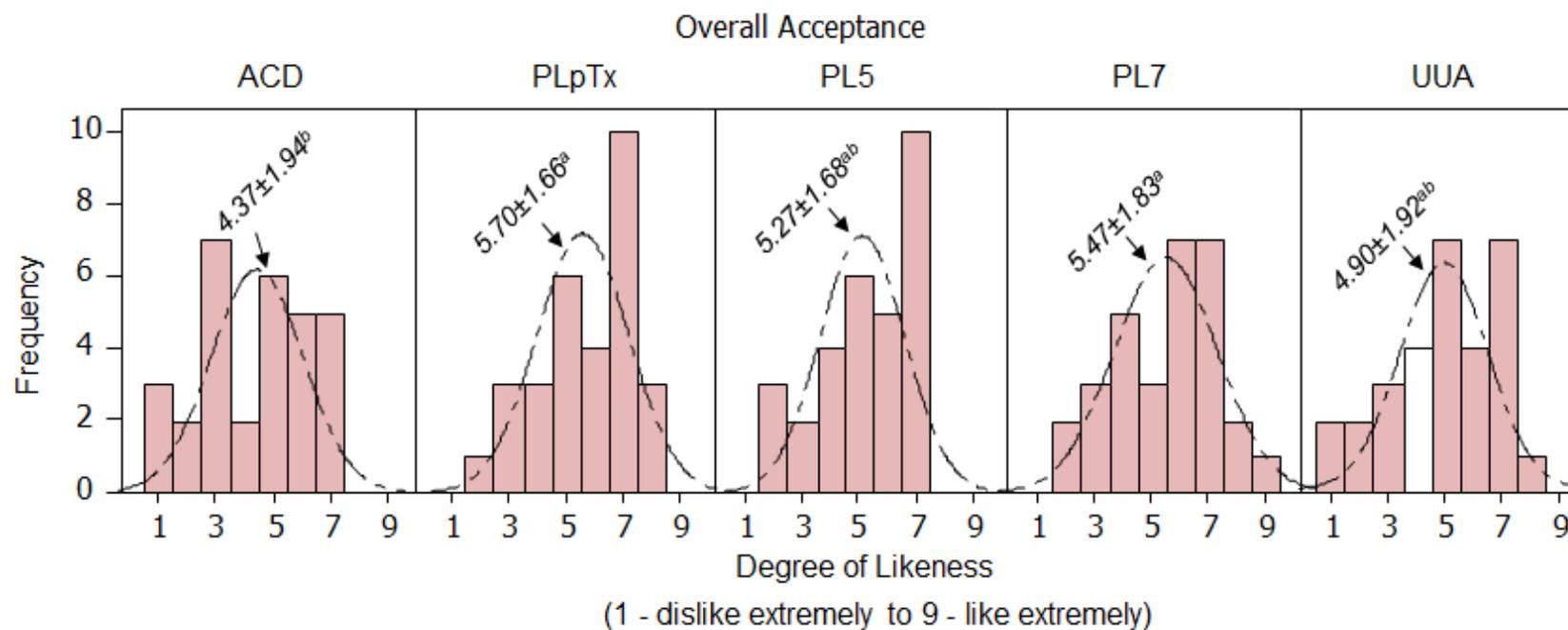


Figure 5.6: Histogram and distribution fit of the Overall acceptability of potential probiotic maize porridges

Mean ± standard deviation, n=30

ACD - Artificially acidified; UUA - Unfermented un-acidified; PL5 and PL7 - Porridges of ground maize slurries fermented by *L. plantarum* strains (NGL5 AND NGL7) isolated from *akamu* sample (M3)

PLpTx - Porridges of ground maize slurries fermented by commercial probiotic *L. plantarum* strain

5.4 Discussion

5.4.1 Proximate composition

Traditionally *akamu* is stored in homes under excess water and is usually decanted daily and replaced with fresh water. Water decanting in *akamu* for sale is followed by pressing out of excess water using muslin bags to obtain the cake that is moulded into balls of different sizes for sale. The observed moisture contents of the traditional *akamu* samples ($\geq 51.39\%$) were higher than the ranges of 6.3 - 10.9% reported in literature for similar fermented cereal products (Akingbala et al., 1994, Aminigo and Akingbala, 2004, Egounlety et al., 2002, Inyang and Idoko, 2006, Oluwamukomi et al., 2005). Variations in moisture could be a function of the amount of water pressed out from the slurry before wrapping in cellophane bags for sale. The amount of water added during the fermentation of the irradiated ground maize slurry in this study was neither decanted nor pressed out from the fermentation slurry. This accounted for the significantly ($p \leq 0.05$) higher moisture content (67.49 - 69.33%) of the starter culture fermented ground maize slurry. Moisture levels are likely to influence the stability of food products. However, the low pH (3.22 - 3.95) of the samples was able to likely inhibit the growth of undesirable microorganisms evidenced in by absence of Enterobacteriaceae, *Yersinia*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Listeria* and *Escherichia coli* in the *akamu* samples (Section 3.3.1.4) and the inhibition of *E. coli* 1077 in the controlled fermentation of the irradiated ground maize slurry (Section 4.3.1.2).

About 65 - 75% of the cereal grain composition have been reported to be carbohydrates (Haard et al., 1999) and the traditional technique of *akamu* production, which involves several washing steps, wet milling and sieving

invariably, yielded a starchy product with very low levels of other nutrients. This was evidenced by the negative correlation between carbohydrate and each of the other nutrient variables (protein, lipid and ash) in the samples. The observed lipid levels (1.77 - 3.68%) were similar to values reported in literature (Akingbala et al., 1994, Akingbala et al., 2003, Aminigo and Akingbala, 2004, Antai and Nzeribe, 1992, Otunola et al., 2007) but lower than values reported by Egounlety et al., (2002), Osundahunsi and Aworh (2003) and Oluwamukomi et al., (2005). The lipid distribution in maize kernel is such that 76 - 83% is found in the germ with 13 - 15% in the aleurone layer (Tan and Morrison, 1979). The removal of the germ and aleurone layers during processing may have contributed to the low lipid content and absence or trace amount of ash respectively. Although, the lipid content of the freshly inoculated starter culture fermented ground maize was significantly ($p \leq 0.05$) greater than the traditional *akamu* samples, there was significant decrease with progression in fermentation such that the lipid content of the starter culture fermented ground maize after 72 h did not vary from those of the *akamu* samples. The low lipid level therefore, may also be due to the oxidation of fatty acids by the microorganism to obtain energy for metabolic activities as reported by Oyarekua, (2011). Low lipid levels are however desirable for product storage stability.

The ash content of the starter culture fermented ground whole maize grain slurries that were not sifted did not vary from its original ground maize but was significantly ($p \leq 0.05$) greater than the ash content of the *akamu* samples. The grain pericarps are known to be major contributors of inorganic component that remains after the incineration of the organic components. Hence, the sieving process in the traditional *akamu* processing that involved the removal of the

pericarp encouraged the trace amount and/or the absence of ash in the *akamu* samples. This may have also contributed to the significant reduction in some of the mineral composition of the *akamu* samples in comparison to the ground maize slurries.

Although, some of the individual *akamu* samples had significantly ($p \leq 0.05$) lower protein contents than the controlled fermentation, the observed increase in the protein content of the controlled fermentation was however not significantly different from the traditional *akamu* samples based on their origin. The protein of maize grain has been reported to consist of the endosperm-specific proteins (zein and G1- and G2-glutelins) found specifically in the endosperm and the basic proteins (salt-insoluble proteins, G3-glutelins and residual proteins) that are found in all other grain fractions and tissues (Landry and Moureaux, 1980). Most likely, the different production processes may have led to some losses of protein and the resultant variations among the *akamu* samples from within the same location. The evaluation of the different amino acid profile of the proteins in the different samples would be useful in ascertaining any differences in protein quality.

According to the report of the Joint WHO/FAO/UNU Expert consultation (WHO, 2007), the daily protein and energy requirement for an infant female of 0.5 years old that is involved in moderate physical activity level were 1.12 g kg^{-1} and 340 KJ kg^{-1} body weight respectively. An infant weighting 7.34 kg would require 8.2 g protein and 2495 KJ energy per day. To meet this energy requirement they would need to consume $136 - 144 \text{ g}$ of *akamu*. However, higher quantities may

be required as the process of the porridge preparation involves cooking and diluting the infant food with large quantities of water to obtain thin gruel.

5.4.2 Mineral composition

Mineral compositions of cereals have been shown to vary widely among cultivars and between regions (Barikmo et al., 2007, Rodríguez et al., 2011, Roohani et al., 2012). The similarity of the mineral composition of the *akamu* samples suggested the use of similar maize variety within Rivers State, Nigeria in the production of the *akamu*. Although, the Mg and S concentration of the traditional *akamu* sample E3 made from white maize variety was significantly ($p \leq 0.05$) the least.

Calcium (Ca) is the primary mineral required for bone and teeth formation and plays important role in many metabolic processes. Nuts and whole grains are good sources of calcium (Berdanier and Zemleni, 2009). In this study, the concentration of calcium ranged from 37.88 - 70.29 and 73.08 - 94.62 mg kg⁻¹ for the traditional *akamu* samples and the *L. plantarum* starter culture fermented ground maize slurry respectively. The recommended calcium intake for infants and children <3 years ranges between 300 and 500 mg per day (FAO, 2001) and 100 g of the samples would meet $\leq 3.2\%$ of the recommended Ca intake. Although deficiency of calcium in children would lead to stunted growth and rickets, primary deficiency is related to the level of calcium absorption in association with vitamin D (Department of Health, 2012).

Copper (Cu) is one of the essential trace elements for humans. It is associated with many enzymes. Whole grains have been classified as excellent sources of copper. In this study, the copper levels in 100 g of the ground maize on dry weight basis (136 - 166 μg) were lower than values of 240 μg 100 g^{-1} for yellow, white and blue maize reported by Fardet et al., (2008). One of the fermented traditional *akamu* samples however, had Cu levels of 239 μg 100 g^{-1} . To meet the dietary reference intake (DRI) of copper for infants' ≤ 1 year (200 - 220 μg day⁻¹) (Berdanier and Zempleni, 2009), about 100 - 200 g of the samples would be required. Although copper absorption is influenced by the presence of divalent minerals in addition to the amount in the food mixture consumed, its deficiency is rare in human consuming variety of foods but could be manifested as anaemia and poor wound healing (Berdanier and Zempleni, 2009).

Iron (Fe) is essential for the formation of blood cells and an integral part of important enzyme systems in various tissues and its deficiency results in anaemia (Berdanier and Zempleni, 2009). The prevalence of Fe deficiency is highest among infants, children, adolescents and women of child bearing age in developing countries (FAO, 2001). The range of Fe concentration in the *akamu* samples and the ground maize were comparable with literature reports (Greffeuille et al., 2011, Fardet et al., 2008). Fe in cereal products are usually present as inorganic compounds and increases in the Fe contents of cereal foods during fermentation had been reported (Osundahunsi and Aworh, 2003). The significantly higher concentration of Fe in the traditional *akamu* could be due to the hydrolytic activities of the various endogenous microbes and possible Fe contamination from the milling equipment as reported by (Greffeuille et al., 2011). Similar increases in Fe and Ca contents of rice flour was attributed to

adventitious contamination during village milling (Chan et al., 2007). According to WHO/FAO, (2004) the recommended nutrient intakes (RNIs) in mg per day for a dietary iron bioavailability of 5% for infants aged 0.5 - 1 year is 18.6. The iron concentration in 100 g of the traditional *akamu* samples would be up to 14.8 - 87.4% of the daily Fe recommendation and $\leq 12.8\%$ from the fermented ground maize slurries. Consumption of over 390 g for the *L. plantarum* strain fermented samples would be needed to meet at least 50% of the RNIs

Potassium (K) is involved in the maintenance of body extra-cellular fluid (ECF) and acid balance, transmitting of nerve impulses to the muscles, muscle contraction and maintenance of blood pressure (Mir-Marqués et al., 2012). The value obtained for K in this study confirmed the report of Prasad et al., (2000) that K in plant is nearly ten-fold higher than the concentration of sodium. The *akamu* samples had lower K concentration than the starter culture fermentation, which could be attributed to the losses in the production method and the decrease in the K content with fermentation time in the starter culture fermentation could be as a result of leaching into the processing water or the utilization of the element by the microbes. Similar reduction observed by Prasad et al., (2000) during vegetable processing was attributed to leaching. The reference K intake for infants' between 0 - 12 months is in the range of 400 to 700 mg day⁻¹ (Berdanier and Zemleni, 2009) and the level of K in the traditional *akamu* samples and the ground maize slurries measured up to 2.7 - 17.8 and 6.8 - 66.6% of the daily requirement. Although the abundance of K in natural foods makes dietary deficiencies uncommon, in the case of protein-energy malnutrition (kwashiorkor) where tissue breakdown as well as diarrhoea

occurs there could be large loss of K, which could lead to heart failure (Prasad et al., 2000, Department of Health, 2012).

Magnesium (Mg) is an important constituent of the skeleton and functions as co-factor in many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis and maintenance of the electrical potential of nervous tissues and cell membranes. Although most unrefined cereal grains are reasonable sources of dietary Mg, corn flour has been reported to have extremely low Mg contents (FAO, 2001). The Mg content of 100 g of the fermented ground maize was found to be greater than the RNI for infants' ≤ 3 years ($36-60 \text{ mg day}^{-1}$) (WHO/FAO, 2004). Probably, due to losses during processing; ≥ 274 g of the traditional *akamu* sample would be required to meet the daily Mg requirement. Deficiency of Mg is rare and may result from prolonged diarrhoea or excessive loss from urine rather than from low intakes (Department of Health, 2012).

Manganese (Mn) concentration of the *akamu* samples and the ground maize slurries did not differ significantly ($p \leq 0.05$) from the unfermented maize flour and were comparable with the report of Fardet et al., (2008). In this study, 100 g of the samples contained Mn levels (0.15 - 0.47 mg) greater than the adequate intake levels for infants aged 0 - 6 months ($0.003 \text{ mg day}^{-1}$) (Berdanier and Zemleni, 2009) but 122 - 400 g of the samples would be required to meet the adequate intake levels for infants and children aged 7 - 12 months (0.6 mg day^{-1}) (Berdanier and Zemleni, 2009). This micro element is an essential mineral needed for bone mineralization, protein and energy metabolism, metabolic regulation and cellular protection from free radical species (Ericson et al., 2007).

Cereals and cereal products are rich sources of Mn (Department of Health, 2012) and its deficiency that is marked with abnormal function of bone and cartilage, and impaired glucose tolerance, poor reproduction performance, growth retardation and congenital malformations in offspring (Goldhaber, 2003) may rarely occur with the consumption of variety of other products.

The concentration of sodium (Na) in the starter culture fermented ground maize was significantly ($p \leq 0.05$) greater than the ground maize flour. Similar increase in the Na content of fermented cereal and legume blend was reported by Oyarekua, (2011). Sodium (Na) is important in the maintenance of the human ECF and plays important role in the functioning of the nerves and muscles and the regulation of blood pressure. The high Na content in the fermented samples could imply the breakdown of certain substances by the fermenting organisms to its Na component. To meet the acceptable intake of Na for infants ≤ 1 year old ($120 - 370 \text{ mg day}^{-1}$) (Berdanier and Zemleni, 2009) would require the consumption of more than 500 g of the samples evaluated in this study, although, deficiency of Na is rarely reported since most processed foods have sodium added to them.

Phosphorus (P) exists as a component of phytic acid mostly in the bran of cereals such that whole-grain products contains more phytates than the refined products (Itkonen et al., 2012). Degradation of phytates in cereal foods during fermentation was reported by Sanni et al., (1999) and this ought to increase the content of inorganic phosphates in the product. However, there was significant decrease in the phosphorus concentrations in the fermented samples with the contents in the *akamu* samples significantly ($p \leq 0.05$) the least. This infers that

there could be losses due to the processing technique: discard of pomace and leaching into the liquid medium or possible utilization of the element by the microbes. The concentration of phosphorus in the samples were comparable with the report by Sanni et al., (1999) and greater than the RDA for infants <0.6 years. Phosphorus plays important role in energy release and metabolic processes, excess however, may produce low levels of calcium in the blood, trigger involuntary muscle convulsion, increase the incidence of cardiovascular diseases and is harmful to bone metabolism (Itkonen et al., 2012, Department of Health, 2012).

Although decrease in phytate component during fermentation has been implicated in the increase of Zn concentrations in cereal products, the concentrations of zinc (Zn) in the unfermented ground maize slurry ($\leq 20.6 \text{ mg kg}^{-1}$) did not differ significantly ($p \leq 0.05$) from their fermented counterpart ($\leq 21.6 \text{ mg kg}^{-1}$). The significantly ($p \leq 0.05$) lowest zinc levels ($\leq 9.9 \text{ mg kg}^{-1}$) of the traditional *akamu* samples highlights losses during processing. The concentrations of Zn in the traditional *akamu* samples were comparable with values reported for traditionally fermented maize by Oyarekua, (2013). The ground maize Zn content were similar to the value reported by Fardet et al., (2008) but higher the values reported by Tang et al., (2013) due likely to origin and maize variety. Zinc's central role in cell division, protein synthesis, and growth is very important for infants and children, any deficiency would result to growth retardation, diarrhoea and impairment of immune defences (FAO, 2001). The recommended Zn intake for infants aged between 0 and 12 months is between $6.6 - 8.4 \text{ mg day}^{-1}$ (WHO/FAO, 2004), 100 g of the samples in this

study would meet ≤ 15 and $\leq 33\%$ for the traditional *akamu* samples and starter culture fermented ground maize slurries respectively.

5.4.3 Dietary fibre

The chromatogram of the SDFS showed high glucose peak, small LMWDF (DP ≥ 3) peak, maltose and xylose peaks in addition to the D-sorbitol internal standard peaks. The glycerol peak was confirmed using a glycerol standard solution and was suspected to be from the amyloglucosidase (AGM) stock and protease solution. The observed high peak area of the glucose residue in comparison to the malto-oligosaccharide (Figure 5.2) has been attributed to the hydrolytic activity of the AMG added during sample digestion as one unit of AMG has the capacity to release 1 micromole of D-glucose from soluble starch (Brunt and Sanders, 2013). For high starch containing food samples it had been reported that the combined pancreatic α -amylase and AMG hydrolysis is unable to convert quantitatively all non-resistant starch and maltodextrin into glucose (Brunt and Sanders, 2013). This was observed in these fermented starchy maize products as shown by LMWDF in Figure 5.2. However, the SDFS computation involved both the R_f and the peak area of the SDFS. The quantified glucose levels that were released ranged between 42.75 ± 0.29 to 54.20 ± 0.16 for *L. plantarum* fermentation after 24 h and the traditional *akamu* sample (M3).

The disaccharide maltose was also produced from the hydrolysis of starch by the α -amylase. This was significantly ($p \leq 0.05$) higher in the unfermented samples, indicating the combined effect of microbial hydrolysis and that of the added enzyme in the fermented samples. The available carbohydrate from the

integrated dietary fibre analysis was therefore composed of glucose and maltose. Xylose, may have originated from the enzymatic hydrolysis of the cell wall polysaccharide where it constituted the cell wall building blocks (Bach Knudsen, 2001). This further explains why the unfermented sample had higher level of xylose. The proximate composition of the samples had shown protein content $\leq 7.45\%$ and the non-detectable level of protein in the DF analysis could be attributed to hydrolytic activity of the protease enzyme.

The IDF (35.10 - 82.05 mg g⁻¹ DM) and SDF (26.97 - 50.80 mg g⁻¹ DM) values reported in this study were inconsistent with the IDF and SDF values of 9.42 and 0.18 mg g⁻¹ respectively reported for unfermented yellow corn by Guo and Beta, (2013). The variation could be a confirmation that fermentation resulted in increased DF content. The enzymatic-gravimetric method used in this study has been reported to be the most suitable for nutritional labelling and quality control purposes as loss of a considerable portion of DF occur with the use of non-enzymatic gravimetric methods (Huang et al., 2013) and the IDF content in this study was comparable with the report of Huang et al., (2013).

In cereal products there is high variation in the content of DF depending on the proportion of the individual kernel parts (Dziedzic et al., 2012). The lowest IDF content of the traditional *akamu* product may be attributed to the removal of most of the bran fragment during wet sieving process. However, the significantly ($p \leq 0.05$) highest SDFS (NDO) content of the *akamu* sample suggested the influence of any other production process. Although, the process of soaking has been reported to cause increases in resistant starch content (Kutoš et al., 2003). It was observed that there were increases in the DF fractions of the *L.*

plantarum starter culture samples with progression in fermentation. The SDF constituted the majority of the DF content of the traditional and the *L. plantarum* fermented sample after 72h.

The DF content of the fermented samples is important due to the physiological benefits attributed to DF. The insoluble fraction of DF activates intestinal peristalsis and is capable of binding bile acids and water which is significant in prevention of diet-induced diseases (Dziedzic et al., 2012), while the soluble fraction of DF have the capacity to increase viscosity, reduce glycemic response and involved in lowered blood cholesterol (Elleuch et al., 2011).

5.4.4 Pasting characteristics

The traditional *akamu* sample and the un-irradiated ground maize had pasting temperatures comparable to typical maize starch (Sandhu et al., 2004, Kuakpetoon and Wang, 2007). Pasting temperature is an indication of the minimum temperature required to cook the sample. This explained why the addition of boiling water (100°C) to traditional *akamu* slurry yields a gelatinized gruel. The undetected pasting temperature of the irradiated ground maize and its *L. plantarum* strain fermented samples implied the sample's inability to form a paste within the range of the experimental temperature (50 - 95°C) as confirmed by the significantly ($p \leq 0.05$) lower peak viscosities (≤ 139.5 cP).

Peak viscosity occurs at the equilibrium point between swelling and the leaching out of amylose component of the starch hence it reflects the water-binding capacity of the starch granules and the flimsiness of swollen granules. The peak viscosity of the traditional *akamu* sample and the un-irradiated ground maize

suggests that their starch structures were such that allowed greater water absorption and granule extension causing increased polymer leaching and subsequent increase in viscosity. Although lipids have been reported to restrict swelling (Singh et al., 2002), the lipid content of the samples as shown in Table 5.1 and Table 5.2 did not show any significant variation. This implies that the variation in viscosity of the samples is more of the starch structure than fat surrounding the granules and restricting swelling. With the increase in viscosity, the mechanical agitation from the paddle will generate shear force greater than that of the granules in starch/water system, thereby causing the swollen granules to loss integrity and rupture followed by decrease in viscosity (Alamri et al., 2013). This breakdown was observed in all the samples.

On cooling however, the final viscosity of the traditional *akamu* sample and the un-irradiated ground maize increased significantly ($p \leq 0.05$) greater than their peak viscosity while that of the irradiated samples decreased. The traditional *akamu* sample and the un-irradiated ground maize could be said to be more stable than the others since the final viscosity marks the ability of the material to form gel after cooking and cooling. During cooling, it is the hydrogen bonding interactions between the amylose and the amylopectin that results in the gelling (Frazier, 2009). Although, the hydrolytic effect of fermentation increases the short chain of amylopectin in the amorphous region which could associate like the amylose to improve the paste viscosity (Yuan et al., 2008), increased viscosity during cooling was only observed for the traditional *akamu* samples and not the starter culture fermented irradiated ground maize.

On the other hand, fermentation has also been implicated in the decrease of final viscosity and setback of corn starch (Yuan et al., 2008) as insufficient concentration of amylose owing to its hydrolysis results in lowered degree of polymerisation. This may explain the significant ($p \leq 0.05$) higher final viscosity and setback of the un-radiated and unfermented sample in comparison to the traditional *akamu* sample. The viscosity of the irradiated fermented samples in this present research however, were in no way comparable with the traditional *akamu* sample which was a product of spontaneous fermentation, thereby suggesting that irradiation rather than fermentation may have caused greater changes in the starch structure.

