STRESS RESPONSES OF LACTIC ACID BACTERIA AND YEASTS ISOLATEDFROM SORGHUM GRUEL AND RETTED CASSAVA AND THEIR APPLICATION IN FOOD FERMENTATION

BY

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ABSTRACT

Lactic Acid Bacteria (LAB) and yeasts are exposed to constant physical and chemical fluctuations in food matrix during fermentation, resulting in stresswhich often reduce the quality of food products. Microorganisms are known to develop adaptive responses to harsh fluctuations. However, these adaptive mechanisms are rarely investigated. This study was designed to investigate the physiological and proteomic responses of LAB and yeasts to acid and oxidative stresses as well as the application of stress-adapted LAB and yeasts in food fermentation.

Lactic acid bacteria and yeasts were isolated and identified from sorghum gruel and rettted cassava using standard methods. The physiological responses of identified LAB and yeasts to acidic, hydrogen peroxide (H_2O_2) stresses and control were measured using turbidimetry method. Proteins (dehydrogenase and stress response proteins) extracted from the LAB and yeasts were separated using SDS-PAGE and two-dimensional gel electrophoresis. Induced and repressed proteins were identified by LC-MS. The best stress-adapted LAB and yeasts were selected as starters in single and in combination for the fermentation of sorghum gruel and retted cassava while spontaneous fermentation served as control. Chemical composition (proximate, mineral, anti-nutritional) and sensory properties of the products were evaluated using standard procedures. Data were subjected to descriptive statistics and ANOVA at $\alpha_{0.05}$.

Sixty-four LAB and seventy-two yeasts isolated from sorghum gruel and retted cassava were identified as *Lactobacillus amylovorus* (13), *L. acidophilus* (8), *L. fermentum* (5), *Pediococcus pentasaceus* (6), *L. plantarum* (11), *L. brevis* (2), *L. paracasei* (7), *Leuconostoc pseudomesenteroids* (2), *Enterococcus faecalis* (10)*, Candida kefyr* (12), *C. glabrata* (19), *C. tropicalis* (12) and *Saccharomyces cerevisiae* (29). The optical-density of *Lactobacillus amylovorus*LS07 (0.235) and *C. kefyr*YS12 (0.367) from sorghum gruel adapted best to pH 1 and to 5mM H_2O_2 0.32 and 0.737 respectively. The optical-density of *Lactobacillus plantarum*LC03 (0.659)and *C. glabrata*YC02 (0.967) from retted cassava had best adaptation at pH 4 andto1mM H_2O_2 0.986 and 0.868 respectively. Bifunctional acetaldehyde CoA/alcohol dehydrogenase showed increased intensity at pH

4 and 5mM H_2O_2 in*L. amylovorus*LS07, while increased intensity of 6-phosphogluconate dehydrogenase was detected in *C. kefyr*YS12 at pH 4 and 1mM H_2O_2. *Lactobacillus plantarum*LC03 showed increased intensity of elongation factor thermo unstable at pH 3 and 5mM H_2O_2. Increased intensity of enolase was observed in *C. glabrata*YC02atpH 4. These suggested increased microbial metabolism, which reduced stress encountered. Sorghum gruel produced with combined starters of *L. amylovorus*LS07 and *C. kefyr*YS12 had the highest crude protein (10.94%) and iron (85.50ppm). Cassava fermented with combined starters of *L. plantarum*LC03and *C. glabrata*YC02 recorded the lowest tannin (0.0007%), phytate (0.0078%), alkaloids (0.14%), cyanide (6.49ppm) and highest overall acceptability (7.92) which were significantly different from values obtained from spontaneous fermentation: 0.0018%, 0.0093%, 0.17%, 7.11ppm, 7.13, respectively. The combined starters yielded foods with improved sensory properties, mineral and reduced anti-nutrient contents.

Isolates from sorghum gruel (*Lactobacillus amylovorus*LS07 and *Candida kefyr*YS12) and retted cassava (*L. plantarum*LC03and *C. glabrata*YC02) showed increased protein production in the presence of acid and oxidative stress. Hence, the stress-adapted organisms as starters are encouraged in food fermentation.

Keywords: Stress adaptation, Induced and repressed enzymes, *Lactobacillus amylovorus*LS07, *Candida kefyr*YS12 and SDS-PAGE

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CERTIFICATION

This is to certify that this work was carried out by Oluwaseun Adeola, Adewara (Matriculation number: 152728) under supervision in the Department of Microbiology, University of Ibadan, Ibadan. Nigeria.

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DEDICATION

To my family, Mr Adesanya Folorunso and Master Oluwamayomipo and Miss Oluwamayomipe Folorunso; and to my mother and father, Mr. and Mrs. Sunday Adewara and siblings

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CHAPTER ONE

INTRODUCTION

1.1 Study background

Foods from plants and animal sources undergo fermentation by microbesfor example lactic acid bacteria (LAB) and yeasts yielding fermented foodswhich have experienced desirable biochemical changes thereby causing significant modifications of the foods (Cambell-Platt, 1987; Chamberlain *et al*., 1997; Adeleke *et al.,* 2010). In production of fermented products, the transformation of sugars to alcohol and CO_2 or acids employing bacteria and/otherwise yeast without oxygen is described as fermentation (William and Dennis, 2011). The oldest method to preserve foods is through fermentation. LAB and yeasts are most commonly accountable for fermenting these food materials (Adeleke *et al.,* 2010).

Generally, microbes must be able to persistseries of stresses inflictedwithin the environment in the course of food production and storage before it can start to perform its bioticfunction of enacting survival (Susan *et al.,* 2011). 'Stress' can be termed as all environmental changes that induce an adaptive reaction. The general requirement for the continued existence and growth of microorganisms depends on the ability to acclimatize to fluctuations in internal and external cellular conditions. The presence of molecular processes of responding, mending and acclimatizing, a lot of of these responses are significantlymaintainedthroughthe natural world, gives the cell the resilence it needs to adapt to its constantly-fluctuatingenvirons, a homeostatic occurrenceknown as stress response (Estruch, 2000).

Temperature extremes, oxidative stress, osmotic, and food matrix stresses are stress conditions experienced during food processing (Susan *et al.,* 2011). Microbes are in nature endowed with plenitude of defense edifices to increase continued existence to harsh environment (De Angelis and Gobbetti, 2004; van Schaik and Abee, 2005; Sugimoto*et al.,* 2008). These defense mechanisms consist of chaperone proteins proteases, systems transportation, enzymes that defend against Reactive Oxygen Species (ROS), proton pumps, decarboxylases and transporters (Corcoran *et al.,* 2008).

Transcription modifications in the characteristic groups of genetic materialwhile responding todiverse stresses are termed as general stress response (Ruis and Schuller, 1995). During general stress responses, about 10% of the genome – wide transcriptional profiling are induced or repressed. Variation in cell roles for example,fold up and breakdown of protein,transference and breakdown of sugars carried out by induced Environmental Stress Response (ESR) genes while the repressed ESR genes are connected with methods associated with development of cell, including break down of RNA, production of nucleotide, secretion and functionality of ribosomes (Gasch, 2002).

Various genes that take part in the environmental stress response imply that the programme defends and retains mixed and different kinds of cellular system in danger in times of stress. Interestingly, many of the genetic material activated during environmental stress response are simply needed for the continued existence of the cell at definite situations, regardless of their usual induction when subjected to diverse stressful environments (Gasch, 2002; Giaever *et al*., 2002).

Responses and remodeling of bacteria to environmental stresses has gained much progress through the significant insights provided from significant researches in proteomics. Remarkably, researches that utilize these methods constantly emphasize the significance of the primary molecular defense processes, uncover stress-related supervisory networks, and yet give more understanding into new systems that work to defend and secure stressed cells thereby stating novel molecular tools for improving probiotics (Champomier-Verges *et al.,* 2002). Kleerebezem*et al.* (2002)affirmed that the best requirements intended for growth and survival of bacteria is scarce when utilized in industries or during fermentation and these procedures can be a source of stress encountered.

Therefore, it is imperative to choose strains which are able to function properly and can tolerate harsh situations which arise in the course of fermentation (Sanders*et al.,* 1998). Hence, this present research was focused on investigating the responses of LAB and yeasts isolated from sorghum gruel (*Ogi)* and fermented cassava (*Gari*) to acid and oxidative

stress. As part of this study, investigations on the proximate composition, nutritional content and sensory attributes of *Ogi* and fermented cassava using selected stress-adapted LAB and yeast was conducted. The underlying hypothesis of this research was that the LAB and yeasts used in this researchelicit distinctive responses as important role in stress adaptation (response to acid and oxidative stress).

1.2. Statement of problem

In the evolution of human cuisine, fermentation process occupies a special place by improving the quality of food produced in addition to prolonging the storage time. Since age-old times, foods produced through fermentation with LAB as well as yeasts form an importantform of human nutrition. Owing to the industrialization in food transformation, the economic importance of these microorganisms in fermentation has increased and they perform a vital function towards the enhancement of sensory and nutrient of fermented products. During processing and storage of fermented foods, LAB and yeastsexperience harsh environmental conditions. These conditions are intended to be harmful tomicrobes that spoil food itemsand microbes that cause disease on food materials.Fermentation changes food characteristics and bring about potential variations within the food environs, and concurrently, serves as a source of stress for the microbes implicated. Actually, each and every time indigenous LAB and yeasts are adjusted and viable in their own environs, the surroundings becomes stressful to the organism. Hence, reduction in microbial viability, reproducibility, organoleptic and fermentative qualities due to exposure of LAB and yeasts to several environmental stress conditions. Fluctuations in temperature, pH, osmotic pressure as well as nutrient availability and oxidation gives rise to inconsistencies in producing fermented products**.**

1.3. Justification for this study

It is important to understand the terms, which are favorable or harmful to the existence of LAB and yeasts, as well as what mechanisms permit their survival and metabolic activities.

In past times, enormous research and study to increase our understanding on the environmental stresses encountered by LAB and yeasts have been carried out. Regardless the habitation of these organisms, they are open to continuous variations in their own environment. As a result, they have settled multifaceted reactions, controlled by the creation of protein complexes, phosphorylation of contingent message transduction approach so as to adjust and continue to exist in the midst of the different stress conditions. Therefore, to ensure continued existence in adverse environment, LAB and yeasts may become accustomed to variations in their present environs by reacting to the inflicted stress. These reactions are diverse and are subject to nature of the microorganisms as well as the stress condition within the environment. Modifications in gene expression especially for those genes whose outcome are necessary to prevent the harmful effectis needful. Studies on successful gene expressionoffers a start off point for comprehending the means by which microorganisms tolerate environmental conditions. Classification of the single genes whose expression is impacted in various conditions will provide a useful understanding of the functions performed by the gene products. Furthermore, a comprehensive knowledge of stress response techniques utilized by LAB and yeasts to survive various stress conditions is of great importance. This may be further employed in making strains resistant and able to grow and survive in the food complexesresulting in improved quality of products.

1.4. Aim of this study

To produce*Ogi*and *Gari* using stress-adapted LAB and yeast strains with improved mineral content, reduced anti-nutrients and enhanced organoleptic properties.

1.5. The specific objectives of this reseach therefore were to:

1. isolate and identify LAB and yeasts from *Ogi* and fermented cassava using convectional and molecular methods.
2. determine the responses (physiological and proteomic) of identified LAB and yeasts subjected to acid and oxidative stress conditions.
3. evaluate the proximate, mineral, anti-nutritional composition, and organoleptic properties of selected stress-adapted LAB and yeast in the production of *Ogi*and *Gari*.

1.6. Research questions

1. What proteins are expressed or repressed during acid and oxidative stress in LAB and yeast isolated from *Ogi* and fermented cassava?
2. How does the use of stress-adapted LAB and yeasts as starter cultures affect the proximate, mineral, anti-nutritional and organoleptic properties of *Ogi* and fermented cassava?

CHAPTER TWO

LITERATURE REVIEW

2.1. History of fermentation

The oldest method of preserving perishable foods which precedes written history is the intentional fermentation of foods by man. According to documentation, fermented foods have been consumed 70 centuries back in Babylon (Battock and Aza-Ali, 1998). There has existed a close relationship amid the human existence, his food and the actions of fermentation of microbes as long as human civilization. The act of fermentation is required in producing fermented products, known as food and beverages exposed to the influence of microbes to produce desired biological and chemical modifications. The microbes accountable for the activities of fermentation may possibly be microrganisms currently inherent on the food material, or could be integrated as starters (Harlander, 1992).

In the olden times, fermented food products formed a vital part of human diet and were mostly produced locally or in small scale industries. A number of these fermented foods are produced at higher dimensions after going through some industrial development (Wood, 1998; Boekhout *et al*., 2003; Clark and Plotka, 2004). Previously, there existed no proven records as regards the efficient, nutritive, scientific as well as quality assurance consequences of locally fermented foods. Nonetheless, about 26 years ago, reports that address issues regarding the autochthonous fermented produce in the regions of the World have risen expeditiously (Steinkraus *et al*., 1993).

All across the globe, food products produced via fermentation are indispensible parts of diets, especially in Africa (Odunfa, 1985). While producing fermented foods, fruits, vegetables, cereals, root crops, legumes and oilseeds, microbes involved in fermentation are utilized in producing these foods. Most developing countries employ fermentation of food material asa highly efficient technique of making and conserving fermented productssince ancient times (Hamad and Fields, 1979; David and Aderibigbe, 2010)

2.2. Food fermentation

The production of local fermented products lingers as a domestic technique and is produced at small scale levels. However, the production of many other fermented foods has been developed through the use of biotechnology and most fermented foods are being produced on a larger commercialized level (Bol and Vos, 1997).Fermented food products are commonly produced with plants and animal materials in association with microbes within the environs, or in cultures maintained and preserved earlier by human effort. Microbes involved in fermentation are universal; clearly, these inhabiting microbes are found everywhere (Chamberlain *et al*., 1997).

The process in which microbes facilitate transformation of sugars into alcohol and lactic acid is known as fermentation (Soni and Sandhu, 1990). The consumption of fermented foods by humans frequently set in microflora that occupy and populate the body of the consumers (Picard *et al.,* 2005).

2.2.1. Functions of food fermentation

The primitive production of food through fermentation has a number of functions including improvement of fare resulting in the enhancement of flavour, savour, improving the quality of consistency in food materials, protection and enhancing food storage through lactic and acetic acid fermentation, alcohol fermentation, alkaline fermentation, augmentationas well asimprovement of diet, enhancing edibility and obtainability of important nutrients, lessened composition of food anti-nutrient, in addition to a significant drop in food preparation time and energy (Steinkraus, 1995).

2.2.1.1. Improved nutritional value and digestibility in food

A vital difference between fermented and unfermented foods is in their superiority in nutritional value and digestibility. Cereal fermentation gives rise to products with better-quality of protein, particularly the availability of amino acid, for example, lysine (Hamad and Fields, 1979). Improved sensory qualities as a result offlavourdevelopment in

varoiusfermented foods is also an important advantage of fermentation (Khertarpaul and Chauhan, 1993).Furthermore, as a result of the production of antibiotic-like substances, hydrogen peroxide, organic acids and decrease in the oxidation-reduction, the growth of potential harmful and dangerous microbes are prevented (Nout, 1994).

2.2.1.2. Reduction of anti-nutrients in food

Reduction of anti-nutrients and production of important nutrients during fermentation has been noted. Due to the utilization of food energy by the fermenting microbes in the process of fermentation, the foods become unsuitable for the inhabitation of spoilage microbes. For example, in producing *Pickling*, major acids released by the dominant microbes during fermentation impede the development of damaging and diseases-causing microbes (Scheinberg *et al.,* 2013). The process of fermentation improves food edibility either throughtransformation of certain food constituents or by predigesting the foods materials. In the same vein, there are some foods that contain some amounts of poisonous compounds for example cassava, which are then possibly transformed to safe products for consumption through fermentation. Similary, a number of coffee beans are peeled through moistened fermentation, rather than through the unmoistened procedure (Battcock and Aza-Ali, 1998).

2.2.1.3. Provides health benefits

Fermented foods are being appreciated around the world and it provides healthy advantages. Fermentation changes food flavour from ordinary to an enjoyable mouth-sourness boosted by fermenting bacteria alongside increase in nutrients. Research investigation have shown that certain LAB dominant in a number of fermented foods, for example *Ogi* and *Kunun-zaki*, cereal fermented foods, may allow the habitation of these microbes in the vagina, consequently eradicating disease causing agents, as well as diminishing the risks of infection (Cadieux *et al.,* 2002). Possible medicinal advantages of consuming *Ogi* include the stimulatory effect of the immune system as a result of the presence of important microbes in the gastrointestinal tract (GIT) which in order words suppresses the development of diseases causing agents. The ability of LAB to proliferate the GIT or the vagina lessens bacterial infection which reduces the rate of contacting HIV (Reid, 2002). This results in reduction of

incidences associated with sexually transmitted diseases (Reid *et al.,* 2001) as well as diaarrhoea (Adebolu *et al.,* 2007).

2.2.2. Types of fermentation

Fermented produce are made worldwide by employing a number ofindustrialmethods, unprocessedresources and microbes. There are different fermentation processes which included the lactic acid, alkali, alcoholic and acetic acid fermentation processes (Soni and Sandhu, 1990). Lactic acid fermentation is majorly brought about by LAB for instance in milk and cereal grains fermentations. Lactic acid fermentation promotes the safekeeping, nutritive worth, storage period as well as appropriateness of a comprehensive diversity of cereal centered foods (Oyewole, 1997). Alkali fermentation usually occurs during the fermentation of food products such as fish and seeds where amino acids and ammonia are produced from protein hydrolysis. Furthermore, during alcoholic fermentation, the end products include ethanol and the predominant organisms at this stage are yeasts while the acetic acid producers which are also important in food fermentation, in the presence of excess oxygen, usually tranforms alcohol to acetic acid (for example the *Acetobacter* species) (McKay and Baldwin, 1990) .

2.2.3. Microorgansims involved in fermentation

The study of microorganisms infermented food produce is complex and broad. Fermentation of these foods is biological and encompasses the effect and co existence of all these microbes symbiotically (Sieuwerts *et al.,* 2008). During the course of fermentation, a number of microbes partake equally whereas others perform in succession with the fluctuating domineering ecosystem (Li *et al.,* 2017). The normal microorganisms that carry out fermentation include species of *Lactobacillus, Bacillus* and yeast. The most frequently occurring yeast during most fermentation is the species of *Saccharomyces*, which produces alcohol as its major end product (Steinkraus, 1998).

2.2.4. Factors affecting microorganisms during fermentation

The diverse groups of bacteria dominant in individual fermented foods are influenced by both biotic and abiotic factors (salt molarity, water activity, acidity, temperature, and the

constituents of the food environment) (Charoenchai *et al.,* 1998). Majority of foods produced by fermentation includingproductscommonly consumed in the West, in additionto lots ofthe products originating frompreviousoriginwhich are less typified appropriately rely onLAB to carry outfermentation (Conway, 1996).

2.3. Lactic acid bacteria

Aguirre and Collins (1993) defined lactic acid bacteria as a wide and large set of bacteria that do not produce spores, could be rod or/and cocci shaped, gram-positive, with no cell motility, catalase-negative microbes which break down starchto produce lactic acid at the end of fermentation. LAB can be classified into two classes based ontheir metabolism of hexoses, namely, homofermentative and heterofermentative LAB. Homofermentative LAB breaks down sugars to produce mainly lactic acid whereas heterofermenters produce lactic acid, ethanol and CO_2 during fermentation (Aguirre and Collins, 1993).

2.3.1. Classification of lactic acid bacteria (LAB)

Lactic acid bacteria are made up of a totality of genera enclosed in Phyllum Firmicutes including genera *Lactobacillus, Carbonobacterium, Lactococcus, Lactosphaera, Millisococcus, Pediococcus, Leuconostoc, Streptoccus, Vagococcus, Enterococcus and Weisella, Tetragenococcus and Oenococcus*(Jay, 2000; Holzapfel *et al.,* 2001).

Classification of LAB basedon sequencing of 16S ribosomal RNA showed that a few classificationsarebased on physical make-up and not related genetically. Some techniques such as molecular procedures, particularly the techniques hinged on polymerase chain reaction, in addition to pulse-field gel electrophoresis, may be considered essential in carrying out precise classification and identification of LAB strains (Gevers *et al.,* 2001).