The low viscosity of the irradiated ground maize slurries fermented by the starter cultures of *L. plantarum* strains would be desirable for infant complementary food for a nutrient-dense product without dilutions with excess water. Although, *akamu* with low viscosity may not be appreciated by some adults and could be considered as spoilt or containing low dry matter content. The dry matter contents of the samples (2.93 g weight) were the same and this implies that certain changes may have taken place in the irradiated samples as compared to the un-irradiated samples. For adults who would prefer much thicker gruel, such high viscosity can be achieved by the addition of more of the ground maize which would imply increase in nutrient composition.

5.4.5 Sensory analysis

The analysis of the sensory attributes: appearance, colour, aroma, Sourness (acidity), flavour, texture (smoothness) and overall acceptability of the *L. plantarum* strains fermentation showed that the samples were liked to varying

degrees by the assessors. *Akamu* would usually be consumed with sugar, salt and/or milk added depending on affordability. The acidified sample was reported to be tart: too strong and leaving a bitter taste while the *L. plantarum* strains fermented samples were characterised by flavours that evoked honey and apples. This suggested the likely influence of microbial metabolites on the flavour of the product that was not achieved by the artificial acidification. The overall acceptabilities of the porridges from the *L. plantarum* strains fermented ground maize slurries were that of moderate likeness.

5.5 Conclusion

This study revealed that the *akamu* samples as sold in the market and the resultant product from the controlled fermentation of the ground maize slurries were starchy foods with pH <4.0 due mainly to the production of lactic acid by the fermenting microorganisms. The ground maize slurries fermented by the *L. plantarum* starters had significantly ($p \leq 0.05$) higher levels of ash and mineral (Ca, K, Mg, Na, S and Zn) than the traditional *akamu* samples.

The traditional *akamu* sample had higher pasting viscosity than the starter culture fermented irradiated ground maize samples, although lowered viscosity would be desirable for infant feeding. Microscopic examination of the extent of granule deformation and disruption in the samples may provide further explanations to the behaviour of the samples.

The traditional *akamu* sample had the lowest IDF but the highest SDFS content, suggesting the likely influence of the traditional production process. A comparative evaluation of the DF content of the different maize fraction and the

effect of variation in processing methods may help in properly attributing the cause of changes in DF composition of processed maize products.

The sensory attributes of the new *akamu* product (porridges from the *L. plantarum* strains fermented slurries) were acceptable to varying degrees by the assessors.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND FUTURE WORK

6.1 General discussion

Maize is one of the most important cereal grains worldwide. It is processed and consumed in a variety of ways. *Akamu* is one of such products obtained from the fermentation of ground maize slurry and is consumed by people of all ages in Nigeria. The traditional *akamu* production technique has been implicated in nutrient losses and contamination that may occur at any stage from production to consumption affects the quality and safety of the product. This study focused on the effect of fermentation with selected microbial inocula on the proximate, mineral and dietary fibre composition, pasting characteristics, sensory properties and safety of *akamu* in addition to the characterisation of the selected microbial inocula for possible probiotic potential in the production of *akamu* as a functional food.

Chapter 3 provided baseline information on the microbial population and fermentation characteristics of identified microorganisms from Nigerian traditional *akamu* samples obtained from Rivers State in Nigeria (section 3.2.1.1). This was achieved using both molecular and conventional microbiological techniques as described in section 3.2.4. A comparison of both methods' results for bacteria revealed that more Lactic acid bacteria (LAB) were identified using the culture-independent methods (PCR-DGGE and sequencing analysis) and some of the LAB that were culturally identified were present in the culture-independent analysis (section 3.3.1.2, 3.3.1.3 and 3.3.1.4). Although identification based on biochemical testing was more related to metabolic

functions than to genetic closeness, due probably to the culture conditions some LAB were identified with the culture-dependent technique. The closeness of 2 strains with similar DNA regions made the classification of such operational taxonomic unit difficult. These highlighted the limitations and strengths of both techniques in confirmation to literature reports (Marshall et al., 2003, Stiles and Holzapfel, 1997, Wakil et al., 2008).

Yeasts were conventionally isolated and their identity confirmed using culture-independent technique (section 3.2.4 and 3.3.1.4). Lactic acid bacteria and yeast were therefore associated with the fermentation of the traditional Nigerian *akamu* samples. The predominant LAB were of the genera *Lactobacillus* and included *Lactobacillus fermentum*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus*. Others included *L. rhamnosus*, *L. casei*, *L. salivarius*, *L. acidophilus* and *L. reuteri* and a strain of *Lactococcus lactis* subsp. *cremoris* (from the genus *Lactococcus*). The presence of these lactic acid bacteria has been reported in some fermented cereal-based foods except for the occurrence of *L. helveticus* in maize fermentation that seemed unique. Identified yeasts included *Candida tropicalis*, *C. albicans*, *Clavispora lusitaniae* and *Saccharomyces paradoxus*.

Yeast do not produce appreciable quantities of lactic acid, their various other volatile compounds (Annan et al., 2003) may however be of importance in the development of appreciable product flavour. However the identification of potential pathogens such as *C. albicans* was an indication of contamination from handlers. Although, the improvement on personal hygiene during *akamu*

production is advisable, economic status of the rural people, poor sanitary conditions and lack of adequate clean water supply may be a deterrent factor.

Fermentation of sterile ground maize slurries by the endogenous LAB isolates from a selected *akamu* sample (M3), revealed that strains of *L. plantarum* (NGL5 and NGL7) amongst others had significantly ($p \leq 0.05$) greater fermentation ability which led to further characterisation of the strains for probiotic potential and their use both as single and mixed starter culture with yeast in the fermentation of irradiated ground maize slurry. As single starter cultures, the *L. plantarum* performed creditably shortening the fermentation time to 12 h with pH ≤ 3.93 when inoculated from freshly washed broth cultures and 6 h with pH ≤ 3.92 as freeze dried cultures (Table 3.9 and Figure 3.6 respectively).

Freeze drying has been reported as a preservation technique that have improved the stability of LAB during storage (Dianawati et al., 2013) and with the use of skimmed milk as a cryoprotectant, the *L. plantarum* strains survived freeze drying in their numbers for efficient fermentation. Although the duration of stability of the *L. plantarum* strains in their freeze dried state was not established in this study, the freeze dried cultures would provide easier means of making the prospective starter cultures available for use at household level in the rural areas of the developing world.

Obtaining a sterile raw material and fermentation environment as it was in this study may not be possible at household level; the confirmation of the presences and relative abundance of the starter cultures of the *L. plantarum* strains that

may be inoculated during traditional *akamu* fermentation using specially designed microbial probes can constitute another research objective.

Different mathematical functions with curve fitting softwares such as Gompertz, Logistics, Baranyi and Roberts' model have been in use in describing microbial growth curves. The suitability of these models for microbical analysis had been investigated and the ability of Baranyi and Roberts' model to better fit experimental data was reported (López et al., 2004). The Baranyi and Roberts' model unlike others estimates the mid-phase as close to linear, hence data set with distinct decrease at any point after the exponential phase before further increases tend to show significant variation from the model fit. This was the case with some of the model fit such as *L. helveticus* - NGL3 and *Sacch. boulardii* SB20 in the mixed culture fermentation with the *L. plantarum* strains.

The likely safety and probiotic effect of the selected microbial inocula were demonstrated in chapter 4. An agar spot method was employed to determine the inhibitory activity of the selected *L. plantarum* strains (NGL5 and NGL7) and *C. tropicalis* (NGY1) isolated from *akamu* samples and their probiotic counterparts: *L. plantarum* (LpTx) and *Sacch. boulardii* SB20. Although this method seemed unique for the yeast, it is a common method used to appropriate the cause of LAB inhibitory activity. Glucose fermentation by LAB yields lactic acid that reduces pH of the medium to levels that can inhibit growth of unwanted microorganisms. Variation in the glucose composition of the media and the incubation condition could lead to the production of other antimicrobial agents such as hydrogen peroxide and bacteriocins. The inhibitory activity of

the *L. plantarum* strains were attributed to acidity because there was no inhibition in the modified MRS media (section 4.3.1.1).

Although the results can be influenced by factors such as inoculum size and growth phase, media, diffusion rate of fermentation product, incubation time and temperature. The inoculum sizes of both the *L. plantarum* strains and the pathogen were standardized after a specified growth period using the same specific growth media for the *L. plantarum* strains and pathogen respectively under their specific incubation conditions. The antimicrobial activity of the LAB due to acidity was confirmed in a challenge test with *Escherichia coli* 1077 in the fermentation of ground maize slurry. The fermentation temperatures had some influence on the survival of *E. coli* 1077 as there was viability of 4 and 5 Log_{10} CFU mL^{-1} after 120 min at 22°C, although complete inhibition was observed after 24 h. Despite the known influence of temperature on acid dissociation and microbial growth, it may be interesting to know whether *E. coli* 1077 expressed genes that enabled its survival in the acid condition at 22°C.

The selected *L. plantarum* strains were able to withstand acid and bile conditions as shown in Figure 4.11 to Figure 4.13. The demonstration of this acid/bile tolerance ability of the *L. plantarum* strains in the modified growth media (MRS broth at pH 2 and MRS broth with 0.3% ox gall bile) and the inoculation of acid stressed cells into the bile media followed the perception that microorganisms in the GIT are usually not alone but in contact with the food substrate in which it is relayed into the gut and in the digestive system cellular stress actually begins with exposure to acid environment in the stomach before entrance into the small intestine where bile concentration is encountered. The

use of ox gall bile for bile tolerance studies have been very common due to its similarities with the human bile juice (Gilliland and Walker, 1990) and the concentration of 0.3% is in the range of human bile concentration of 0.3 to 0.5% (Vinderola and Reinheimer, 2003), while pH 2 is well within the range of the acidity of the human stomach (pH 1.5 to 4.5) (Lin et al., 2006).

The *L. plantarum* strains exhibited significant ($p \leq 0.05$) hydrophobicity, auto-aggregation capabilities and adhesion to porcine mucin and Caco-2 cells (section 4.3.2 to 4.3.5). Adhesion to solvents has often been used in assessing bacterial hydrophobicity due to the affinity of bacterial cell wall charges to basic (electron donor) and/or acidic (electron acceptor) solvents (Bellon-Fontaine et al., 1996). The adhesions of the *L. plantarum* strains to mono polar solvents with maximal affinity for chloroform was an indication of more electron donor (-COOH) groups on the bacterial cell surface. Hydrophobicity has been reported as an important factor that governs adhesion of bacteria to various surfaces (Zita and Hermansson, 1997, Liu et al., 2004), as was confirmed in its correlation with bacteria cell-to-cell adhesion. Hydrophobicity and adequate mass achieved through aggregation offers competitive advantage important for bacterial persistence in the gut (Vinderola and Reinheimer, 2003, Xie et al., 2012, Jankovic et al., 2003) and auto-aggregation has been suggested to be necessary for adhesion to intestinal epithelial and mucosal cells (Kos et al., 2003, Collado et al., 2007a, Collado et al., 2007b, Ouwehand et al., 1999).

The use of porcine mucin in demonstrating bacteria adhesion to mucosal surface is embedded in the fact that mucin is the main structural component of the viscoelastic gel-like substance that covers the mammalian epithelial surface

(mucus) (Jonsson et al., 2001, Svensson and Arnebrant, 2010), it harbours glycan-rich domains that provide preferential binding sites for bacteria (Juge, 2012) and the human and porcine mucin have been reported to share similar conserved motifs and structure (Chen et al., 2004).

The *in-vitro* inability of the *L. plantarum* strains to hydrolyse gelatine into its peptide or amino acid units and breakdown red blood cells implies they would neither derange the protective mucus lining of the GIT nor cause cessation of the underlying epithelial layer which could create pathway for infections (Kalui et al., 2009). These positive effects could aid in proffering certain health benefits without any possible virulent factor.

Chapter 5 covered the proximate composition and mineral content of the ground maize slurry fermented under controlled condition with the selected *L. plantarum* strains in comparison to the traditional *akamu* samples obtained as described in section 3.2.1.1 from Nigeria, integrated total dietary fibre composition and pasting properties of a selected *akamu* sample (M3) and ground maize slurry fermented by *L. plantarum* strain NGL5 and the sensory properties of the *L. plantarum* strains fermented ground maize slurry. The result showed that both products were predominantly starchy foods. The new production technique significantly ($p \leq 0.05$) influenced increased ash, TDF and mineral (Ca, K, Mg, Na, S and Zn) contents against the traditional samples, due probably to the sieving processes that excluded most of the grain outer kernel layers. The selected traditional *akamu* sample (M3) had the least IDF content due probably to the same reason.

The levels of some of the minerals obtained in the samples were less than the recommended intakes, it may be wrong however to assess adequacy based on that as the actual nutrient requirement for each individual may actually be less than the recommended intakes and factors such as age, height, weight, sex and physical activities through out the day may as well influence nutrient requirement (Department of Health, 2012). The sensory analysis data revealed that the product sensory attributes: appearance, colour, aroma, Sourness (acidity), flavour, texture (smoothness) were liked to varying degrees despite that the assessors were persons not familiar with the fermented *akamu* product. They were however consumers of different other lactic acid bacteria fermented products like yughort and kushuk (a preboiled LAB fermented wheat product).

The AOAC 2011.25 integrated total dietary fibre assay has been modified and approved for the determination of insoluble, soluble and total dietary fibre (IDF, SDF and TDF respectively) in plant materials, food and food ingredients (McCleary et al., 2011). This method quantitated the water-insoluble dietary fibre (IDF) separately from the water-soluble fibre (SDF). The two different components of the SDF: dietary fibre precipitated in 78% aqueous ethanol or IMS (SDFP) and the dietary fibre that remained soluble in 78% aqueous ethanol or IMS (SDFS) were separately quantitated. With the previous AOAC method 2009.01, the IDF and the SDFP were quantified as one and known as HMWDF, although the quantification was not based on the molecular weights of the fibre fractions (Megazyme, 2012). The HPLC analysis in the determination of SDFS was calibrated using D-glucose as D-glucose provided an LC refractive index (RI) response equivalent to the response factor for the non-digestible oligosaccharides that make up the SDFS. This reference factor was then used

in quantifying SDFS of the samples. The results of the analysis for the traditional *akamu* sample, and the unfermented and the fermented ground maize slurry therefore showed the IDF, SDFP and the SDFS contents of the samples from where the SDF and the TDF were obtained.

Among the sugars (xylose, glucose and maltose) obtained (Table 5.6), the amount of glucose was significantly ($p \leq 0.05$) higher in confirmation of the hydrolytic activities that take place during the digestion of samples. Dietary fibre consists of a mixture of chemical entities and the choice of analytical method for fibre investigation will depend on the composition of the particular fibre (Elleuch et al., 2011). The soluble dietary fibre in starchy foods is composed mainly of resistant malto-dextrins. The heat and enzymatic treatment during the analysis had resulted in the hydrolysis of the malto-dextrin main α -(1,4)-D-glucose and the branched α -(1,6)-D-glucose chains used for the quantification of the soluble dietary fibre mass.

Fermentation resulted in increased SDFS content of the samples as the unfermented samples had significantly ($p \leq 0.05$) the least SDFS level and an increase was observed with progression in fermentation. This increase in SDFS content of the fermented samples is an important prebiotic attribute as soluble fibre had been found to be beneficial to gut health. Maathuis et al., (2009) in an *in-vitro* study found that soluble maize fibre increased the production of short chain fatty acids (SCFA) and the concentration of bifidobacteria and some *Lactobacillus* spp. while the putrefactive metabolites from protein metabolism were reduced. The health benefit of soluble maize fibre were confirmed in healthy adult men with the positive effect as minimal GIT upset, reduction in

faecal putrefactive compounds, stool bulking and increased bifidogenic potential (Vester Boler et al., 2011). This suggests further studies for the investigation of the health effect of the DF components of the *akamu* samples.

6.2 General Conclusion

The findings of this study in realisation of the stated specific objectives were summarised as follows:

Stage 1: Baseline data on the quality and microbial population of Nigerian spontaneously fermented *akamu* samples

1. *Akamu* was characterised with a mixed population of lactic acid bacteria (LAB) and yeast as fermenting microorganisms
2. The predominant LAB were *Lactobacillus fermentum*, *L. plantarum*, *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus* while the isolated yeasts were *Candida tropicalis*, *C. albicans*, *Clavispora lusitaniae* and *Saccharomyces paradoxus*
3. The *akamu* samples had pH <4 due mainly to lactic acid production
4. The product was a starchy food having less of other nutrients

Stage 2: Selection of microbial inoculant for the controlled fermentation of *akamu*

5. Among the lactic acid bacteria: 2 strains of *Lactobacillus plantarum* (NGL5 and NGL7); 3 strains of *L. helveticus* (NGL1, NGL3 and NGL8); 2 strains of *L. acidophilus* (NGL2 and NGL6) and a strain of *Lactococcus lactis* subsp. *lactis* (NGL4) isolated from the selected Nigerian

spontaneously fermented *akamu* sample (M3), the *L. plantarum* strains showed strong fermentation ability at 30°C after 24 h (pH \leq 3.63, TTA \geq 0.70% and lactic acid level \leq 80.78 mmol L⁻¹). Others had low level of lactic acid $<$ 7 mmol L⁻¹, pH \geq 5.31 and TTA $<$ 0.37% except for the fermentation with *Lactococcus lactis* subsp. *lactis* that had pH and TTA of 4.40 and 0.43% respectively

6. The yeast: *Candida tropicalis* (NGY1) isolated from the Nigerian *akamu* sample (M3) and the probiotic *Saccharomyces boulardii* SB20 did not produce appreciable amount of acid for any significant pH decrease
7. The selected *L. plantarum* strains: NGL5 and NGL7 isolated from *akamu* sample M3 and their probiotic counterpart (LpTx) in a mixed starter culture with the yeasts (*C. tropicalis* NGY1 or the probiotic *Sacch. boulardii* SB20) had significant ($p \leq 0.05$) lactic acid production (63.88 mmol L⁻¹) and decreased pH (\leq 3.61) after 24 h

The two *L. plantarum* strains (NGL5 and NGL7) either as single or mixed starter cultures with yeast demonstrated significant fermentation abilities and were selected for further characterisation.

Stage 3: Characterisation (antimicrobial and probiotic potential) of the selected inoculant

8. The *L. plantarum* strains inhibitory activity against five relevant foodborne pathogens: *Bacillus cereus* NCIMB 11925, *Escherichia coli* 1077, *Listeria monocytogenes* NCTC 7973, *Salmonella enteritidis* NCTC 5188, and *Staphylococcus aureus* NCTC 3750 on MRS agar plates was mainly due to acid production.

9. *E. coli* 1077 in the co-inoculation fermentation of ground maize slurries with the *L. plantarum* strains at 30°C (pH≤3.48) was inhibited after 24 h
10. *E. coli* 1077 inoculated into the *L. plantarum* strains already fermented ground maize slurries at 30°C (pH≤3.48) was inhibited after 180 min
11. *E. coli* 1077 decreased below detection limit after 20 mins of inoculation into porridges of the fermented ground maize slurries (pH 3.41)
12. The selected *L. plantarum* strains (NGL5 and NGL7) survived acid and bile condition, aggregated, adhered to mucin and Caco-2 cells without hydrolysis of gelatine and red blood cells

It could be suggested that fermentation of *akamu* with the characterised *L. plantarum* strains (NGL5 and NGL7) would improve the product safety and the probiotic potential of the *L. plantarum* strains that has been demonstrated in the *in-vitro* studies can be of advantage to the consumers.

Stage 4: Product evaluation

13. The *L. plantarum* strain fermented ground maize slurries did not differ significantly ($p \leq 0.05$) from the Nigerian spontaneously fermented *akamu* samples in terms of their protein, lipid and energy content but had higher contents of ash, mineral, IDF and SFS contents and the lowest pasting viscosity
14. Sensory attributes: appearance, colour, aroma, Sourness (acidity), flavour, texture (smoothness) and overall acceptability of the porridges were liked to varying degrees and had a moderate generally acceptance by more than 75% of the assessors.

Although the proximate composition of the new product (the *L. plantarum* strain fermented ground maize slurries) was not significantly improved upon in comparison to the traditional *akamu* product, the acceptability of the sensory attributes of the new product was very crucial in meeting one of the technological requirements (fermentation without alteration of the products desired sensory properties) for potential probiotics in the production of functional foods. The products DF components: IDF and SDF would be relevant as prebiotics in the improvement of gut health.

Considering the findings of this study, it could be suggested that this new *akamu* product is a potential functional food. Although *in-vivo* studies need to be carried out to ascertain and authenticate beneficial health effects on the consumers.

6.3 Future work

There are still many aspects within this traditionally fermented maize ecosystem that may require further studies such as:

1. Determination of microbial successional changes during the traditional fermentation of *akamu* using both culture-dependent and culture-independent microbiological methods
2. Establishment of the total yeast population present in traditionally fermented *akamu* using culture-independent techniques
3. Investigation of the fermentation end products of the established yeasts for the verification of the actual roles of yeast in the product

4. Further characterisation of the *akamu* LAB strains in non sterile condition and validation of their probiotic potential using *in-vivo* studies
5. Determination of the ability of selected *akamu* LAB(s) to improve immune modulatory properties
6. The effect of control fermentation on the keeping quality of the product
7. Comparative evaluation of the dietary fibre content of the different maize fraction and the effect of variation in processing methods on the dietary fibre composition of fermented maize food. This would further clarify the cause of changes in DF composition of processed maize products
8. *In-vitro* assessment of the prebiotic component of *akamu* and its ability to sustain growth of probiotics
9. The production of ready to eat functional *akamu* porridges in tetrapacks that can be bought of the shelves in the markets
10. The production of *akamu* powder with documented methodology for the preparation of the porridges that can be communicated to end users in developing countries

REFERENCES

- ADAMS, M. R. & MOSS, M. O. 2000. *Food Microbiology*, Cambridge, United Kingdom, The Royal Society of Chemistry.
- ADAMS, M. R. & NICOLAIDES, L. 1997. Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control*, 8, 227-239.
- ADEBAWO, O. O., AKINGBALA, J. O., RUIZ-BARBA, J. L. & OSILESI, O. 2000. Utilization of high lysine-producing strains of *Lactobacillus plantarum* as starter culture for nutritional improvement of *Ogi* *World Journal of Microbiology and Biotechnology*, 16, 451-455.
- ADEYEMI, I. A. 1983. Dry-milling of sorghum for *Ogi* manufacture. *Journal of Cereal Science*, 1, 221-227.
- ADEYEMI, I. A. & BECKLEY, O. 1986. Effect of period of maize fermentation and souring on chemical properties and amylograph pasting viscosity of *Ogi*. *Journal of Cereal Science*, 4, 353-360.
- AFIFI, Z. E. M., NASSER, S. S., SHALABY, S. & ATLAM, S. A. E. 1998. Contamination of weaning foods: Organisms, channels, and sequelae. *Journal of Tropical Pediatrics*, 44, 335-337.
- AIDOO, K. E., NOUT, R. M. J. & SARKAR, P. K. 2006. Occurrence and function of yeasts in Asian indigenous fermented foods. *FEMS Yeast Research*, 6, 30-39.
- AKINGBALA, J. O., ADEYEMI, I. A., SANGODOYIN, S. O. & OKE, O. L. 1994. Evaluation of amaranth grains for *Ogi* manufacture. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, 46, 19-26.
- AKINGBALA, J. O., AKINWANDE, B. A. & UZO-PETERS, P. I. 2003. Effects of color and flavor changes on acceptability of *Ogi* supplemented with okra

- seed meals. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, 58, 1-9.
- AKINGBALA, J. O., ROONEY, L. W. & FAUBION, J. M. 1981. A laboratory procedure for the preparation of *Ogi*, a Nigerian fermented food. *Journal of Food Science*, 46, 1523-1526.
- AKINRELE, L. A. 1970. Fermentation studies on maize during the preparation of a traditional African cake food. *Journal of the Science Food and Agriculture* 21, 619-625.
- ALAMRI, M. S., MOHAMED, A. A. & HUSSAIN, S. 2013. Effects of alkaline-soluble okra gum on rheological and thermal properties of systems with wheat or corn starch. *Food Hydrocolloids*, 30, 541-551.
- ALELJUNG, P., SHEN, W., ROZALSKA, B., HELLMAN, U., LJUNGH, Å. & WADSTRÖM, T. 1994. Purification of collagen-binding proteins of *Lactobacillus reuteri* NCIB 11951. *Current Microbiology*, 28, 231-236.
- ALI, A. A. & MUSTAFA, M. M. 2009. Isolation, characterization and identification of lactic acid bacteria from fermented sorghum dough used in Sudanese *Kisra* preparation. *Pakistan Journal of Nutrition* 8, 1814-1818.
- AMINIGO, E. R. & AKINGBALA, J. O. 2004. Nutritive composition and sensory properties of *Ogi* fortified with okra seed meal. *Journal of Applied Science & Environmental Management*, 8, 23-28.
- AMPE, F., BEN OMAR, N. & GUYOT, J. P. 1999. Culture-independent quantification of physiologically-active microbial groups in fermented foods using rRNA-targeted oligonucleotide probes: application to *Pozol*, a Mexican lactic acid fermented maize dough. *Journal of Applied Microbiology*, 87, 131-140.

- ANDERSSON, A. A. M., ANDERSSON, R., PIIRONEN, V., LAMPI, A.-M., NYSTRÖM, L., BOROS, D., FRAŚ, A., GEBRUERS, K., COURTIN, C. M., DELCOUR, J. A., RAKSZEKI, M., BEDO, Z., WARD, J. L., SHEWRY, P. R. & ÅMAN, P. 2013. Contents of dietary fibre components and their relation to associated bioactive components in whole grain wheat samples from the HEALTHGRAIN diversity screen. *Food Chemistry*, 136, 1243-1248.
- ANGELOV, A., GOTCHEVA, V., KUNCHEVA, R. & HRISTOZOVA, T. 2006. Development of a new oat-based probiotic drink. *International Journal of Food Microbiology*, 112, 75-80.
- ANNAN, N. T., POLL, L., SEFA-DEDEH, S., PLAHAR, W. A. & JAKOBSEN, M. 2003. Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. *Journal of Applied Microbiology* 94, 462-474.
- ANTAI, S. & NZERIBE, E. 1992. Suitability of using sieved or unsieved maize mash for production of "OGI" — A fermented cereal food. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, 42, 25-30.
- AOAC 1995. *Official Methods of Analysis*, Arlington, USA, Association of Analytical Chemists.
- AOAC 2005. *Official methods of analysis of Association of Official Analytical Chemists International*, Gaithersburg, Maryland, USA, AOAC International.
- APOSTOLOU, E., KIRJAVAINEN, P. V., SAXELIN, M., RAUTELIN, H., VALTONEN, V., SALMINEN, S. J. & OUWEHAND, A. C. 2001. Good

- adhesion properties of probiotics: a potential risk for bacteremia? *FEMS Immunology and Medical Microbiology*, 31, 35-39.
- AREMU, C. Y. 1993. Nutrient composition of corn OGI prepared by a slightly modified traditional technique. *Food Chemistry*, 46, 231-233.
- AREMU, M. O., OSINFADE, B. G., BASU, S. K. & ABLAKU, B. E. 2011. Development and nutritional quality evaluation of Kersting's Groundnut-Ogi for African weaning diet. *American Journal of Food Technology*, 6, 1021-1033.
- ASHENAFI, M. 1991. Growth of *Listeria monocytogenes* in fermenting *Tempeh* made of various beans and its inhibition by *Lactobacillus plantarum*. *Food Microbiology*, 8, 303-310.
- AXELSSON, L. 2004. Lactic acid bacteria: Classification and physiology. In: SALMINEN, S., VON WRIGHT, A. & OUWEHAND, A. (eds.) *Lactic acid bacteria: Microbiological and functional aspects*. 3rd revised ed. New York: Marcel Dekker, Inc.
- BACCIGALUPI, L., DI DONATO, A., PARLATO, M., LUONGO, D., CARBONE, V., ROSSI, M., RICCA, E. & DE FELICE, M. 2005. Small surface-associated factors mediate adhesion of a food-isolated strain of *Lactobacillus fermentum* to Caco-2 cells. *Research in Microbiology*, 156, 830-836.
- BACH KNUDSEN, K. E. 2001. The nutritional significance of "dietary fibre" analysis. *Animal Feed Science and Technology*, 90, 3-20.
- BAKARE, S., SMITH, S. I., OLUKOYA, D. K. & AKPAN, E. 1998. Comparison of the survival of diarrhoeagenic agents in two local weaning foods (*Ogi* and *Koko*). *Journal of Tropical Paediatrics*, 44 332-334.

- BANIGO, E. O. I. & MULLER, H. G. 1972. Manufacture of *Ogi* (a Nigerian fermented cereal porridge): Comparative evaluation of corn, sorghum and millet. *Canadian Institute of Food Science and Technology Journal*, 5, 217-21.
- BAO, Y., ZHANG, Y., ZHANG, Y., LIU, Y., WANG, S., DONG, X., WANG, Y. & ZHANG, H. 2010. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. *Food Control*, 21, 695-701.
- BARANYI, J. & ROBERTS, T. A. 1994. A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*, 23, 277-294.
- BARIKMO, I., OUATTARA, F. & OSHAUG, A. 2007. Differences in micronutrients content found in cereals from various parts of Mali. *Journal of Food Composition and Analysis*, 20, 681-687.
- BARNETT, J. A. & PANKHURST, R. J. (eds.) 1974. *A new key to the yeasts: A key for identifying yeasts based on physiological tests only*, New York: North-Holland Pub. Co
- BELLON-FONTAINE, M. N., RAULT, J. & VAN OSS, C. J. 1996. Microbial adhesion to solvents: a novel method to determine the electron-donor/electron-acceptor or Lewis acid-base properties of microbial cells. *Colloids and Surfaces B: Biointerfaces*, 7, 47-53.
- BERDANIER, C. D. & ZEMPLINI, J. 2009. *Advanced nutrition: macronutrients, micronutrients, and metabolism*, London, CRC Press
- BJÖRCK, I., ÖSTMAN, E., KRISTENSEN, M., MATEO ANSON, N., PRICE, R. K., HAENEN, G. R. M. M., HAVENAAR, R., BACH KNUDSEN, K. E., FRID, A., MYKKÄNEN, H., WELCH, R. W. & RICCARDI, G. 2012.

Cereal grains for nutrition and health benefits: Overview of results from in vitro, animal and human studies in the HEALTHGRAIN project. *Trends in Food Science and Technology*, 25, 87-100.

BLANDINO, A., AL-ASEERI, M. E., PANDIELLA, S. S., CANTERO, D. & WEBB, C. 2003. Cereal-based fermented foods and beverages. *Food Research International*, 36, 527-543.

BRENNAN, C. S. & CLEARY, L. J. 2005. The potential use of cereal (1→3,1→4)-β-d-glucans as functional food ingredients. *Journal of Cereal Science*, 42, 1-13.

BROWN, L., ROSNER, B., WILLETT, W. W. & SACKS, F. M. 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *The American Journal of Clinical Nutrition*, 69, 30-42.

BRUNT, K. & SANDERS, P. 2013. Improvement of the AOAC 2009.01 total dietary fibre method for bread and other high starch containing matrices. *Food Chemistry*, 140, 574-580.

BUCHANAN, R. E. & GIBBONS, N. E. (eds.) 1974. *Bergey's manual of determinative bacteriology*, Baltimore, USA: The Williams and Wilkins Company.

BVOCHORA, J. M., REED, J. D., READ, J. S. & ZVAUYA, R. 1999. Effect of fermentation processes on proanthocyanidins in sorghum during preparation of *Mahewu*, a non-alcoholic beverage. *Process Biochemistry*, 35, 21-25.

BYARUHANGA, Y. B., BESTER, B. H. & T.G, W. 1999. Growth and survival of *Bacillus cereus* in *Mageu*, a sour maize beverage. *World Journal of Microbiology and Biotechnology* 15, 239-333.

- CALDERON, M., LOISEAU, G. & GUYOT, J. P. 2003. Fermentation by *Lactobacillus fermentum* Ogi E1 of different combinations of carbohydrates occurring naturally in cereals: consequences on growth energetics and [alpha]-amylase production. *International Journal of Food Microbiology*, 80, 161-169.
- CAMPBELL-PLATT, C. 1987. *Fermented foods of the World: A Dictionary and Guide*, London, Butterworths.
- CAPLICE, E. & FITZGERALD, G. F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, 50, 131-149.
- CARRILLO, E., PRADO-GASCÓ, V., FISZMAN, S. & VARELA, P. 2013. Why buying functional foods? Understanding spending behaviour through structural equation modelling. *Food Research International*, 50, 361-368.
- CHAN, S. S. L., FERGUSON, E. L., BAILEY, K., FAHMIDA, U., HARPER, T. B. & GIBSON, R. S. 2007. The concentrations of iron, calcium, zinc and phytate in cereals and legumes habitually consumed by infants living in East Lombok, Indonesia. *Journal of Food Composition and Analysis*, 20, 609-617.
- CHANDALIA, M., GARG, A., LUTJOHANN, D., VON BERGMANN, K., GRUNDY, S. M. & BRINKLEY, L. J. 2000. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *New England Journal of Medicine*, 342, 1392-1398.
- CHARALAMPOPOULOS, D., PANDIELLA, S. S. & WEBB, C. 2002a. Growth studies of potentially probiotic lactic acid bacteria in cereal-based substrates. *Journal of Applied Microbiology*, 92, 851-859.

- CHARALAMPOPOULOS, D., WANG, R., PANDIELLA, S. S. & WEBB, C. 2002b. Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology*, 79, 131-141.
- CHAVAN, J. K. & KADAMS, S. S. 1989. Nutrient improvement of cereals by fermentation. *Critical Reviews Food Science and Nutrition*, 28, 349-400.
- CHEN, Y., ZHAO, Y. H., KALASLAVADI, T. B., HAMATI, E., NEHRKE, K., LE, A. D., ANN, D. K. & WU, R. 2004. Genome-wide search and identification of a novel gel-forming mucin MUC19/Muc19 in glandular tissues. *American Journal of Respiratory Cell and Molecular Biology*, 30, 155-165.
- CHERIGUENE, A., CHOUGRANI, F., BEKADA, A. M. A., EL SODA, M. & BENSOLTANE, A. 2007. Enumeration and identification of lactic microflora in Algerian goats' milk. *African Journal of Biotechnology*, 6, 1854-1861.
- CHINNICI, F., SPINABELLI, U., RIPONI, C. & AMATI, A. 2005. Optimization of the determination of organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. *Journal of Food Composition and Analysis*, 18, 121-130.
- COLLADO, M. C., MERILUOTO, J. & SALMINEN, S. 2007a. Measurement of aggregation properties between probiotics and pathogens: *In vitro* evaluation of different methods. *Journal of Microbiological Methods*, 71, 71-74.
- COLLADO, M. C., SURONO, I., MERILUOTO, J. & SALMINEN, S. 2007b. Indigenous *Dadih* lactic acid bacteria: Cell-surface properties and interactions with pathogens. *Journal of Food Science*, 72, M89-M93.

- CZERUCKA, D. & RAMPAL, P. 2002. Experimental effects of *Saccharomyces boulardii* on diarrheal pathogens. *Microbes and Infection*, 4, 733-739.
- DALIÉ, D. K. D., DESCHAMPS, A. M. & RICHARD-FORGET, F. 2010. Lactic acid bacteria - Potential for control of mould growth and mycotoxins: A review. *Food Control*, 21, 370-380.
- DE VRIES, M. C., VAUGHAN, E. E., KLEEREBEZEM, M. & DE VOS, W. M. 2006. *Lactobacillus plantarum*-survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal*, 16, 1018-1028.
- DEAN, M., SHEPHERD, R., ARVOLA, A., VASSALLO, M., WINKELMANN, M., CLAUPEIN, E., LÄHTEENMÄKI, L., RAATS, M. M. & SABA, A. 2007. Consumer perceptions of healthy cereal products and production methods. *Journal of Cereal Science*, 46, 188-196.
- DEL RE, B., SGORBATI, B., MIGLIOLI, M. & PALENZONA, D. 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Letters in Applied Microbiology* 31, 438-442.
- DELGADO, S., FLÓREZ, A. & MAYO, B. 2005. Antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* species from the human gastrointestinal tract. *Current Microbiology*, 50, 202-207.
- DEPARTMENT OF HEALTH 2012. Manual of Nutrition. 12th ed. London: TSO (The Stationary Office).
- DEVRIES, J. W. 2003. On defining dietary fibre. *Proceedings of the Nutrition Society*, 62, 37-43.
- DHINGRA, D., MICHAEL, M., RAJPUT, H. & PATIL, R. T. 2012. Dietary fibre in foods: a review. *Journal of Food Science and Technology*, 49, 255-266.

- DIANAWATI, D., MISHRA, V. & SHAH, N. P. 2013. Stability of microencapsulated *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. *cremoris* during storage at room temperature at low aw. *Food Research International*, 50, 259-265.
- DIKE, K. S. & SANNI, A. I. 2010. Influence of starter culture of lactic acid bacteria on the shelf life of *agidi*, an indigenous fermented cereal product. *African Journal of Biotechnology*, 9, 7922-7927.
- DING, W. K. & SHAH, N. P. 2007. Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. *Journal of Food Science*, 72, M446-M450.
- ĐORĐEVIĆ, T. M., ŠILER-MARINKOVIĆ, S. S. & DIMITRIJEVIĆ-BRANKOVIĆ, S. I. 2010. Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry*, 119, 957-963.
- DUTCOSKY, S. D., GROSSMANN, M. V. E., SILVA, R. S. S. F. & WELSCH, A. K. 2006. Combined sensory optimization of a prebiotic cereal product using multicomponent mixture experiments. *Food Chemistry*, 98, 630-638.
- DZIEDZIC, K., GÓRECKA, D., KUCHARSKA, M. & PRZYBYLSKA, B. 2012. Influence of technological process during buckwheat groats production on dietary fibre content and sorption of bile acids. *Food Research International*, 47, 279-283.
- EDEMA, M. O. & SANNI, A. I. 2006. Micro-population of fermenting maize meal for sour maize bread production in Nigeria. *Nigerian Journal of Microbiology*, 20, 937-946.
- EDEMA, M. O. & SANNI, A. I. 2008. Functional properties of selected starter cultures for sour maize bread. *Food Microbiology*, 25, 616-625.

- EGOUNLETY, M., AWORH, O. C., AKINGBALA, J. O., HOUBEN, J. H. & NAGO, M. C. 2002. Nutritional and sensory evaluation of tempe-fortified maize-based weaning foods. *International Journal of Food Science and Nutrition*, 53, 15-27.
- EHRMANN, M. A. & VOGEL, R. F. 2005. Molecular taxonomy and genetics of sourdough lactic acid bacteria. *Trends in Food Science & Technology* 16, 31-42.
- ELLEUCH, M., BEDIGIAN, D., ROISEUX, O., BESBES, S., BLECKER, C. & ATTIA, H. 2011. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, 124, 411-421.
- ELYAS, S. H. A., EL TINAY, A. H., YOUSIF, N. E. & ELSHEIKH, E. A. E. 2002. Effect of natural fermentation on nutritive value and *in vitro* protein digestibility of pearl millet. *Food Chemistry*, 78, 75-79.
- ERCOLINI, D. 2004. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *Journal of Microbiological Methods*, 56, 297-314.
- ERICSON, J. E., CRINELLA, F. M., CLARKE-STEWART, K. A., ALLHUSEN, V. D., CHAN, T. & ROBERTSON, R. T. 2007. Prenatal manganese levels linked to childhood behavioral disinhibition. *Neurotoxicology and Teratology*, 29, 181-187.
- ESREY, S. A. & FEACHEM, R. G. 1989. Interventions for the control of diarrhoeal diseases among young children: promotion of food hygiene. Geneva, Switzerland: World Health Organization [WHO]1989. 22 p. (WHO/CDD/89.30).
- FANG, F., LI, Y., BUMANN, M., RAFTIS, E. J., CASEY, P. G., COONEY, J. C., WALSH, M. A. & O'TOOLE, P. W. 2009. Allelic variation of bile salt

- hydrolase genes in *Lactobacillus salivarius* does not determine bile resistance levels. *Journal of Bacteriology*, 191, 5743-5757.
- FAO 2001. Human vitamin and mineral requirements: Report of a joint FAO/WHO expert consultation. Bangkok, Thailand: FAO, Rome.
- FAO/WHO 2002. Guidelines for the evaluation of probiotics in food. A Joint FAO/WHO Working Group Report. London
- FARDET, A., ROCK, E. & RÉMÉSY, C. 2008. Is the in vitro antioxidant potential of whole-grain cereals and cereal products well reflected *in vivo*? *Journal of Cereal Science*, 48, 258-276.
- FERNÁNDEZ, M. F., BORIS, S. & BARBÉS, C. 2005. Safety evaluation of *Lactobacillus delbrueckii* subsp. *lactis* UO 004, a probiotic bacterium. *Research in Microbiology*, 156, 154-160.
- FIELDS, M. L., HAMAD, A. M. & SMITH, K. D. 1981. Natural lactic acid fermentation of corn meal. *Journal of Food Science*, 46, 900-905.
- FOOKS, L. J. & GIBSON, G. R. 2002. *In vitro* investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiology Ecology*, 39, 67-75.
- FRAIZER, W. C. & WESTHOFF, D. C. 1995. *Food Microbiology*, New Delhi, England, Tata McGraw-Hill Publishing Company Limited.
- FRAZIER, R. A. 2009. Food Chemistry. In: CAMPBELL-PLATT, G. (ed.) *Food Science and Technology*. Oxford, UK: Wiley-Blackwell.
- FUENTES-ZARAGOZA, E., RIQUELME-NAVARRETE, M. J., SÁNCHEZ-ZAPATA, E. & PÉREZ-ÁLVAREZ, J. A. 2010. Resistant starch as functional ingredient: A review. *Food Research International*, 43, 931-942.

- GAMEL, T. H. & ABDEL-AAL, E.-S. M. 2012. Phenolic acids and antioxidant properties of barley wholegrain and pearling fractions. *Agricultural and Food Science*, 21, 118-131.
- GIANNOCCARO, E., WANG, Y.-J. & CHEN, P. 2008. Comparison of two HPLC systems and an enzymatic method for quantification of soybean sugars. *Food Chemistry*, 106, 324-330.
- GILLILAND, S. E., STALEY, T. E. & BUSH, L. J. 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct *Journal of Dairy Science*, 67, 3045-3051.
- GILLILAND, S. E. & WALKER, D. K. 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. *Journal of Dairy Science*, 73, 905-911.
- GIRAFFA, G., CHANISHVILI, N. & WIDYASTUTI, Y. 2010. Importance of lactobacilli in food and feed biotechnology. *Research in Microbiology*, 161, 480-487.
- GIRARD, V. & MOUREZ, M. 2006. Adhesion mediated by autotransporters of Gram-negative bacteria: Structural and functional features. *Research in Microbiology*, 157, 407-416.
- GOBBETTI, M. & CORSETTI, A. 1997. *Lactobacillus sanfransisco* a key sourdough lactic acid bacterium: A review. *Food Microbiology*, 14, 175-187.
- GOLDHABER, S. B. 2003. Trace element risk assessment: essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology*, 38, 232-242.
- GRAJEK, W., OLEJNIK, A. & SIP, A. 2005. Probiotics, prebiotics and antioxidants as functional foods. *Acta Biochimica Polonica.* , 52, 665-671.

- GRANDE, M. J., LUCAS, R., ABRIOUEL, H., VALDIVIA, E., OMAR, N. B., MAQUEDA, M., MARTÍNEZ-BUENO, M., MARTÍNEZ-CAÑAMERO, M. & GÁLVEZ, A. 2006. Inhibition of toxicogenic *Bacillus cereus* in rice-based foods by enterocin AS-48. *International Journal of Food Microbiology*, 106, 185-194.
- GREFFEUILLE, V., POLYCARPE KAYODÉ, A. P., ICARD-VERNIÈRE, C., GNIMADI, M., ROCHETTE, I. & MOUQUET-RIVIER, C. 2011. Changes in iron, zinc and chelating agents during traditional African processing of maize: Effect of iron contamination on bioaccessibility. *Food Chemistry*, 126, 1800-1807.
- GUILLERMOND, A. (ed.) 1920. *The Yeasts*, New York: John Wiley & Sons.
- GUO, W. & BETA, T. 2013. Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. *Food Research International*, 51, 518-525.
- HAARD, N. F., ODUNFA, S. A., LEE, C.-H., QUINTERO-RAMÍREZ, R., LORENCE-QUIÑONES, A. & WACHER-RODARTE, C. 1999. Cereals: Rationale for fermentation. *Fermented cereals: A global perspective*. Rome: FAO.
- HALM, M., HORNBAEK, T., ARNEBORG, N., SEFA-DEDEH, S. & JESPERSEN, L. 2004. Lactic acid tolerance determined by measurement of intracellular pH of single cells of *Candida krusei* and *Saccharomyces cerevisiae* isolated from fermented maize dough. *International Journal of Food Microbiology*, 94, 97-103.
- HALM, M., LILLIE, A., SØRENSEN, A. K. & JAKOBSEN, M. 1993. Microbiological and aromatic characteristics of fermented maize doughs

- for *Kenkey* production in Ghana. *International Journal of Food Microbiology*, 19, 135-143.
- HALM, M. & OLSEN, A. 1996. The inhibitory potential of dominating yeasts and moulds in maize fermentation. *In*: HALM, M. & JAKOBSEN, M. (eds.) *Traditional food processing in Africa 3rd biennial seminar on African fermented food*. KVL, Copenhagen, Demark.
- HALM, M., OSEI-YAW, A., HAYFORD, A. E. & AMOA-AWUA, W. K. A. 1996. Experiences with the use of a starter culture in the fermentation of maize for '*Kenkey*' production in Ghana. *World Journal of Microbiology and Biotechnology*, 12, 531-536.
- HARRIGAN, W. F. (ed.) 1998. *Laboratory Methods in Food Microbiology*, London: Academic Press Ltd.
- HELLAND, M. H., WICKLUND, T. & NARVHUS, J. A. 2004. Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. *International Dairy Journal*, 14, 957-965.
- HELLSTRÖM, A. M., VÁZQUES-JUÁREZ, R., SVANBERG, U. & ANDLID, T. A. 2003. Biodiversity and phytase capacity of yeasts isolated from Tanzanian *Togwa*. *International Journal of Food Microbiology*, 136, 352-358.
- HEVIA, A., MARTÍNEZ, N., LADERO, V., ÁLVAREZ, M. A., MARGOLLES, A. & SÁNCHEZ, B. 2013. An extracellular serine/threonine-rich protein from *Lactobacillus plantarum* NCIMB 8826 is a novel aggregation-promoting factor with affinity to mucin. *Applied and Environmental Microbiology*, 79, 6059-6066.