In recent times, methodologies that are culture-independent were utilized. This is aimed at discovering intestinal microbiota (Zoetendal *et al.,* 2002). Other approaches such as the temperature and denaturating gradient gel electrophoresis employed in examining the 16S ribosomal DNA gene and amplicons in feacal samples presented strong and effective

methods in ascertaining as well as studying the faecal sample of bacterial population (Zoetendal *et al.*, 1998).

2.3.2. Occurrence of LAB in food fermentation

LABhave been isolated from many fermented foods (Caplice and Fitzgerald, 1999; Lonvaud-Funel, 2001; Lui, 2003; Milanowski *et al.,* 2017). Wang *et al.* (2016)studied the characterization of LAB from typicaldairy products. The largest taxonomic group reported was *Lactococcus lactis* subsp. *lactis* with the percentage occurrence of 32%, while the second most frequent species was *L. plantarum*, consisting of 12.3% of the total isolates, followed by *Leuc. mesenteroides* comprising for 11.33% of all the isolates.

LAB occurs inherently in fermented fares (Caprice and Fitzgerald, 1999; Angelov *et al*., 2017) as well as in soils (Ekundayo, 2014; Lavanya and Dayakar, 2017), water, manure (Idham *et al.,* 2016) and sewage (Holzapfel *et al.,* 2001). LAB have also been found and isolated in humans (Eidaman and Szilagyi, 1979; Martins *et al.,* 2003),also in animals (Gilliland *et al*., 1975; Schrezenmeir and de Vrese, 2001).

2.3.3. Activities of LAB in food fermentation

Lactic acid bacteria bring about decomposition of foods for example meat, beverage and fish which may eventually spoil the food product (Jay, 2000; Lui, 2003). On the other hand, LAB can also be employ toflavour and texturize some food materials, for preservation of foods, and as starter cultures in food fermention (Caprice and Fitzgerald, 1999).*Lactobacillus lactis* and *Streptococcus thermophiles* hinder the activities of damaging and infectious bacteria in food in addition to maintaining the beneficial potentials of foodstuff materials thereby extending the average storage period of fermented products (Heller, 2001).

Production of organic acids (for example lactic acid) by LABreduces the acidity of their growth environment, thereby enabling themcarry out their antimicrobial activity (Caplice and Fitzgerald, 1999). Acidic fermentation convertscarbon-based acids into lipids that are soluble, which afterwards disperse effortlessly into the cytoplasm across the cell membrane

(Gottschalk, 1998). Some compounds such as acetaldehyde, diacetyl, hydrogen peroxide, bacteriocins polysaccharides and carbon dioxide are produced efficiently during fermentationand may act as antimicrobial agents (Caprice and Fitzgerald, 1999; Rodrigues *et al.,* 2003).

2.3.4. Nutritional and beneficial traits of LAB

Through the past century, there has been a repeated attention placed on researching the nutritional and beneficial traits of LAB and their end products. Lactic acid bacteria as well as the resultant foods produced by them offer numerous health in addition to medicinal advantages to the end users (Bengmark, 2000; Reid *et al.,* 2001).

2.3.4.1. LAB as probiotics

Lactobacillus species has been employed as probiotic. For example, *Lactobacillus acidophilus* has been exploited as an important probiotics since it is known to be the dominant LAB in the intestines. However, a widespread variety of lactobacillus has been utilized in preparing probiotics consisting of diverse species of *Lactobacillus* (Steintrause, 1995; Vinderola *et al.,* 2002).

The use of LAB as probiotics therefore necessitates a good and safe evaluation. The useful and effective characteristics of each strain have been reviewed and recorded thoroughly (Holzapfel *et al.,* 2001). The potential of probiotics to remain in the GIT as well as attachment and modification of immune responses are largely accepted as health stimulating and non-pathogenic properties (Reid *et al.,* 2003). The significance of probiotic consumption, for example, bulging and obstructing the gastrointestinal tract has been invesitigated (Gibson and Fuller, 2000). Bacteria could alter the constituent of food or organic secretions transforming these materials into other secondary consitutents which may be harmful to the host (Ishibashi and Yamazaki, 2001).

LAB is considered majorly a class of healthy microbes (Collins *et al*., 1998). Marteau *et al.* (1995) described probiotic to mean ‘for life’ as microorganisms have been confirmed to offer medical benefits in humans and animals. Steinkrause (1995) viewed probiotics as activemicroorganisms, beneficial to the body of the host by enhancingstabilityof

microorganisms in the intestinal guts. Similarly, Agerholm-Larsen *et al.* (2000) proposed that probiotics are living microbes ingested to provide medical benefits, furthermore offering qualitative nutritional balanced diet, they communally conserve an intricate stability between the gastrointestinal area and defense systems whereas prebiotics termed as food constituents which foster increased development as well as activities of important abdominal bacteria, hence obtaining awareness and significance as useful foods quickly.

2.3.4.2. LAB as starters

The most significant and vital bacteria in the fermentation of food materials are LAB(Campbell-Platt, 1987). They are ultimately significant in fare fermentation. Markedly, aside the need of LAB as starters, following the standpoint of welfare, scientific usefulness and funding, several precise qualities need to be well-thought-out while choosing LAB for various fermentation of food products. Therefore, the yardsticks for choosing LAB as starters in fare fermentation are subject to the category of the wanted qualities of the produce, required activities of metabolism, and qualities of the final products in addition to technology employed. Microbial preparations of significant qualities of the minimum amount of microorganisms included into a fresh food so as to yield fermented food substances through speeding-up and driving the course of fermentation is termed as 'starter cultures'. LAB performs a vital function during fermentation and is affected by an extended and sound account of utilization when producing fermented products (Wood and Holzapfel, 1995; Caplice and Fitzgerald, 1999).

2.3.4.3. LAB and their endproducts as antimicrobial agents

Lactic acid bacteria can acidify the crude food materials quickly by producing acids, alcohols, hydrogen peroxide, aldehydes and ketones, carbon dioxide used singly or in combine form (De Vuyst and Vandammne, 1994; Ari *et al.,* 2012). These compounds may promote the prevention of harmful or damaging microbes and consequently improving the storage period, enhancing consistency and providing pleasing organoleptic profile of the fermented foods (Ekwem, 2014). Olukoya *et al.* (1994) investigated the production of a better-quality *Ogi*known as*DogiK*, which had healthful characteristicsand antagonistic

properties againstcertain enteric microbes when *Lactobacillus* species were used as starter culture.

2.3.4.4. Proper functioning of the digestive system

For normal functioning of the digestive system, it is necessary to have the presence of microbial flora of *Lactobacillus* species. The eradication or grave disturbance of these microbes leads to diarrhea or constipation; hence the maintainance of beneficial bacteria is desirable in the digestive system (Pathmakanthan *et al.,* 2000). According to laboratory trials carried out by Lei and Jakobsen (2004), lactic acid bacteria gotten fromKoko, a type of cereal gruel, may be able to tolerate the problems created by the digestive area and may well inhabit the digestive area successfully.

However, in experimental tests conducted in human, Lei and Jakobsen(2004) proved that sour water obtained from Koko decreased cases of diarrhea recorded in young ones. Reports have shown that children who were fed with fermented maize were found to be significantly less prevalent to feacal enteric bacteria compared to children who did not feed with the fermented cereal meal (Tetteh *et al.,* 2004). Therefore, cereal components in the formulation of probiotic foods as fermentable substrates using LAB as starters, supplementation with dietary fibre and/or material encapsulations have remained investigated (Venter, 2007; Arena *et al.,* 2014).

2.3.5. Safe use of LAB

A common status (generally regarded as safe, GRAS) is usually given to LAB,for example*Lactococci*and *Lactobacilli* species. Previously, LAB have been subjected to lethal dose quanitification using mice by way of oral administration and the LD50 result obtained was reported as >1010 cfu/kg, subject to the strain (Ishibashi and Yamazaki, 2001). The safe use and benefits of two *Bifidobacteriumlongum* strains isolated from humans were evaluated on healthy adult volunteers and the result revealed no negative results plus the immune factors quantified remained with no unwanted alterations (Makelainen *et al.,* 2003).

Even though the beneficial aspects of LAB to health are unquestionable, some of these bacteria have been linked with illnesses and diseases. Aguirre and Collins (1993) reported the involvement of *Lactobacilli*species in clinical illness associated with humans over a period of 50 years where about 68 reports were investigated. Similarly, about 27 cases and 18 cases of illnesses associated with *Leuconostoc* and *Pediococcus* species respectively have been reported. There has also been report cases of illness associated with *Enterococci* species. It therefore signifies that LAB are opportunists that are not able to instigate infection in normal healthy individuals.

The possible peril of continued survival of transmissible genes from antibiotic resistance amid species of *Lactobacilli* has remained of ultimate interest (Lindgren, 1999). Certain species of LAB such as *Lactobacillus reuteri, L. rhamnosus, L. acidophilus* as well asspecies of *Leuconostoc* are frequently exploited within food processing industries otherwise those that are inherent in natural unprocessed food materials are unaffected by the use of glycopeptide antibiotics (Goldstein *et al.,* 2000).Transfer of genes encoding antibiotic resistance into a strain which is susceptible by way of a mobile and active genes is possible (Shlaes*et al.,* 1989; Noble *et al.,* 1992) as an example, plasmids (Teuber *et al.*, 1999), in addition to transposons (Hill *et al.,* 1985; Arthur *et al.,* 1993) to give novel and different bacterial strains which are more resistant (Danielson and Wind, 2003).

Coupled transposable elements are frequently present in *Streptococci* and *Enterococci* in addition to some strains of *Lactococcuslactis* have been stated to comprise a transposable element located in the chromosomes (Rauch and de Vos, 1992; Immonen and Saris, 1998). Lactic acid bacteria plasmids may not typically transfer transmissible antibiotics resistance genes however they can pick up conjugative plasmids and transposons. Plasmids can generally get integrated inside the DNA(Steele and McKay, 1989; Rauch and de Vos, 1992). Some form of hazard is associated with plasmid-linked antibiotic resistance (Lindgren, 1999).

2.4. Yeasts and fermentation

Yeasts impactation upon manufacture, value and wholesomeness of fermented products is closely related to their ecosystem and biologic actions (Scott and Sullivan, 2008). The intensified interest of yeasts, their occurrence and importance in fermented foods has been encouraged by current innovations in comprehending the biochemistry, nomenclature, ecosystem, structure and molecular biology (Kurtzman and Fell, 2006). This has brought about a greater knowledgeas well as a better understanding of their functions of yeasts during fermentation relating to several fermented foods (Fleet, 2007).

Whether oxygen is available or not, yeasts are able to develop in the fermenting medium. Energy, biomasses and CO_2 are the end products of the breakdown of sugars in the presence of oxygen. Meanwhile, in anaerobic condition (alcoholic fermentation), carbohydrates are transformed to intermediary end-products (Verstrepen *et al*., 2004). Additionally, during alcoholic fermentation, sugars are gradually employed to yield the required energy for the cell to grow and survive, which is referred to fermentation (Ringbom *etal*., 1996; Feldmann, 2005).

Fermentation of fare substances to be consumed by humans is strongly achieved by the genus *Saccharomyces*, particularly, *Saccharomyces cerevisiae* (Faria-Oliveira *et al.,* 2013). Through phenotypic selection, following some era of confined concomitance, these species have advanced to yield foods with enhanced sensory composition enjoyable to humans. Nevertheless, with the increased level of variety in the environment, it is thought to discover yeasts having modern as well as additional significant features intended for the industry in several and undiscovered places (Steensels and Verstrepen, 2014; Conceicao *et al*., 2015).

Yeasts can be found in different environment other than fermented foods (Nayak, 2011; Syal and Vohra, 2013; Mishra *et al.,* 2018). Several other niches that yeast can be found on include plants (Limtong *et al.,* 2014; Canto *et al.,* 2017), animals (Alvarez-Perez *et al.,* 2016), soils (Hilber-Bodmer *et al.,* 2017), water (Montanari *et al.,* 2018), skin (Lorch *et al*., 2018), gastrointestinal tract of animals (Al-Temimay and Hasan, 2016; Istiqomah *et al.,*

2018), as well as aquatic animals (Zaky *et al.,* 2014; Dhaliwal and Chandra, 2016). Some of these yeasts are able to survive and grow in acute and harsh environments such as high salt concentrations, low pH or severely cold temperatures (Gadanho *et al.,* 2006; Kejzar *et al*., 2013; Tsuji *et al.,* 2013).

2.4.1. Interaction of yeast with other microbes during fermentation

Traditional fermentation involved diverse microorganisms and the value of the resultant product is known by the mutual growth and metabolic action of the whole microbial population. Meaningful interactions of species and strains that influenced changes in the inhabitation of the ecology, even within yeasts community exists. There has been an uprise in the variation and multifariousness of these microbial interactions (Viljoen, 2006; William and Dennis, 2011).

Occurrence of yeasts in diverse fermented diets prepared using either plant or animal source material is abundant. The existence of yeast singly or in combination with fungi or LAB gives substantial impact on characteristics of food such as flavour, consistency, odour and nutritional worth (Aidoo *et al.,* 2006).

2.4.1.1. Yeast and yeast interaction during fermentation

The fermentation of food materials have recorded notable occurrence of a system of yeast to yeast interaction in most ecological unit. As fermentation proceeds, the interaction is evident with the observable increase in the development and mortality frequencies of varied yeasts types and varieties of organisms contained in individual genus (Scott and Sullivan, 2008). The processes instigating these environmental changes are more than a few. These could be due to diverse intensities in the transportation of nutrients and intake by various types and varieties of microbes, their responsiveness to by-products of metabolism as well as reactions to dangerous poisons (Fleet, 2003).

2.4.1.2. Yeast and bacteria interaction during fermentation

The relationship that exist among yeasts and bacteria are mostly comprehended as a repressive effects of the action of yeasts as regards the bacteria as result ofthe ethanol

produced by the yeast cells; conversely, the associationsreveal more complexicity than expected. During wine fermentation, yeast cells dies and lyse thereby releasing important nutrients that promote the growth of vital bacteria involved in wine fermentations (Fleet, 2003; Alexandre *et al.,* 2004).

Production of ethanol by yeasts in the course of fermentations of cocoa beans encourages the development and multiplication of acetic acid bacteria (Ardhana and Fleet, 2003). The production of acetic acid by the bacteria (acetic acid bacteria) is needed and aimed at destroying the cocoa seeds plus triggerring internal breakdown of cocoa seeds, which produces the development of flavour observed in chocolate (Scwan and Wheals, 2003; Ardhana and Fleet, 2003). Some yeast utilizes the organic acid present in some food products thereby increasing the food acidityand hence, reducing the growth of food-spoiling and disease-causing bacteria (Viljoen, 2006).

2.4.1.3. Yeast and fungi interaction in fermentation

Symbiotic association among yeast and fungi has not been comprehensively investigated, with the exception of bio control perspectives. In the course of grape juice fermentations, the successive growth of fungal population on fermenting grapes yields constituents that deter the development of yeast (Fleet, 2003). By contrast, some yeast improves the growth of *Penicillum* species in the course ofproducing and maturing cheese (Hansen *et al.,* 2001). Quite a lot of yeasts have potent effect of producing antifungal agents which possess the possibility for biological control of fungi. Commercial formulations of some species of yeasts are currently obtainable aimed at controlling fruits, vegetables and grain spoilage fungi before and after harvest (Fleet, 2003).

2.4.2. Isolation and characterization of yeasts involved in food fermentation

Yeasts are eukaryotescategorized under the Mycota Kingdom. Yeasts have existed for many decades, since they had been involved in fermentation. After isolating and identifying yeasts by Pasteur, in the 19th century, industrial production and commercial use of yeast began. These days, owing to the scientific facts and realistic characterization of yeast, the isolation and industrialized manufacture of yeasts possessing explicit characteristics

towardsgratifying the requirements expected forindustrial production have been made possible (Pimentel *et al.,* 1994; Pimental and Morse, 2003).

The understanding of the prevalance and importance of yeasts in fermented foods is underlying to the ability of isolating and characterizing yeasts into their genera, species and strain categories. The use of molecular methods is rendering the study of yeast ecosystem far more striking as well as more appropriate than in the past; nonetheless, traditional techniques has continued to persist (Fernandez-Espinar *et al.,* 2006; Fleet and Balia, 2006). While identifying novel samples of yeast was tedious requiring about 80-100 characterization tests, the identification process is more rapidly attained using the method of sequencing of DNA. The DNA testing of the encoded genes and testing of the ITS1-ITS2 region of ribosomal RNA, in addition to supplementary genes, is recognized for a lot. Previous reports of several novel genera and species have been made possible with many developing account of sequence-phylogenetic data which has brought about a comprehensive review of yeast nomenclature and grouping (Kurtzman and Fell, 2006).

The analysis of the ITS1-ITS2 region using the restriction fragment length polymorphism in the identification of yeasts are less costly and faster and the outcome of investigationshave been properly documented (Fernandez-Espinar *et al.,* 2006). Genetic probes (nucleic aicd) and PCR (real time) used as identification techniques showed great success for a few genera, for instance *S. cerevisiae, B. bruxellensis* and *Z. bailii* (Rawsthrone and Phister, 2006). A novel cytometric analysis (probe flow) described for a number of *Candida* species have demonstrated huge success (Page and Kurtzman, 2005).Kurtzman *et al*. (2011) reported the high diversity of yeast species as closely related to the division of nearly 1500 species.

2.4.3. Utilization of yeasts as health-promoting microbes

Holzapfel *et al.* (2001) investigated that a few number of yeasts strains isolated from fermented foods, for instance *S. boulardii* and *S. cerevisiae*can be utilized as probiotics. The capability of health-promoting yeasts to persist within the GIT and relate in a hostile way with residing pathogenic microbes have been reported and displayed (Lourens-Hattingh and Viljeon, 2001). Effective utilization of yeasts as health-promoting microbes depends on

qualities includingmass strength, structural variability,nutritive pliability,also adaptation to stressful conditions, potential to generate enzymes, antioxidant, anticancer and antimicrobialactions and the capability to yield beneficialmetabolic products (Fredlund *et al.,* 2002).

2.4.4. The safe use of yeast in fermentation

For day-to-day living, individuals ingest substantial portion of yeasts without antagonistic effect upon their wellbeing. Yeasts are hardly related with occurrences of foodborne diseases, food poisoning or infections, unlike bacteria and viruses. Nonetheless, care is necessary, as well as more investigation regarding the use and application of yeast (Fleet and Balia, 2006).

Yeasts exist as non-antagonistic, infective organisms, but a few species live as opportunistic disease causing agents responsible for variety of infection (Hazen and Howell, 2003). The persons that happen to be vulnerable to these infections are those with deteriorated well-being and immune systems, tumor, acquired immune deficiency syndrome, and ill- and hospital-admitted persons, as well as persons going through therapy with immune suppressant medications, wide range drugs and radiation chemotherapy for cancer patients. Increasing news report about yeast infections is as a result of the increasing proportion of such persons in the community. Moreover, the figure of yeast species found present in food materials is on the increase (for example, *Candida krusei, Saccharomyces cerevisiae, Pichia anomola, Candida famata* and species of *Rhodotorula*) (Hobson, 2003; Fleet and Balia, 2006).

There has been notable infection caused by *S. cerevisiae*in immune compromised persons (de Llanos *et al.,* 2006).Hospitalized patients are believed to become imperiled to elevated concentration of yeasts via the biofilms created on waste pipe and other invasive machines, Contact with yeasts may probably come from the body of workers in the hospital as well as the meals taken into the environments around the hospital (Fleet and Balia, 2006).

Therefore, there is need to buildmore effectiverelationships amid the functionality of fares in supporting yeast-related disease through more investigations. Also, detailed information is necessary to know the existence and development of yeasts in the GIT into the circulatory system, and of course, the common existence of yeasts in environs of medical centers. Furthermore, it is important to investigate the cases in which a non causing disease agent for instance, *Saccharomyces cerevisiae* turn out to be disease causative (Fleet, 2007).

2.5. Cereal-based fermented food

In Africa, an extensive diversity of cereal-made fermented fares are produced from grains for example, maize, sorghum and millet which serve as well-known material for production. The makeup of cereal grains includes an embryo (germ), an epidermis containing the endosperm and a seed coat (husk). The endosperm produces starch grains of distinct masses (Hoseney, 1992) whereas the seed is packed with nutrients including amino acids, sugars, lipids, minerals (Nikolov, 1993).