- HOLLMANN, J., THEMEIER, H., NEESE, U. & LINDHAUER, M. G. 2013. Dietary fibre fractions in cereal foods measured by a new integrated AOAC method. *Food Chemistry*, 140, 586-589.
- HOLZAPFEL, W. 1997. Use of starter cultures in fermentation on a household scale. *Food Control*, 8, 241-258.
- HOLZAPFEL, W. H. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*, 75, 197-212.
- HOLZAPFEL, W. H. & SCHILLINGER, U. 2002. Introduction to pre- and probiotics. *Food Research International*, 35, 109-116.
- HOUNHOUGAN, D. J., NOUT, M. J. R., NAGO, M. C., HOUBEN, J. H. & ROMBOUUTS, F. M. 1999. Use of starter cultures of *Lactobacilli* and yeast in the fermentation of *Mawe*, an African Maize product. *Tropical Science*, 39, 220-226.
- HUANG, Z., YE, R., CHEN, J. & XU, F. 2013. An improved method for rapid quantitative analysis of the insoluble dietary fiber in common cereals and some sorts of beans. *Journal of Cereal Science*, 57, 270-274.
- HUGGETT, A. C. & SCHLITER, B. 1996. Research needs for establishing the safety of functional foods. *Nutrition Reviews*, 54, S143-S148.
- INYANG, C. U. & IDOKO, C. A. 2006. Assessment of the quality of ogi made from malted millet. *African Journal of Biotechnology* 5 2334-2337.
- ITKONEN, S. T., EKHOLM, P. J., KEMI, V. E. & LAMBERG-ALLARDT, C. J. E. 2012. Analysis of in vitro digestible phosphorus content in selected processed rye, wheat and barley products. *Journal of Food Composition and Analysis*, 25, 185-189.

- JANKOVIC, I., VENTURA, M., MEYLAN, V., ROUVET, M., ELLI, M. & ZINK, R. 2003. Contribution of Aggregation-Promoting Factor to Maintenance of Cell Shape in *Lactobacillus gasser* 4B2. *Journal of Bacteriology*, 185, 3288-3296.
- JESPERSEN, L. 2003. Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages. *FEMS Yeast Research*, 3, 191-200.
- JESPERSEN, L., HALM, M., KPODO, K. & JAKOBSEN, M. 1994. Significance of yeasts and moulds occurring in maize dough fermentation for 'Kenkey' production. *International Journal of Food Microbiology*, 24, 239-248.
- JOHANSSON, M. L., SANNI, A., LÖNNER, C. & MOLIN, G. 1995. Phenotypically based taxonomy using API 50CH of *Lactobacilli* from Nigerian *Ogi*, and the occurrence of starch fermenting strains. *International Journal of Food Microbiology*, 25, 159-168.
- JONES, D. S., ADAIR, C. G., MAWHINNEY, W. M. & GORMAN, S. P. 1996. Standardisation and comparison of methods employed for microbial cell surface hydrophobicity and charge determination. *International Journal of Pharmaceutics*, 131, 83-89.
- JONSSON, H., STRÖM, E. & ROOS, S. 2001. Addition of mucin to the growth medium triggers mucus-binding activity in different strains of *Lactobacillus reuteri* in vitro. *FEMS Microbiology Letters*, 204, 19-22.
- JUGE, N. 2012. Microbial adhesins to gastrointestinal mucus. *Trends in Microbiology*, 20, 30-39.
- KALUI, C. M., MATHARA, J. M. & KUTIMA, P. M. 2010. Probiotic potential of spontaneously fermented cereal based foods - A review. *African Journal of Biotechnology*, 9, 2490-2498.

- KALUI, C. M., MATHARA, J. M., KUTIMA, P. M., KIIYUKIA, C. & WONGO, L. E. 2009. Functional characteristics of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* from *Ikii*, a Kenyan traditional fermented maize porridge. *African Journal of Biotechnology*, 8, 4363-4373.
- KANKAANPÄÄ, P., SÜTAS, Y., SALMINEN, S. & ISOLAURI, E. 2003. Homogenates derived from probiotic bacteria provide down-regulatory signals for peripheral blood mononuclear cells. *Food Chemistry*, 83, 269-277.
- KAVANAGH, K. (ed.) 2005. *Fungi: Biology and Application*, Chichester, England: John Wiley and Sons Limited.
- KEBEDE, A. 2007. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of *Borde*, an Ethiopian cereal beverage. *African Journal of Biotechnology*, 6, 1469-1478.
- KEDIA, G., VÁZQUEZ, J. A. & PANDIELLA, S. S. 2008. Enzymatic digestion and *in vitro* fermentation of oat fractions by human *Lactobacillus* strains. *Enzyme and Microbial Technology*, 43, 355-361.
- KINGAMKONO, R., SJÖGREN, E., SVANBERG, U. & KAIJSER, B. 1994. pH and acidity in lactic-fermenting cereal gruels: Effects on viability of enteropathogenic microorganisms *World Journal of Microbiology and Biotechnology* 10, 664-669.
- KINGAMKONO, R., SJÖGREN, E., SVANBERG, U. & KAIJSER, B. 1995. Inhibition of different strains of enteropathogens in a lactic-fermenting cereal gruel. *World Journal of Microbiology*, 11, 299-303.
- KINGAMKONO, R. R., SJÖGREN, E. & SVANBERG, U. 1998. Inhibition of enterotoxin production by, and growth of enteropathogens in a lactic

- acid-fermenting cereal gruel *World Journal of Microbiology and Biotechnology*, 14, 661-667.
- KIRJAVAINEN, P. V., OUWEHAND, A. C., ISOLAURI, E. & SALMINEN, S. J. 1998. The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiology Letters*, 167, 185-189.
- KIVANÇ, M., YILMAZ, M. & ÇAKIR, E. 2011. Isolation and identification of lactic acid bacteria from *Boza*, and their microbial activity against several reporter strains. *Turkey Journal of Biology*, 35, 313-324.
- KLEIN, G., PACK, A., BONAPARTE, C. & REUTER, G. 1998. Taxonomy and physiology of probiotic lactic acid bacteria. *International Journal of Food Microbiology* 41 103-125.
- KONDOH, M., HAYASHI, A., OKAMORI, M., MOTONAGA, C., ENOMOTO, T., CHENG, L. & SHIMADA, T. 2009. Effects of *Enterococcus* sp. isolated from deep seawater on inhibition of allergic responses in mice. *British Journal of Nutrition*, 102, 3-7.
- KOS, B., ŠUŠKOVIĆ, J., VUKOVIĆ, S., ŠIMPRAGA, M., FRECE, J. & MATOŠIĆ, S. 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Bacteriology*, 94, 981-987.
- KPODO, K., SØRENSEN, A. K. & JAKOBSEN, M. 1996. The occurrence of mycotoxins in fermented maize products. *Food Chemistry*, 56, 147-153.
- KRISTENSEN, M., JENSEN, M. G., RIBOLDI, G., PETRONIO, M., BÜGEL, S., TOUBRO, S., TETENS, I. & ASTRUP, A. 2010. Wholegrain vs. refined wheat bread and pasta. Effect on postprandial glycemia, appetite, and subsequent ad libitum energy intake in young healthy adults. *Appetite*, 54, 163-169.

- KRUTULYTE, R., GRUNERT, K. G., SCHOLDERER, J., LÄHTEENMÄKI, L., HAGEMANN, K. S., ELGAARD, P., NIELSEN, B. & GRAVERHOLT, J. P. 2011. Perceived fit of different combinations of carriers and functional ingredients and its effect on purchase intention. *Food Quality and Preference*, 22, 11-16.
- KUAKPETOON, D. & WANG, Y.-J. 2007. Internal structure and physicochemical properties of corn starches as revealed by chemical surface gelatinization. *Carbohydrate Research*, 342, 2253-2263.
- KUNENE, N. F., HASTINGS, J. W. & VON HOLY, A. 1999. Bacterial populations associated with a sorghum-based fermented weaning cereal. *International Journal of Food Microbiology*, 49, 75-83.
- KUTOŠ, T., GOLOB, T., KAČ, M. & PLESTENJAK, A. 2003. Dietary fibre content of dry and processed beans. *Food Chemistry*, 80, 231-235.
- KUZNESOF, S., BROWNLEE, I. A., MOORE, C., RICHARDSON, D. P., JEBB, S. A. & SEAL, C. J. 2012. WHOLEheart study participant acceptance of wholegrain foods. *Appetite*, 59, 187-193.
- LANDRY, J. & MOUREAUX, T. 1980. Distribution and amino acid composition of protein groups located in different histological parts of maize grain. *Journal of Agricultural Food Chemistry* 28, 1186-1191.
- LAWAL, A. K., OYEDOYIN, O. B. & OLATUNJI, O. O. 2009. Fate of pathogenic bacteria during fermentation of cereal porridge ("Ogi")-a weaning food formula. *Nigerian Food Journal*, 27, 19-26.
- LEE, C.-H. 2009. Food Biotechnology. In: CAMPBELL-PLATT, G. (ed.) *Food Science and Technology*. Oxford, UK: Blackwell Publishing Ltd.
- LEI, V., FRIIS, H. & MICHAELSEN, K. F. 2006. Spontaneously fermented millet product as a natural probiotic treatment for diarrhoea in young children:

- An intervention study in Northern Ghana. *International Journal of Food Microbiology*, 110, 246-253.
- LEI, V. & JAKOBSEN, M. 2004. Microbiological characterization and probiotic potential of *Koko* and *Koko* sour water, African spontaneously fermented millet porridge and drink. *Journal of Applied Microbiology*, 96, 384-397.
- LENAERTS, J. 2004. Cereal fermentation: An assest to developing African countries. *Microbiologist*, 5, 45.
- LEROI, F. & PIDOUX, M. 1993. Characterization of interactions between *Lactobacillus hilgardii* and *Saccharomyces florentinus* isolated from sugary *Kefir* grains. *Journal of Applied Bacteriology*, 74, 54-60.
- LIN, W.-H., HWANG, C.-F., CHEN, L.-W. & TSEN, H.-Y. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food Microbiology*, 23, 74-81.
- LIN, W.-H., WU, C.-R., FANG, T. J., LEE, M.-S., LIN, K.-L., CHEN, H.-C., HUANG, S.-Y. & HSEU, Y.-C. 2011. Adherent properties and macrophage activation ability of 3 strains of lactic acid bacteria. *Journal of Food Science*, 76, M1-M7.
- LIN, W. H., YU, B., LIN, C. K., HWANG, W. Z. & TSEN, H. Y. 2007. Immune effect of heat-killed multistrain of *Lactobacillus acidophilus* against *Salmonella typhimurium* invasion to mice. *Journal of Applied Microbiology*, 102, 22-31.
- LIU, Y., YANG, S.-F., LI, Y., XU, H., QIN, L. & TAY, J.-H. 2004. The influence of cell and substratum surface hydrophobicities on microbial attachment. *Journal of Biotechnology*, 110, 251-256.

- LÓPEZ, S., PRIETO, M., DIJKSTRA, J., DHANOA, M. S. & FRANCE, J. 2004. Statistical evaluation of mathematical models for microbial growth. *International Journal of Food Microbiology*, 96, 289-300.
- LOPEZ, Y., GORDON, D. T. & FIELDS, M. L. 1983. Release of phosphorus from phytate by natural lactic acid fermentation. *Journal of Food Science*, 48, 953-954.
- LORRI, W. & SVANBERG, U. 1994. Lower prevalence of diarrhoea in young children fed lactic acid-fermented cereal gruels. *Food and Nutrition Bulletin* 15, 57-63.
- LU, Z.-H., LI, L.-T., MIN, W.-H., WANG, F. & TATSUMI, E. 2005. The effects of natural fermentation on the physical properties of rice flour and the rheological characteristics of rice noodles. *International Journal of Food Science & Technology*, 40, 985-992.
- MAATHUIS, A., HOFFMAN, A., EVANS, A., SANDERS, L. & VENEMA, K. 2009. The effect of the undigested fraction of maize products on the activity and composition of the microbiota determined in a dynamic in vitro model of the human proximal large intestine. *Journal of the American College of Nutrition*, 28, 657-666.
- MADOROBA, E., STEENKAMP, E. T., THERON, J., HUYS, G., SCHEIRLINCK, I. & CLOETE, T. E. 2009. Polyphasic taxonomic characterization of lactic acid bacteria isolated from spontaneous sorghum fermentations used to produce *Ting*, a traditional South African food. *African Journal of Biotechnology*, 8, 458-463.
- MAJHENIÇ, A. Ç., LORBERG, P. M. & ROGELJ, I. 2007. Characterisation of the *Lactobacillus* community in traditional *Karst* ewe's cheese. *International Journal of Dairy Technology*, 60, 182-190.

- MARAGKOUDAKIS, P. A., MIARIS, C., ROJEZ, P., MANALIS, N., MAGKANARI, F., KALANTZOPOULOS, G. & TSAKALIDOU, E. 2006a. Production of traditional Greek yoghurt using *Lactobacillus* strains with probiotic potential as starter adjuncts. *International Dairy Journal*, 16, 52-60.
- MARAGKOUDAKIS, P. A., ZOUMPOPOULOU, G., MIARIS, C., KALANTZOPOULOS, G., POT, B. & TSAKALIDOU, E. 2006b. Probiotic potential of *Lactobacillus* strains isolated from dairy products. *International Dairy Journal*, 16, 189-199.
- MARKLINDER, I. & LONNER, C. 1994. Fermented oatmeal soup - Influence of additives on the properties of a nutrient solution for enteral feeding. *Food Microbiology*, 11 505-513.
- MARSHALL, M. N., COCOLIN, L., MILLS, D. A. & VANDERGHEYNST, J. S. 2003. Evaluation of PCR primers for denaturing gradient gel electrophoresis analysis of fungal communities in compost. *Journal of Applied Microbiology*, 95, 934-948.
- MARTIN-LAURENT, F., PHILIPPOT, L., HALLET, S., CHAUSSOD, R., GERMON, J. C., SOULAS, G. & CATROUX, G. 2001. DNA extraction from soils: Old bias for new microbial diversity analysis methods. 67, 4397.
- MASUDA, K. & KAWATA, T. 1985. Reassembly of a regularly arranged protein in the cell wall of *Lactobacillus buchneri* and its reattachment to cell walls: chemical modification studies. *Microbiology and Immunology*, 29, 927-938.
- MATHARA, J. M., SCHILLINGER, U., GUIGAS, C., FRANZ, C., KUTIMA, P. M., MBUGUA, S. K., SHIN, H. K. & HOLZAPFEL, W. H. 2008. Functional

characteristics of *Lactobacillus* spp. from traditional *Maasai* fermented milk products in Kenya. *International Journal of Food Microbiology*, 126, 57-64.

MATTILA-SANDHOLM, T., MÄTTÖ, J. & SAARELA, M. 1999. Lactic acid bacteria with health claims-interactions and interference with gastrointestinal flora. *International Dairy Journal*, 9, 25-35.

MBATA, T. I., IKENEBOMEH, M. J. & EZEIBE, S. 2009. Evaluation of mineral content and functional properties of fermented maize (Generic and specific) flour blended with bambara groundnut (*Vigna subterranean L*). *African Journal of Food Science*, 3, 107-112.

MCCLEARY, B. V., DE VERIES, J. W., RADE, J. I., COHEN, G., PROSKY, L., MUGFORD, D. C., CHAMP, M. & OKUNMA, K. 2011. Collaborative Study Report: Determination of insoluble, soluble and total dietary fibre (Codex definition) by an enzymatic-gravimetric method and liquid chromatography. *AACC International report*. AACC International, Inc.

MEGAZYME 2012. Integrated total dietary fibre assay procedure including resistant starch and non-digestible oligosaccharides K-INTDF 06/12. Bray, Co. Wicklow, Ireland: Megazyme International.

MENSAH, P. 1997. Fermentation - The key to food safety assurance in Africa? *Food Control* 8 271-278.

MENSAH, P. P., TOMKINS, A. M., DRASAR, B. S. & HARRISON, T. J. 1990. Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. *The Lancet*, 336, 140-143.

MERCADE, M., LINDLEY, N. D. & LOUBIÈRE, P. 2000. Metabolism of *Lactococcus lactis* subsp. *cremoris* MG 1363 in acid stress conditions. *International Journal of Food Microbiology*, 55, 161-165.

- MICHIDA, H., TAMALAMPUDI, S., PANDIELLA, S. S., WEBB, C., FUKUDA, H. & KONDO, A. 2006. Effect of cereal extracts and cereal fiber on viability of *Lactobacillus plantarum* under gastrointestinal tract conditions. *Biochemical Engineering Journal*, 28, 73-78.
- MILES, A. A. & MISRA, S. S. 1938. The estimation of the bactericidal power of the blood. *Journal of Hygiene*, 38, 732-49.
- MIR-MARQUÉS, A., CERVERA, M. L. & DE LA GUARDIA, M. 2012. A preliminary approach to mineral intake in the Spanish diet established from analysis of the composition of university canteen menus. *Journal of Food Composition and Analysis*, 27, 160-168.
- MOLERO, G., DÍEZ-OREJAS, R., NAVARRO-GARCÍA, F., MONTEOLIVA, L., PLA, J., GIL, C., SÁNCHEZ-PÉREZ, M. & NOMBELA, C. 1998. *Candida albicans*: genetics, dimorphism and pathogenicity. *International Journal of Microbiology*, 1, 95-106.
- MONTEAGUDO-MERA, A., RODRÍGUEZ-APARICIO, L., RÚA, J., MARTÍNEZ-BLANCO, H., NAVASA, N., GARCÍA-ARMESTO, M. R. & FERRERO, M. Á. 2012. *In vitro* evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin. *Journal of Functional Foods*, 4, 531-541.
- MOTARJEMI, Y. 2002. Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology*, 75, 213-229.
- MOTARJEMI, Y., KAFERSTEIN, F., MOY, G. & QUEVEDO, F. 1993. Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bulletin of the World Health Organization*, 71, 79-92.

- MUGULA, J. K., NARVHUS, J. A. & SØRHAUG, T. 2003a. Use of starter cultures of lactic acid bacteria and yeasts in the preparation of *Togwa*, a Tanzanian fermented food. *International Journal of Food Microbiology*, 83, 307-318.
- MUKISA, I. M., PORCELLATO, D., BYARUHANGA, Y. B., MUYANJA, C. M. B. K., RUDI, K., LANGSRUD, T. & NARVHUS, J. A. 2012. The dominant microbial community associated with fermentation of *Obushera* (sorghum and millet beverages) determined by culture-dependent and culture-independent methods. *International Journal of Food Microbiology*, 160, 1-10.
- MUYANJA, C. M. B. K., NARVHUS, J. A., TREIMO, J. & LANGSRUD, T. 2003. Isolation, characterisation and identification of lactic acid bacteria from *Bushera*: A Ugandan traditional fermented beverage. *International Journal of Food Microbiology*, 80, 201-210.
- MUYZER, G. 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology*, 2, 317-322.
- MUYZER, G., DE WAAL, E. C. & UITTERLINDEN, A. G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59, 695-700.
- NAGO, C. M., TÉTÉGAN, E., MATENCIO, F. & MESTRES, C. 1998a. Effects of maize type and fermentation conditions on the quality of Beninese traditional *Ogi*, a fermented maize slurry. *Journal of Cereal Science*, 28, 215-222.
- NAGO, M. C., HOUNHOUGAN, J. D., AKISSOE, N., ZANOUE, E. & MESTRES, C. 1998b. Characterization of the Beninese traditional *Ogi*, a fermented

- maize slurry: physicochemical and microbiological aspects. *International Journal of Food Science & Technology*, 33, 307-315.
- NGUYEN, T. T. T., LOISEAU, G., ICARD-VERNIÈRE, C., ROCHETTE, I., TRÈCHE, S. & GUYOT, J.-P. 2007. Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: A new method for preparing high energy density complementary foods for young children. *Food Chemistry*, 100, 623-631.
- NIKOLIC, M., LÓPEZ, P., STRAHINIC, I., SUÁREZ, A., KOJIC, M., FERNÁNDEZ-GARCÍA, M., TOPISIROVIC, L., GOLIC, N. & RUAS-MADIEDO, P. 2012. Characterisation of the exopolysaccharide (EPS)-producing *Lactobacillus paraplantarum* BGCG11 and its non-EPS producing derivative strains as potential probiotics. *International Journal of Food Microbiology*, 158, 155-162.
- NIVEN, S. J., BEAL, J. D. & BROOKS, P. H. 2004. The simultaneous determination of short chain fatty acid, monosaccharides and ethanol in fermented liquid pig diets. *Animal Feed Science and Technology*, 117, 339-345.
- NOUT, M. J. R. 1991. Ecology of accelerated natural lactic fermentation of sorghum-based infant food formulas. *International Journal of Food Microbiology*, 12, 217-224.
- NOUT, M. J. R. 2009. Rich nutrition from the poorest – Cereal fermentations in Africa and Asia. *Food Microbiology*, 26, 685-692.
- NOUT, M. J. R. & MOTARJEMI, Y. 1997. Assessment of fermentation as a household technology for improving food safety: a joint FAO/WHO workshop. *Food Control*, 8, 221-226.

- NOUT, M. J. R., ROMBOUTS, F. M. & HAVELAAR, A. 1989b. Effect of accelerated natural lactic fermentation of infant good ingredients on some pathogenic microorganisms. *International Journal of Food Microbiology*, 8, 351-361.
- NYATOTI, V. N., MTERO, S. S. & RUKURE, G. 1997. Pathogenic *Escherichia coli* in traditional African weaning foods. *Food Control*, 8, 51-54.
- OBASI, N. E. & WOGU, C. O. 2008. Effect of soaking on proximate compositions and anti-nutritional factors of yellow maize. *Nigerian Food Journal*, 26, 69-79.
- OBINNA-ECHEM, P. C. 2009. Effect of starter cultures on the pH, short chain fatty acids and ethanol levels of fermented maize food 'Akamu'. *Nigerian Food Journal*, 27, 27-35.
- OBINNA-ECHEM, P. C., KURI, V. & BEAL, J. 2014. Evaluation of the microbial community, acidity and proximate composition of *Akamu*, a fermented maize food. *Journal of the Science of Food and Agriculture*, 94, 331-340.
- ODUGBEMI, T., OYERINDE, J. P. O., ODUJINRIN, O. M. T., AKITOYE, C. O. & ESUMEH, F. I. 1991. Bacteriological study of cooked *Ogi* (fermented cereal weaning food) and its potential safety in a rural Nigerian community. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87, 234-235.
- ODUNFA, S. A., ADENIRAN, S. A., TENIOLA, O. D. & NORDSTROM, J. 2001. Evaluation of lysine and methionine production in some *Lactobacilli* and yeasts from *Ogi*. *International Journal of Food Microbiology*, 63, 159-163.
- OGUNTOYINBO, F. A. & NARBAD, A. 2012. Molecular characterization of lactic acid bacteria and in situ amylase expression during traditional fermentation of cereal foods. *Food Microbiology*, 31, 254-262.