Cereals are a major component of human food in Africa. Nutritional experts have paid attention to cereal based foods from maize, sorghum and millet sources (Guyot, 2012). These cereals have high content of dietary fibrefor example, beta glucan possessing health promoting role. Researches on the clinical and epidemiological studies on the importance of consuming diets rich in dietary fibre for example oat-based foods, barley have indicated an effectivemanagement of heart-related illnesses in humans (Shimzu *et al.,* 2008; Beck *et al.,* 2010). Duchonova *et al.* (2013) suggested manifoldadvantages of utilizing cereal grains for producing numerous new cereal diet targeted at a specific population. Furthermore, cereals are good fermentable source material for the growth of health-promoting microbes (Charalampopoulos *et al.,* 2009). Cereals contain insoluble dietary fibre that stimulates the growth of colonic *Lactobacilli* and *Bifidobacteria* which act as probiotics (Chavan and Kadam, 1989; Charalampopoulos *et al.,* 2002; Duchonova and Sturdik, 2010).

Depending on the food material used and the mode of consumption, cereal products are given several names known as *koko, akamu, agidi, eko, kamu* and *fura*, and these names are particular along the West African coastal region (Caplice and Fitzgerald, 1999).

2.5.1. Ogi- cereal based fermented food

Ogi is produced from fermentation of cereal grains for example, millet, sorghum or maize utilized as source material to initiate fermentation. In West Africa, it is considered as the major weaning meal for infants though, also being taken by older persons (Onyekwere *et al.,* 1993, Moss *et al.,* 1993; Banigo, 1993).

To make *Ogi*, the grains are steeped, ground and filtered (Steinkraus, 1998). Previous report on the nutritive quality of *Ogi*revealed that phytate is broken down to liberate phosphorous during fermentation (Lopez *et al.,* 1983) and a notable increase in some vitamins, such as riboflavin and niacin contents (Kuboye, 1985). On the other hand, Akinrele and Bassir (1967) and Adeyemi (1983) reported that 20-30% of available nutrients in the cereal grains are lost in time while producing*Ogi*, especially losing the grain germ and protein granulesin the course of wet-milling and wet-sieving.

In producing *Ogi*, the cereal grains are soaked for a period of 24-72 h. In the course of fermentation, LAB, yeasts and molds are involved in the fermenting the source food items, where, *L. plantarum* dominates most amid other microorganisms. Others include *Corynebacterium* species, which hydrolyze the corn starch, while *Candida* and *Saccharomyces* species promote the flavouring of *Ogi* (Caplice and Fitzgerald, 1999).

Depending on the cereal employed, the appearance of *Ogi* may be creamy-white for maize, dirty grey for millet and red-brown for sorghum (Onyekwere *et al.,* 1993; Banigo, 1993). The product *Ogi*possesses a tangyflavour comparedwith yoghurt as well as a characteristic and unique aroma, which differscompared to commonly fermented foods from cereal grains (Chavan and Kadam, 1989).

2.6. Cassava-based fermented foods

Cassava tuber (*Manihot esculenta*) is regarded a principal tuber plant for not less than 800 million inhabitants dwelling in emerging hot nations (Burns *et al.,* 2010). Approximately 70% of the cassava tubers produced around the world is consumed by humans, while 30% is

employed in producing feeds for animals as well as for producing important industrial items, for example, alcohol, glucose and starchy materials (El-Sharkawy, 2004). It also currently been utilized in producing bioethanol (Narina and Odeny, 2011).

Cassava possessesvitalagriculturalbenefitsincludingincreased production in nutrient deficient lands, opposition to famine and plant diseases, storability in the topsoil after maturation, as well as a relatively increased production of starch, compared to other carbohydrate based foods (Cooke and Coursey, 1981; Zhang *et al.,* 2010). However, there are two other deficiencies associated with cassava roots. First, the presence of gynogenic glycoside linamarin (Okafor *et al.,* 1984) and methyl linamarin hydrolysed by linamarase thereby releasingpoisonous cyanide HCN (Egwim *et al.,* 2013). Secondly, cassava roots arelow in protein content, havingapproximately 1% protein (Cooke and Coursey, 1981).

The sweet or bitter varieties of cassava are commonlyknown.Low cyanogen content found mostly in the peels of cassava is notable with the sweet varieties while the bitter varieites contain higher cyanogen which is evenly distributed throughout the roots (Egwim *et al.,* 2013). In humans, the average lethal dose of cyanide is approximately 1-3 mg/kg per body weight (Emsley, 2008).

It is necessary to determine the cyanogenic potential of cassava tubers and products for example cassava flour or *Gari*, in order to prevent further outbreaks of diseases associated with cassava consumption (Kostinek, 2007). A simplified method employing the usuage of picrate paper kit for determining cyanogenic potential of cassava products is available for the use mostly on the field by relatively unskilled persons in the developing countries (Egan *et al.,* 1998).

2.6.1. Gari-cassava fermented food

Gari a popular fermented food made from cassava is consumed by millions of people throughout Western Africa (Okafor and Ejiofor, 1990). About 241 million tons of total global cassava was produced in 2009 (Bull *et al.,* 2011).

2.6.2. Fermentation of cassava for the production of Gari

Gari is prepared by the fermentationof grated cassava. In the course ofprocessing (grating) of the root, the internal enzyme (linamarse) is liberated hence breaking down the exposed linamarin. The enzyme is insufficient in breaking down the glycoside entirely; thereforeremnants are normallypassedonto the *Gari*(Okafor *et al.,* 1998). Consumption of cassava food for lengthy periods especially with low quantity of protein diets have shown traces of glycosides which isassociated withillnesses such asthyromegaly and tropical ataxic neuropathy (Osuntokun, 1973).

Studies have shown that consumers may be exposed to some quantities of cyanide after consuming *Gari*;however the quantitytaken into the body from a mealtime is relatively minute and not likelyto trigger severefood poisoning. On the other hand, continued consumption of cyanohydrins is presumed to break down at the higher pH point to produceequivalentmolarity of cyanide in the digestive tube. The lengthyperiodit takes for cyanide absorption in the blood may imply that constant consumption of *Gari*may possiblyallow the accumulation of cyanide in the blood (Oluwole *et al.,* 2002). Most often, signssuch as nausea, abdominal pains, dizziness, headache and feebleness may be exhibited as a result of cyanide poisoning from constant intake of cassava contaningexcessivequantiity of cyanogens (Egwim *et al.,* 2013).

2.6.3. Involvementof microorganismsduring fermentation of cassava for the production of Gari

Once fermentationcommences*Geotricum* and *Candida*species begin action upon the fermented cassava thereby increasing the acidity of the food item resulting in the death of a number of microbes that cannot survive the new acidic environment. Next, strains of *Corynebacteria lactis*which are able to withstand the increased acidity continue fermentation resulting in the hydrolysis of most of the unsafe and poisonous chemicals (Egwim *et al.,* 2013). Lactic acid bacteria such as *Leuconostoc mesenteroides, Lactobacillus fermentum* and *Lactobacillus plantarum*are involved in fermenting casssava to produce *Gari* (Oguntoyinbo, 2007). It is nowadaysrelatively acknowledged that the aroma of *Gari* is

generatedthrough actions of LAB and yeasts in the course of fermentation, several of which have been discovered to be a source of linamarase (Okafor and Ejiofor, 1990; Giraud *et al*., 1993).

2.7. Stress responses

For microorganisms to grow and function optimally, it is important to have anexplicit and a well-stablized inner environment. Similarly, for cells to operate effectively, the inner surroundings of the cell have to be adequately and continuouslysustained. Conversely, variations in the exteriorsurroundings can bring about change of cellular perturbations which can interrupt activities within the innersurroundings. These variations in the surroundingsmay inhibit the optimumperformance of enzyme, interfere with metabolism, and weakenthe structureswithin the cells, chemical gradients perturbationsresulting intotalvariability(Gasch, 2002).

An organism's survival and continued existence is its capability to acclimatize to instabilities internally and externally. The presence of genetic responses, reformation and acclimatization, significantly preserved through life, provides the cell with the malleability required to acclimatize with its unstable environment, this is referred to as stress response (Estruch, 2000).

It is recognized that development and proliferation of microbes is limited by its own activity and is repeatedly sub-optimal in the wild when compared with control requirements in research laboratories. Stress conditions could be natural (such as acidity, starvation frequently produced during the growth of cells), or environmentally induced stress conditions (for example, temperature rise, oxidation or osmotic shock) (Foster, 1999).

With the purpose of surviving adverse consequences that stress brings, microbesbuildpromptmicroscopic reactions to fix the harmas well assafeguard against additionalcontactwithsimilar and newtypes of stress conditions. The mostconsideredreactionto stress is the production of few numbers of proteins, known as stress proteins. Although, post transcriptional processesmay perform a vital function in

regulating the response to stress conditions, investigationregarding this subject has been primarilyconcentratedon transcription, and different stress provoked transduction components have been known and characterized (Ruis and Schuller, 1995).

It is generallypresumed that the production of proteins in response to stress is aimed at continued existence and modification of the cells toharshstate. The presence of provoked adaptation appears to providetemporarymanagementof gentle stress condition causingtherise in toleratingsubsequent, usuallyharmful, amount of similar stress. The bestexplanationto occurrences of stress proteinsearlierprovoked by gentle stress improvesthe adaptation of the cell to stress conditions. Nevertheless, the associationinvolving the stimulation of distinct proteins and adaptationto stress may not be proven at all times and it has been the center of delibration (Estruch, 2000).

In the process of detecting and transfering stress signs into the nucleus, a genomic reorganizationtakes placewhich indicates a reduction in the manifestation of protective genes and production of protein which enhances the mieu of genes that encode stress proteins. Some of these stress proteins include molecular chaperones, (accountable for conservation of protein folding, transcription factors which are responsible for modulating genes expression), protein involved in membrane transportation and detoxification and repair pathways, metabolism of nutrient and production of osmolyte. An effective cell adaptation to the new stress conditions as well as damage repair caused on the cell indicates the survival and recommencement of growth. Even thoughexplicit stress conditions provokediverse cellular reactions, fundamentalprogrammes for genetic expression typical to all ecological stress responses are notable (Estruch, 2000).

2.7.1. Stress responses in yeasts

Yeast cells have evolved to be exceptionally capable in surviving sudden and harsh fluctuations in the external surrounding. Naturally, yeasts cells have to handle inconsistencies in acidity, temperature, osmolarity of their environs, as well as surviving stress conditions in the occurrence of toxic and radiation chemicals, and protracted episodes of nutrient starvation(Gasch, 2002). The growth of yeast cells during stressful conditions

necessitates the internal system management, however the programming of the cells is necessary for its survival since this relies on the challenges the cells have to deal with externally (Gasch, 2002; Gasch and Washburne, 2002).

Yeast cells initial a multidimensional response involving a momentary halt of usual cellular ways all through a phase of restructuring of internal environment, usually in response to unexpected alteration in growth requirement. The extent of cellular arrangement is clearly associated with response to environmental stresses. For example, to counter the effect of glucose starvation, proteins may be present on the surface of the cell to sense the shortage of glucose and transduction pathways in various sections of the cell (Johnston, 1999; Estruch, 2000; Igual and Estruch, 2000).

2.7.2. Stress responses in LAB

There is a dearth of data regarding the stress-induced processes involving *invivo* studies for boosting the existence of these organisms during food production, irrespective of the widespread utilization of LAB. More knowledge on the adaptive reactions of LAB is essential since fermentation process frequently expose these microbes to unfavourable environmental state (Gasch, 2002). During industrial processes, lactic acid bacteria should be able to withstand the harmful conditions encountered, for instance, in the course of handling starter cultures, storage (spray drying, freeze drying or freezing) and throughout the fermentation, bringing about dynamic changes in the environment. These occurences emphasize the necessity for strong lactic acid bacteria as they may have to live and develop in diverse unfavourable environments while exhibiting explicit roles (for example during stationary phase or storage) (van de Guchte *et al.,* 2002).

LAB developed definite processess to react and persist during environmental pressures and variations (stress-sensing structure and defenses). Infact, microbes have particular regulators assigned to every single regulated gene and adjust their expression based to the environment. Defenses against stresses are excellent illustrations of such intergrated regulation systems. The incorporation of responses to stress conditions is achieved by a grid of regulations which permit the cells to respond to several and complicated shifts in the

environment (Estruch, 2000). Lactic acid bacteria respond to stress conditions in distinctive approaches subject to the strains type, species and type of stress condition encountered. Acid, heat, oxidative and cold stresses are the best studied stresses, although studies carried out on cold stresses are centered on a particular set of proteins rather than investigating the full response to stress (Serrazanetti *et al.,* 2009).

Understanding the method utilized by LAB in tolerating byproducts of their individual metabolism and the reaction to acidic foods is of enormous significance. In lactic acid bacteria, one of the utmost operational systems for resisting acidic environs is with the use of Glutamate Decarboxylase (GAD) (Kapteyn *et al.,* 2001). Researches have suggested that the roles of aromatic L-amino acid decarboxylase for regulating cellular pH in the internal and external environs is through utilization of existing H^+ during carboxylation feedback (Cotter and Hill, 2003).

Consequently, treatment of LAB in mild acid concentrations stimulates an Acid Tolerance Response (ATR). The induced system consists of balanced cellular pH, defense and remending approaches. Gene as well as proteins implicated in pH regulation, defense or overhauling of cells play a important part in tolerating acidic stress, nevertheless this function can also encompass new and varied common acid tolerance mechanisms(Causton *et al.,* 2001). Furthermore, a defined and more detailed research was worked upon to determine the consequenes of lactic acid stress on *Lactobacillus plantarum* by transcriptive characterization (Pieterse *et al.,* 2005).

2.8. Acid stress

Acidic environs are a stress condition whichaltersthe metabolic activity of yeast cell resulting incell death, reduced cell viability and capability to carry out fermentation (Carmelo *et al.,* 1998), even though strains utilized in industries exihibit more tolerance to acid and heat stresscompared to strains used in the research laboratory (Brosnan *et al.,* 2000).Acid stress condition as a result of induction of both inorganic and organic acids brings about some form of gene expression in yeast (Kapteyn *et al.,* 2001; Kawahata *et al.,* 2006).

Acid stress responses have been investigated in yeast cells previouslysubjectedto weak organic acid, food preservatives and herbicides (Piper *et al.,* 2001; Cabral *et al*., 2004; Kawahata *et al.,* 2006). When yeast are exposed to weak acids, they exihibit decreased penetrability of membrane, expulsion of anion, an improvedcapacity to breakdown preservatives (Mollapour and Piper, 2011) and expression of genes (Causton *et al.,* 2001).

Findings frominvestigation and functional screening of genetic material associated withhydrochloric, acetic and lactic acids stress responses showed that acidic conditions affect cell wall architecture, the genomic expressionassociated with the breakdown of metals, vacuolar H+-ATPase (V-ATPase) and HOG MAPK protein levels (Kawahata *et al*., 2006).

2.9. Oxidative stress

Responding to oxidative stress can beexpressed asoccurrenceswherebycells react to changes in its oxidation-reductioncondition. As a result of aerobic growth, cells are frequentlysubjected to reactive oxygen species (ROS); active oxidants able of causingwide-rangingharmto the cells especially on the DNA, protein and lipid contents. Owing to these, life forms ranging from microorganisms to human beings have createdmethods to maintainthiol oxidation-reduction equilibrium in the cells. This is accomplished by restraining the buildup of oxidants, metabolism regulation of iron and copper, stimulatingthe pathways for thiol oxidation-reduction and throughmending of harm caused (Toledano *et al.,* 2003).

Hydrogen peroxide (H_2O_2) which are single and distinct compounds have been reportedly used in many studies as a model oxidant during oxidative stress.Other typesoxidant can be used to initiate ‘oxidative stress’, provided the cells is able to alter the reactive oxygen species by means of oxidant-specific responses (Temple *et al*., 2005). Hydrogen peroxide is the commonest oxidant utilized as a model to initiate oxidative stress condition, owing to its easiness and simplicity of use as well as its solubility in water and absolute stability. Hydrogen peroxide is a universal molecule produced in the course of aerobic cellulose

respiration subsequently, exposing H_2O_2 to several biotic and ecological elements results in the destruction of cells thereby stimulating oxidative stress (Veal *et al.,* 2007).

2.9.1. Oxidative stress in LAB

Lactic acid bacteria are non-loving oxygen or little-loving oxygen microbes that can convert some pyruvate produced to lactate, hence, regenerating NAD^+ from NADH produced in the course of glycolysis(van de Gutche *et al.,* 2002; Lechardeur *et al.,* 2011). Oxygen is not necessary for their growth; it is mostly thought that LAB may not be able to utilize O_2 for accepting a negatively charged particle (van de Gutche *et al.,* 2002). Nonetheless, a number ofLABpossesses nicotinamide adenine dinucleotide oxidase system while others may utilize anefficientelectron transport chainwith the existence of hematin (Lechardeur *et al.,* 2011).

Significant alterations in the breakdown of carbohydrate in LAB can occur due to induced aeration, regardless of the toxic effects of oxygen. The outcome of the activityof fermentation is majorly influenced by availaibility of oxygen. Generally, LAB are capable of developing fairly with the existence of oxygen; yet, increased proliferation of LAB is observed under anaerobic conditions. Lactic acid bacteria may begin respiration metabolism, where the metabolism of the cell such as the oxygen condition, pH, capacity to develop and survive with existence of hematin and oxygen is being adjusted (Rezaiki *et al.,* 2004).

In the course of fermentation, hydrogen peroxide is produced with available oxygen.Many LAB possessenzymes which can stop and remove the lethal effects of oxidative stress (van de Guchte *et al.,* 2002). As regardsdeterring the occurence of reactive oxygen species, the wide-range response is to eradicate available oxygen. Previously, *L. helveticus* have been reported to change the composition of lipids present within thecell membrane while responding to oxidative stress. Really, there was a notable increase in the activities of the oxygen consuming desaturase system thereby reducing the damage of free radicals to the cell (Guerzoni *et al.,* 2001).

Usually, reaction of LAB to oxidative stress is related; though, responsesmay possibly be

influenced by species and strains types as well as, on the capability of the bacterial population to break down catalase (Serranzanetti *et al.,* 2009).Identifying some genetic materialas well as their individual encoded proteins has revealed a great deal of contribution to oxidative stress resistance in *Lactococcus lactis*. Furthermore, intiating these genetic materials depend on the periods of growth, for example, whether at exponential or stationary period, hence, their products provide resistance to multiple stress conditions (Duwat *et al.,* 2000). Resistance approaches to common stress condition might possibly provide defenceto stresses caused by oxidation. Ideally, isolation of a number of resilient variants of *Lactococcus lactis*showing resistence and defenceto stresses caused by oxidation has been conducted (Rallu *et al.,* 2000).

2.9.2. Oxidative stress in yeast

Yeast engages in dealing withproducedROS when growing aerobically. By relating the form of proteins stimulated in reaction to hydrogen peroxide or O_2^-, possibility of diverse ROS stimulating various and distinct groups of genes has been studied. Even though, there exist proteins that are explicitly provoked by hydrogen peroxide or menadione, a majorconnection occurs amist both responses (Jamieson *et al.,* 1994).

Oxidative stress caused by free radicals in the course of aerobic metabolism mightperform the function of replicating older yeast cells resulting in gradualdecline of the yeast cells with continued fermentation, thereby accumulating a greater precentage of mature yeast cells (Powell *et al.,* 2000). End-products of available radical lipid responses activated by means ofROSrises from noxiousness of lipid peroxidation, in addition to toxicity from formation of aldehyde compounds, also peroxyl and alkoxyl radicals (Esterbauer, 1993; Girotti *et al*., 1998; Iwai *et al.,* 2010).

Yeast employs diverse responses in overcoming oxidative stress namely enzymatic and non-enzymatic responses (Costa *et al.,* 1997; Trotter and Grant, 2005). Responses to oxidative stress vary throughout the process of food fermentation, for instance, in the course of fermenting wort in a semi-defined medium, with decreasing levels of oxygen, the actions of cellular superoxide dismutase and catalase fall abruptly (Clarkson *et al*., 1991). Delayed

advancement in the cell division is another way yeasts cells respond to exposure to ROS. This allows repair of damage on any macromolecular without passing through their genetically identified cells (Shackelford *et al.,* 2000). Therefore, exposure to ROS and the resultant oxidative stress results in reprogrammed death in yeasts cell (Madeo *et al.,* 2002).