- OHENHEN, R. E. 2002. *Enrichment of Ogi, A corn meal product*. Ph.D PhD, University of Benin.
- OHENHEN, R. E. & IKENEBOMEH, M. J. 2007. Shelf Stability and Enzyme Activity Studies of *Ogi*: A corn meal fermented product. *Journal of American Science*, 3, 38-42.
- OLASUPO, N. A., SCHILLINGER, U., NARBAD, A., DODD, H. & HOLZAPFEL, W. H. 1999. Occurrence of nisin Z production in *Lactococcus lactis* BFE 1500 isolated from *Wara*, a traditional Nigerian cheese product. *International Journal of Food Microbiology*, 53, 141-152.
- OLUKOYA, D. K., EBIGWEI, S. I., OLASUPO, N. A. & OGUNJIMI, A. A. 1994. Production of DogiK: an improved *Ogi* (Nigerian fermented weaning food) with potentials for use in diarrhoea control. *Journal of Tropical Paediatrics*, 20, 108-113.
- OLUWAMUKOMI, M. O., ELEYINMI, A. F. & ENUJIUGHA, V. N. 2005. Effect of soy supplementation and its stage of inclusion on the quality of *ogi* - a fermented maize meal. *Food Chemistry*, 91, 651-657.
- OMAR, N. B. & AMPE, F. 2000. Microbial community dynamics during production of the Mexican fermented maize dough *Pozol*. *Applied and Environmental Microbiology*, 66, 3664-3673.
- OMEMU, A. M. 2011. Fermentation dynamics during production of *Ogi*, a Nigerian fermented cereal porridge. *Report and Opinion*, 3, 8-17.
- OMEMU, A. M., OYEWOLE, O. B. & BANKOLE, M. O. 2007. Significance of yeasts in the fermentation of maize for *Ogi* production. *Food Microbiology*, 24, 571-576.
- OSUNDAHUNSI, O. F. & AWORH, O. C. 2003. Nutritional evaluation, with emphasis on the protein quality of maize-based complementary foods

- enriched with soya bean and cowpea *Tempe*. *International Journal of Food Science and Technology*, 38, 809-813.
- OSUNGBARO, T. O. 1990. Effect of fermentation period on amylose content and textural characteristics of "Ogi" (a fermented maize porridge). *Journal of Fermentation and Bioengineering*, 70, 22-25.
- OTITOJU, G. T. O. 2009. Effect of dry and wet milling processing techniques on the nutrient composition and organoleptic attributes of fermented yellow maize (*Zea mays*). *African Journal of Food Science* 3, 113-116.
- OTUNOLA, E. T., OGUNSOLA, O. & ABIOYE, V. F. 2006. Effect of the addition of Tempeh on some properties of 'Agidi', a West African fermented maize gel. *International Journal of Food and Agricultural Research*, 3, 119-128.
- OTUNOLA, E. T., SUNNY-ROBERTS, E. O. & SOLADEMI, A. O. 2007. Influence of the addition of Okra seed flour on the properties of Ogi, a Nigerian fermented maize food. In: TIELKES, E. (ed.) *Book of abstracts, Conference on International Research on Food Security, National Resource Management and Rural Development "Tropentag 2007"*. Witzhausen, Cuvillier Verlag Göttingen.
- OUWEHAND, A. C., KIRJAVAINEN, P. V., SHORTT, C. & SALMINEN, S. 1999. Probiotics: mechanisms and established effects. *International Dairy Journal*, 9, 43-52.
- OUWEHAND, A. C. & SALMINEN, S. J. 1998. The health effects of cultured milk products with viable and non-viable bacteria *International Dairy Journal*, 8, 749-58.
- OWUSU-KWARTENG, J., AKABANDA, F., NIELSEN, D. S., TANO-DEBRAH, K., GLOVER, R. L. K. & JESPERSEN, L. 2012. Identification of lactic

- acid bacteria isolated during traditional *fura* processing in Ghana. *Food Microbiology*, 32, 72-78.
- OYAREKUA, M. A. 2011. Evaluation of the nutritional and microbiological status of co-fermented cereals/cowpea 'OGI'. *Agricultural and Biology Journal of North America*, 2, 61-73.
- OYAREKUA, M. A. 2013. Effect of co-fermentation on nutritive quality and pasting properties of maize/cowpea/sweet potato as complementary food. *African Journal of Food, Agriculture, Nutrition and Development*, 13, 7171-7191.
- OYEYIOLA, G. P. 1990. Microbiological and biochemical changes during the fermentation of maize (*Zea mays*) grains for G. P. Oyeyiolamasa production. *World Journal of Microbiology and Biotechnology*, 6, 171-177.
- PAN, W.-H., LI, P.-L. & LIU, Z. 2006. The correlation between surface hydrophobicity and adherence of *Bifidobacterium* strains from centenarians' faeces. *Anaerobe*, 12, 148-152.
- PANG, G., XIE, J., CHEN, Q. & HU, Z. 2012. How functional foods play critical roles in human health. *Food Science and Human Wellness*, 1, 26-60.
- PARENTE, E., CIOCIA, F., RICCIARDI, A., ZOTTA, T., FELIS, G. E. & TORRIANI, S. 2010. Diversity of stress tolerance in *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum*: A multivariate screening study. *International Journal of Food Microbiology*, 144, 270-279.
- PATEL, H. M., PANDIELLA, S. S., WANG, R. H. & WEBB, C. 2004. Influence of malt, wheat, and barley extracts on the bile tolerance of selected strains of lactobacilli. *Food Microbiology* 21, 83-89.

- PEDERSEN, L. L., OWUSU-KWARTENG, J., THORSEN, L. & JESPERSEN, L. 2012. Biodiversity and probiotic potential of yeasts isolated from *Fura*, a West African spontaneously fermented cereal. *International Journal of Food Microbiology*, 159, 144-151.
- PEPE, O., BLAJOTTA, G., ANASTASIO, M., MOSCHETTI, G., ERCOLINI, D. & VILLANI, F. 2004. Technological and molecular diversity of *Lactobacillus plantarum* strains isolated from naturally fermented sourdoughs. *Systematic and Applied Microbiology*, 27, 443-453.
- PLESSAS, S., FISHER, A., KOURETA, K., PSARIANOS, C., NIGAM, P. & KOUTINAS, A. A. 2008. Application of *Kluyveromyces marxianus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *L. helveticus* for sourdough bread making. *Food Chemistry*, 106, 985-990.
- POUTANEN, K. 2012. Past and future of cereal grains as food for health. *Trends in Food Science and Technology*, 25, 58-62.
- PRASAD, N. N., SIDDALINGASWAMY, M., PARAMESWARIAH, P. M., RADHAKRISHNA, K., RAO, R. V., VISWANATHAN, K. R. & SANTHANAM, K. 2000. Proximate and mineral composition of some processed traditional and popular Indian dishes. *Food Chemistry*, 68, 87-94.
- RAGAEI, S., ABDEL-AAL, E.-S. M. & NOAMAN, M. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, 98, 32-38.
- RAGAEI, S., GUZAR, I., DHULL, N. & SEETHARAMAN, K. 2011. Effects of fiber addition on antioxidant capacity and nutritional quality of wheat bread. *LWT - Food Science and Technology*, 44, 2147-2153.

- RAHMAN, S., BIRD, A., REGINA, A., LI, Z., PHILIPPE RAL, J., MCMAUGH, S., TOPPING, D. & MORELL, M. 2007. Resistant starch in cereals: Exploiting genetic engineering and genetic variation. *Journal of Cereal Science*, 46, 251-260.
- RASOAMANANA, R., EVEN, P. C., DARCEL, N., TOMÉ, D. & FROMENTIN, G. 2013. Dietary fibers reduce food intake by satiation without conditioned taste aversion in mice. *Physiology & Behavior*, 110–111, 13-19.
- REDDY, G., ALTAF, M., NAVEENA, B. J., VENKATESHWAR, M. & KUMAR, E. V. 2008. Amylolytic bacterial lactic acid fermentation - A review. *Biotechnology Advances*, 26, 22-34.
- ROBERTS, C. L., KEITA, Å. V., PARSONS, B. N., PROROK-HAMON, M., KNIGHT, P., WINSTANLEY, C., O'KENNEDY, N., SÖDERHOLM, J. D., RHODES, J. M. & CAMPBELL, B. J. 2013. Soluble plantain fibre blocks adhesion and M-cell translocation of intestinal pathogens. *The Journal of Nutritional Biochemistry*, 24, 97-103.
- ROBIN, F., SCHUCHMANN, H. P. & PALZER, S. 2012. Dietary fiber in extruded cereals: Limitations and opportunities. *Trends in Food Science and Technology*, 28, 23-32.
- RODRÍGUEZ, L. H., MORALES, D. A., RODRÍGUEZ, E. R. & ROMERO, C. D. 2011. Minerals and trace elements in a collection of wheat landraces from the Canary Islands. *Journal of Food Composition and Analysis*, 24, 1081-1090.
- ROOHANI, N., HURRELL, R., WEGMUELLER, R. & SCHULIN, R. 2012. Zinc and phytic acid in major foods consumed by a rural and a suburban population in central Iran. *Journal of Food Composition and Analysis*, 28, 8-15.

- ROOS, S. & JONSSON, H. 2002. A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. *Microbiology*, 148, 433-442.
- ROOS, S., KARNER, F., AXELSSON, L. & JONSSON, H. 2000. *Lactobacillus mucosae* sp. nov., a new species with *in vitro* mucus-binding activity isolated from pig intestine. *International Journal of Systematic and Evolutionary Microbiology* 50 251-258.
- ROSENQUIST, H. & HANSEN, Å. 1998. The antimicrobial effect of organic acids, sour dough and nisin against *Bacillus subtilis* and *B. licheniformis* isolated from wheat bread. *Journal of Applied Microbiology*, 85, 621-631.
- RUIZ-MOYANO, S., MARTÍN, A., BENITO, M. J., CASQUETE, R., SERRADILLA, M. J. & CÓRDOBA, M. D. G. 2009. Safety and functional aspects of pre-selected lactobacilli for probiotic use in Iberian dry-fermented sausages. *Meat Science*, 83, 460-467.
- RYAN, L., THONDRE, P. S. & HENRY, C. J. K. 2011. Oat-based breakfast cereals are a rich source of polyphenols and high in antioxidant potential. *Journal of Food Composition and Analysis*, 24, 929-934.
- SAARELA, M., MOGENSEN, G., FONDÉN, R., MÄTTÖ, J. & MATTILA-SANDHOLM, T. 2000. Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology*, 84, 197-215.
- SALMERON, I., FUCIÑOS, P., CHARALAMPOPOULOS, D. & PANDIELLA, S. S. 2009. Volatile compounds produced by the probiotic strain *Lactobacillus plantarum* NCIMB 8826 in cereal-based substrates. *Food Chemistry*, 117, 265-271.
- SALMINEN, S., NYBOM, S., MERILUOTO, J., COLLADO, M. C., VESTERLUND, S. & EL-NEZAMI, H. 2010. Interaction of probiotics and

- pathogens - Benefits to human health? *Current Opinion in Biotechnology*, 21, 157-167.
- SALMINEN, S., VON WRIGHT, A., MORELLI, L., MARTEAU, P., BRASSART, D., DE VOS, W. M., FONDÉN, R., SAXELIN, M., COLLINS, K., MOGENSEN, G., BIRKELAND, S.-E. & MATTILA-SANDHOLM, T. 1998. Demonstration of safety of probiotics — a review. *International Journal of Food Microbiology*, 44, 93-106.
- SÁNCHEZ, B., GONZÁLEZ-TEJEDO, C., RUAS-MADIEDO, P., URDACI, M. C. & MARGOLLES, A. 2011. *Lactobacillus plantarum* extracellular chitin-binding protein and its role in the Interaction between chitin, Caco-2 cells, and mucin. *Applied and Environmental Microbiology*, 77, 1123-1126.
- SANDERS, M. E. 2009. How do we know when something called “Probiotic” is really a probiotic? A guideline for consumers and health care professionals. *Functional Food Reviews*, 1, 3-12.
- SANDERS, M. E., GIBSON, G. & GILL, H. S. 2007. Probiotics: Their potential to impact human health. *Council for Agricultural Science and Technology*, 36, 1-19.
- SANDHU, K. S., SINGH, N. & KAUR, M. 2004. Characteristics of the different corn types and their grain fractions: physicochemical, thermal, morphological, and rheological properties of starches. *Journal of Food Engineering*, 64, 119-127.
- SANNI, A. I. & LONNER, C. 1993. Identification of yeasts isolated from Nigerian traditional alcoholic beverages. *Food Microbiology*, 10, 517-523.
- SANNI, A. I., ONILUDE, A. A. & IBIDAPO, O. T. 1999. Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. *Food Chemistry*, 65, 35-39.

- SANNI, A. I., SEFA-DEDEH, S., SAKYI-DAWSON, E. & ASIEDU, M. 2002. Microbiological evaluation of Ghanaian maize dough co-fermented with cowpea. *International Journal of Food Science and Nutrition*, 53, 367-373.
- SANOJA, R. R., MORLON-GUYOT, J., JORE, J., PINTADO, J., JUGE, N. & GUYOT, J. P. 2000. Comparative characterization of complete and truncated forms of *Lactobacillus amylovorus* alpha -amylase and role of the C-terminal direct repeats in raw-starch binding. *Applied and Environmental Microbiology*, 66, 3350-3356.
- SANTOYO, M. C., LOISEAU, G., SANOJA, R. R. & GUYOT, J. P. 2003. Study of starch fermentation at low pH by *Lactobacillus fermentum* Ogi E1 reveals uncoupling between growth and [alpha]-amylase production at pH 4.0. *International Journal of Food Microbiology*, 80, 77-87.
- SAULNIER, D. M. A., SPINLER, J. K., GIBSON, G. R. & VERSALOVIC, J. 2009. Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. *Current Opinion in Biotechnology*, 20, 135-141.
- SCHÄR-ZAMMARETTI, P. & UBBINK, J. 2003. The cell wall of lactic acid bacteria: surface constituents and macromolecular conformations. *Biophysical Journal* 85, 4076-4092.
- SCHILLINGER, U., GUIGAS, C. & HEINRICH HOLZAPFEL, W. 2005. *In vitro* adherence and other properties of lactobacilli used in probiotic yoghurt-like products. *International Dairy Journal*, 15, 1289-1297.
- SCHILLINGER, U. & LÜCKE, F.-K. 1987. Identification of *Lactobacilli* from meat and meat products. *Food Microbiology*, 4, 199-208.
- SCHLEGEL, H. G. 1986. *General Microbiology*, London, Cambridge University Press.

- SEFA-DEDEH, S., KLUVITSE, Y. & AFOAKWA, E. O. 2001. Influence of fermentation and cowpea steaming on some quality characteristics of maize-cowpea blends. *African Journal of Science and Technology*, 80, 71-80.
- SHAHIDI, F. & CHANDRASEKARA, A. 2013. Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods*, 5, 570-581.
- SHETTY, P. H. & JESPERSEN, L. 2006. *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends in Food Science and Technology*, 17, 48-55.
- SILVA, T., RETO, M., SOL, M., PEITO, A., PERES, C. M., PERES, C. & MALCATA, F. X. 2011. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. *LWT - Food Science and Technology*, 44, 1349-1354.
- SINGH, J., SINGH, N. & SAXENA, S. K. 2002. Effect of fatty acids on the rheological properties of corn and potato starch. *Journal of Food Engineering*, 52, 9-16.
- SITI HAJAR, M. D., NOORHISHAM, T. K. & NURINA, A. 2012. Yeast identification from domestic ragi for food fermentation by PCR method. *International Food Research Journal* 19, 775-777.
- SLAVIN, J. 2003. Why whole grains are protective: biological mechanisms. *Proceedings of the Nutrition Society*, 62, 129-134.
- SONGRÉ-OUATTARA, L. T., MOUQUET-RIVIER, C., ICARD-VERNIÈRE, C., ROCHETTE, I., DIAWARA, B. & GUYOT, J. P. 2009. Potential of amylolytic lactic acid bacteria to replace the use of malt for partial starch

- hydrolysis to produce African fermented pearl millet gruel fortified with groundnut. *International Journal of Food Microbiology*, 130, 258-264.
- STILES, M. E. & HOLZAPFEL, W. H. 1997. Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, 36, 1-29.
- SUCCI, M., TREMONTE, P., REALE, A., SORRENTINO, E., GRAZIA, L., PACIFICO, S. & COPPOLA, R. 2005. Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *FEMS Microbiology Letters*, 244, 129-137.
- SULLIVAN, P., ARENDT, E. & GALLAGHER, E. 2013. The increasing use of barley and barley by-products in the production of healthier baked goods. *Trends in Food Science & Technology*, 29, 124-134.
- SUN, J., LE, G.-W., SHI, Y.-H. & SU, G.-W. 2007. Factors involved in binding of *Lactobacillus plantarum* Lp6 to rat small intestinal mucus. *Letters in Applied Microbiology* 44, 79-85.
- SVANBERG, U. & LORRI, W. 1997. Fermentation and nutrient availability. *Food Control*, 8, 319-327.
- SVANBERG, U., SJÖGREN, E., LORRI, W., SVENNERHOLM, A. M. & KAIJSER, B. 1992. Inhibited growth of common enteropathogenic bacteria in lactic-fermented cereal gruels. *World Journal of Microbiology and Biotechnology*, 8, 601-606.
- SVENSSON, O. & ARNEBRANT, T. 2010. Mucin layers and multilayers — Physicochemical properties and applications. *Current Opinion in Colloid & Interface Science*, 15, 395-405.

- TAN, S. & MORRISON, W. 1979. The distribution of lipids in the germ, endosperm, pericarp and tip cap of amylo maize, LG-11 hybrid maize and waxy maize. *Journal of the American Oil Chemists' Society*, 56, 531-535.
- TANG, M., HE, X., LUO, Y., MA, L., TANG, X. & HUANG, K. 2013. Nutritional assessment of transgenic lysine-rich maize compared with conventional quality protein maize. *Journal of the Science of Food and Agriculture*, 93, 1049-1054.
- TEJERO-SARIÑENA, S., BARLOW, J., COSTABILE, A., GIBSON, G. R. & ROWLAND, I. 2012. In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids. *Anaerobe*, 18, 530-538.
- TENIOLA, O. D., HOLZAPFEL, W. H. & ODUNFA, S. A. 2005. Comparative assessment of fermentation techniques useful in the processing of *Ogi*. *World Journal of Microbiology and Biotechnology* 21, 39-43.
- TENIOLA, O. D. & ODUNFA, S. A. 2001. The effects of processing methods on the levels of lysine, methionine and the general acceptability of *Ogi* processed using starter cultures. *International Journal of Food Microbiology*, 63, 1-9.
- TENIOLA, O. D. & ODUNFA, S. A. 2002. Microbial assessment and quality evaluation of *Ogi* during spoilage. *World Journal of Microbiology and Biotechnology*, 18, 731-737.
- TESTER, R. F. & MORRISON, W. R. 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, 67, 551-557.
- TOPPING, D. 2007. Cereal complex carbohydrates and their contribution to human health. *Journal of Cereal Science*, 46, 220-229.

- TOWO, E., MATUSCHEK, E. & SVANBERG, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and *in vitro* accessible iron. *Food Chemistry*, 94, 369-376.
- TRIAS, R., BAÑERAS, L., BADOSA, E. & MONTESINOS, E. 2008. Bioprotection of Golden Delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *International Journal of Food Microbiology*, 123, 50-60.
- TUO, Y., HANLI, Y., LIANZHONG, A., ZHENGJUN, W., BENHENG, G. & WEI, C. 2013. Aggregation and adhesion properties of 22 *Lactobacillus* strains. *Journal of Dairy Science*, 96, 4252-4257.
- UCHIDA, H., KINOSHITA, H., KAWAI, Y., KITAZAWA, H., MIURA, K., SHIIBA, K., HORII, A., KIMURA, K., TAKETOMO, N., ODA, M., YAJIMA, T. & SAITO, T. 2006. *Lactobacilli* binding human A-antigen expressed in intestinal mucosa. *Research in Microbiology*, 157, 659-665.
- UMOH, V. & FIELDS, M. J. 1981. Fermentation of corn for Nigerian *Agidi*. *Journal of Food Science*, 46, 903-905.
- VALENZUELA, A. S., RUIZ, G. D., OMAR, N. B., ABRIOUEL, H., LÓPEZ, R. L., CAÑAMERO, M. M., ORTEGA, E. & GÁLVEZ, A. 2008. Inhibition of food poisoning and pathogenic bacteria by *Lactobacillus plantarum* strain 2.9 isolated from ben saalga, both in a culture medium and in food. *Food Control*, 19, 842-848.
- VAN DER A KÜHLE, A., JESPEREN, L., GLOVER, R. L., DIAWARA, B. & JAKOBSEN, M. 2001. Identification and characterization of *Saccharomyces cerevisiae* strains isolated from West African sorghum beer. *Yeast*, 18, 1069-1079.

- VAN DER AA KÜHLE, A., SKOVGAARD, K. & JESPERSEN, L. 2005. In vitro screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology*, 101, 29-39.
- VERMEIREN, L., DEVLIEGHERE, F. & DEBEVERE, J. 2004. Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology*, 96, 149-164.
- VESTER BOLER, B. M., ROSSONI SERAO, M. C., BAUER, L. L., STAEGER, M. A., BOILEAU, T. W., SWANSON, K. S. & FAHEY, G. C. 2011. Digestive physiological outcomes related to polydextrose and soluble maize fibre consumption by healthy adult men. *British Journal of Nutrition*, 106, 1864-1871.
- VIEIRA-DALODÉ, G., JESPERSEN, L., HOUNHOUIGAN, D. J., LANGE, M. P., NAGO, M. C. & JAKOBSEN, M. 2007. Lactic acid bacteria and yeasts associated with *Gowé* production from sorghum in Bénin. *Applied Journal of Microbiology*, 103, 342 - 349.
- VIEIRA-DALODÉ, G., MADODÉ, Y. E., HOUNHOUIGAN, J., JESPERSEN, L. & JAKOBSEN, M. 2008. Use of starter cultures of lactic acid bacteria and yeasts as inoculum enrichment for the production of *Gowé*, a sour beverage from Benin. *African Journal of Microbiology Research*, 2, 179-186.
- VINDEROLA, C. G. & REINHEIMER, J. A. 2003. Lactic acid starter and probiotic bacteria: a comparative “*in vitro*” study of probiotic characteristics and biological barrier resistance. *Food Research International* 36, 895-904.

- VITAGLIONE, P., NAPOLITANO, A. & FOGLIANO, V. 2008. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology*, 19, 451-463.
- VIZOSO-PINTO, M. G., FRANZ, C. M. A. P., SCHILLINGER, U. & HOLZAPFEL, W. H. 2006. *Lactobacillus* spp. with *in vitro* probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology*, 109, 205-214.
- WAKIL, S. M., ONILUDE, A. A., ADETUTU, E. M. & BALL, A. S. 2008. PCR-DGGE fingerprints of microbial successional changes during fermentation of cereal-legume weaning foods. *African Journal of Biotechnology*, 7, 4643-4652.
- WANG, B., WEI, H., YUAN, J., LI, Q., LI, Y., LI, N. & LI, J. 2008. Identification of a surface protein from *Lactobacillus reuteri* JCM1081 that adheres to porcine gastric mucin and human enterocyte-like HT-29 cells. *Current Microbiology*, 57, 33-38.
- WEICKERT, M. O., MÖHLIG, M., SCHÖFL, C., ARAFAT, A. M., OTTO, B., VIEHOFF, H., KOEBNICK, C., KOHL, A., SPRANGER, J. & PFEIFFER, A. F. H. 2006. Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. *Diabetes Care*, 29, 775-780.
- WHO 2007. Protein and amino acid requirements in Human nutrition: Report of a Joint FAO/WHO/UNU Expert Consultation (WHO Technical Report Series; no.935). Geneva.
- WHO/FAO 2004. Vitamin and mineral requirements in human nutrition. 2nd ed. Geneva, Switzerland: WHO.
- WOOD, B. J. B. & HOLZAPFEL, W. H. 1995. *The genera of lactic acid bacteria in the lactic acid bacteria.*, London, Blackie Academic and Professional.

- WRIGHT, A. V. & AXELSSON, L. 2012. Lactic acid bacteria: An introduction. *In*: LAHTINEN, S., SALMINEN, S., OUWEHAND, A. & WRIGHT, A. V. (eds.) *Lactic acid bacteria: Microbiological and functional aspect*. 4th ed. Boca Raton, USA: CRC Taylor and Francis group.
- XIE, N., ZHOU, T. & LI, B. 2012. Kefir yeasts enhance probiotic potentials of *Lactobacillus paracasei* H9: The positive effects of coaggregation between the two strains. *Food Research International*, 45, 394-401.
- YARTEY, J., HARISSON, E. K., BRAKOHIAPA, L. A. & NKRUHMAH, F. K. 1993. Carbohydrate and electrolyte content of some home-available fluids used for oral rehydration in Ghana. *Journal of Tropical Pediatrics*., 39, 234-239.
- YOUSIF, N. M. K., DAWYNDT, P., ABRIOUEL, H., WIJAYA, A., SCHILLINGER, U., VANCANNEYT, M., SWINGS, J., DIRAR, H. A., HOLZAPFEL, W. H. & FRANZ, C. M. A. P. 2005. Molecular characterization, technological properties and safety aspects of enterococci from 'Hussuwa', an African fermented sorghum product. *Journal of Applied Microbiology*, 98, 216-228.
- YOUSIF, N. M. K., HUCH, M., SCHUSTER, T., CHO, G.-S., DIRAR, H. A., HOLZAPFEL, W. H. & FRANZ, C. M. A. P. 2010. Diversity of lactic acid bacteria from *Hussuwa*, a traditional African fermented sorghum food. *Food Microbiology*, 27, 757-768.
- YUAN, M.-L., LU, Z.-H., CHENG, Y.-Q. & LI, L.-T. 2008. Effect of spontaneous fermentation on the physical properties of corn starch and rheological characteristics of corn starch noodle. *Journal of Food Engineering*, 85, 12-17.
- ZAR, J. H. (ed.) 1999. *Biostatistical Analysis*, London, UK: Prentice Hall.