Research hypothesis have shown that the induction of reactive oxygen species is a vital factor for the apoptotic pathway since yeasts apoptosis are activated as result of exposure to H_2O_2 or GSH exhaustion in GSH1 mutants (Madeo *et al.,* 1999). Consequently, the promotion of yeast apoptosis have been reported to be a result of various stress conditions, lots of which are related to generated ROS (Pereira *et al.,* 2008).

The mode in which yeast respond to ROS can also be by changing the appearance of genes that encode antioxidant resistancemethodsas well as genes that encode enzymes responsible for mending and detoxifying resultant destruction to the cell (Costa *et al.,* 1997). This establishes the core for adaptive inducible responses where cells indulged with small amount of oxidant can acclimatize to turn out to be impervious to consequent and also dangerous treatments. In *S. cerevisiae*, where there has been a broad investigation in the adaptive responses to stress conditions, it is currently known that there are varied cell responses that enable the continued existence of the cell after contact with oxidants for example H_2O_2, or results of oxidative injury (Turton *et al.,* 1997). Responses of yeasts to different oxidant treatments may be described by the kind of proteins produced. In concordance with this thought, a broad range of transcriptional and translational reprogramming is apparent in the course of adaptable remedies (Shenton *et al*., 2006).

2.10. Proteomics

The "-omics" era of research has gradually risen since the rise of genomic sequences and microarray technologies for deoxyribonucleic acid (DNA) isolation. The logical continuation of widely-used transcriptional profiling methodologies is also termed Proteomics. It is essential to characterize and name the set of proteins encoded by the gene which is described as the proteome, in order to identify and understand the term proteomics

(Wilkins *et al*., 1996). The entire product translated from genomic sequences, resultant proteins arising from processing of post-transcriptional and post-translational, in addition to complexes produced from these biomolecules is refered to proteome (Ahrens *et al.,* 2010). The proteome of a cell is active and its changes in profile are in concordance to the physiological position and stages of cell differentiation, besides its vastintricacy (Jensen, 2004).

The investigation on the multiprotein structures of microorganisms, the entire protein make-up of its gene, alongside comprehending vital goals of some individual proteins in addition to their function as a fraction of a better conserved system is also known as proteomics. Proteomics is an essential constituent of current techniques for biology methodologies, with the objective of typifying the behaviour of the system more preferably than the behaviour of a single constituent (Wilkins *et al.,* 1996). Proteins are exposed to many modifications, for example post-translational and other modifications by ecological agents, hence the measurement of messenger ribonucleic acid (mRNA) using the technique DNA microarrays is not the best approach as it provides insufficient data(Ahrens *et al.,* 2010). Proteins are accountable for the structure, production of energy, interactions, and activities as well as splitting up of all cells, are therefore essential to a detailed understanding and interpretation of systems biology (Karpievitch *et al.,* 2010).

2.10.1. Mass spectrometry in the field of proteomics

Institutions of mass spectrometry (for example matrix-assistant laser desorption/ionization mass spectrometry andelectrosparay ionization mass spectrometry)allowedtransformation of the functional investigation of biological materials (Karas and Hillenkemp, 1988; Fenn *et al.,* 1989). Due to flexibility, sensitivity, speed, reliability and accuracy of tandem mass spectrometry (MS/MS), it is currently exploited more regularly for protein sequencing as an alternative to the typical Edman degradation technique. Biological mass spectrometry has steered to the beginning of proteomics by permitting protein analysis on large-scale (Yates, 1998; Aebersold and Goodlett, 2001).

The combination of high-resolution technique of 2 DE and mass spectrometry for separation

of protein has presented current research an indispensable method for identifying proteins in proteomics (Anderson and Anderson, 2002), and post-translational modification (Lisacek *et al.,* 2001; Claverol *et al.,* 2002). Novel techniques using an online multidimensional liquid chromatography separation of protein or peptide mixture has significantly covered the extensiveness and extent of protein examination comparative to analysis carried out exclusively by liquid chromatography mass spectrometry (Wall *et al*., 2000; MacCoss *et al.,* 2002; Florens *et al.,* 2002).

2.10.2. Tandem mass spectrometry

Protein are then usually identified by primarily matching the features of the experimental and notable mass spectrometry to a list of estimated or formerly known features (For example by MS/MS) or based on earlier investigation of well identified samples), in bottom-up proteomics. The generally utilized method is tandem mass spectrometry alongside search from data catalogue (Nesvizhskii *et al.,* 2007), where the pattern of fragmented peptide are matched to theoretic arrangements as provided in the databank using programs namely; X Tandem (Craig and Beavis, 2004), Mascot (Perkins *et al.,* 1999) and Sequest (Eng *et al.,* 1994).

Based on the protein mass and time used for elution, or together with MS/MS break-up arrangements, identifications of proteins can be made with high-resolution LC-MS instruments (Pasa-Tolic *et al.,* 2004). Sequencing of *de novo* peptide (Standing, 2003) and a combination of the *de novo* and database searching methods (Tabb *et al.,* 2003; Frank and Pevznar, 2005) are alternatives to database-searching.

In tandem mass spectrometry, original charged particles carrying many peaks in an image is splitted and imaged once more, whereas in collision-induced dissociation (CID), original charged particles may be splitted through collision using an unaligned gas (Sleno and Volmer, 2004). Successive mass spectrometry examination quantifies the ratio of mass to charge also, the strength of broken particles (resultant and original particles), thereby generating a breakup form. Collision-induced dissociation generally result into the

production of *b* and *y* particles via fragmentation of the amide bond about the backbone of the peptide.

The b-ions are produced as soon as the charged particles are reserved by the amino-end pieces, while on the other hand, as soon as the charged particles are reserved by the carboxyl-end pieces; the y-ions are produced (Sobott *et al*., 2009). Breakup forms for example a, c, x and z types are viable. The making of z- and c- ions is noticed in electron capture dissociation leaving the lateral chains whole. The arrangement of disintegration is comparable to the peptide identification, as a result, amino acid sequencing are thus, estimated. Assuming the peptide sequence is included within the search database, the noted fragmentation pattern should correspond with its theoretical pattern.

The establishment of search database is achieved by asserting a catalog of proteins which may have some proteins present in a sample. The whole known proteome, for example, in human studies can be stated with a FASTA folder which can at that point be utilized to produce sequences from peptide fragment by promoting protein digestion using the enzyme: trypsin. A hypothetical disintegration arrangement is then formed for each resultant peptide. Several software programs such as SEQUEST, X-Tandem and Mascot are available for database matching. Each program has its unique method used in opening the area in the middle of experimental and theoretic bands; also similarities in their outome may exist (Searle *et al.,* 2008).

Based on an exceptionally exact mass measurements and LC elution times, high-quality and solvable liquid chromatography mass spectrometry tools are used for identification of peptides. It is necessary to have a database containing both theoretic or formerly studied mass and elution time quantification mass and time tags in order to compare with the high-resolution LC-MS data especially when using mixed perspective (Pasa-Tolic *et al.,* 2004). Hybrid approaches allow for higher throughput analysis, since MS/MS is simple but time-intensive, exposing just subgroups of the sample to MS/MS fragmentation arrangement is usually used for the creation of a mass and tag database. Low levels of sampling problems

related with LC-MS/MS are prevented using numerous liquid chromatography mass spectrometry datasets establishing the databases.

2.10.3. Ionization methods

In current studies in proteomics, involving the identification of protein, two major ionization methods are availabile and exploited which include; electrospray ionization (ESI) and the matrix assisted laser desorption/ionization (MALDI). (Zaluzec *et al*., 1995; Nguyen and Fenn, 2007).

2.10.3.1. Matrix assisted laser desorption/ionization (MALDI)

When employing the MALDI, a carbon-based matrix, commonly alpha-cyano-hydroxycinnamic acid is used to crytallize the peptides. For the formation of peptide ions, the matrix ions and sublimates move the charge to analytes once the laser bombardment is completed (Zaluzec *et al*., 1995).

2.10.3.2. Electrospray ionization (ESI)

The ESI is different from the MALDI because the analyte containing an aqueous solution is made to go through a tubular pointer subjected to intense power. Liquid is then expelled as a spurt through electric drops that generates chemical constituents in charged structures when the diluent has been vaporized by a heat up immobile gas flow (Nguyen and Fenn, 2007).

2.10.4. Types of mass analyzers

Ions formed in the source region are fast-tracked into the mass analyzer through an electric field. The ions are separated by the mass analyzer based on their mass to charge ratio. Mass anaalyzers are selected depending on the resolution, range of masses, scan frequency and detection limits needed for an application (Price, 1991; Siuzdak, 1994). Irrespective of the technique of ionization employed, the masses of the ions are evaluated inside an analyser following their passage through an air-freed cylinder. Commonest types of analyzers used include time of flight (TOF), ion trap (IT) and the quadrupole (Q) (Siuzdak, 1994; May *et al.,* 2011).

2.10.4.1. Time of flight (TOF)

With the use of the time of flight analyzers, resultant ions obtained using the MALDI, are sped up by a potential in the midst of two electrodes then passed across a pipeline at rates proportional inversely to its masses. The interval spent to obtain detection from ionization is applied in deriving the value of the ratio of mass to charge. Ion signals passed into the analog signal which is converted by the detector is translated and deciphered at a workplace. This gives a chart of the mass to charge ratio against the ion count concentration commonly known as mass spectrometry spectrum (Wollnik, 1993). Databases for example MASCOT (Perkins *et al.,* 1999) and SEQUEST (Eng *et al.,* 1994) are now being utilized and compared to generated signals based on available information in the databases, and protein of interest is identified.

2.10.4.2. Ion trap (IT)

With the ion trap analyzers, in three-dimensional electric current, ions of interest which have been sieved and entangled are slowly liberated in a mass to charge rising order. An example of an ion trap analyzer which possesses an extra magnetic field thereby compelling ions to show circular movement with elevated rate of occurence with the use of a fourier transform is referred to as a fourier transfer ion cyclotron resonances (FT-ICRs) (May *et al.,* 2011).

An alternative type of ion trap analyzer is the orbitrap; whereby there is an oscillation of ions along and from place to place in a particular curved terminal. The frequency of vacillations is proportionally equal to the radicand of the mass to charge ratio which is ratio determined with high-level precision (Hu *et al.,* 2005; Walther and Mann, 2010). This knowledge has moved towards the combination methods containing for instance, an ion trap, or the combination of a Fourier transfer ion cyclotron resonances and an ion trap, two autonomous mass spectrometers which may merge (Walther and Mann, 2010).

2.10.4.3. Quadrupole (Q)

A quadrupole mass analyser is made up of four rods placed parallel to each other, with the opposite set of rods connected to each other electrically. Based on the stability of the ion's movement through the oscillating electric field, the ions are separated. When a radio frequency (RF) voltage is allowed to get in between a pair of the opposite rods within the quadrupole, the field is generated (Loo, 2003; Clark, 2017). On the other hand, direct current voltage is introduced to the second pair of opposite rods. For ions to get to the detector, ions of a particular mass-to-charge ratio will have a srable movement into the quadrupole present electric field. The quadrupole filters the masses of the ions of a specific mass-to-charge ratio as along as the radio frequency and direct current voltages are fixed properly(Loo, 2003).

Single quadrupole mass spectrometer possesses one mass analyser which qualifies ions produced in the instrument source through fragmentation of the static molecular ions or fragmented ions. The triple quadrupole mass spectrometer, on the other hand, has two quadrupole analyser, separated by collision cell. Through the process known as collision-induced dissociation, the intial ions are selected, fragmented and passed to the detector (Somogyi, 2008; Clark, 2017).

2.11. Protein separation technique

Separating proteins using 1D and 2D electrophoresis, proteins need to be firstly separated and extracted from desired materials. To obtain good electrophoretic results, an appropriate protein extraction is fundamental(Anderson and Anderson, 2002). Regarding the numerous kindsof protein extraction, as well asthe origin of bioticspecimens, the method of extracting samples requires personal modifications.Irrespective of the modifications, proteins require proper solubilzation, disaggregration, denaturing and treatment using a disulphide bond reducing agent (de Marqui *et al.,*2006).

2.11.1. One dimensional (1D) gel electrophoresis

One dimensional gel electrophoresis also known as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is an great technique for separation, detection and monitoring of protein during purification as well as obtaining the consistency

of purified fractions (Garfin, 1990). SDS PAGE makes available important facts regarding the mass, amount and purity of the protein sample. As a result of the interaction of particles within the encompassing gel environment, which functions as a molecular filter, the movement of particles are retarded. The degree of difference observed inthe movement of the component proteins is the resultant effect arising from the opposing interactions of the electrical force and molecular filtration (Gallagher, 2012).

When preparing samples for SDS PAGE, proteins are treated with hot sodium dodecyl sulphate (SDS) which denatures the proteins by attaching firmly to the hydrophobic core of the protein, thereby giving the complexes formed a negative chargeequivalent to its mass. Most SDS PAGE is carried out in vertical compartments in gel slabs formed in the middle of two plates. The use of the slab make room for uniformity, hence, providing direct comparism between different samples on the same gel (Garfin, 1990; Gallagher, 2012)

2.11.2. Two dimensional (2D) electrophoresis

Two-dimensional electrophoresis (2DE) is another method forproteinseparation (Klose and Kobalz, 1995;Gorg *et al*., 2004; Weiss and Gorg, 2009), which was initiallyproposed and described in 1975 (Klose, 1975; O'Farrel, 1975).

In standardtwo dimensional electrophoresis, proteins are normally splitted and separatedaccording to two successive steps. The first step, referred to isoelectric focusing (IEF). In this IEF step,particlestravel in the strips containing the polyacrylamide gel using an immobilized (Bjellqvist *et al.,* 1982) or amphoteric buffer-generated (Gorg *et al.,* 2004; Berth *et al*., 2007) pH gradient up until they get to their isoelctric point (IP); the point at which their charge is equivalent to zero.

In the second step, known as the sodium dodecyl sulfate polyacrylamide gel electrophoresis, separation of proteins is done intracks vertical to the isoelectic focusing in gel which issplitted-upbased on their sizes. SDS PAGE is not different from theone dimensional electrophoresis, in thatparticles are introducedright onto the SDS-PAGE and splitted-up in correspondence to their masses. When an electric current is put on, the proteins which are

negatively charged move in the direction of the positive charged electrode while proteins having low molecular masses move more rapidlyand quickly through the gel than proteins with higher molecular masses. Multiple copies of the proteins will usuallytraveltogetherand end up placed and fixed in massparticularly on aspot on the gel (Gorg *et al*., 2004; Weiss and Gorg, 2009).

2.12. Staining and detection of gels

Staining materials in form of marketable accessible stains are used for staining gels so as to allow visibility of the protein bands and spots on the 1 D and/or 2D gel correspondingly. About 100-2000 spots can be visible on a two dimensional gel electrophoresis, each of which contain about a single to quite a lot of proteins, besides specific post-translational modifications can be identified effortlessly as either vertical or horizontal allied groups of spots (Steinberg, 2009).

With the help of computerized instruments, the backdrop of the gel is removed out after gel image digitalization, the visible spots are contrasted, and the collected facts are standarized and analysed numerically for evaluaiton of proteinconcentration (Dowsey *et al.,* 2003).In the 1-D gels, a simpler protocol is used in which interested bands and spots or full runs are excised and analysed (Rezaul *et al.,* 2005).

2.13. Identification of proteins

Identification and description of protein is the crucial goal of proteomic analysis. In order to identify a protein, proteins of interest or full runs found in the 1-D gel bands and 2-D gel spots, with the aid of the enzyme, trypsin, to digest the protein into peptides, which can be analysed by matrix-assisted laser desorption ionization mass spectrometry (MALDI MS) or liquid chromatography mass spectrometry (LC-MS). The in-gel trysin digestion involves cutting out target protein bands or spots, removal of stains from gels, reduction and alkylation of protein,digestion, followed by extraction of peptides for mass spectrometry (Shevchenko *et al.,* 2006; Huynh *et al.,* 2009).

2.14. Sequencing of peptides

The sequencing of the peptides are accomplished in the course of a second mass spectrometry, after determining the ratio of mass to charge of the whole peptide: by overlapping using an inactive gas either collision-induced dissociation or by electron transference, further abundant peptides are selected explicitly which then go through fragmentation; the benefit of electron transference is that it preserves post-translational modifications in proteins in the course of a mechanised investigation (Hu *et al.,* 2005). The initial fragmentation of peptide occur primarily near its structure, which is typically among the amide nitrogen and the carbonyl oxygen, consequently, resulting in the generation of two ion groups designated as y and b. Lists of mass to charge percentages intended for discrete fragments whose mass difference match to an amino acids is given as the resultant MS/MS spectrum. The estimation of mass scaling pieces from the y particle set or the b particle set permits for deduction of the sequence of protein. Identification of protein can be done with the results of the several peptides (Walther and Mann, 2010).

Sequencing of *de novo* peptide is a different approach to database search (Standing, 2003). Assembling sequences of amino acid centered on the direct inspection of arranged spectrum is the main entity of de novo sequencing. The likely fragmentation ions and masses for a particular amino acid sequence are quantified, in addition to the frequency estimated through which every category of disintegrate electrically charged particles are produced. Sequencing of DNA thus attempts discovering orders whereby a notable spectral arrangement is highly possible. It is important to have a prior knowledge of sequences; it is the vital distinction from database search approaches (Standing, 2003). While studying human samples, for example, using the databank-probe, the entire complement of protein found in human, FASTA file is loaded first and having entry to the protein pattern obtained afterwards. In the case of new arrangent of genes, any protein pattern could be regarded. The relevance of this is noted when investigating organisms that have partial data on their gene (Ram *et al.,* 2005).

CHAPTER THREE

MATERIALS AND METHOD

3.1. Materials

3.1.1. Collection of samples

Dried sorghum grains (*Sorghum bicolar*) were obtained from Bodija market, Ibadan, Nigeria while fresh cassava tubers (*Manihot esculenta*) were obtained from water area, University of Ibadan road, Ibadan, Nigeria and were immediately transported to the laboratory for traditional production of *Ogi* and *Gari* respectively.

3.2. Methods

3.2.1. Laboratory preparation of *Ogi*

Ogi was madeby way of soaking-wet the sorghum grains in water for fermentation for a periodof 72 h. The wet soft grains were cleaned,ground andfiltered. Fermentation of the filtered*Ogi* was done at room temperature (28+2^{o}C) for about 72 h. For isolation of LAB and yeasts, samples were taken aseptically at 12 h interval in the course of fermenting the filtered *Ogi* (Omemu *et al.,* 2007). The flowchart for the production of *Ogi* is presented in Figure 3.1.

3.2.2. Laboratory preparation of *Gari*

The laboratory preparation of *Gari* involves the step by step process of peeling the cleaned cassava tubers, followed by washing of the tubers and grating (Chelule, 2010). The mash obtained was placed in a table cloth and weighed down using a weighty item to eliminate waste from the mash while fermentation proceeded at room temperature for 2-4 days. At 12 h intervals, fermenting mash was taken out aseptically for isolation of LAB and yeasts.

Dewatered and fermented chunk was pounded, sifted and then the resultant semi-dry well mash was heated and roasted in a pot.Flowchart for the production of *Gari*is presented in Figure 3.2.

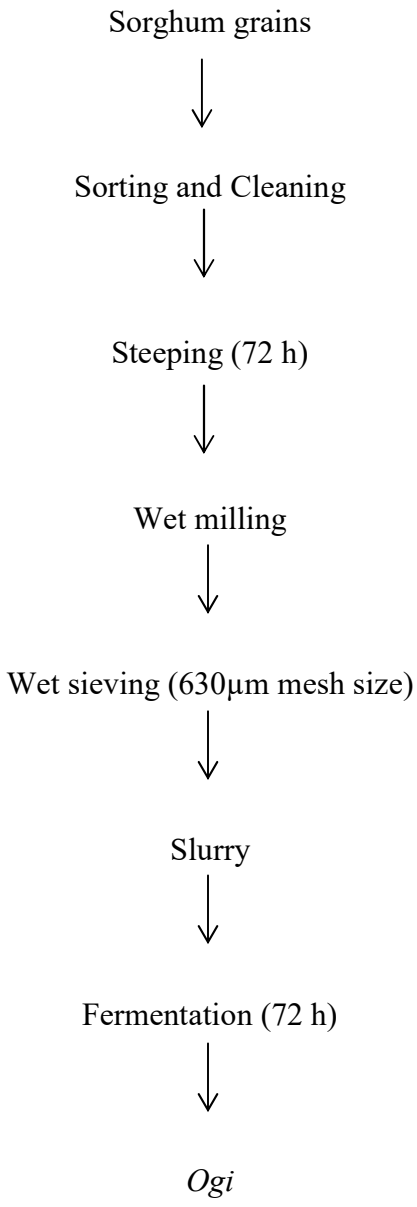


Figure 3.1: Production of *Ogi* (Omemu *et al.*, 2007).