- ZHANG, D., LI, R. & LI, J. 2012. *Lactobacillus reuteri* ATCC 55730 and L22 display probiotic potential in vitro and protect against Salmonella-induced pullorum disease in a chick model of infection. *Research in Veterinary Science*, 93, 366-373.
- ZHANG, Y., ZHANG, L., DU, M., YI, H., GUO, C., TUO, Y., HAN, X., LI, J., ZHANG, L. & YANG, L. 2011. Antimicrobial activity against *Shigella sonnei* and probiotic properties of wild lactobacilli from fermented food. *Microbiological Research*, 167, 27-31.
- ZHENG, G. H., SOSULSKI, F. W. & TYLER, R. T. 1998. Wet-milling, composition and functional properties of starch and protein isolated from buckwheat groats. *Food Research International*, 30, 493-502.
- ZHOU, J. S., GOPAL, P. K. & GILL, H. S. 2001. Potential probiotic lactic acid bacteria *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019) do not degrade gastric mucin *in vitro*. *International Journal of Food Microbiology*, 63, 81-90.
- ŽILIĆ, S., HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, V., DODIG, D., MAKSIMOVIĆ, V., MAKSIMOVIĆ, M. & BASIĆ, Z. 2011. Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *Journal of Cereal Science*, 54, 417-424.
- ZITA, A. & HERMANSSON, M. 1997. Determination of bacterial cell surface hydrophobicity of single cells in cultures and in wastewater in situ. *FEMS Microbiology Letters*, 152, 299-306.

APPENDICES

Appendix A3.1: The type of media, amount of media powder, distilled water and the supplements used in the media preparation for microbial cultivation, isolation and enumeration

Media	Weight (g)	Distilled water (mL)	Supplements
Agar			
Baird Parker Agar (BPA) BPA base (CM0275)	31.5	500	Egg yolk Terrulite (SR0054) - 25 mL added to base
Blood agar Columbia agar base (CM0331)	19.5	475	Defibrinated sheep blood (SR0051) 25 mL added to base
de Man, Rogosa and Sharpe (MRS) agar (CM0361) Gelatine media	62	1000	
Nutrient agar (CM0003)	28	1000	Gelatin (48723- Fluka) - 4 g in NA base
Listeria selective agar Listeria selective agar base(CM0856)	27.75	500	Listeria selective supplement (SR0260E) A vial added to base
MacConkey (CM0007)	25.75	500	
Malt Extract (ME) agar (CM0059)	50	1000	
MRS agar with 0.001% cyclohexamide MRS agar base	62	1000	Cyclohexamide (C1988)* 0.1 g in 9.9 mL of 95% ethanol. Filter sterilized and 1 mL added to MRS agar base
Nutrient agar (NA) (CM0003)	28	1000	

Continues

Appendix A3.1 *Continued*

Media	Weight (g)	Distilled water (mL)	Supplements
Nutrient agar supplemented with sucrose Nutrient agar (CM0003)	28	100	Sucrose 7% (7 g in NA base) 0.1% (0.1 g in NA base)
Rose Bengal Chloramphenicol Agar (RBCA) RBCA base (0549B)	32	1000	Chloramphenicol (C078) (0.01% selective supplement)* 1 g in 9 mL of 95% ethanol. Filter sterilized and 1 mL added to RBCA base
Taylor's xylose lysine desoxycholate agar (XLD) (CM0469) Yersinia selective agar	26.5	500	
Yersinia selective agar base (CM653)	29	500	Yersinia selective supplement (SR109) A vial added to base
Broth			
Malt Extract broth (CM0057)	26	500	
Malt Extract broth supplemented with Cyclohexamide Malt Extract broth (CM0057)	26	500	Cyclohexamide (C1988)* 0.1 g in 9.9 mL of 95% ethanol. Filter sterilized and 1 mL added to ME broth
MRS (pH 2) MRS broth (CM0359)	31	500	adjusted pH with HCl - 5 mol L ⁻¹
MRS broth (CM0359)	26	500	
MRS with 0.3% Ox gall bile MRS broth (CM0359)	15.6	300	Ox gall bile (LP0056) 0.9 g added to MRS agar before dissolving in distilled water
Nutrient broth (CM0001)	13	1000	

*Supplements were obtained from Sigma-Aldrich, Gillingham, Dorset, England

Appendix A3.2: Media preparation for the differentiation of oxidation and fermentation of carbohydrates by the isolated LAB and yeast

The media for the isolated LAB involved a basal medium and the different carbohydrate solutions.

The required amounts of the component of the basal medium of MRS broth without glucose and meat extract but containing a pH indicator were:

Bacteriological peptone	10 g
Yeast extract	5 g
Polyoxyethylene (20) Sorbitan monooleate (Tween 80)	1 g
Dipotassium hydrogen phosphate	2 g
Sodium acetate	5 g
Triammonium citrate	2 g
Magnesium sulphate, hydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.2 g
Manganese sulphate, hydrate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$)	0.05 g
Bromocresol purple	0.004%
Distilled water	1 L

The weight out components were transferred into a beaker containing the distilled water, mixed thoroughly by stirring on a Bibby HB501 magnetic stirrer (Bibby Sterilin Limited, Stone Staffordshire, England) and dispensed in 150 mL into 250 mL autoclave bottles and sterilized by autoclaving at 121°C for 15 min. The pH of the media was adjusted to 7.3 ± 0.02 during stirring using 0.1 mol L^{-1} NaOH or HCl as the case may be.

The sugars (arabinose, cellobiose, esculine, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, mellibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, sucrose, tetraose and xylose) and starch were prepared as 10% solution (4 g in 40 mL distilled water) and sterilized by filtration. Each substrate was aseptically added to the prepared basal medium to give a final concentration of 2% (i.e. 30 mL of 10% substrate in 150 mL of basal medium) and then dispensed in 9.8 mL into UBs with inverted Durham tubes. In the case of glucose, it was added to the base media before autoclaving.

For the isolated yeast, each of the substrate media was composed of:

Yeast extract	5 g
Substrate	20 g
Bromocresol purple	0.004%
Distilled water	1 L

The weight out components were transferred into a beaker containing the distilled water, mixed thoroughly by stirring on a Bibby HB501 magnetic stirrer (Bibby Sterilin Limited, Stone Staffordshire, England) and dispensed in 9.8 mL into UBs with inverted Durham tubes, covered and autoclaved at 121°C for 15 min.

Appendix A3.3: Media for the hydrolysis of Arginine

This media had similar composition as the MRS basal media for LAB carbohydrate fermentation as in Appendix A3.1 except that triammonium citrate was replaced with trisodium citrate and 3 g of arginine was added.

The components were thoroughly mixed in the distilled water by stirring on a magnetic stirrer and then dispensed in 9.8 mL into UBs with inverted Durham tubes before autoclaving at 121°C for 15 min.

Appendix A3.4: Composition of the media for yeast ascospore formation and resistance to cyclohexamide

For the test for ascospore formation by yeast, cultures were subcultured twice in media A and the to media B.

Media A:

Nutrient agar	14 g
Glucose	20 g
Tartaric acid	2.5 g
Distilled water	500 mL

Media B

Bacteriological peptone	5 g
Glucose	0.2 g
Anhydrous sodium acetate	0.7 g
Agar	7.5 g
Distilled water	500 mL

Appendix A3.5: Preparation of HPLC reagents and standards

Analyte	*Purity (%)	*Molecular weight (g)	*Density (g mL ⁻¹)	†Quantity required
Liquids				
Acetic acid	99.7	60.05	1.049	57.47 mL
Butyric acid	99	88.11	0.986	90.26 mL
Ethanol	99.8	46.07	0.789	58.51 mL
Lactic acid	88	90.08	1.209	84.67 mL
Perchloric acid	70	100.46	1.670	85.94 mL
Propionic acid	99	74.08	0.992	75.43 mL
Solids				
Citric acid				
Anhydrous	99.7	192.10		192.68 g
Monohydrate		210.15		210.77 g
Dipotassium	99.9	138.21		138.35 g
Carbonate				
Fructose	≥99	180.16		180.16 g
Glucose	99.5	180.20		181.11 g
Maltose	95	360.3		379.26 g
Sucrose	≥99.5	342.30		342.30 g

*Information as contained on product label

† The quantity required for each analyte was computed based on the fact that:

For solids: A molar solution of the substance would contain gram molecular weight of the substance in 1 L of solvent and considering the sample purity, $Q_2 = (C_1Q_1)/C_2$. Where Q_1 = Amount of substance in absolutely pure state (molecular weight), C_1 = Absolute purity of sample (100%), Q_2 = Amount required with the actual purity of sample and C_2 = Actual sample purity. An example with glucose:

$$Q_2 = (100 \times 180.2) / 99.5 = 181.11 \text{ g}$$

For the liquids: The density give the amount of substance contained in 1 mL of pure solvent enabling computation of the volume of the substance solution containing the gram molecular weight. And using the formula: $C_1V_1=C_2V_2$, (where V_1 = volume of solution containing the molecular weight of substance and V_2 = Actual volume of solution required considering the actual purity of solution. the required volume of the impure solution for 1 M solution is obtained.

An example with lactic acid:

1.209 g of lactic acid is contained in 1 mL pure solvent

90.08 g will be contained in $(90.08/1.209) \times 1 \text{ mL} = 74.508 \text{ mL}$.

Hence, 74.508 ml of 100% of lactic acid in 1 L of distilled H₂O will yield 1 Molar solution of lactic acid

Given that $C_1V_1=C_2V_2$

In this case, $C_1=100\%$, $V_1= 74.508 \text{ mL}$, $C_2=88\%$ and V_2 is unknown

$$V_2 = (100/88) \times 74.508 \text{ mL}$$

$$= 84.668 \text{ mL}$$

Making up 8.467 mL of 88% lactic acid with distilled H₂O to mark in 100 mL volumetric flask will yield 1 Molar solution.

For the organic acids: Lactic, acetic, propionic, butyric and citric acids, a mixed standard solution of 200 mmol L⁻¹ was obtained by pipetting 1 mL of each of the 5 organic acids together in a tube.

Lesser concentrations of the mixed standard solution were prepared by was subsequently obtained from appropriate dilution of the 1 M solution. (200 mmol =1:5 dilution).

Appendix A3.6: Preparation of Spike samples for HPLC analysis

The maize slurry was prepared by adding 20 ml of sterile distilled water to 10 ± 0.1 g of irradiated ground maize in a 100 ml beaker. The slurry was mixed thoroughly with a stirring rod. Different concentrations of the organic acid (acetic, citric, butyric, lactic and propionic acids) standards in the maize slurry were obtained as follows:

100 mmol = 1 mL of 200 mmol of the organic acid mixed standard solution + 1 mL of the maize slurry in a 10 ml centrifuge bottle

50 mmol =1 mL of 100 mmol of the organic acid mixed standard solution + 1 mL of the maize slurry in a 50 mL centrifuge bottle

One and half millilitres of the spiked samples was transferred into eppendorff tubes The spiked samples were homogenised on a Multi-mixer (Fisher scientific, UK) for 10 s and then centrifuged at 13000 g for 20 min (Sanyo-MSE MSB010.CX2.5 Micro Centaur Centrifuge, Alconbury, UK). A part of the samples were acid treated for protein precipitation as follows: 500 μ L of the sample supernatant were added to 500 μ L of 0.5 mol L^{-1} of HClO_4 and 250 μ l of acid treated supernatant were neutralized with equal volume of 1 mol L^{-1} of K_2CO_3 . 20 μ L of 7% H_2SO_4 was added to 100 μ L of either an acid treated or untreated sample in 400 μ L of Milli-Q water. Samples were then filtered through MF-millipore microfiltre (0.20 μm pore size) into vials and sealed with crimp cap (11mm, Ruber/PTFE, Fisher Scientific, UK) and stored at -80°C until needed for analysis.

Appendix A3.7: pH of ground maize slurries fermented by LAB identified from *akamu* sample (M3)

Isolates	Sample codes	Time (h)								Rate (h ⁻¹)
		0	3	6	12	24	36	48	72	
Un-inoculated	Control	5.99±0.01 ^a	6.00±0.01 ^b	6.00±0.01 ^c	5.99±0.01 ^f	6.00±0.00 ^h	5.99±0.01 ^d	6.00±0.10 ^d	5.96±0.05 ^f	0.00
<i>Lactobacillus helveticus</i>	AL1	6.23±0.02 ^d	6.12±0.05 ^c	5.80±0.18 ^{bc}	5.64±0.03 ^{bc}	5.45±0.09 ^f	4.96±0.74 ^{bc}	3.98±0.73 ^c	3.25±0.08 ^{abc}	-0.03 ^a
<i>L. acidophilus</i>	AL2	6.22±0.04 ^d	6.12±0.02 ^c	5.77±0.11 ^{bc}	5.66±0.08 ^{cd}	5.48±0.07 ^f	5.38±0.01 ^{cd}	3.85±0.09 ^{bc}	3.25±0.02 ^{abc}	-0.03 ^a
<i>L. helveticus</i>	AL3	6.06±0.04 ^{ab}	5.98±0.01 ^b	5.94±0.02 ^c	5.95±0.02 ^{ef}	5.88±0.01 ^h	5.87±0.03 ^d	5.86±0.01 ^d	5.83±0.02 ^e	-0.01
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	AL4	6.09±0.05 ^{bc}	5.96±0.01 ^{ab}	5.95±0.04 ^c	5.93±0.04 ^{ef}	4.40±0.00 ^d	4.37±0.02 ^b	4.02±0.01 ^c	3.74±0.01 ^d	-0.07
<i>L. plantarum</i>	AL5	6.05±0.01 ^{ab}	5.99±0.03 ^b	5.97±0.04 ^c	5.87±0.01 ^e	3.63±0.01 ^c	3.51±0.23 ^a	3.44±0.01 ^{abc}	3.33±0.02 ^c	-0.10
<i>L. acidophilus</i>	AL6	6.06±0.05 ^{ab}	5.95±0.02 ^{ab}	5.97±0.01 ^c	5.95±0.01 ^{ef}	5.69±0.03 ^g	4.92±0.03 ^{bc}	3.63±0.05 ^{abc}	3.32±0.00 ^{bc}	-0.02
<i>L. plantarum</i>	AL7	6.18±0.03 ^{cd}	6.12±0.03 ^c	5.59±0.15 ^{ab}	5.55±0.02 ^b	3.27±0.04 ^a	3.15±0.15 ^a	3.10±0.02 ^a	3.23±0.01 ^{ab}	-0.12
<i>L. helveticus</i>	AL8	6.16±0.01 ^{cd}	6.10±0.01 ^c	5.85±0.10 ^{bc}	5.74±0.03 ^d	5.31±0.04 ^e	4.63±0.02 ^b	3.23±0.02 ^{ab}	3.18±0.01 ^a	-0.04 ^a
Back Slop Fermentation	BSF	6.09±0.03 ^{bc}	5.90±0.02 ^a	5.49±0.05 ^a	4.18±0.02 ^a	3.46±0.01 ^b	3.42±0.01 ^a	3.42±0.02 ^{abc}	3.72±0.01 ^d	-0.11

*values with the same superscript in the same column do not differ significantly ($p \leq 0.05$). N=3±SD

Rate = (value after 24 h – Value after 0 h)/24 h

Appendix A3.8: Total titratable acidity (as %Lactic acid) of ground maize slurries fermented by LAB identified from *akamu* sample (M3)

Isolate	Sample codes	Time (h)								Rate (h ⁻¹)
		0	3	6	12	24	36	48	72	
Un-inoculated	Control	0.13±0.06 ^a	0.20±0.00 ^a	0.20±0.00 ^a	0.20±0.00 ^c	0.20±0.00 ^c	0.20±0.00 ^c	0.23±0.06 ^d	0.23±0.06 ^c	0.00 ^c
<i>Lactobacillus helveticus</i>	AL1	0.20±0.00 ^a	0.23±0.06 ^a	0.27±0.06 ^a	0.27±0.06 ^{bc}	0.37±0.12 ^{bc}	0.40±0.10 ^{bc}	0.57±0.29 ^{cd}	1.37±0.50 ^a	0.01 ^{bc}
<i>L. acidophilus</i>	AL2	0.23±0.06 ^a	0.23±0.12 ^a	0.23±0.06 ^a	0.23±0.06 ^{bc}	0.30±0.10 ^c	0.30±0.00 ^{bc}	0.53±0.21 ^{cd}	1.00±0.17 ^{ab}	0.00 ^c
<i>L. helveticus</i>	AL3	0.20±0.00 ^a	0.20±0.00 ^a	0.20±0.00 ^a	0.23±0.06 ^{bc}	0.33±0.06 ^c	0.30±0.00 ^{bc}	0.33±0.06 ^d	0.23±0.06 ^c	0.01 ^c
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	AL4	0.23±0.06 ^a	0.23±0.06 ^a	0.23±0.06 ^a	0.27±0.06 ^{bc}	0.43±0.15 ^{bc}	0.47±0.12 ^b	0.60±0.00 ^{bcd}	0.67±0.12 ^{bc}	0.01 ^{bc}
<i>L. plantarum</i>	AL5	0.20±0.00 ^a	0.27±0.06 ^a	0.23±0.06 ^a	0.30±0.00 ^{bc}	0.70±0.17 ^{ab}	0.97±0.12 ^a	1.07±0.06 ^a	1.33±0.06 ^a	0.02±0.01 ^{ab}
<i>L. acidophilus</i>	AL6	0.20±0.00 ^a	0.23±0.06 ^a	0.20±0.00 ^a	0.20±0.00 ^c	0.27±0.12 ^c	0.40±0.10 ^{bc}	0.87±0.15 ^{abc}	1.37±0.31 ^a	0.00 ^c
<i>L. plantarum</i>	AL7	0.20±0.10 ^a	0.27±0.06 ^a	0.27±0.06 ^a	0.33±0.06 ^{ab}	0.90±0.20 ^a	0.90±0.00 ^a	0.97±0.06 ^{ab}	1.23±0.06 ^{ab}	0.03±0.01 ^a
<i>L. helveticus</i>	AL8	0.23±0.06 ^a	0.27±0.06 ^a	0.27±0.06 ^a	0.20±0.00 ^c	0.30±0.10 ^c	0.30±0.00 ^{bc}	0.80±0.10 ^{abc}	1.23±0.06 ^{ab}	0.00 ^c
Back Slop Fermentation	BSF	0.23±0.06 ^a	0.23±0.06 ^a	0.33±0.06 ^a	0.43±0.06 ^a	0.93±0.06 ^a	0.93±0.06 ^a	1.10±0.10 ^a	0.83±0.06 ^{ab}	0.03 ^a

*values with the same superscript in the same column do not differ significantly (p<0.05). N=3±SD. Rate = (value after 24 h – Value after 0 h)/24 h

Appendix A3 9: pH and Total titratable acidity (as %Lactic acid) of ground maize slurries fermented by yeasts identified from *akamu* sample (M3)

Isolates	Sample codes	Time (h)								Rate (h ⁻¹)
		0	3	6	12	24	36	48	72	
pH										
Un-inoculated	Control	5.99±0.01 ^a	6.00±0.01 ^a	6.00±0.01 ^a	5.99±0.01 ^a	6.00±0.00	5.99±0.01	6.00±0.01	5.96±0.05	0.00 ^e
<i>Candida tropicalis</i>	AY1	6.26±0.01 ^c	6.21±0.01 ^b	6.19±0.01 ^b	6.13±0.03 ^b	5.71±0.01	5.43±0.01 ^a	5.33±0.03	5.27±0.01	-0.02 ^{bc}
<i>C. albicans</i>	AY2	6.25±0.04 ^c	6.23±0.01 ^b	6.21±0.01 ^b	6.17±0.01 ^b	5.92±0.01	5.39±0.03 ^a	5.17±0.01	5.16±0.02	-0.01 ^d
<i>C. albicans</i>	AY3	6.09±0.03 ^b	6.01±0.02 ^a	5.97±0.01 ^a	5.92±0.03 ^a	5.55±0.02	5.22±0.01	4.99±0.01	4.97±0.02	-0.02 ^c
<i>C. albicans</i>	AY4	6.05±0.04 ^{ab}	6.01±0.03 ^a	5.97±0.03 ^a	5.94±0.04 ^a	5.42±0.00	4.93±0.03	4.89±0.02	4.89±0.02	-0.03 ^b
Back Slop Fermentation	BSF	6.09±0.03 ^b	5.90±0.02	5.49±0.05	4.18±0.02	3.46±0.01	3.42±0.02	3.42±0.02	3.72±0.01	-0.11 ^a
Total titratable acidity										
Un-inoculated	Control	0.13±0.06 ^a	0.20±0.00 ^c	0.20±0.00 ^a	0.20±0.00 ^c	0.20±0.00	0.20±0.00	0.23±0.06 ^a	0.23±0.06 ^a	0.00
<i>Candida tropicalis</i>	AY1	0.20±0.00 ^a	0.30±0.00 ^{ab}	0.30±0.00 ^a	0.33±0.03 ^{ab}	0.50±0.00	0.43±0.06 ^a	0.53±0.06 ^a	0.47±0.06 ^{ab}	0.01 ^b
<i>C. albicans</i>	AY2	0.23±0.06 ^a	0.33±0.06 ^a	0.33±0.12 ^a	0.33±0.03 ^{ab}	0.33±0.06 ^{ab}	0.53±0.06 ^a	0.57±0.12 ^a	0.60±0.10 ^a	0.00
<i>C. albicans</i>	AY3	0.20±0.00 ^a	0.20±0.00 ^c	0.27±0.06 ^a	0.30±0.00 ^{bc}	0.30±0.00 ^b	0.43±0.06 ^a	0.50±0.26 ^a	0.30±0.10 ^{bc}	0.00
<i>C. albicans</i>	AY4	0.20±0.00 ^a	0.20±0.00 ^c	0.23±0.06 ^a	0.23±0.03 ^{bc}	0.40±0.00 ^a	0.43±0.06 ^a	0.40±0.10 ^a	0.43±0.06 ^{abc}	0.01 ^{ab}
Back Slop Fermentation	BSF	0.23±0.06 ^a	0.23±0.06 ^{bc}	0.33±0.06 ^a	0.43±0.03 ^a	0.93±0.06	0.93±0.06	1.10±0.10	0.83±0.06	0.03

*values with the same superscript in the same column do not differ significantly (p≤0.05). N=3±SD

Rate = (value after 24 h – Value after 0 h)/24 h

Appendix A3.10: pH and titratable acidity (% lactic acid) of ground maize slurries fermented by freeze dried *Lactobacillus plantarum* strains starter cultures

Parameter	Samples	Time (h)				
		0	6	12	24	48
pH	FLpTx	6.00±0.01 ^a	3.89±0.01 ^c	3.66±0.01 ^d	3.42±0.01 ^e	3.43±0.01 ^e
	FL5	6.00±0.01 ^a	3.92±0.01 ^c	3.67±0.01 ^d	3.38±0.00 ^f	3.33±0.01 ^g
	FL7	5.96±0.05 ^b	3.88±0.01 ^c	3.66±0.01 ^d	3.40±0.01 ^{ef}	3.37±0.01 ^{fg}
Titratable acidity	FLpTx	0.18±0.00 ^a	0.60±0.05 ^{bc}	0.87±0.05 ^e	1.23±0.05 ^{fg}	1.35±0.09 ^{gh}
	FL5	0.18±0.00 ^a	0.51±0.05 ^b	0.69±0.05 ^{cd}	1.20±0.05 ^f	1.50±0.05 ⁱ
	FL7	0.18±0.00 ^a	0.57±0.05 ^{bc}	0.78±0.05 ^{de}	1.17±0.00 ^f	1.47±0.05 ^{hi}