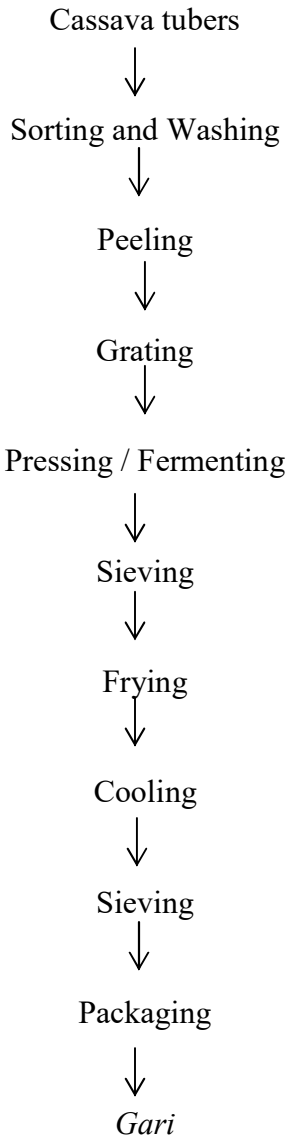


Figure 3.2: Production of *Gari* (Chelule *et al.,* 2010).

3.3. Culture media

Isolation and enumeration of LAB and yeast were done on MRS (De Mann Rogosa Sharpe) broth and agar media and YPD (Yeast Extract Peptone Dextrose) broth and agar media respectively. The media were weighed and made into a solution according to manufacturer instruction as stated in the Appendix 1. The resultant solution was homogenized by stirring using a magnetic stirrer for 10 minutes. Media were autoclaved at 121^{o}C for 15 minutes and thereafter allow coolingto 45^{o}C prior to using the media (Lammert, 2007).

3.4. Enumeration and isolation of microorganisms

3.4.1. Enumeration and isolation of LAB and yeast from laboratory prepared Ogi

One gram of laboratory prepared *Ogi* was transferred to 9 ml of sterile distilled water and mixed carefully for 5-10 seconds to give 10^{-1} dilution. One (1) ml of 10^{-1} dilution was transferred to 9 ml of sterile distilled water and mixed carefully for 5-10 seconds to give 10^{-2} dilution. Serial dilution was carried out sequentially to 10^{-5} dilution. Using a fresh sterile pipette, 0.1 ml of 10^{-4} and 10^{-5} dilution were inoculated onto the surface of MRS agar plates (for isolation of LAB) and YPD agar plates (for isolation of yeast), a sterile stick spreader was used to spread the inoculum over the surface of the plates while ensuring that the liquid does not come in contact with the outer edges of the plates.MRS plates were cultured underanaerobic condition at 37^{o}C for duration of 48 h while the yeast plates were cultured aerobicallyat 30^{o}C for duration of 24 – 48 h. After incubation, distinctive LAB and yeasts colonies with different morphology for example color, dimension and form were handpicked arbitrarily from the agar plates (MRS and YPD) as probable LAB and yeasts isolates respectively. Pure isolates of LAB and yeast were achieved by recurrent sub-culturing on MRS and YDP agar plates respectively (Lammert, 2007).

3.4.2. Enumeration and isolation of LAB and yeast from laboratory prepared fermented cassava

One gram of laboratory prepared fermented cassava was transferred to 9 ml of sterile distilled water and mixed carefully for 5-10 seconds to give 10^{-1} dilution. One (1) ml of 10^{-1} dilution was transferred to 9 ml of sterile distilled water and mixed carefully for 5-10 seconds to give 10^{-2} dilution. Serial dilution was carried out sequentially to 10^{-5} dilution. Using a fresh sterile pipette, 0.1 ml of 10^{-4} and 10^{-5} dilution were inoculated onto the surface of MRS agar plates (for isolation of LAB) and YPD agar plates (for isolation of yeast), a sterile stick spreader was used to spread the inoculum over the surface of the plates while ensuring that the liquid does not come in contact with the outer edges of the plates. MRS plates were cultured under anaerobic condition at 37^{o}C for duration of 48 h while the yeast plates were cultured aerobically at 30^{o}C for duration of 24 – 48 h. Afterincubation, distinctiveLAB and yeasts colonies with different morphologyfor examplecolor,dimension and form were handpickedarbitrarily from the agar plates (MRS and YPD) as probable LAB and yeasts isolates respectively. Pure isolates of LAB and yeast were achieved by recurrentsub-culturing on MRS and YDP agar plates respectively (Lammert, 2007).

3.5. Preservation of pure cultures

Pure cultures of LAB isolates were sub-cultured onto fresh MRS slants; then incubated at 30^{0}C until growth becomes visible. On the other hand, the pure culture of the yeast isolates were sub cultured into slants of YPD agar. The cultures were kept at 4^{o}C for later routine usuage and at intervals of 4 weeks, the stock LAB isolates were sub cultured (Lammert, 2007).

3.6. Classification of LAB isolates

LAB isolates were classified and typifiedusing macroscopic and microscopic properties, as well as biochemical and physiological tests as follows:

3.6.1. Macroscopic examination

The cultural characteristics of each LAB and yeasts were examined. The appearance, colour, shapes and sizes of respective colonies were then noted.

3.6.2.Microscopic examination

3.6.2.1.Gram staining

Smears of the LAB isolates were stained. This was done by flooding the smear with crystal violet stain for 60 seconds, and drained after 30 seconds. Two drops of Gram's iodine was then applied on the smear, which act as the mordant. The solution was left for 60 seconds on the slide and washed off carefully under tap water. Next, 95% ethanol was applied and used to wash the slide quickly until it appeared free of the violet stain, after which it was rinsed and thereafter, flooded with safranin for duration of 30 seconds. Afterwards, the slides were rinsed, then dried. Oil immersion was applied on the smear and used to determine the gram reaction and the cellular characteristics (Coico, 2005).

3.6.3.Biochemical tests

3.6.3.1.Catalase test

LAB isolates of 18 h old were used for the catalase test. Drops of newly prepared hydrogen peroxide (3%) were added onto the plates (Seeley and Van Demark, 1972). A catalase positive reaction was noted by the development of bubbling white gas while the absence of development of bubbling white gas shows a negative reaction.

$2H_2O_2 \longrightarrow 2H_2O + O_2$

3.6.3.2.Spore stain

Onto uncontaminated glass slides, smears of pure LAB cultures were prepared and masked with Malachite green reagent and boiled thereafter left to cool for a period of 5 minutes. Next, slides were carefully rinsedusing water, thereafter stained using safranin in time duration of 1 minute. Afterwards, the slides were rinsed and allowed to dry. The existence and non-existence of spores were observed under the microscope and noted.

3.6.4.Physiological test for LAB isolates

3.6.4.1.Growth at different temperatures

LAB cultures (18 h old) were introduced into 5 ml of MRS broth and cultured in incubators set on 15^{o}C and 45^{o}C, for 2-4 days; turbidity in tubes after incubation were considered positive while tubes that did not show turbidity were considered negative (Samelis *et al.,* 1994).

3.6.4.2.Growth at different pH

Five ml of MRS broth previously modified to acidic pH of 3.9 and alkaline pH of 9.4 were inoculated using 18-24 h old cultures and incubated micro-aerobically for at 30^{o}C for 48 h; turbid tubes were compared to the uninoculated tubes (control) and regarded as positive, while unturbid tubes as negative (Samelis *et al.,* 1994).

3.6.4.3.Growth at 4%, 6% and 8% NaCl

MRS broth in tubes containing 4%, 6% and 8% NaCl were distributed into bolt lid tubes and sterilized. MRS broth prepared in tubes using 4%, 6% and 8% sodium chloride was distributed into bolt lid tubes and sterilized. Inoculation of fresh cultures (18 h old) of LAB isolates was into the cooled sterile medium and incubation micro-aerobically at 30^{o}C for 3-4 days was carried out. Positive results were regarded as tubes having greater turbidity than before while negative results were recorded with unturbid and clear tubes. Uninoculated tubes served as control (Samelis *et al.,* 1994).

3.6.4.4.Gas production (CO_2) from glucose

The production of gas from glucose was done using MRS broth having 1% glucose which was distributed into tubes holdingoverturned Durham tubes and sterilized. LAB isolates were introduced into the cooled, sterile medium and incubated micro-aerobically at 30^{o}C for 3-4 days. Uninoculated tubes served as control. The production of gas (CO_2) was observed by the presence of small spaces at the uppermostpart of the overturned Durham tubes and such tubes were considered positive, while negative tubes did not show the presence of small spaces at the uppermost part of the overturned Durham tubes (Samelis *et al.,* 1994).

3.6.5.Carbohydrate fermentation tests for LAB isolates

Fermentation of carbohydrates using lactose, D-raffinose, fructose, L-arabinose, ribose, galactose, sucrose, maltose and D-xylose was tested on the LAB isolates. Bromo-cresol purple broth base (Appendix 1) was used as an essential basic medium. The sugar solution

was made sterile by filtering the prepared solution using a 0.2 µm millipore filter (final concentration of 1%) and then aseptically added into the sterile bromo-cresol purple broth base. Fresh LAB isolates (18 h old) were introduced into the medium containing the sugars and incubated micro-aerobically at 30^{o}C for 3-4 days. Uninoculated tubes served as control. Utilization of the sugars was accessed by the observed changes in the bromo-cresol purplebase medium from purple to yellow and such tubes were regarded as positive, while tubes where the bromo-cresol purple broth base remained purple indicated the non-utilization of such sugars by the LAB isolates and the results were regarded as negative (Kandler and Weiss, 1986).

3.7.Characterization of yeasts isolates

The yeasts isolates were characterized employing their morphological and physiological tests as well astheir pattern of sugar fermentation:

3.7.1.Morphological characteristics

The morphological features of the yeasts isolates was carried out by the macroscopic examination of the surface of the colonies on the Yeast Extract Peptone Dextrose (YPD) agar plates to determine the shape, color, texture, degree of growth and opacity of the isolates. Actively growing yeasts cells were streaked onto freshly made YPD agar plates and cultured at 30^{o}C for duration of 5 days. Wet mount was prepared for the yeast isolates by picking single colonies of individual yeasts isolates from the YPD plates onto clean slides, stained with lacto-phenol cotton blue and examined microscopically to determine the individual cell morphology of the yeast isolates (Barnett *et al.,* 2000).

3.7.2.Physiological tests for yeast isolates

3.7.2.1.Growth at different temperatures

Five ml of YPD broth were inoculated with fresh yeast culture (18 h old) and incubated in incubators at 25^{o}C, 30^{o}C and 37^{o}C for 2-4 days. Turbidity in tubes was considered as positive while unturbid tubes were recorded as negative. Tubes which were left uinoculated served as control (Kurtzman *et al*., 2011).

3.7.2.2.Growth at 50% and 60% Glucose

Tubes holding 5 ml of YPD broth complemented with 50 and 60% (w/v) glucose were inoculated with fresh yeast culture (18 h old) and incubated at 30^{o}C for 2-4 days. Turbidity in tubes was considered as positive while unturbid tubes as negative. Uninoculated tubes served as control (Kurtzman *et al.,* 2011).

3.7.2.3.Urea hydrolysis

Five ml of filter-sterilized urease broth were inoculated with fresh yeast culture (18-24 h) and incubated at 30^{o}C for 5-7 days. Color changes observed in tubes from light yellow to light pink showed positive results while tubes that showed no color change were considered negative. Tubes left uninoculated were used as the control (Kurtzman *et al.,* 2011).

3.7.2.4.Growth at 0.1% and 0.01% cyclohexamide

Yeast samples to be tested were grown at 0.1% and 0.01% cyclohexamide. Yeast nitrogen base agar plates with filter sterile cyclohexamide made up to a definitemolarity of0.1% (100mg/ml) and 0.01% (10mg/ml). Thereafter, yeast cultures (18-24 h old) were introduced into the prepared media and cultured at 30^{o}C for 5-7days. Observation of growth on the plates was considered as positive results while absence of growth on the plates was seen and recorded as negative results. Uninoculated tubes served as the control (Kurtman *et al.,* 2011).

3.7.2.5.Starch formation

Isolates of freshly cultured yeasts were introduced onto yeast extract peptone agar plates containing previously added starch then, incubated at 30^{o}C for for 7-14 days thereafter flooded with Lugol's Iodine. Results that were positive to starch formation revealed a dark blue to green color change while the observation of no color development was considered as negative (Kurtzman *et al.,* 2011).

3.7.2.6.Growth in 1% acetic acid

A 4mm-loopful of 18-24 h yeast cell suspension was inoculated on acetic acid agar plates by streaking across the plate. The plates were cultured at 30^{o}C, examined following 3-6 days of

incubation for development of colonies. Plates observed with the growth of yeast colonies were recorded as positive results while plates showing no growth of colonies were recorded as negative. Uninoculated tubes served as control (Kurtzman *et al.,* 2011).

3.7.3. Nitrogen assimilation of the yeast isolates

The yeast isolates were tested for the assimilation of 0.78g potassium nitrate and 0.26g sodium nitrite. Yeast isolates were inoculated onto yeast carbon base broth and incubated at 30^{o}C for 5-7 days to allow the entire consumption of nitrogeneos compounds brought from the pre-cultured media. A drop of yeast cell suspension was inoculated onto the basal medium containing the nitrogen compounds and incubated at 30^{o}C for 7-14 days. Observation of growth on plates after incubation indicated the assimilation of the nitrogen compoundsby the yeast isolates and results were considered positive while the absence of growth on the plates was considered as negative results indicating the non assimilation of nitrogen compounds (Kurtzman *et al.,* 2011).

3.7.4. Carbohydrate fermentation tests for yeasts isolates

Fermentation of carbohydrates by the yeasts isolates was tested on these sugars as follows; L-arabinose, maltose, fructose, lactose, ribose, sucrose, galactose, D-xylose, D-raffinose. Bromothymol Blue broth base (Appendix 1) was used as the essential basic media. The sugar solution was made sterile by filtering the solution employing a millipore filter measuring 0.2 µm and aseptically introduced into the sterile bromothymol blue broth base to give a final concentration of 1%. Yeast culture of 18-24 h old were inoculated into medium containing the sugars and incubated at 30^{o}C for 3-4 days. Uninoculated tubes served as the control. Utilization of the sugars was accessed by the observation of color change of bromothymol blue from deep blue to yellow which were considered as positive, while the tubes in which bromothymol blue remained blue indicated that the suagrs were not utilized by the yeasts isolates and were considered negative (Kurtzman *et al.,* 2011).

3.8. Lactic acid bacteria and yeast identification employing MALDI TOF MS

3.8.1.Sample preparation- Formic acid Extraction Method

Fresh LAB and yeast cells were transferred from MRS and YPD plates respectively into an eppendorf tube measuring 1.5 mlusing the pipet end point and thoroughly stirred in water

(300 µl). Next, the introduction of absolute ethanol (900 µl) which was then thoroughly mixed, and centrifuged at 16 160 g for 2 mins followed by discarding the supernatant. The obtained pellet was centrifuged again to eliminate all the remaining ethanol by circumpectly removing the pellet without disturbance. The pellet obtained was dried at 25^{o}C for an hour. Afterwards 70% v/v formic acid (5 µl) was introduced into the pellet and thoroughly mixed prior to adding of 5 µl of pure acetonitrite followed by centrifuging (4 800 rpm for duration of 2 mins). The supernatant (1 µl) obtained was laid on a spot on the stainless steel plate (matrix assisted laser desorption ionization typical plate) and dried at 25±2^{o}C. The matrix solution (1 µl) was overlaid on every single sample, dried at 25±2^{o}C. Once the sample was dried, analysis of target plate using the MALDI TOF MS was carried out. Samples were prepared in duplicate according to the method of Gorton *et al.* (2014).

3.8.2. Mass spectrometry

The MALDI TOF MS Instrument (Bruker, Germany) pre-programmed FlexControl 3.0 method MBT_FC.par (Bruker, Germany), the mass spectra were gotten on an AutoFlex III Smartbeam of the machine. The flex control method stipulated the following parameter settings as; nitrogen laser potencyfixedat 337nm, was considerablybeyond the limit for desorption/ionization, voltage of ion sources one and two were set at 20 kV and 25 kV respectively and the pulsed-ion extraction time was set at 100 ns. An average of 600 shots was delivered at one point for every singlespot containing the sample and the final spectrum was an average accumulation of all spectra gathered from minimum six variouspositions on a particularspot containing the samples. The protein molecular weight detection limit was set within the range of 2000 and 20000 Da. The spectra range was externally adjusted with the use of the customary combination calibrant namely *Escherichia coli* isolates together with RNase Aprotein and myoglobin protein to validate the authencity of mass spectrum data produced by the MALDI TOF MS Instrument (Gorton *et al.,* 2014). In order to obtain a consistent calibration of the MALDI TOF MS equipment, the mass spectral report of the standard calibrant mixture was employed and consequently acquired using the instrument's pre-programmed FlexControl 3.0 method, MBT_autoX.axe (Bruker, Germany).

3.8.3. Generation of spectrum and data analysis

Dried formulations on the MALDI stainless steel target plate were subjected to laser pulses, which caused the shift of energy to the analyte molecules from the matrix, which was desorbed into the gaseous phase. In order to accelerate the ionized molecules to the mass spectrometer, electric potentials were used. Given the mass-to-charge ratio, the biomarkers were split upand the resulting spectra were compared to the reference spectra of microorganisms previously present in the MALDI TOF Database. Hence, the MALDI Bruker Daltonics Biotyper software usescorrespondingpatterns to relate mass spectra which are not identified yet usingpreviously collected and deposited recordswithin the Bruker Daltonics mass spectra database. About 3740 microorganisms' reference spectra are presently held in the Bruker Daltonics database (Gorton *et al.,* 2014). According to the manufacturer, the bruker Daltonics Biotyper 3.0 software stipulate log (score) values as guidelines for the identification to species and genus level (Gorton *et al.,* 2014) as presented in Table 3.1.

3.9. Identification of LAB and yeasts isolated from *Ogi* and fermented cassava

Isolates of LAB and yeast were classified according to their morphology, biochemical and physiological properties, sugar fermentation tests in addition to MALDI-TOF MS analysis.

3.10. Screening of LAB and yeasts isolated from *Ogi* and fermented cassavasubjected to stress

The screening of LAB and yeast isolated from *Ogi* and fermentedcassava subjected to acid and oxidative stress was carried out as described by Brandao *et al. (*2014)

3.10.1. LAB and yeast subjected to acid stress at different pH concentrations

The screening of LAB cultures subjected to acid stress at different pH concentrations was carried out by cultivating the cells to static phase in MRS broth at 30^{o}C for 24 h. Optical density (OD_{600}) was quantified and fine-tuned to 1.0 corresponding to 1.0 x 10^{7} cfu/ml. Five microliters of five-fold serially diluted cells was spotted onto freshly prepared MRS plates with pH previously adjusted to 1, 2, 3 and 4 appropriately. The plates were incubated micro-aerobically at 30^{o}C for 2 days (Brandao *et al* 2014). The growth of cells was examined by observing the incubated plates and observations recorded as: w = 1-19%; + = 20-49%; ++ = 50-79%; +++ = 80-89%; ++++ = 90-100%.

Table 3.1: Guidelines for the intepretation of MALDI TOF MS score values

Range	Description	Indicator colour
2.300-3.000	Extremely possible species	Green
2.000-2.299	Safe genus and possible identification of species	Green
1.700-1.999	Possible identification of species	Yellow
0.000-1.699	No dependable identification	Red

The screening of yeast cultures subjected to acid stress at different pH concentrations was carried out by cultivating the cells to static phase in YPD broth at 30^{o}C for 24 h. Optical density (OD_{600}) was quantified and fine-tuned to 1.0 corresponding to 1.0 x 10^{7} cfu/ml. Five microliters of five-fold serially diluted cells was spotted onto freshly prepared YPD plates with pH previously adjusted to 1, 2, 3 and 4 appropriately. The plates were incubated at 30^{o}C for 2 days (Brandao *et al* 2014). The growth of cells was examined by observing the incubated plates and observations recorded as: w = 1-19%; + = 20-49%; ++ = 50-79%; +++ = 80-89%; ++++ = 90-100%.

3.10.2. LAB and yeast subjected to oxidative stress at different concentrations of H_2O_2

The screening of LAB cultures subjected to oxidative stress at different hydrogen peroxide concentrations was conducted by cultivating the cells to static phase in MRS broth at 30^{o}C for 24 h. Optical density (OD_{600}) was quantified and fine-tuned to 1.0 corresponding to 1.0 x 10^{7} cfu/ml. Five microliters of five-fold serially diluted cells was spotted onto freshly prepared MRS plates containing 1mM, 3mM and 5mM H_2O_2 appropriately. The plates were incubated micro-aerobically at 30^{o}C for 2 days (Brandao *et al.,*2014). The growth of cells was examined by observing the incubated plates and observations recorded as: w = 1-19%; + = 20-49%; ++ = 50-79%; +++ = 80-89%; ++++ = 90-100%.

The screening of yeast cultures subjected to oxidative stress at different hydrogen peroxide concentrations was conducted by cultivating the cells to static phase in YPD broth at 30^{o}C for 24 h. Optical density (OD_{600}) was quantified and fine-tuned to 1.0 corresponding to 1.0 x 10^{7} cfu/ml. Five microliters of five-fold serially diluted cells was spotted onto freshly prepared YPD plates containing 1mM, 3mM and 5mM H_2O_2 appropriately. The plates were incubated at 30^{o}C for 2 days (Brandao *et al.,*2014).. The growth of cells was examined by observing the incubated plates and observations recorded as: w = 1-19%; + = 20-49%; ++ = 50-79%; +++ = 80-89%; ++++ = 90-100%.

3.11. Physiological response of LAB and yeast isolated from *Ogi* and fermentedcassava tostress

LAB and yeast isolates were subjected to the following stress conditions:

i. acid stress at different pH concentrations and
ii. oxidative stress at different concentrations of hydrogen peroxide (H_2O_2)

3.11.1. LAB and yeast subjected to acid stress at different pH concentrations

LAB cultures were grown in MRS broth (Sigma Aldrich) overnight, then 5µL of the culture was sub-cultured into 250µL of MRS broth which has been modified to pH 1, 2, 3 and 4 in micro-plates. The micro-plates were cultured micro-aerobically at 30^{o}C for duration of 24 h. The experiment was conducted in triplicate. After incubation, the growth of cultures was read in the microplate reader (Becker Coulter) at 620nm at 30^{o}C (Brandao *et al.,* 2014).

Similarly, the yeast cultures were grown in YPD broth (Sigma Aldrich) overnight, then 5µL of the culture was sub-cultured into 250µL of YPD broth which has been adjusted to pH 1, 2, 3 and 4 in micro-plates. Culturing of the micro-plates was done at 30^{o}C for duration of 24 h. Experimental trials was conducted as set of three. After incubation, the growth of cultures was read in the microplate reader (Becker Coulter) at 620nm at 30^{o}C (Brandao *et al.,* 2014).

3.11.2. LAB and yeast subjected to oxidative stress at different concentrations of H_2O_2

LAB cultures were grown in MRS broth (Sigma Aldrich) overnight, then 5µL of the culture was sub-cultured into 250µL of MRS broth containing 1mM, 3mM and 5mM H_2O_2 in micro-plates. The micro-plates were cultured micro-aerobically at 30^{o}C for duration of 24 h. Experiments were conducted in set of three. After incubation, the growth of cultures was read in the microplate reader (Becker Coulter) at 620nm at 30^{o}C (Brandao *et al*., 2014).

Similarly, the yeast cultures were grown in YPD broth (Sigma Aldrich) overnight, then 5µL of the culture was sub-cultured into 250µL of YPD broth containing 1mM, 3mM and 5mM

H_2O_2 in micro-plates. The micro-plates were cultured at 30^{o}C for duration of 24 h. Experimental trials was conducted as set of three. After incubation, the growth of cultures was read in the microplate reader (Becker Coulter) at 620nm at 30^{o}C (Brandao *et al.,* 2014).

3.12. Proteomic response of LAB and yeast isolated from *Ogi*and fermented cassava to stress

In the examination of the proteomic response of LAB and yeast isolated from *Ogi* and fermented cassava to stress conditions (acid and oxidative stress), the following were carried out;

i. extraction of proteins from selected strains of LAB and yeast,
ii. quantification of protein in the lactic acid bacteria and yeast protein extract,
iii. SDS PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) and two dimensional electrophoresis,
iv. protein detection and analysis of image
v. identification of protein from gel bands and spotsusing the in-gel trypsin digestion

3.12.1. Extraction of proteins from screened LAB and yeast strains exposed to stress (acid and oxidative stress) (modified protocol)

MRS broth adjusted to acid stress at pH 1, 2, 3, 4; oxidative stress at 1mM, 3mM, 5mM H_2O_2) and MRS broth without stress conditions (control) were inoculated with 18-24 h cultures of LAB and cultured at 30^{o}C for duration of 24 h. In the same vein, 18 -24 h yeast cultures were inoculated in YPD broth adjusted to acid stress at pH 1, 2, 3, 4; oxidative stress at 1mM, 3mM, 5mM H_2O_2) and YPD broth without stress conditions (control) and cultured at 30^{o}C for duration of 24 h. After incubation, LAB and yeast cells were taken and centrifuged at 4200 rpm (Beckman Coulter, Allegra X-22 Centrifuge) for 20 mins, while the resulting liquid wasthrown out. Collected residueswere washed thrice in phosphate buffer saline (PBS) solution (10 ml) (Appendix 2), centrifuged at 4200 rpm for 10 mins each. The weight of each cell pellet in the eppendorf tubes were obtained and estimated to give the volume of thiourea lysis buffer (4% Chaps, 2M Thiourea, 1 % DTT, 7M Urea, 2% carrier ampholytes pH 3-10, 1M Tris base, 10mg Protease inhibitor) (Apendix 2) to be added to each cell pellet. Lysing of cells were carried out using acid washed glass beads of about 212 to 300 μm diameter with thorough vortexing on table mixer (Maxi Mix II, Barnstead

Thermolyne, USA) at a maximum speed approximately 2500 rpm with intermittent placement of samples on ice cubes for 1 min. The cells previously lysed were subsequently re-solubilized by ultra-sonication (Ultrasonic bath- Bio-equip 393 × 407) on ice for 6 rounds at 15 secs each. Samples were cooled on ice for a minute in between sonication to completely solubilize precipitated proteins. Removal of cell remains was done bycentrifuging the cells at 4800rpm for duration of 10 mins at 4^{o}C. Resultant supernatant which contain the protein remained at -80^{o}C until for storage until further analysis (Becerra *et al*., 2001).

3.12.2. Quantification of protein extracted from LAB and yeasts isolates from Ogi and fermented cassava

Given the procedure described by Bradford (1976), the molarity of protein was conducted using Bovine serum albumin (BSA) as basic stock solution. Assays were performed in 250µL microplate and the linear range for BSA was 10-1000µg/ml. BSA (5mg/ml) was diluted using thiourea lysis buffer used for the extraction of protein and is given in Table 3.2.

Five microlitres of each protein standard and unknown protein samples were pipetted into the microplate well and 250 µL of the Coomassie Blue G-250 dye reagent (Bio-Rad) was introduced into each well and harmonized by mixing comprehensively and further depressing the plunger repeatedly followed by incubation at 25^{o}C for 30mins. Water served as the blank samples (0µg/ml). The assay was performed in duplicate. After incubation, samples were read using a microplate reader (Promega GloMax 2640, USA) at 600nm. The resulting absorbance from the protein standard was used to plot a chart representing the absorbance (600nm) verse the concentration (µg/ml) as a standard curve. Protein concentration of each unknown sample was obtained from the resulting equation generated from the standard curve (Appendix 6).

3.12.3.Sodium dodecyl sulphate polyarcylamide gel electrophoresis (SDS PAGE) and two dimensional electrophoresis (2 DE)

3.12.3.1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

After extracting protein from LAB and yeasts isolated from *Ogi* and fermented cassava

which had been subjected to acid and oxidative stress, SDS PAGE was conducted. The SDS electrophoretic system consists of the chamber, cover with attached power cables, electrode assembly, cell dam for holding buffer, stands for casting frames, 15-well combs and glass plates of thickness 1.5mm (Bio-Labs). 12% acrylamide resolving gel and stacking gels were

Table 3.2: Quantification of protein standard (5mg/ml)

Concentration of protein (µg/ml)	Volume of BSA (µl)	Volume of Thiourea/lysis buffer (µl)
0	0	1000
10	10	990
30	30	970
50	50	950
100	100	900
200	200	800
300	300	700
400	400	600
600	600	400
800	800	200
1000	1000	0

used for the SDS PAGE and prestained molecular marker brand range (Bio Labs). The glass plates were assembled and the resolving gel (Appendix 2) was prepared, mixed carefully to prevent the development of bubbles. The gel solution was emptied carefullyamidst the glass plates allowinga quarter of the area open and intended for pouring the stacking gel. Some quantity of water was carefully introduced to the uppermostpart of the electrophoretic set up containing the resolving gel, then allowed to polymerize for duration of 30 mins. The water was discarded and washed gently with double distilled water after polymerization. The stacking gel (Appendix 2) was prepared and poured using a pipette until overflow, the comb was immediately inserted and allowed to polymerize for at least 60 mins. The combs were carefully removed after polymerization, followed by placement of the gel into the electrophoretic chamber filled with 1X running buffer (Appendix 2). Samples preparation was done by mixing sample buffer (10 µl)(Appendix 2) plus protein sample (10 µl) in the ratio of 1:1, and heated at 70^{o}C using a heating block for a period of 10 mins followed by centrifuging the mixture at 4 800rpm for about 2 mins. The molecular marker and prepared protein samples were carefully introduced into every single well as required, and the gels was allowed to run on 50 mA meant for 2 minigels, 100 volts for 2 h. As soon as the dye line got to the lowermostpart of the gel, the electrophoretic run was ended followed by disassembling of the gel sandwich and removal of the gels from the glass plates for staining (Anderson and Anderson, 1978).

3.12.3.2. Two-dimensional electrophoresis

Two-dimensional electrophoresis was done on protein samples extracted from selected yeast isolated from '*Ogi'* and fermented cassava subjected to acid and oxidative stress. Immobiline Dry Strips were rehydrated (IPG Strip; Amersham Pharmacia Biotech) in the Immobiline Dry Strips Rehydration trays (Amersham Pharmacia Biotech). The IPG Strips utilized in this study measured 7 cm in length with pH ranging from 3- 10. Protein samples (12 µl) was centrifuged at 4 800rpm for a period of 10 mins, and then addedinto 140 µl of

the rehydration buffer (Protein solubilizer- urea (8 M), Chaps (2%), DTT (2 M), Appro Zoom @ carrier ampholytes, trace bromophenol blue),which was then introduced into the IPG dry strips and left at 25^{o}C overnight prior the commercement of the first dimensionelectrophoresis. The remoisturized strips were focused with the use of the Multiphor II electro

phoresis unit at 20^{o}C at gradient mode. This step is referred to as the isoelectric focusing (IEF). remoisturized strips were focused at 0-250 V aimed at 15 mins, next 250- 4000 V aimed at 1 h and lastly 4000 V for duration of 8- 10 000 V/ h with a total of 8 kVh stored. To ensure fine focusing of strips, a second focusing was carried out on the same strips as described above. The remoisturized strips wereutilizedinstantly after focusing for the second phase of electrophoresis; otherwise, the strips were kept at -80^{o}C pendingwhen it will be needed. Before the start of the second dimension,sodium dedocyl sulphate polyacrylamide gel electrophoresis, the strips were placed in the first equilibration buffer (Appendix 2) for the 15 mins, and then transferred into the second equilibration buffer (Appendix 2)for another 15 mins. In each case, 2 ml of the equilibration solutions was used. The equilibrated strips were carefully placed in the well for the second dimension SDS PAGE performed using 12% acrylamide revolving gel and stacking gels and afterwards prestained molecular marker brand range (Bio Labs). About 1 ml of overlay Agarose (Appemdix 2) was added into the well containing the rehydrated strips to prevent strips from moving up and down while running the gels. After the second dimension electrophoresis, the gels were carefully detachedout of the glass-plates forstaining (O' Farrell, 1975; Anderson and Anderson, 1978).

3.12.4. Protein detection and image analysis

Staining of the gels obtained from the SDS PAGE and 2-DE was done using Coomassie brilliant blue R-250. Next, gels were soaked in excess staining solution (Appendix 2) for a period of 1 h followed by destaining of the gels using large excess of the destaining solution (Appendix 2). New destaining liquid was introduced a number of times up until the surrounding of the stained gel was satisfactorily removed and bands were more observable (Westermeier, 2006). Well satisfactorily destained gels were visualized using the imager, G: Box (Syngene).

3.12.5. Identification of protein from gel band and spots using in-gel trypsin digestion

For the identification of proteins from gel bands and spots of the SDS PAGE and 2-DE respectively, the following steps were employed:

i. excision and destaining of gel bands and spots
ii. reduction of disulfide and alkylation of free cysteines
iii. trypsin digestion
iv. peptide extraction
v. desalting and enrichment of peptides
vi. liquid chromatography electron spray ionization mass spectrometry (LC-ESI-MS) analysis

3.12.5.1. Excision and destaining of gel bands and spots

A clean scalpel was used to excise the protein bands and spots (Coomassie stained) of interest from SDS PAGE and 2-DE gels. The bands and spots were cut into pieces (1 x 1 to 2 x 2 mm) and placed in a clean low binding micro centrifuge tubes. 100 µl of water was introduced into tubes containing gels and allowed to sit for 5 mins, and then water was removed and discarded. Furthermore, 100 µl of 100mM ammonium bicarbonate: acetonitrile/water (1:1 v/v) was placed in tubes holding gels and allowed to sit for 15 mins, and then ammonium bicarbonate: acetonitrile/water was removed and discarded. Pure acetonitrile (ACN) of about 50 µl was introduced into the tubes containing gels and kept for 5 mins, and then (ACN) was removed and discarded. Gels pieces appeared shrunken and opaque. Repeated washing was done on the gel pieces using100 µl of 100 mM ammonium bicarbonate: acetonitrile/water (1:1 v/v) for duration of 15 mins, and discarded, and then the addition of 50 µL acetonitrile (ACN) for 5 mins until gel pieces were colorless. Drying of the gels bits was done with the use of vacuum centrifuge for 5-10 mins (Shevchenko *et al.,* 2006).

3.12.5.2. Reduction of disulfide and alkylation of free cystienes

A volume of 10mM dithiothreitol (DTT) in 100mM ammonium bicarbonate was introduced into tubes containinggels, and then vortexed, followed by a brief centrifugation. The treated gels were incubated for 45 mins at 56^{o}C. The tubes were cooled at ambient temperature, followed by removal of resultant liquid which is then discarded.Next,55mM iodoacetamide (IAA) in 100mM ammonium bicarbonate (20 µl) was introduced into the tubes holdingthe gels, then vortexed and centrifuge briefly, and incubated in a dusky room for 30 mins at 25^{o}C, the supernatant was then removed and discarded. Thereafter, 100mM ammonium bicarbonate; acetonitrile/ water (100 µL) at ratio 1:1 v/v was introduced into tubes containing gels and allowed to sit for 15 mins, and then ammonium bicarbonate: acetonitrile/water was removed and discarded. Furthermore, the addition of ACN (50 µl)into the tubes containing gels was done and allowed to sit for about 5 mins, and then (ACN) was removed and discarded. Gels pieces appeared shrunken and opaque. The vacuum centrifuge was employed in drying the gel pieces for 5-10 mins (Aebersold and Goodlett, 2001; Shevchenko *et al.,* 2006).

3.12.5.3. Enzymatic digestion

The enzymatic digestion of protein in gel was done using trypsin, 20-25 ng/µl (Promega). The gel enzyme stock solution was prepared in 1:1000 in 100 mM ammonium bicarbonate to obtain a 10- 20 µg\ml working solution. 20 µl of gel enzyme working mixture was introduced into tubes containing gels, thenincubatedupon ice aimed at incubation period of 1 h. Theadded enzyme mixtureused to digest the gels was taken away and discarded, and thenintroduction of 50 mM ammonium bicarbonate (10 µl) into thegel fragment, then placed in a water bath for an incubation period overnight (Shevchenko *et al.,* 2006).

3.12.5.4. Peptide extraction

The resultant liquid containing the peptide is transferred to a new micro centrifuge tube (labelled tube 1). A volume of 100 µl of extraction solution (45% water/50% acetonitrile/5%

formic acid) was introduced into the tubes holding the gel pieces, and then, vortexed for about 10 mins, followed by sonication for 5 mins and brief centrifugation (14 800 rpm for about 1 min). The resultant liquid (supernatant) is extracted and added into tube 1. A volume of 100 µl of the solution extracted was imtroduced into the tubes holding the gel pieces, and then, vortexed for 10 mins, sonicated for 5 mins thencentrifuged at 14 800 rpm for duration of 1 min. The extracted liquid was transferredintotube 1.This was repeated again and then the volume oftube 1 was reduced to 20 µl using the vacuum centrifuge (Shevchenko *et al.,* 2006).

3.12.5.5. Desalting and enrichment of peptides

Desalting of peptide was carried out employing the reversed-phase chromatography method using the Zip Tip µC18 Cartridge column. The column was fastened to a 20 µl micropipette set at 7 µl. Withdrawal of acetonitrile was done cautiously, while the column was lowered into the acetonitrile and pipetted out taking caution to prevent the introduction of air bubbles into the Zip Tip. Repetition of the previous stage was done 7-8 times and finally the acetonitrile was pipetted out. Water measuring about 7 µl was slowly taken out across the Zip Tip cautiously. Repetition of the previous stage was done 10 times to make sure that the entire acetonitrile had been completely removed. 20 µl of extracted peptide sample solution was dispersed into 20 µl of 0.5% formic acid. Precaution was taken towards ensuring that the lower side of the tube containing the extracted peptides was washed properly to ensure maximum dissolution of the peptides. The Zip-Tip was cautiouslyloaded with the peptide liquid and slowly introducedwithin a clean empty tube. This was repeatedseverally (8-10 times) to certifythat the peptides were retained on the Zip-Tip. Formic acid (0.5%) was used to wash the Zip-Tip severally (8-10 times) to perform the desalting and washing of the peptides. After pipetting the wash solution, the Zip-Tip was slowly filled with 0.5% formic acid: water/acetonitrile 1:1v/v (extraction solution 1). The extracted solution (extract 1) was pipetted into a clean empty tube (tube A). The Zip-Tip was then filled with acetonitrile (extraction solution 2) and after short duration of 10 seconds, the extracted solution (extract 2) was introduced into tube Aholding the previous extract. The collective extracts in tube A was collected and used for identification of protein (Naldrett *et al.,* 2005).

3.12.5.6. Liquid chromatography electrospray ionization mass spectrometry analysis (LC-ESI-MS)

The samples containing the peptide already digested with trypsin were removed and evaluated using LCQ classic ion trap mass spectrometer.In brief, 365 x 100 µm fused silica tubing was drawn using a laser puller (P-2000) to make a 5 micron tip, and afterwards collected using a POROS 10 R2 10 µm hydrophobic packaging object to a bed length measuring 10-15 cm with the use of a high-pressure helium container. Loading of the sample was done by inserting the samples into the pillar, followed by placement in the Eppendorf tube consisting the peptide sample solution hooked unto an elevated pressure container, closing the container, and introducing the blunted edge of the pillar over a swagelock suitable into the eppendorf tube. In order to compel the solution onto the pillar, high-pressure container was pressurized to 400-500 psi. Consequently, the tryptic digest solution was put back to suspension and spinned in a centrifuge, the resulting liquid was then compelled into the cylinder so as to reduce clogging of the column. To detach the bound tryptic peptides, a 30 min linear gradient (0-60% buffer B (Appendix 2)) was used. With the use of acontrollable capillary tube(75 m pre-column), the speed of the current was decreased from 150 l/min to about 300 nl/min. An HP 1100 was made use of to generate the current and speed and the remaining peptide were taken out from the former run employing a small 15-min gradient (Shevchenko *et al.,* 2006).