Values that share the same superscript in the same column for each parameter do not differ significantly ($p \leq 0.05$). N=3±SD

FLpTx - Samples fermented by commercial probiotic *L. plantarum* strain (LpTx)

FL5 & FL7 - Samples fermented by Nigerian fermented maize *L. plantarum* strains (NGL5 & NGL7)

Appendix A4.1: Preparation of 50 mmol L⁻¹ Na₂CO₃ buffer and 100 µg mL⁻¹ porcine mucin solution

50 mmol L⁻¹ Na₂CO₃ buffer

Molecular weight of Na₂CO₃ = 105.99 g

1 mol L⁻¹ = 105.99 g in 1 L of solvent (Distilled H₂O)

1 mmol L⁻¹ = 0.10599 g in 1 L of solvent (Distilled H₂O)

50 mmol L⁻¹ = 0.10599 g x 50 mmol L⁻¹

= 5.2995 g in 1 L of solvent (Distilled H₂O)

100 µg mL⁻¹ porcine mucin solution

1000 000 µg = 1 g and 1000 mL = 1 L

Therefore, 100 µg of porcine mucin in 1 mL of Na₂CO₃ buffer is equivalent to

100 000 µg of porcine mucin in 1000 mL of Na₂CO₃ buffer

= 0.1 g of porcine mucin in 1 L of Na₂CO₃ buffer

Appendix A4.2: Diameter (mm) of *Lactobacillus plantarum* strains inhibition halos over five relevant foodborne pathogens (n=3 ±SD)

<i>L. plantarum</i> strains	LAB growth conditions	Foodborne pathogens					Averages
		<i>B. cereus</i> NCIMB 11925	<i>E. coli</i> 1077	<i>L. monocytogenes</i> NCTC 7973	<i>S. Enteritidis</i> NCTC 5188	<i>Staph. aureus</i> NCTC 3750	
NGL5	Aerobic	15.4±0.3 ^b	19.9±0.3 ^a	12.8±0.1 ^b	20.7±0.6 ^b	15.0±0.7 ^b	16.8ⁿ
NGL7	Aerobic	13.3±0.1 ^c	17.2±0.2 ^c	10.6±0.2 ^c	19.1±0.6 ^{cd}	12.5±0.1 ^c	14.5
LpTx	Aerobic	13.7±0.6 ^c	17.9±1.0 ^{bc}	12.1±0.2 ^b	18.9±0.3 ^d	14.2±0.4 ^b	15.4
Averages		14.1^y	18.3	11.8	19.6^x	13.9^{yz}	15.6
NGL5	Anaerobic	17.4±0.3 ^a	19.8±0.3 ^a	13.4±0.5 ^a	20.2±0.6 ^{bc}	16.5±1.0 ^a	17.5^m
NGL7	Anaerobic	17.1±0.3 ^a	19.0±0.2 ^{ab}	12.7±0.4 ^b	19.8±0.3 ^{bcd}	15.5±0.4 ^{ab}	16.8ⁿ
LpTx	Anaerobic	15.3±0.3 ^b	19.5±0.4 ^a	13.7±0.3 ^a	23.9±0.1 ^a	14.5±0.2 ^b	17.3^m
Averages		16.6	19.4^x	13.4^z	21.3	15.5	17.2

Values that share the same superscript in the same column do not differ significantly (p≤0.05). Averages with the same superscript do not differ significantly.

Appendix A4.3: Correlation coefficient between pH, TTA, levels of *Lactobacillus plantarum* and *Escherichia coli* 1077 in the co-inoculation fermentation

Time (h)	Temp (°C)	r_{ap}	r_{lp}	r_{la}	r_{ep}	r_{ea}	r_{el}
0	22	0.13(0.68)	-0.62(0.03)	-0.31(0.33)	0.19(0.56)	0.40(0.20)	-0.02(0.95)
	30	-0.08(0.81)	0.50(0.10)	0.09(0.79)	-0.31(0.33)	-0.02(0.96)	-0.61(0.04)
3	22	-0.30(0.34)	-0.77(0.00)*	0.06(0.86)	0.12(0.72)	0.18(0.57)	-0.40(0.20)
	30	-0.63(0.03)	-0.32(0.30)	0.11(0.74)	0.30(0.34)	-0.78(0.00)	0.17(0.61)
6	22	-0.40(0.20)	-0.70(0.01)*	0.24(0.45)	-0.24(0.45)	0.40(0.20)	0.01(0.97)
	30	-0.35(0.27)	-0.89(0.00)*	0.19(0.55)	0.77(0.00)*	-0.29(0.36)	-0.65(0.02)
9	22	0.00(1.00)	-0.93(0.00)*	0.11(0.73)	0.11(0.74)	0.25(0.44)	-0.21(0.51)
	30	-0.58(0.05)	-0.99(0.00)*	0.52(0.09)	0.88(0.00)*	-0.47(0.13)	-0.89(0.00)*
12	22	0.07(0.83)	-0.98(0.00)*	-0.07(0.83)	0.55(0.06)	0.46(0.13)	-0.54(0.07)
	30						
24	22	-0.93(0.00)*	-1.00(0.00)*	0.93(0.00)*	0.96(0.00)*	-0.85(0.00)*	-0.95(0.00)*
	30	-0.94(0.00)*	-1.00(0.00)*	0.95(0.00)*	1.00(0.00)*	-0.94(0.00)*	-1.00(0.00)*
48	22	-0.97(0.00)*	-0.99(0.00)*	0.97(0.00)*	0.90(0.00)*	-0.90(0.00)*	-0.87(0.00)*
	30	-0.98(0.00)*	-1.00(0.00)*	0.97(0.00)*	1.00(0.00)*	-0.97(0.00)*	-1.00(0.00)*

Correlation coefficient (P-value)

*p≤0.01 was an indication of correlation between variables.

r_{ap} = correlation between TTA and pH while *L. plantarum* and *E. coli* viable count were held constant

r_{lp} = correlation between *L. plantarum* viable count and pH while TTA and *E. coli* viable count were held constant

r_{la} = correlation between *L. plantarum* viable count and TTA while pH and *E. coli* viable count were held constant

r_{ep} = correlation between *E. coli* viable count and pH while TTA and *L. plantarum* viable count were held constant

r_{ea} = correlation between *E. coli* viable count and pH while TTA and *L. plantarum* viable count were held constant

r_{el} = correlation between *L. plantarum* and *E. coli* viable count while TTA and pH were held constant

Appendix A4.4: Absorbance from Versa Max microplate reader (Molecular Devices, UK) for mucin binding assay

Incubation condition	LAB	OD _{ml}			OD _m			Adhesion*		
		i	ii	iii	i	ii	iii	i	ii	iii
30°C 2h	NGL5	0.0806	0.0848	0.0807	0.0476	0.0486	0.0471	0.6921	0.7437	0.7146
	NGL7	0.0753	0.0798	0.0776	0.0476	0.0486	0.0471	0.5808	0.6409	0.6487
	LpTx	0.1173	0.1161	0.1181	0.0476	0.0486	0.0471	1.4626	1.3873	1.5092
	<i>L. reuteri</i> NCIB 11951	0.0833	0.0813	0.0818	0.0476	0.0486	0.0471	0.7488	0.6717	0.7379
30°C 20 h	NGL5	0.1810	0.1566	0.1459	0.0783	0.0779	0.0778	1.3106	1.0103	0.8761
	NGL7	0.1443	0.1440	0.1340	0.0783	0.0779	0.0778	0.8421	0.8485	0.7231
	LpTx	0.1199	0.1068	0.1073	0.0783	0.0779	0.0778	0.5306	0.3710	0.3798
	<i>L. reuteri</i> NCIB 11951	0.1463	0.1413	0.1473	0.0783	0.0779	0.0778	0.8677	0.8139	0.8941
4°C 20h	NGL5	0.0581	0.0485	0.0474	0.0461	0.0467	0.0466	0.2603	0.0385	0.0172
	NGL7	0.0567	0.0762	0.0517	0.0461	0.0467	0.0466	0.2299	0.6317	0.1094
	LpTx	0.0537	0.0481	0.0458	0.0461	0.0467	0.0466	0.1649	0.0300	-0.0172
	<i>L. reuteri</i> NCIB 11951	0.1276	0.0929	0.1141	0.0461	0.0467	0.0466	1.7679	0.9893	1.4485

OD_{ml} = Absorbance of mucin coated wells inoculated with the LAB

OD_m = Absorbance of mucin with only the buffer solution.

*Adhesion = (OD_{ml} - OD_m)/OD_m

Appendix A5.1: Ethical Approval Consent

Faculty of Science and Technology

Smeaton 009, Plymouth

To:	Patience Obinna-Echem	From:	Paula Simson
cc:			Secretary to Human Ethics Committee
Your Ref:		Our Ref:	scitech:\x:\human ethics:
Date:	1 November 2012	Phone Ext:	84503

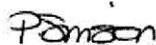
Application for Ethical Approval

Thank you for submitting the ethical approval form and details concerning your project:

'Sensory evaluation of probiotic maize porridge'

I am pleased to inform you that this has been approved.

Kind regards



Paula Simson

Appendix A5.2: E-mail invitation for participation in sensory evaluation of probiotic maize porridge

Volunteers required to help with sensory evaluation

You are invited to take part in the sensory evaluation of probiotic porridges on **Wednesday 5th of December 2012**

If you could spare me some time (approx. 10 minutes), either between **10.00 am -12.00 noon** or between **1.00 to 3.00 pm**, please would you come to the Food and Nutrition Unit during these times, located on the ground floor of Link Building. (Entrance located in the foyer at the South end of Davy Building)

The aim of this study is to evaluate the sensory qualities and acceptability of unfermented and differently fermented maize porridges. The product contains ground maize, lactic culture (non-dairy) and water. Persons with allergies to any of these ingredients would be excluded, no personal information would be taken and you are free to withdraw from the panel anytime.

Thank you very much for your anticipated participation

Obinna-Echem, Patience C.

School of Biological Sciences

patience.obinna-echem@plymouth.ac.uk/07901272624

Appendix A5.3: Briefing Sheet

Title of Research:

Sensory evaluation of probiotic maize porridges

Aim:

The aim of this study is to evaluate the sensory qualities and general acceptability of unfermented and differently fermented maize porridges.

Name of Investigators:

Patience C. Obinna-Echem, Dr. Jane Beal and Dr. Victor Kuri

Ingredients: **ground maize, lactic culture (*L. plantarum*) and water**

Allergy advice: Samples contain maize

You will be served some samples of porridge made from unfermented and differently fermented ground maize and you will be required to evaluate them: taste one sample at the time and indicate your opinion from strongly like, neither like nor dislike to strongly dislike about each product attributes by ticking (√) in the correct box.

Please feel free to leave your name and any comments on the space provided or on the reverse of the sheet.

Consent information:

The objectives of this research have been explained to me:

- ◆ I do not have food allergies to any components in these products.
- ◆ I know that I am free to withdraw from this panel at any time, and I have right to withdraw my data.
- ◆ I know that personal information will not be kept after the exercise
- ◆ Photos might be taken during the evaluation, without showing the faces. Please, indicate in case you do not like your photo to be taken.
- ◆ I am aware that risk assessment was conducted to preclude potential risks during this work.
- ◆ Under these circumstances, I agree to participate in the panel.

If you are dissatisfied with the way the research is conducted, please contact the principal investigator in the first instance: e-mail patience.obinna-echem@plymouth.ac.uk (telephone 01752 584604). If you feel the problem has not been resolved please contact the secretary to the Faculty of Science and technology Human Ethics Committee: Mrs Paula Simson 01752584503.

Thank you for your participation in the panel

Appendix A5.4: Consent form

Research Title: Sensory evaluation of probiotic maize porridges

I have read the briefing sheet and considered the consent information.

Under these circumstances, I agree to participate in the panel.

N o.	Panellists	Signature	Comment
1			
2			
3			
4			
5			
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Appendix A5.5: Ballot sheet

Sensory evaluation of probiotic maize porridges

Panellists code/initial:

Product code:

Please evaluate each sample and indicate your opinion about each attribute by marking (√) in a suitable box in each attribute row. Please make sure that your results are placed under the correct code.

Attributes	Liking scale								
	← Strongly dislike			Neither like nor dislike			Strongly like →		
Aroma									
Appearance									
Colour									
Flavour									
Sourness (Acidity)									
Texture (Smoothness)									
Overall acceptability									

Additional comments:

Appendix A5.6 Comparison of the samples protein and energy values expressed as percentages of the daily protein and energy requirements

Nutrient	Samples	Nutrient composition in the samples		Requirements				Percentages of Requirements met							
		Minimum	Maximum	0.5 years		2.5 years		0.5 years				2.5 years			
				Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
Protein	<i>Akamu</i>	<i>Protein (g 100g⁻¹)</i>		<i>Protein (g day⁻¹)</i>				47.5	110.6	47.5	110.6	47.3	110.1	46.6	108.6
	SFGM	3.19	7.43	6.72	6.72	6.75	6.84	94.0	104.2	94.0	104.2	93.6	103.7	92.4	102.3
Energy	<i>Akamu</i>	<i>Energy (KJ 100g⁻¹)</i>		<i>Energy (KJ day⁻¹)</i>				86	91.4	84.8	90	55.2	58.7	57.5	61.1
	SFGM	1729	1837	2010	2040	3132	3006	80.9	89.3	79.8	88	51.9	57.3	54.1	59.7

SFGM- Starter culture (*L. plantarum* strains) fermented ground maize slurry

The daily protein and energy requirement were computed from values adopted from (WHO, 2007) at assumed body weight of 6 and 9 kg for ages 0.5 and 2.5 years respectively.

Appendix A5.7: Comparison of the samples mineral levels with the daily requirements

Elements	Samples	Element concentrations in samples (mg 100g ⁻¹)		Requirements			Percentages of Requirement met by samples					
		Minimum	Maximum	0-6 Months	7-12 Months	1-3 years	0-6 Months	7-12 Months	1-3 years			
Ca	Akamu	3.788	7.029				1.3	2.3	0.9	1.8	0.8	1.4
	SFGM	7.308	9.462	300	400	500	2.4	3.2	1.8	2.4	1.5	1.9
Cu	Akamu	0.115	0.239				57.5	119.5	52.3	108.6	33.8	70.3
	SFGM	0.111	0.201	0.2	0.22	0.34	55.5	100.5	50.5	91.4	32.6	59.1
Fe	Akamu	2.747	10.144				14.8	54.5	23.7	87.4	21.8	80.5
	SFGM	1.716	2.386	18.6	11.6	12.6	9.2	12.8	14.8	20.6	13.6	18.9
K	Akamu	19.133	71.163				4.8	17.8	2.7	10.2	0.6	2.4
	SFGM	204.423	266.423	400	700	3000	51.1	66.6	29.2	38.1	6.8	8.9
Mg	Akamu	7.887	21.932				21.9	60.9	14.6	40.6	13.1	36.6
	SFGM	72.732	86.295	36	54	60	202.0	239.7	134.7	159.8	121.2	143.8
Mn	Akamu	0.151	0.468				5033.3	15600.0	25.2	78.0	12.6	39.0
	SFGM	0.27	0.308	0.003	0.6	1.2	9000.0	10266.7	45.0	51.3	22.5	25.7
Na	Akamu	21.008	23.591				17.5	19.7	5.7	6.4	2.1	2.4
	SFGM	20.968	30.834	120	370	1000	17.5	25.7	5.7	8.3	2.1	3.1
P	Akamu	58.049	112.766				58.0	112.8	21.1	41.0	12.6	24.5
	SFGM	171.597	186.83	100	275	460	171.6	186.8	62.4	67.9	37.3	40.6
S	Akamu	55.734	72.301									
	SFGM	76.561	79.841									
Zn	Akamu	0.55	0.992				8.3	15.0	6.5	11.8	6.6	12.0
	SFGM	1.654	2.157	6.6	8.4	8.3	25.1	32.7	19.7	25.7	19.9	26.0

*No available for the requirements for sulphur. SFGM-Samples of starter culture fermented ground maize slurries

Appendix A5.8: Anderson-Darling Normality test for the evaluated sensory attributes

Treatment	Statistics	Sensory parameters						Overall Acceptance
		Aroma	Appearance	Cololour	Flavour	Sourness	Texture	
ACD	A-Squared	0.76	1.67	1.21	0.46*	0.88	0.9	0.8
	P-value	0.043	<0.005	<0.005	0.238	0.021	0.019	0.034
PLpTx	A-Squared	1.3	1.06	1.65	0.6	0.54*	0.93	1.06
	P-value	<0.005	0.007	<0.005	0.106*	0.154	0.016	0.007
PL5	A-Squared	1.08	1.73	1.56	0.74	0.68*	1.43	1.3
	P-value	0.007	<0.005	<0.005	0.048	0.067	<0.005	<0.005
PL7	A-Squared	0.89	1.47	2.17	0.84	1.06	1.41	0.68*
	P-value	0.02	<0.005	<0.005	0.027	0.007	<0.005	0.069
UUA	A-Squared	1.31	1.27	1.19	1.21	0.93	1.08	0.7*
	P-value	<0.005	<0.005	<0.005	<0.005	0.016	0.007	0.059

*Values not normally distributed (P>0.05)

ACD-Artificially acidified

UUA-Unfermented un-acidified

PL5 and AL7- Porridges of ground maize slurry fermented by *L. plantarum* strains isolated from traditional *akamu* sample

PLpTx- Porridges of ground maize slurry fermented by commercial probiotic *L. plantarum* strain

POSTER PRESENTATIONS



Probiotic potential of *Lactobacillus plantarum* strains isolated from fermented maize food

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Introduction

Lactobacillus plantarum is a versatile lactic acid bacterium, that is commonly associated with human gastrointestinal tract (GIT)¹ and also found in various indigenous fermented foods². Probiotic bacteria play crucial role in maintaining normal microbiota in the GIT and are required for preventing intestinal disorder and microbial infections³. This has led to increased interest not just in the development of food products containing beneficial Lactobacilli but the characterisation of non-intestinal isolates for possible health promoting benefits. In order to exert health benefits, probiotics must withstand passage through the GIT, adhere to intestinal mucosa and epithelial cells⁴. Bacteria survival at low pH and in the presence of bile salts and adhesion to mucin has been evaluated in vitro using acid and bile salt supplemented media and mucin extracts from human and animals^{3, 5}.

This study was therefore aimed at evaluating the acid/bile tolerance and mucin binding potential of two strains of *Lactobacillus plantarum* isolated from Nigerian fermented maize food 'akamu'.

Methodology

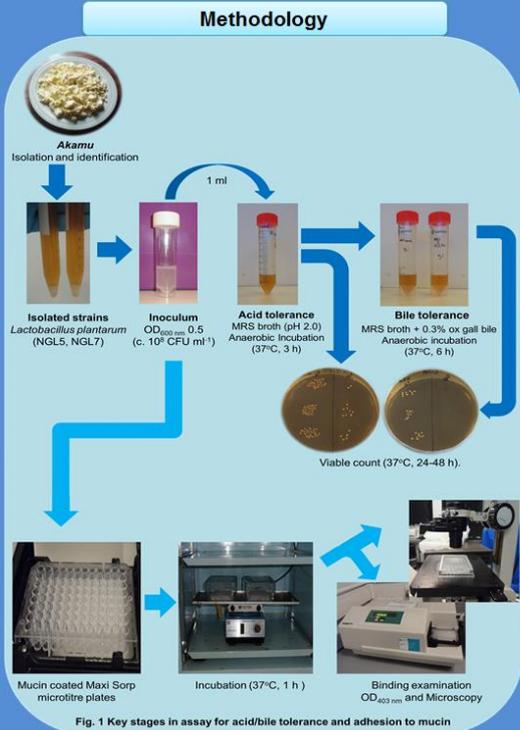
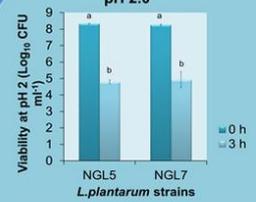


Fig. 1 Key stages in assay for acid/bile tolerance and adhesion to mucin

Results

pH 2.0



0.3% ox gall bile

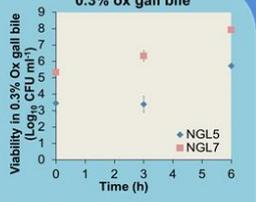
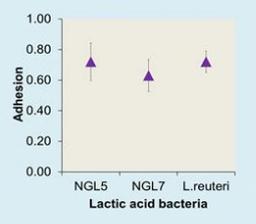


Figure 2 Effect of exposure to acid and bile condition on the viability of two strains of *L. plantarum* isolated from 'akamu' - a Nigerian fermented maize food. (n=3 ± Sd).

There was significant reduction in viability of both strains from 8 to 4 Log CFU ml⁻¹ in the acid condition. However, subsequent incubation in bile environment resulted in significant growth enhancement to 6 and 8 Log CFU ml⁻¹ for NGL5 and NGL7 respectively.

Adhesion



Microscopic images



Figure 3 Adhesion of *L. plantarum* strains to porcine mucin immobilised into microtitre plates. Adhesion = (A_{muc} - A_{lab}) / A_{muc} - OD of Mucin+LAB, A_{muc} - OD of mucin. *L. reuteri* was used as standard. (n=3 ± Sd).

Both *L. plantarum* strains showed high binding ability to porcine mucin with no significant difference to that of *L. reuteri*.

Conclusion

Both *L. plantarum* strains were able to withstand acid and bile conditions and had strong adhesion to porcine mucin. This suggests they may be capable of colonizing the gut.

Further studies

Adhesion of the *L. plantarum* strains to epithelial cell culture.



The research was funded by Rivers State Scholarship Board, Rivers State, Nigeria.

References

- de Vries MC, Vaughan EE, Kleerebezem M, et al. *Lactobacillus plantarum* - survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal* 2006; **16**: 1018-1028
- Kalui CM, Mathara JM, Kutima PM. Probiotic potential of spontaneously fermented cereal based foods - A review. *African Journal of Biotechnology* 2010; **9**: 2490-2498
- Baccigalupi L, Di Donato A, Parlato M, et al. Small surface-associated factors mediate adhesion of a food-isolated strain of *Lactobacillus fermentum* to Caco-2 cells. *Research in Microbiology* 2005; **156**: 830-836
- FAO/WHO Guidelines for the evaluation of probiotics in food, London Ontario, Canada. 2002.
- Roos S, Jonsson H. A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. *Microbiology* 2002; **148**: 433-442

Poster B1.3. In European Marie Curie Sponsored Conference and Master Classes, 10-11th September 2012. Keele University, UK.

Foodborne pathogen inhibition by strains of *Lactobacillus plantarum* and *Candidia tropicalis* isolated from Nigerian maize fermented porridge 'akamu'

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Introduction

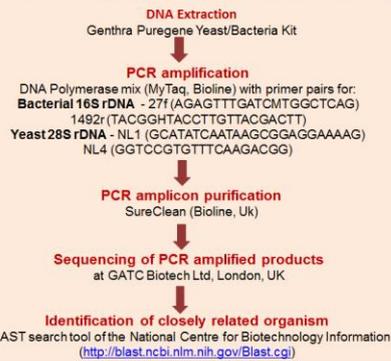
A wide spectrum of microorganisms is involved in the fermentation of Nigerian maize food 'akamu' but a few types of lactic acid bacteria (LAB) and yeasts predominate. LABs ferment carbohydrate producing organic acids with a concomitant decrease in pH which is very crucial in the preservation and safety of the food. Yeasts such as *Sacch. boulardii* exert a protective effect against pathogens through different mechanisms including the production of antimicrobial protease and protein (Tasteyre et al. 2002; van der Aa Kühle et al. 2005). This study investigated the inhibitory potency of two strains of *L. plantarum* (NGL5 and NGL7) and *C. tropicalis* (NGY1) isolated from 'akamu' against *B. cereus* NCIMB 11925, *E. coli* 1077, *L. monocytogenes* NCTC 7973, *S. enteritidis* 5188, and *Staph. aureus* 3750 in comparison to commercial probiotics *L. plantarum* (LpTx) and *Sacch. boulardii* SB20.