In the course of the liquid chromatography analysis, the tandem mass spectra were created using data dependent MS/MS so that the three primary very intensive ions were carefully chosen from the complete mass spectrometry test. The acquisition of redundant MS/MS data occurs during search, hence to minimize such occurrences, a three-min forceful exclusion was employed. Consequently, the mass spectra were then matched to the mass spectra in MASCOT (Perkins *et al.,* 1999) searches for the identity of protein. Database probing was carried out with MASCOT ALGORITHM using the MSDB record on a GPS workplace. The search factors utilized includes:

Search type : MS/MS ion Search

Taxonomy : species

Enzymes : Trypsin

No of maximum-missed cleavages : 1

Fixed modification : Carbamidomethyl cystiene

Variable modification: Oxidation (M)

Values of masses : Monoisotopic

Mass of protein : Unrestricted

Tolerance of peptide mass : ± 10ppm

Tolerance of fragment mass : ± 0.1 Da

3.13. Starter culture selection for the production of *Ogi*and *Gari*

Selection of stress-adapted LAB and yeasts as starter cultures for producing *Ogi* and *Gari*was based on their physiological response to;

i. acid stress at pH 1, 2, 3, 4 and
ii. oxidative stress at 1mM H_2O_2, 3mM H_2O_2, and 5mM H_2O_2

3.14. Preparation and inoculation of laboratory produced*Ogi* and *Gari* with starter cultures

3.14.1. Preparation and inoculation of laboratory produced Ogi with starter cultures

Laboratory preparation of '*Ogi'*was carried out and inoculated with starters of LAB and yeasts following the method as described by Okafor *et al*. (1998). Pure cultures of *Lactobacillus amylovorus*, *Candida kefyr* and the combination of *Lactobacillus amylovorus* and *Candida kefyr* were used as starters for the laboratory prepared*Ogi*.

Sorghum grains were washed and soaked in water of 5% disodium bisulphite solution for 24 h to eliminate microbial contaminants. The grains were drained and washed several timesin sterile distilled water and soaked in sterile distilled water for a period of 48 h (Okafor *et al*., 1998). The softened grains were further cleaned, wet milled and wet sieved. For the inoculation of the wet-milled sorghum, a loopful of *Lactobacillusamylovorus* and *Candida*

kefyr was scrapped from MRS agar slant and Yeast Extract Peptone agar slant respectively and shaken and placed in sterilized distiled water (2 ml) and brought to dilution to give an ODequal to 7.0 x 10^7cfu/ml. This was mixed with 100 g of the wet-milled sorghum and 500 ml of sterilized water in 1L sterilized flask and comprehensivelystirred with the use of a flamed-sterile knife. For the combination of *Lactobacillusamylovorus* and *Candida kefyr*, 1 ml of the prepared inoculum(total of 2ml) was stirred and mixed with 100 g of the wet-milled sorghum and 500 ml of sterile water in a 1L sterile conical flask. The wet-milled '*Ogi'* inoculated with *Lactobacillus* spp, *Candida kefyr* and the combination of *Lactobacillusamylovorus* and *Candida kefyr* were left toferment at 28±2^{o}C for duration of 72 h. The pH of inoculated wet *Ogi* samples was read and taken at the beginning and at the completion of fermentation, samples were also taken for proximate, mineral and anti-nutrient analysis. Additionally, the organoleptic characteristics of the samples were done.

3.14.2. Preparation and inoculation of laboratory producedGari with starter cultures

Laboratory preparation of *Gari*using grated cassava was carried out and inoculated with starters of LAB and yeasts following the method as described by Okafor *et al*. (1998). Pure cultures of *Lactobacillusplantarum*, *Candida glabrata* and the combination of *Lactobacillusplantarum* and *Candida glabrata* were used to inoculate the laboratory prepared grated cassava for the production of *Gari*.

Mature cassava roots werewashedand peeledusing a flame-sterilespatula, soaked in water of 5% disodium bisulphite solution for 3 h to eliminate microbial contaminants. The peeled cassava tubers were drained and washed several times in sterile distilled water followed by grating. In order to inoculate the grated cassava, a loopful of *Lactobacillus plantarum* and *Candida glabrata* were scrapped from MRS agar slant and Yeast Extract Peptone agar slant respectively and shaken and placed in sterile distilled water (2 ml) and brought to dilution to give an OD equal to 7.0 x 10^7cfu/ml. This was mixed with 200 g of grated cassava in 1L sterile conical flask and comprehensivelystirred using a spatula (flamely-sterilized). For combination of *Lactobacillus plantarum* and *Candida glabrata*, 1ml of prepared inoculum (total of 2 ml) was stirred with 200 g of grated cassava in 1L sterile conical flask. The grated cassava inoculated with *Lactobacillus plantarum*, *Candida glabrata*and the combination of

Lactobacillus plantarum and *Candida glabrata*were left to commerce fermentation at 28±2^{o}C for duration of 72 h. The pH of the inoculated gratedcassava samples was read and recorded at the beginning and completion of fermentation; samples were also taken for proximate, mineral and anti-nutrient analysis. Assessment of the organoleptic properties of the samples was performed (Okafor *et al*., 1998).

3.15. Assessment of fermentation

After fermentationof thelaboratory prepared *Ogi*produced with single starter cultures of*Lactobacillusamylovorus*, *Candida kefyr* and the combination of *Lactobacillusamylovorus* and *Candida kefyr*, as well as the spontaneously produced *Ogi*, samples were taken out for assessment.

In the same vein, laboratory prepared*Gari* produced with single starter cultures of*Lactobacillus plantarum*, *Candida glabrata*and the combination of *Lactobacillus plantarum* and *Candida glabrata*, as well as the spontaneouslyfermented cassavawere taken out for assessment at the end of fermentation.

The extent of fermentation was assessed employing the parameters including pH; proximate composition; mineral contents and anti-nutritional contents as well as the organoleptic properties.

3.15.1. pH

Out of the fermenting media, 10 ml of the wet *Ogi* and fermented cassavainoculated with the starter cultures was aseptically set apart into clean sterile bottles and while reading using a Jenway pH meter, the pH of the media was taken and recorded.

3.15.2. Proximate composition

The proximate compositions examined include moisture content, fibre (NDF (neutral deteregent fibre) and ADF (acid detergent fibre), ash content, fat (ether extract), Nitrogen Free Extracts (NFE) and crude protein. Experimental trialwas conducted in set of two.

3.15.2.1. Moisture content determination

Determination of the moisture content of the fermented samples was done giventhe technique stated by AOAC (2006). About 5 g of samples were placed in clean dry scale pans, then weighed and allowed to dryup in an oven set at 80^{o}C for 24 h in order to obtain an uniform weight. Dried sample was then brought out and cooled in a desiccator, followed by reweighing. Moisture content is calculated as:

$$\text{Moisture content} = \frac{(B-A)-(C-A)}{(B-A)}$$

Where:

A = weight of dry scale pans

B = weight of balance pans + wet sample

C = weight of balance pans + dry samples

3.15.2.2. Ash content determination

Five grams of fermented samples were positioned in a previouslyweighed ceramic crucible and the weight was taken again. The crucible was tranfered to a humidifiedoven for 6 h at 600^{o}C to reduce to ashes, referred to as the ash content. The oven was let to cool-off to 200^{o}C and kept at this temperature for about 20 mins. The crucible was positioned in a desiccator with a stopper top, let to get cold, and then weighed again to know the weight of the ash content (AOAC, 2006).

The percentage ash content was evaluated as:

$$\text{Ash content \%} = \frac{100 \times A-B}{C}$$

Where:

A = weight of crucible + sample

B = weight of crucible + ash

C = weight of sample

3.15.2.3. Determination of fat content

Employing the Soxhlet technique, the fat content of the fermented products were ascertained About 5 g of the fermented products was measured and placed in a pan and then transferred to the oven to remove water and then reweighed again. In order to getapproximately 10 refluxes per hour, more petroleum ether was introducedinto the samples and boiled for 5-6 h to remove the fat. The sample was then dried in a rotor evaporator to remove the ether and weighed (AOAC, 2006). Percentage fat content was evaluated as:

$$\text{Fat content \%} = \frac{100 \times (C-A) - (B-A)}{D}$$

Where:

A = weight of clean dry pans

B = weight of clean dry pans + dry fermented products

C = weight of clean dry pans + fat

D = weight of sample

3.15.2.4. Determination of crude fibre content

The crude fibre content was done by determining the neutral detergent fibre (NDF) and the acid detergent fibre (ADF).

3.15.2.4.1. Acid detergent fibre (ADF) determination

Based on reports from AOAC (2006), the acid detergent fibre content was determined. About 1 gram of the fermented product was introduced into a pan, next was the introduction of 100 ml of acid detergent solution (Appendix 2) with some drops of n-octanol. The sample was heated and allowed to boil and refluxed for 60 mins from onset of boiling. Thereafter, filtration and washing of the sample was done thrice using boiled water and then and there twice with cold acetone. The sample was dried for 80 mins at 105^{o}C and then cooled in a desiccator. The dried sample was reweighed and the acid detergent fibre was calculated as:

Acid detergent fibre (ADF) %= $\frac{100 \times (B+A) - B}{C}$

Where:

A = weight of residue

B = weight of pan

C = weight of fermented sample

3.15.2.4.2. Determination of neutral detergent fibre (NDF)

Based on the technique described by AOAC (2006), the neutral detergent fibre content was evaluated. About 1 g of the fermented product was introduced into a pan followed by 100 ml of neutral detergent solution (Appendix 2) was added and then addition of sodium sulfite (0.5 g) as well as droplets of n-octanol. The sample was heated and allowed to boil, thereafter; sieved and cleaned thrice using boiling water and twice using cold acetone. Sample was dried for 80 mins at 105^{o}C and then cooled in a desiccator. The resutling sample was reweighed and used to calculate the neutral detergent fibre as follows:

Neutral detergent fibre (NDF) %=$\frac{100 \times (B+A) - B}{C}$

Where:

A = weight of residue

B = weight of crucible

C = weight of sample

3.15.2.5. Crude protein determination

The composition of crude protein was donegiven to the Kjeldahl's technique. This method estimatesthe absolute nitrogen in the sample once it had beenincororated in sulphuric acid with the use of a mercury catalytic agent. Thefermented product(1 g) was measured and placed in a kjeldahl bottle, followed by the addition of potassium sulphate (10 g), mercuric oxide (0.7 g) as well as sulphuric acid (20 ml). The bottle was positioned and inclined in a digester, thereafter boiled tillresultant liquid was transparent. Sample was allowed to get cold and deionized water (90 ml) was gradually introduced. When the sample was cooled, sodium sulphate (25 ml)was introduced and agitated, next was the introduction of glass

beads and 40% sodium hydroxide (80 ml) whereas the bottle was kept slanted. The occurrence of two distinct layers was noted. The bottle was attached to the distillation unit and boiled.The distillate (50 ml)comprising ammonia was gathered and taken, and then 50 ml of the indicator solution was added for titration using chlorhydric solution as standard (AOAC, 2006). The crude protein was evaluated as:

$$\text{Nitrogen in sample \%} = \frac{100 \times (A \times B) \times 0.0014}{C}$$

Crude protein % = nitrogen in sample x 62.5

Where:

A = chlorhydric acid in titration (ml)

B = normality of standard acid

C = weight of sample

3.15.2.6. Nitrogen free extract (NFE) determination

The nitrogen free extract of the fermented sample was determined by calculation as follows:

$$\text{Nitrogen free extract \%} = \frac{\text{Crude protein of the sample}}{6.25}$$

3.15.3. Mineral content determination

The mineral content of the fermented samples were evaluated based on the technique previously reported by AOAC (2006). For the measurement of absorbance, the A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena Germany) was employed using the air/acetylene flare and the corresponding hollow-cathode standard lamps. The mineral analysed includepotassium (K), manganese (Mn), iron (Fe), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), phosphorous (P) and copper (Cu).

3.15.4. Anti-nutrient determination

3.15.4.1. Tannin determination

Two grams (2.0 g) of the fermented samples was measured into 50 ml beaker, next was the introduction of 50% methanol (20 ml) which was then sealed off using a paraffin and positioned in a pre-heated water bath at temperature of 77-80^{o}C for about 1 h. The mixture

was comprehensivelyshudderedso as to achieve uniformity. Numerical filtration of the extract was achieved with the use of a filter paper (Whatman No 41) and then placed in a bottle. Thereafter, water (20 ml), Folin-Denis reagent (2.5 ml) and 17% $NaCO_3$(10 ml) were introduced and stirred rigorously. Gradual addition of water was done until it attained the 100 ml point of the flask. The mixture was thoroughly mixed and let to stay for 20 mins. The development of a blue-green colour was observed and the level of intake of the tannic acid control solutions were taken from the 21D spectrophotometer employing760nm wavelength (AOAC, 1980). The tannin content percentage was computed as given below:

$$\text{Tannin content \%} = \frac{\text{absorbance of sample x average gradient factor x dilution factor}}{\text{Weight of smaple x 10 000}}$$

3.15.4.2. Determination of phytate

The fermented samplemeasuring about 2 g was located into a bottle(250 ml) and 2% hydrochloric acid (100 ml) was added for deep immersion and assimilation of each sample for duration of 3 h. The mixture was then percolated using a mesh paper. Next, 50 ml of the filtrate was positioned in a tapering bottle measuring 0.50 ml while adding distilled water (107 ml) to the sample to give appropriate acid value. 0.3% ammonium thiocyanate (NH_4SCM) solution (10 ml) was introduced into every singlemixture as indicated. Titration of the mixture was done using the standard Iron (III) chloride containing 0.00195 g Iron per ml. The resultant product appearedbrown-yellow which lasted only 5 mins (AOAC, 1990). The phytic acid percentage was evaluated as:

$$\text{Phytic acid \%} = \frac{\text{titre value x 0.00195 x 1.19 x 100 x 3.55}}{\text{Weight of sample}}$$

3.15.4.3. Total alkaloids determination

Two grams of the fine texturedfermented product was measured into a bottle measuring 100 ml, next was the introduction of 80% absolute alcohol (20 ml) thereby makingan even paste by stirring. The resultant mixture was moved into a 250 ml bottle with the addition of alcohol to make up 100 ml. Meanwhile, 1 g of MgO was then introduced and the solution wasassimilated in a steaming water bathfor about 1 h 30 minsin an outwardly flowing air

condensing unit with periodic stirring. The solution was sieved with the use of a small funnel and the resultant solidgotten was movedinto the bottle and reassimilated using alcohol of about 50 ml for a period of 30 mins. Afterwards, the alcohol was vaporized and the resultant solid was cleanedthree times with boiled water, next, three drops of 10% HCl was introduced into the solution. The resultant mixture was moved into a 250 ml bottle; zinc acetate (5 ml) and potassium ferrocyanide solution (5 ml) were introducedthenstirredcomprehensively to provide a smooth and harmonized solution. Filtration was done using a new filter paper after the mixture in the flask was left for some minutes. The resultantliquid was moved to a separationsyphon; alkaloids existent was obtained bythoroughly stirring with five consecutiveparts of chloroform. The resultant residue was dispersed intoboilingpurified water (10 ml) and moved into a tube (kjeldahl); followed by addingsucrose (0.20 g) and concentrated H_2SO_4(10 ml) and selenium (0.02 g) to digest the mixture to achievea colourless solution (AOAC, 1990). The percentage of nitrogen was ascertained by Kjeldahl distillation techniquewhereas the total alkaloid was computed as:

Total alkaloid % = N% x 3.26

3.15.4.4. Determination of cyanide content

Cyanide composition of the fermented product was ascertained according to the technique by Bradbury *et al.* (1999). Fermented product(0.1 g) was measured and positionedinside a plain malleable flaskwith a screwable lid and 0.1 M phosphate buffer (0.5 ml) (pH 6) was introduced with a tube. A coloured sheet (yellow) containing esters of picric acid connected to a malleablelayer was placed instantly inside ahorizontal-bottomed malleableflask holding the sample as well as the buffer ensuring that the yellow-coloured sheet containing esters of picric acid such that the sheet does not touch the solution in the flask. The bottle was instantlyfastenedusing tight-fitted cap. An absolute solution referred to as the blank was made as describe above in another tight-fitted flask. The linamarin typicalmixture was made by weighing linamarin (10 mg into 0.1M phosphate buffer (10 ml)) at pH 6. Dilution of the linamarin standard stock solution was carried out toobtainranges of 25 – 100 mg/kg(i.e. 25, 50, 75 and 100) concentrations utilized instandardizing and calibrating the spectrophotometer prior to measurement. Preparation and treatment of linamarin paper with

a concentration of 50 mg/kg was achieved in similar manner as the samples and placed in a different plain malleable flask that containedlinamarase enzymes plus phosphate buffer, the flask was quickly sealed. The flasks holding the blank, samples and the linamarin stock paper remained for duration of 16-24 h at 25^{o}C. Afterwards,individual flasks wereunlocked, followed by the removal of the sheet containing esters of picric acid now positioned inside a testtube. Distilled water (5 ml) was introduced into the tubesholding the picrate paper and gently mixed occasionally. Absorbances of every single solution in the tubes as well as the linamarin stock solution weredeterminedwith regard to the blank on a spectronic21D spectrophotometer using a wavelength of 510nm.The absolutecomposition of cyanide was estimatedwith the use of the given formular below:

Cyanide composition = 396 x absorbance

3.15.5. Organoleptic properties of laboratory prepared*Ogi*and *Gari* with selected stress-adapted LAB and yeasts

The organoleptic properties of *Ogi* and *Gari* produced using selected LAB and yeast was conducted to test product acceptability. The assessment of the organoleptic properties was performed by twenty member-panelfamiliarwith drinking*Ogi* and *Gari*by means of the 9-point hedonic scale methodvarying from 9 signifying like exceptionally to 1 indicating dislike exceptionally. Each individual was asked to examine and assess the laboratory produced *Ogi*samples singly thereby pointing out the extent of preference for the samples provided on the survey form (Appendix 7). Also, accessing individual were requested to examine and assess the laboratory produced *Gari*samples singly thereby pointing out the extent of preference for the samples provided on the survey form (Appendix 8).Evaluation of the fermented samples was examined for parameters including appearance, texture, flavour, and general acceptability (Peryam and Pilgrim, 1957; Lim, 2011).

3.16. Statistical analysis

The data obtained from the proximate, mineral, anti-nutrient content and organoleptic properties of *Ogi* and *Gari*produced using the stress-adapted LAB and yeasts(as starter cultures) were analysed statistically employing ANOVA and means were separated using Duncan multiple range test at $\alpha_{0.05}$(Agrestic, 2013).