Methodology

Isolation of LAB and Yeast from akamu sample

- 10 g of akamu was homogenised in 90 ml of Phosphate Buffered Saline (PBS)
- Serially diluted and spread plated on MRS agar for LAB and RBCA for yeasts
- MRS agar plates were incubated at 37°C for 48 under aerobic and anaerobic conditions while the RBCA were incubated at 25°C for 48 h
- Predominant colonies from plates of higher dilutions were purified by streak plating in same growth media and condition.
- Purified colonies were phenotypically identified using gram stain, catalase reaction and sugar fermentation pattern.

Molecular identification of the LAB and Yeast isolates

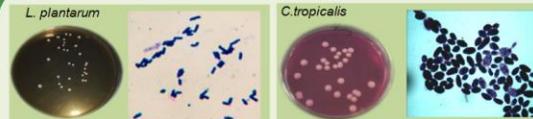


Inhibition assay using agar spot method

- 10 µl of LAB cell suspensions (10⁸ CFU ml⁻¹) were spotted on plates with MRS, MRS with 0.2% glucose (MRS.02), and MRS with 0.2% glucose and without Tween 80 (MRS.02-T80) and incubated anaerobically and anaerobically at 37°C for 24 h. The reduction of glucose, anaerobic incubation and removal of Tween 80 was to check for acid, H₂O₂ and bacteriocin production respectively
- 10 µl of yeasts cell suspensions (10⁷ CFU ml⁻¹) were spotted on Malt extract agar plates and incubated at 25°C for 24 h
- The plates were overlaid with 10 ml of soft Nutrient agar (7.5 g L⁻¹) inoculated with 33 µl of each pathogen cell suspension (10⁸ CFU ml⁻¹) and incubated at the pathogen's optimal growth conditions.
- The inhibition halos average diameter (mm) from 3-4 different angles were obtained using an analogue sliding Vernier Calliper 120 mm (Germany)

Results

Cultural characteristics



Colony and Gram stain appearance of the LAB and yeast isolates

Molecular Identification

Significant sequence alignments for the LAB NCBI Blast

Accession	Description	Max score	Total score
NC_004567.2	<i>Lactobacillus plantarum</i> WCFS1, complete genome	1818	9085
NC_014554.1	<i>Lactobacillus plantarum</i> subsp. <i>plantarum</i> ST-III chromosome, complete genome	1818	9091
NC_012984.1	<i>Lactobacillus plantarum</i> JDM1, complete genome	1818	9091
NC_008497.1	<i>Lactobacillus brevis</i> ATCC 367, complete genome	1620	8086

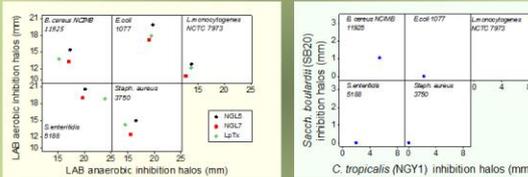


PCR amplified product

Antimicrobial activity



No pathogen inhibition by *C. tropicalis* nor the *L. plantarum* strains in the modified MRS agar for H₂O₂ and bacteriocin production



Diameter of inhibition halos (mm) by *L. plantarum* strains (NGL5, NGL7 & LpTx) and yeasts over foodborne pathogens

Generally, inhibition was significantly ($P \leq 0.05$) greater in the LAB anaerobic growth condition. NGL5 exerted significantly the greatest inhibitory potency over all the pathogens. *S. enteritidis* and *E. coli* were inhibited the most by the LAB while *L. monocytogenes* was the most significantly inhibited by the yeasts. *Sacch. boulardii* exerted more inhibition than *C. tropicalis*. *Staph. aureus* was resistant to both yeasts.

Conclusion

The isolated LAB and yeast were identified as *L. plantarum* and *C. tropicalis* respectively. Probiotic *Sacch. boulardii* SB20 exhibited the maximum inhibition in relation to yeast activity against all pathogens except for *Staph. aureus*, the mechanism is yet unclear. The *L. plantarum* strains exerted significant inhibition against the five relevant foodborne pathogens mainly due to acid production as there was no inhibition observed in the modified MRS agar plates. The result of this study suggests that LAB activities would contribute to the product safety.

Further studies

Evaluation of antimicrobial activities of the *L. plantarum* strains in the food product fermentation

The research was funded by Rivers State Scholarship Board, Rivers State, Nigeria.

References

- Tasteyre, A., Barc, M.-C., Karjalainen, T., Bourlioux, P. and Collignon, A. (2002) Inhibition of in vitro cell adherence of *Clostridium difficile* by *Saccharomyces boulardii*. *Microbial Pathogenesis* 32, 219-225.
- van der Aa Kühle, A., Skovgaard, K. and Jespersen, L. (2005) In vitro screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology* 101, 29-39.



Poster 1.11. In *The 10th Plymouth University Postgraduate Society Conference series for 2012-2013, 21st November 2012*. p.14. Plymouth University, Plymouth, UK.



Inhibitory potency of *Lactobacillus plantarum* strains isolated from Nigerian akamu

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Introduction

Akamu is a lactic acid fermented cereal-based starchy food that constitutes a major infant complementary food in most Africa countries. Uncontrolled fermentation makes *akamu* a variable commodity in terms of safety and nutritional value. Reports have shown that 15-70% diarrhoea episode among infants and children <5 years has been associated with African weaning foods, 25% of which were caused by *E. coli* (Motarjemi et al., 1993; Mensah 1997; Nyatoti et al., 1997). The aim of this study therefore, was to determine whether the safety of *akamu* can be improved by selected LAB inoculant.

Methods

Identification of Lactic acid bacteria (LAB) isolated from *akamu*

- LAB were isolated from *akamu* sample using conventional microbiological method.
- Identity of the isolates were confirmed using polymerase chain reaction (PCR) and sequencing analysis which involved:
 - DNA Extraction
 - PCR amplification and purification
 - Sequencing of PCR products
 - Identification of closely related organism
 - From the BLAST search tool of the National Centre for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>)

Inhibition assay using agar spot method (Majhenić et al. 2007)

- 10 µl of LAB cell suspensions (10^9 Log CFU ml⁻¹) were spotted on MRS and modified MRS agar plates: MRS with 0.2% glucose (MRS.2) and 0.2% glucose and without Tween 80 (MRS.2-T80). The modification was to check for acid, H₂O₂ and bacteriocin production respectively.
- Plates were incubated anaerobically at 37°C for 24 h.
- Thereafter, each of the plates were overlaid with respective pathogen (3.3×10^3 Log CFU ml⁻¹) in 10 ml of soft Nutrient agar
- After 24 h of incubation in the pathogens optimal growth conditions, diameter (mm) of inhibition halos was taken.

Fermentation of ground maize slurry and *E. coli* 1077 exclusion



50 g irradiated ground maize → 18 ml inoculated slurry → Fermentation (28±2°C; 24 h)



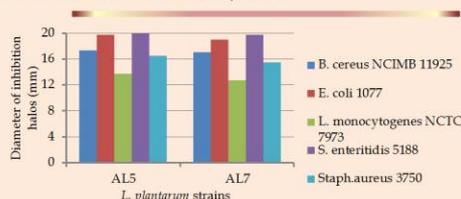
E. coli 1077 inhibition was carried out by

- Co-inoculating *L. plantarum* strain with *E. coli*
- Inoculating *E. coli* into 24 h already fermented ground maize slurry

Results

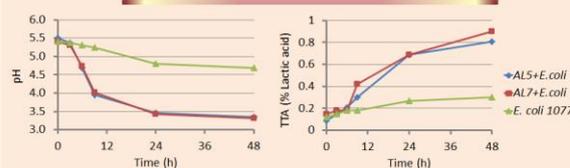
Identity of isolate

- A total of eight lactic acid bacteria (AL1-AL8) were isolated from the *akamu* sample
- Comparison of the nucleotide sequences of isolate AL5 and AL7 to those in the GenBank showed close relation to *L. plantarum* strains with 99% sequences homology
- AL5 and AL7 were considered as strains of *L. plantarum*



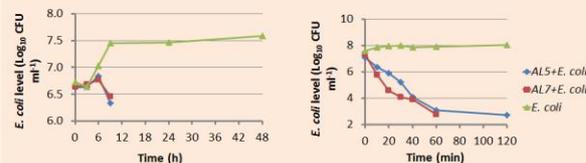
Agar spot pathogen inhibition

- No pathogen inhibition in the modified MRS agar
- Diameter of inhibition halos varied from 12.70±0.35 to 20.20±0.61 mm for *L. monocytogenes* and *S. enteritidis* respectively
- *E. coli* 1077 and *S. enteritidis* were the most significantly inhibited



pH and Titratable acidity of ground maize slurry co-inoculated *E. coli* 1077 (n=3±SD)

- pH and acidity of the test sample was 3.32±0.02 and 0.81±0.09% respectively after 48 h but ≥4.5 and 0.35% in the control



E. coli 1077 level in co-inoculation fermentation

- *E. coli* decreased below detection limit after 12 h and completely inhibited after 24 h, but increased in fermentation without *L. plantarum* strains

E. coli 1077 level in 24 h fermentation

- pH was 5.38 and 5.22 in test and control sample after 24 h when *E. coli* was inoculated
- *E. coli* was not detected after 180 min

Conclusions

This study showed that pathogen inhibition was mainly due to acidity. The *L. plantarum* strains were able to ferment ground maize slurry with pH 5.32±0.02 and acidity ~0.69±0.05 after 24 h. *E. coli* 1077 was completely inhibited in co-inoculated sample after 24 h and 140 min in 24 h *L. plantarum* fermented slurry. The use of the *L. plantarum* strain can therefore contribute towards product safety.



Further studies

Aggregation and adhesion potential of the *L. plantarum* strains

The research was funded by Rivers State Scholarship Board, Rivers State, Nigeria.

References

- Majhenić, A.Č., Lorberg, P.M. and Rogelj, I. (2007) Characterisation of the *Lactobacillus* community in traditional Karst ewe's cheese. *International Journal of Dairy Technology* **60**, 182-190.
- Mensah, P. (1997) Fermentation - The key to food safety assurance in Africa? *Food Control* **8** 271-278.
- Motarjemi, Y., Kafarstein, F., Moy, G. and Quevedo, F. (1993) Contaminated weaning food: a major risk factor for diarrhoea and associated malnutrition. *Bulletin of the World Health Organization* **71**, 79-92.
- Nyatoti, V.N., Mtero, S.S. and Rukure, G. (1997) Pathogenic *Escherichia coli* in traditional African weaning foods. *Food Control* **8**, 51-54.

Poster P6. In Centre for Agricultural and Rural Sustainability Annual Conference, 19th June, 2013. p.30. Parsons Building, Duchy College, Stoke Climsland.



Probiotic potential of Nigerian fermented maize-*Lactobacillus plantarum*

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Introduction

Interest in development of food products containing beneficial *Lactobacilli*, has led to the characterisation of non-intestinal isolates for possible health promoting benefits. To exert health-enhancing benefits, probiotics must possess certain characteristics (1). This study was aimed at investigating the potential of *Lactobacillus plantarum* strains isolated from Nigerian fermented maize food - akamuto withstand acid and bile, to aggregate and adhere to epithelial cells.

Methods

Identification and selection of lactic acid bacteria (LAB)

- LAB were isolated from akamuto sample using conventional microbiological methods.
- Identity of the isolates were confirmed using polymerase chain reaction (PCR) and commercial sequencing as follows:
 - DNA extraction with Gentra Puregene Yeast/Bacteria Kit.
 - PCR amplification using DNA Polymerase mix (MyTaq, Bioline, UK) with primer pairs: 27f (AGAGTTTGATCMTGGCTCAG) and 1492r (TACGGHACCTGTTACGACTT) for the Bacterial 16S rDNA.
 - Purification of PCR amplified products using SureClean (Bioline, UK).
 - Sequencing of PCR amplified products at GATC Biotech Ltd, London, UK.
 - Identification of closely related organism using the BLAST search tool of the National Centre for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).
- The LAB were screened based on fermentation studies that were set up at 30°C for 24 h by inoculating 150 mL batches of sterile ground maize slurry (1.2 w/v) with 1 mL of washed cell suspensions. Characterisation was based on pH, titratable acidity and organic acid profiles.

Acid and bile tolerance Assay

- A 24 h colony of the *Lb. plantarum* strains from MRS agar were grown in MRS broth for 24 h.
- Cells were harvested by centrifugation (Rotina 46, Hettich, Germany) at 4000 g for 10 min, washed twice in Phosphate buffered saline (PBS) and re-suspended in PBS.
- 1 mL of the cell suspension ($c.10^8$ CFU mL⁻¹) was inoculated into 9 mL of modified MRS broth (pH 2.0 with 5 M HCl) and incubated anaerobically (AnaeroGen AN0025A, Oxoid, UK) at 37°C for 3 h.
- The acid stressed cells were washed with PBS, re-suspended in 10 mL MRS broth with or without 0.3% ox gall bile (Oxoid, UK) and incubated anaerobically at 37°C for 6 h.
- The initial and viable counts after the respective incubation periods were enumerated by plating out serial dilutions on MRS agar plates with incubation at 37°C for 24 h.

Aggregation Assay

- Auto-aggregation: 4 mL of washed bacterial cell suspension were centrifuged (6000 g for 10 min at 4°C), re-suspended in same volume of their culture supernatant diluents and incubated at 37°C.
- Co-aggregation: 2 mL of the LAB and pathogen (*E. coli* 1077 and *S. enteritidis* 5188) were mixed and incubated at 37°C without agitation.
- Absorbance (A_{600}) of 1 mL of upper suspension after 5 h was measured against same volume of PBS as blank.
- Auto-aggregation (%) = $1 - (A_{600}/A_{600}^{initial}) \times 100$; Co-aggregation (%) = $\frac{[(A_p + A_m)/2] - A_{(p+m)}}{(A_p + A_m)/2} \times 100$
- Where: A_p , A_m and $A_{(p+m)}$ represent the absorbance of the pathogen, LAB and mixture of pathogen and LAB respectively.

Adhesion to Caco-2 cells

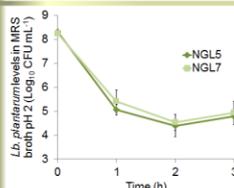
- Caco-2 monolayers were prepared in six-well tissue culture plates using the method of Xie et al., (2)
- Adhesion assay was performed following the method by Maragkoudakis et al., (3). Briefly:
 - 100 μ L of bacterial cell suspension ($c.10^8$ CFU mL⁻¹) was added to the washed Caco-2 monolayers and incubated for 1 h at 37°C in 5% CO₂.
 - Wells were aspirated and the monolayers washed 6 times with PBS to release unbound bacteria.
 - Adhered bacterial cells were lifted with 1 mL of 1% Triton X-100 (BDH, England) into 9 mL of PBS and enumerated using the drop method of Mile & Misra (4)

Results

Genomic identity and fermentation ability of the LAB isolates

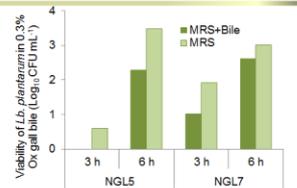
LAB codes	Genomic ID	Sequence homology (%)
NGL1, NGL3 & NGL8	<i>Lb. helveticus</i>	92.95 & 98
NGL2 & NGL6	<i>Lb. acidophilus</i>	97 & 100
NGL4	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	99
NGL5 & NGL7	<i>Lb. plantarum</i>	99

- The *Lb. plantarum* strains NGL5 & NGL7 demonstrated fermentation ability with significant ($p \leq 0.05$) decrease in pH from ≥ 6.05 to ≤ 3.63 and lactic acid levels ≤ 80.78 mmol L⁻¹ after 24 h. Others had pH ≥ 5.54 with none detectable level of lactic acid except for NGL4 with pH of 4.40 and lactic acid level of 7.92 mmol L⁻¹.
- The *Lb. plantarum* strains were therefore selected for probiotic potential assays.



Levels of *Lb. plantarum* strains (NGL5 & NGL7) in MRS broth at pH 2 (n=3±SD).

- Although, there was significant reduction in the levels of both *Lb. plantarum* strains at pH 2, viable counts ≥ 4.80 Log₁₀ CFU mL⁻¹ were obtained after 3 h.



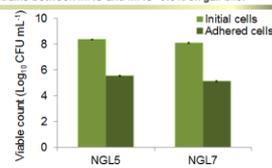
Viability of the *Lb. plantarum* strains in MRS and MRS+0.3% ox gall bile (n=3±SD).

- NGL5 cells did not show quick recovery after 3 h.
- Growth was enhanced by 2.28 and 2.61 Log₁₀ CFU mL⁻¹ for NGL5 and NGL7 respectively after 6 h.
- No significant ($P \leq 0.05$) differences in the viability of both strains between MRS and MRS+0.3% ox gall bile.

Aggregation capabilities of *Lb. plantarum* strains (n=3 ± SD after 5 h)

<i>Lb. plantarum</i> strains	Auto-aggregation (%)	Co-aggregation with pathogens (%)
NGL5	29.61±0.11	-1.15±0.44 3.21±0.29
NGL7	24.96±0.36	2.12±1.27 1.20±0.32

- The *Lb. plantarum* strains were able to auto-aggregate ($\geq 25\%$) but had no co-aggregation ($< 4\%$) with the pathogens.
- Auto-aggregation provides greater mass necessary for exertion of certain health benefits and may aid the prevention of pathogen colonization in the gut.



Adhesion of *Lb. plantarum* strains on Caco-2 cells

- More than 60% (≥ 5 Log CFU mL⁻¹) of the initial Log count (8 Log CFU mL⁻¹) adhered to the Caco-2 cells suggesting the ability of the *Lb. plantarum* strains to utilize the cells binding sites.

Conclusions

This study revealed that the *Lb. plantarum* strains were able to withstand acid and bile conditions, auto-aggregated and adhered to Caco-2 cells. This suggested that the *Lb. plantarum* strains may be able to survive passage through the gastrointestinal track (GIT) and colonize the gut which may have beneficial health effects.

Further studies

Adhesion to intestinal mucosa, gelatinase and haemolytic activities.

Acknowledgement

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References

- FAO/WHO (2002) *Guidelines for the evaluation of probiotics in food*. London Ontario, Canada.
- Maragkoudakis, P.A., Zoumpopoulou, G., Miaris, C., Kalantzopoulos, G., Pot, B. and Tsakalidou, E. (2006) Probiotic potential of *Lactobacillus* strains isolated from dairy products. *International Dairy Journal* **16**, 189-199.
- Xie, N., Zhou, T. and Li, B. (2012) Kefir yeasts enhance probiotic potentials of *Lactobacillus paracasei* H9: The positive effects of coaggregation between the two strains. *Food Research International* **45**, 394-401.
- Miles, A.A. and S.S. Misra, *The estimation of the bactericidal power of the blood*. *Journal of Hygiene*, 1938, **38**, p. 732-49.

Poster 5. In *Society for Applied Microbiology, Summer Conference 1-4 July 2013* p.25. The Hilton, Cardiff, UK.

ORAL PRESENTATIONS' ABSTRACTS

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Isolation and identification of lactic acid bacteria and yeast involved in the spontaneous fermentation of *Akamu* and their screening for suitability as starter cultures in controlled fermentation

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Abstract

Aims

To identify predominant lactic acid bacteria (LAB) and yeast present in spontaneously fermented starchy maize food (*Akamu*) and screen for suitability in controlled fermentation of maize flour.

Methods and results

Appropriate dilutions of *Akamu* sample was plated on de Man, Rogosa and Sharpe (MRS) agar and Rose Bengal Chloramphenicol Agar (RBCA) for isolation of LAB and yeast respectively. Identification was based on morphological and phenotypic characteristics. Fermentation of maize flour (1:2 w/v) at 28±2 °C for 72 h was with one millilitre of microbial inocula (c.10⁶ and 10⁸ CFU ml⁻¹ for yeast and LAB). LAB: *Lactobacillus Jensenii*, *Lact. acidophilus*, *Lact. helveticus*, *Leuconostoc mesenteroids*, *Lact. plantarum*, and yeasts: *Saccharomyces cerevisiae*, *Candidia tropicalis*, *Zygosaccharomyces lactis* and *Candidia mecedomiensis* were identified. One isolate of *Lact. plantarum* had significantly (p<0.05) lower pH (6.18±0.01- 3.27±0.02) and highest rate of lactic acid production (80.16 mM L⁻¹ d⁻¹) within 24 h of fermentation. pH values of yeast isolate samples differed significantly. *Sacch. cerevisiae* had significantly highest percentage acidity of 0.50±0.00 at 24 h.

Conclusions

This study revealed a microbial population dominated by LAB and yeasts. *Lact. plantarum* had favourable spectra of end product during the fermentation of the sterile maize flour

Significance of study

Lact. plantarum has presumably high potential as starter culture and should be subjected to further studies to establish safety and probiotic potentials.

Antimicrobial activity of selected Nigerian *akamu*-*Lactobacillus plantarum*

P.C. Obinna-Echem

Abstract

Akamu is a lactic acid fermented cereal-based food that constitutes a major infant complementary food in most Africa countries. Uncontrolled fermentation makes *akamu* a variable commodity in terms of quality and safety; hence this study was aimed at determining whether the safety of *akamu* can be improved by fermentation with selected microbial inoculant. The microbial community of traditional *akamu* samples were investigated using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. Conventionally isolated lactic acid bacteria: *Lactobacillus helveticus* (NGL1, NGL3 and NGL8), *L. acidophilus* (NGL2 and NGL6), *Lactococcus lactis* subsp. *lactis* (NGL4) and *L. plantarum* (NGL5 and NGL7) were characterised based on fermentation abilities. Fermentation studies were set up at 30°C for 72 h by inoculating 150 mL batches of sterile ground maize slurry (1:2 w/v) with 1 mL of washed cell suspensions ($c.10^8$ CFU mL⁻¹). The two *L. plantarum* strains produced significantly ($p \leq 0.05$) higher lactic acid levels >73 mmol L⁻¹ and low pH ≤ 3.63 than the others after 24 h. Antimicrobial activity of the *L. plantarum* strains was against *Salmonella enteritidis* 5188, *Escherichia coli* 1077, *Bacillus cereus* NCIMB 11925, *Staphylococcus aureus* 3750 and *Listeria monocytogenes* NCTC 7973) using an agar spot assay and against *E. coli* 1077 in ground maize slurry fermented at 22 and 30°C. There was no inhibition in modified MRS agar: 0.2% glucose and 0.2% glucose without Tween 80. Inhibition halos in MRS agar varied from 10.6 ± 0.2 to 23.9 ± 0.1 mm. Decrease in *E. coli* levels in fermentation correlated with decrease in pH and increase in acidity. *E. coli* was inhibited after 180 min and 24 h in the 24 h *L. plantarum* fermented slurry and the co-inoculation assay respectively. This study revealed the common occurrence of *L. fermentum*, *L. plantarum*, and *L. delbrueckii* subsp. *bulgaricus* and *L. helveticus* in the *akamu* LAB population. *L. plantarum* strains had favourable spectra of fermentation end product after 24 h and their antimicrobial activity was mainly due to acidity, suggesting that the use of the *L. plantarum* strain can contribute towards product safety.

Keywords: *Akamu*, *L. plantarum*, fermentation, antimicrobial activity, foodborne pathogens

JOURNAL PUBLICATION

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