CHAPTER FOUR

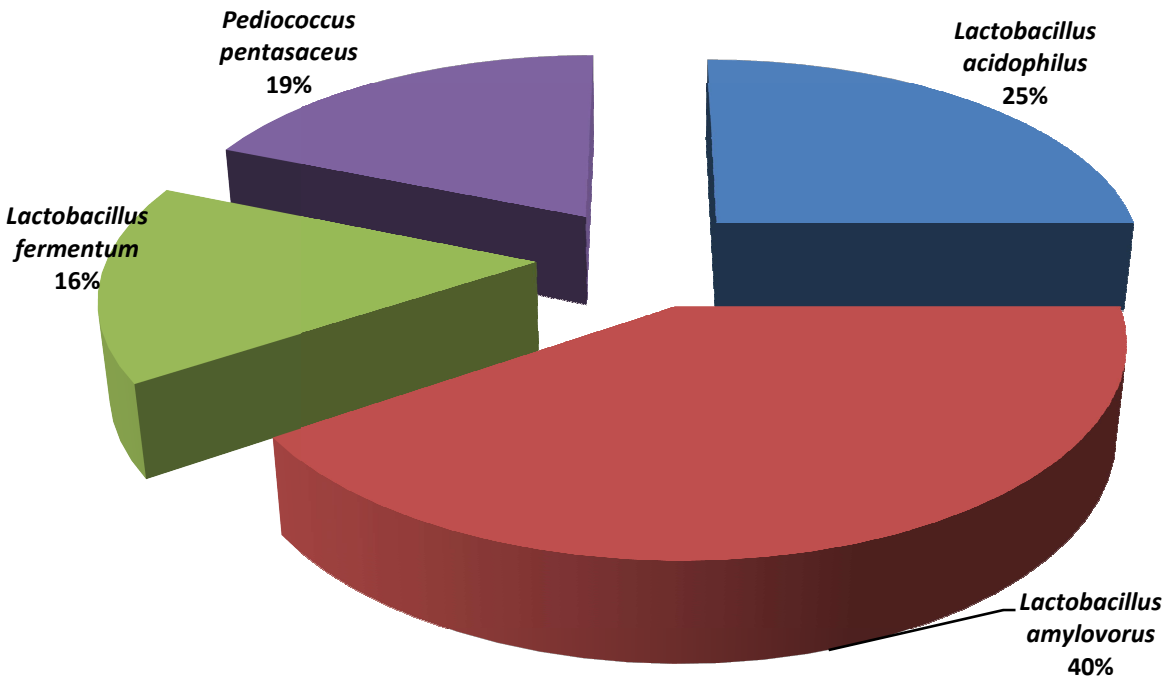
RESULTS

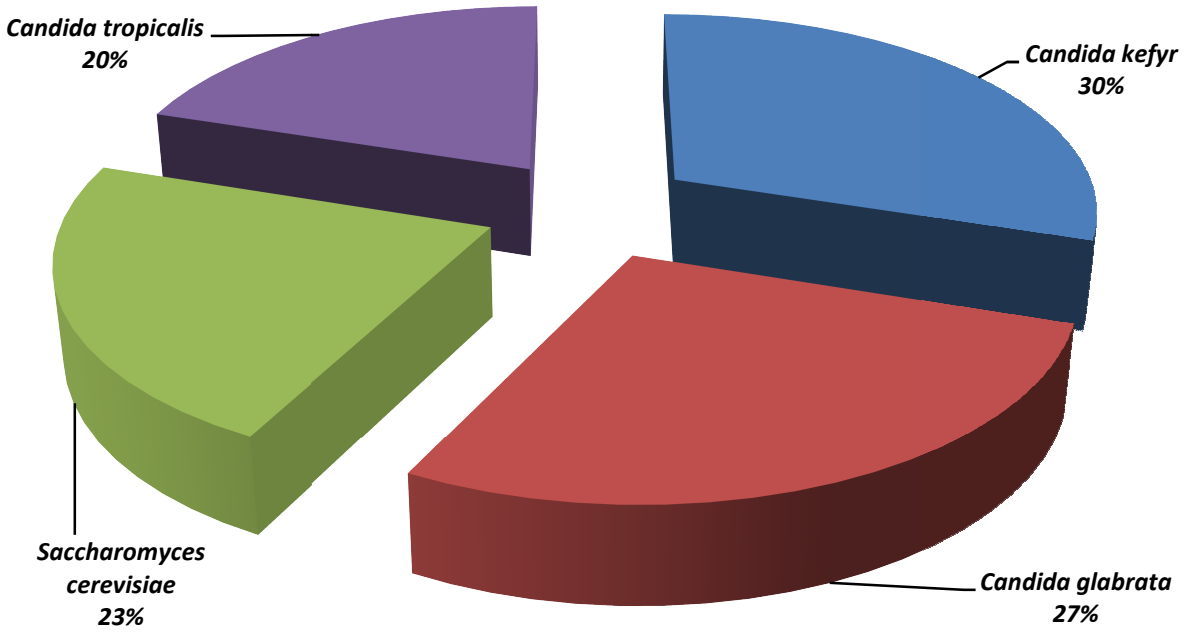
Sixty-four LAB were identified as *Lactobacillus amylovorus* (13), *L. acidophilus* (8), *L. fermentum* (5), *Pediococcus pentasaceus* (6), *L. plantarum* (11), *L. brevis* (2), *L. paracasei* (7), *Leuconostoc pseudomesenteroids* (2), *Enterococcus faecalis* (10)from sorghum gruel and fermented cassava. The percentage occurrence of the LAB isolates in the traditionally produced *Ogi* is as shown in Figure 4.1. It was noted that *Lactobacillus amylovorus* recorded the highest incidence (40%) while the lowest percentage occurrence of 16% was observed in *Lactobacillus fermentum.*

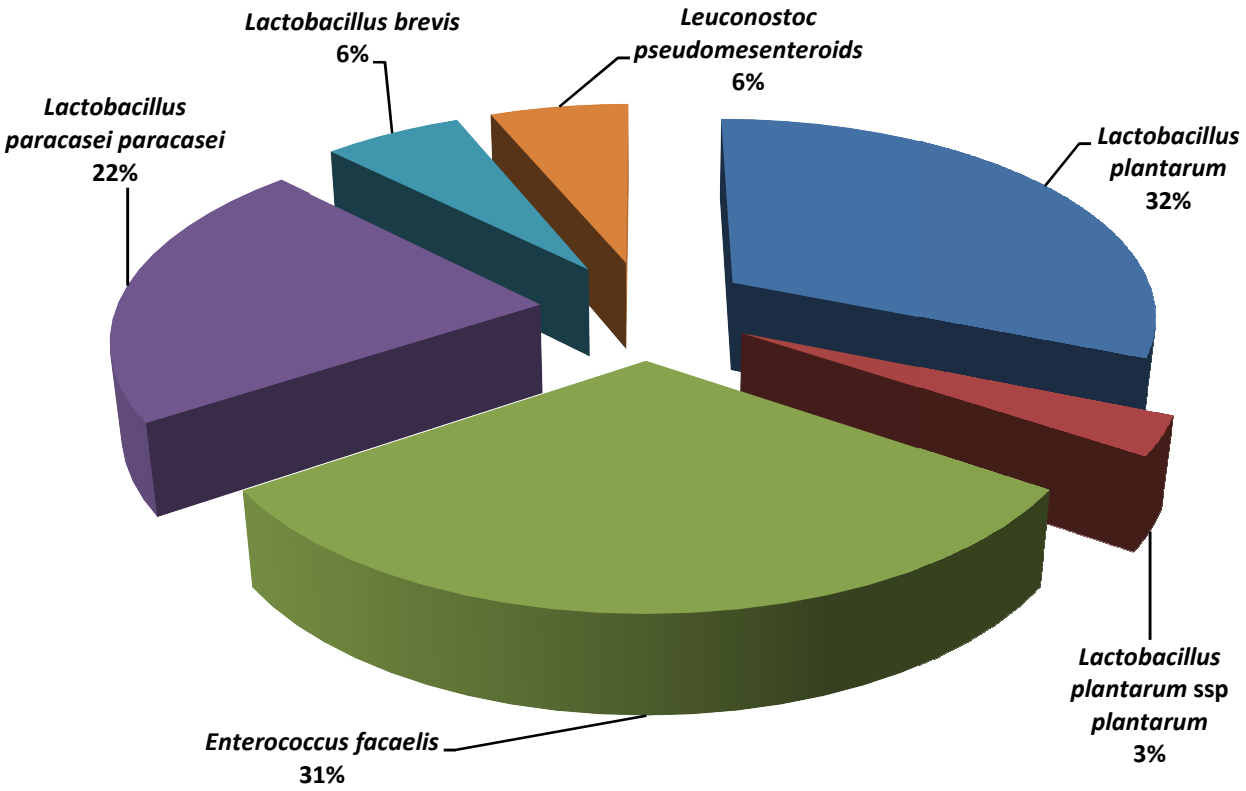
A total of seven-two yeasts were identified as *Candida kefyr* (12), *C. glabrata* (19), *C. tropicalis* (12), and *Saccharomyces cerevisiae* (29) from sorghum gruel and fermented cassava. Figure 4.2 shows the percentage occurrence of the yeasts in the traditionally produced *Ogi.Candida kefyr* had the highest occurrence of 30% while the lowest percentage occurrence was observed in *Candida tropicalis* (20%).

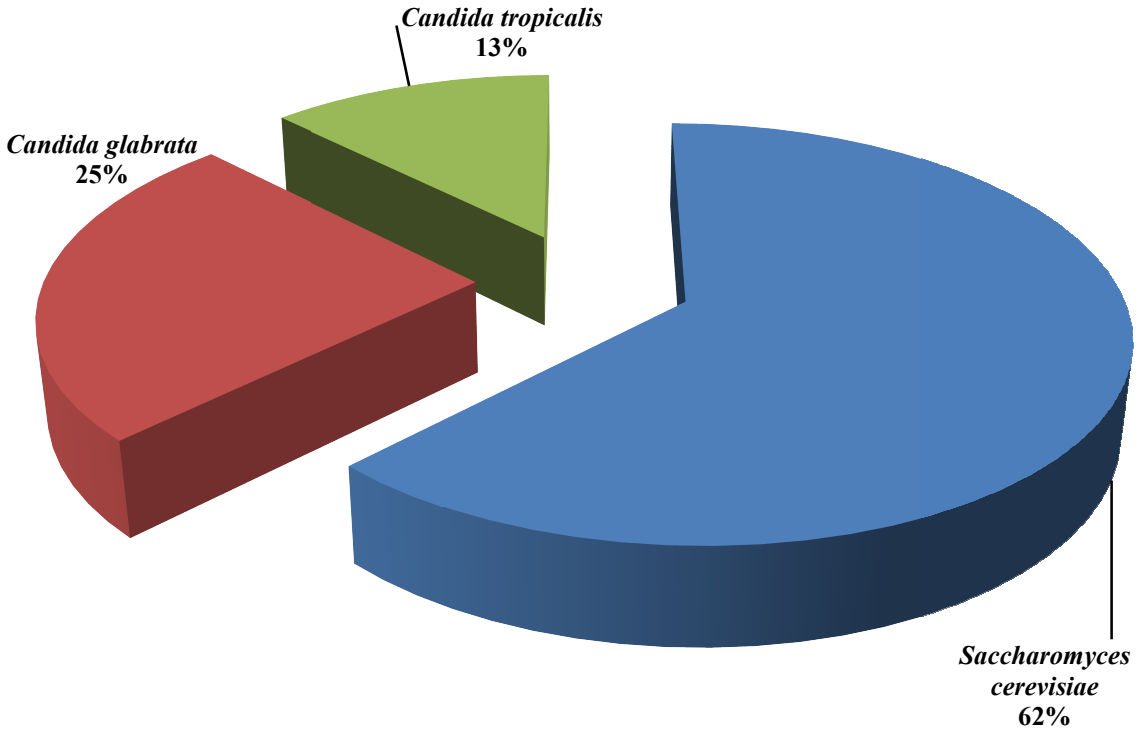
The percentage occurrence of LAB isolated from traditionally produced fermented cassava is shown in Figure 4.3. It was noted that *Lactobacillus plantarum* (32%) showed the highest fraction of incidence however the lowest percentage occurrence was observed in *Lactobacillus plantarum* ssp *plantarum* (3%).

However, the yeasts isolated from traditionally produced fermentedcassava were identified as *Saccharomyces cerevisiae* with the highest percentage occurrence of 62%, *Candida glabrata* with the percentage occurrence of 25%, and *Candida tropicalis* with the lowest percentage occurrence of 14% as shown in Figure 4.4.


Pediococcus pentasaceus 19%
Lactobacillus acidophilus 25%
Lactobacillus fermentum 16%
Lactobacillus amylovorus 40%


Candida tropicalis
20%
Candida kefyr
30%
Saccharomyces
cerevisiae
23%
Candida glabrata
27%


Lactobacillus brevis
6%
Leuconostoc pseudomesenteroids
6%
Lactobacillus paracasei paracasei
22%
Lactobacillus plantarum
32%
Lactobacillus plantarum ssp plantarum
3%
Enterococcus facaelis
31%


Candida tropicalis
13%
Candida glabrata
25%
Saccharomyces
cerevisiae
62%

The entire LAB isolated from *Ogi* were gram positive. They have rod and cocci shapes, do not produce spores, and tested negative to catalase. Physiological tests showed that most of the LAB investigated was heterofermentative and a few were homofermentative. Very few isolates grew at 45^{o}C and 15^{o}C and all grew at 4% NaCl. Most LAB isolates grew at 6% and 8% NaCl. Sugar fermentation test was used to differentiate the species level and it was noted that the entire isolates utilized arabinose, fructose, galactose, maltose, glucose and sucrose moderately, however, showed variations in the fermentation of Raffinose, Xylose, Ribose, and Lactose. MALDI-TOF MS was further utilized in identifyingthe LAB isolates into species level. MALDI-TOF score observed for the isolates ranged from 2.088 to 1.842 in most of the LAB isolates while lower scores were observed in a few of the isolates Tables 4.1 and 4.2.

Yeast isolates from traditionally produced *Ogi* appeared medium sized, white to creamy-white in colony and oval shaped when viewed microscopically. Physiological test on the yeast isolates show that all the yeasts grew at 25^{o}C, 30^{o}C and 37^{o}C and indicated positive growth in the presence of 50% and 60% glucose. All the yeasts tested negative to urease test and did not hydrolyze starch. While all of the yeast strains did not use the nitrogen sources nor grow in the existence of 1% acetic acid, some of the yeasts showed weak growth in 0.1% and 0.01% cyclohexamide. Sugar fermentation tests on the yeasts isolates revealed that all the yeasts did not utilize arabinose but showed varied results in the fermentation of celiobiose, lactose, galactose, melezitose, maltose, sucrose, raffinose, xylose and trehalose. Furthermore, the MALDI score for most of the yeast isolates ranged from 2.078 to 1.842 while some of the yeast recorded lower MALDI scores (Tables 4.3 and 4.4).

Table 4.1: Morphological, biochemical and physiological test on LAB isolated from traditionally produced *Ogi.*

Isolate code	Colony Morphology	Cell Morphology	Gram stain	Catalase test	Spore stain	HM/HE test	15ºC	45ºC	4% NaCl	6% NaCl	8% NaCl	pH 3.9	pH 9.4
LS01	Small tiny creamy -white	Cocci in cluster	+	-	-	HM	+	W	+	W	W	+	W
LS02	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS03	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS04	Small tiny creamy -white	Cocci in cluster	+	-	-	HM	+	W	+	W	W	+	W
LS05	Small tiny creamy -white	Cocci in cluster	+	-	-	HM	+	W	+	W	W	+	W
LS06	Moderate round creamy-white	Long rods	+	-	-	HE	+	+	+	+	+	+	W
LS07	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS08	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS09	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS10	Small tiny creamy -white	Cocci in cluster	+	-	-	HM	+	W	+	W	W	+	W
LS11	Moderate round creamy-white	Long rods	+	-	-	HE	+	+	+	+	+	+	W
LS12	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS13	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS14	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS15	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS16	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS17	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS18	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS19	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS20	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS21	Small tiny creamy -white	Cocci in cluster	+	-	-	HM	+	W	+	W	W	+	W
LS22	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS23	Moderate round creamy-white	Long rods	+	-	-	HE	+	+	+	+	+	+	W
LS24	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS25	Moderate round creamy-white	Long rods	+	-	-	HE	+	+	+	+	+	+	W
LS26	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS27	Medium sized, white, smooth-rough	Short rods	+	-	-	HM	-	+	+	+	W	+	W
LS28	Moderate round creamy-white	Long rods	+	-	-	HE	+	+	+	+	+	+	W
LS29	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS30	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W
LS31	Small tiny creamy -white	Cocci in cluster	+	-	-	HM	+	W	+	W	W	+	W
LS32	Medium- sized, round creamy-white	Short rods in chain or singly	+	-	_	HM	W	-	+	+	W	+	W

Key: - = negative; + = positive; W = weak growth; HM = Homofermentative; HE = Heterofermentative

Table 4.2:Sugar fermentation test and MALDI-TOF scoring on LAB isolated from traditionally produced *Ogi*

Isolate code	Ara	Fruc	Gala	Gluc	Lact	Mal	Rafn	Rib	Sucr	Xyl	MALDI-TOF Score	Probable Identity
LS01	+	+	+	+	-	+	-	-	+	+	1.681	*Pediococcus pentasaceus*
LS02	+	+	+	+	+	+	-	-	+	-	1.982	*Lactobacillus acidophilus*
LS03	+	+	+	+	-	+	-	-	+	-	1.890	*Lactobacillus amylovorus*
LS04	+	+	+	+	-	+	-	-	+	+	1.692	*Pediococcus pentasaceus*
LS05	+	+	+	+	-	+	-	-	+	+	1.596	*Pediococcus pentasaceus*
LS06	+	+	+	+	+	+	+	+	+	+	1.770	*Lactobacillus fermentum*
LS07	+	+	+	+	-	+	-	-	+	-	2.190	*Lactobacillus amylovorus*
LS08	+	+	+	+	+	+	-	-	+	-	1.921	*Lactobacillus acidophilus*
LS09	+	+	+	+	+	+	-	-	+	-	1.788	*Lactobacillus acidophilus*
LS10	+	+	+	+	-	+	-	-	+	+	1.779	*Pediococcus pentasaceus*
LS11	+	+	+	+	+	+	+	+	+	+	1.944	*Lactobacillus fermentum*
LS12	+	+	+	+	-	+	-	-	+	-	1.842	*Lactobacillus amylovorus*
LS13	+	+	+	+	+	+	-	-	+	-	1.895	*Lactobacillus acidophilus*
LS14	+	+	+	+	+	+	-	-	+	-	1.752	*Lactobacillus acidophilus*
LS15	+	+	+	+	-	+	-	-	+	-	2.088	*Lactobacillus amylovorus*
LS16	+	+	+	+	-	+	-	-	+	-	1.612	*Lactobacillus amylovorus*
LS17	+	+	+	+	+	+	-	-	+	-	1.571	*Lactobacillus acidophilus*
LS18	+	+	+	+	+	+	-	-	+	-	1.747	*Lactobacillus acidophilus*
LS19	+	+	+	+	-	+	-	-	+	-	1.890	*Lactobacillus amylovorus*
LS20	+	+	+	+	-	+	-	-	+	-	2.073	*Lactobacillus amylovorus*
LS21	+	+	+	+	-	+	-	-	+	+	1.681	*Pediococcus pentasaceus*
LS22	+	+	+	+	-	+	-	-	+	-	1.882	*Lactobacillus amylovorus*
LS23	+	+	+	+	+	+	+	+	+	+	1.448	*Lactobacillus fermentum*
LS24	+	+	+	+	-	+	-	-	+	-	1.671	*Lactobacillus amylovorus*
LS25	+	+	+	+	+	+	+	+	+	+	1.549	*Lactobacillus fermentum*
LS26	+	+	+	+	-	+	-	-	+	-	1.723	*Lactobacillus amylovorus*
LS27	+	+	+	+	+	+	-	-	+	-	1.824	*Lactobacillus acidophilus*
LS28	+	+	+	+	+	+	+	+	+	+	1.803	*Lactobacillus fermentum*
LS29	+	+	+	+	-	+	-	-	+	-	2.076	*Lactobacillus amylovorus*
LS30	+	+	+	+	-	+	-	-	+	-	1.789	*Lactobacillus amylovorus*
LS31	+	+	+	+	-	+	-	-	+	+	1.903	*Pediococcus pentasaceus*
LS32	+	+	+	+	-	+	-	-	+	-	2.221	*Lactobacillus amylovorus*

Key:

Ara= Arabinose; Fruc= Fructose; Gala= Galactose; Gluc= Glucose; Lac=Lactose; Mal= Maltose; Rafn= Raffinose; Rib= Ribose; Sucr= Sucrose; Xyl= Xylose; - = Negative; + = Positive

Table 4.3:Morphological, biochemical and physiological test on yeasts isolated from traditionally produced *Ogi.*

Isolate code	Colony Morphology	Cell Morphology	25°C	30°C	37°C	50% Glucose	60% Glucose	Urease test	Potassium nitrate	Sodium nitrite	1% acetic acid	0.01% cyclo-hexamide	0.1% cyclo-hexamide	Starch Hydrolysis
YS01	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS02	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS03	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS04	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS05	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS06	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS07	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS08	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS09	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS10	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS11	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS12	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS13	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS14	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS15	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS16	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS17	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS18	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS19	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS20	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS21	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS22	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS23	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS24	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS25	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS26	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS27	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS28	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS29	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS30	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS31	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS32	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS33	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YS34	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS35	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS36	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS37	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS38	Medium-sized, creamy, smooth edges, rough surface	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS39	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YS40	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-

Key: - = Negative; + = Positive; W = weak growth

Table 4.4: Sugar fermentation test and MALDI-TOF scoring on yeasts isolated from traditionally produced *Ogi*

Isolate code	Ara	Cel	Gala	Gluc	Lact	Mal	Mel	Rafn	Sucr	Tre	Xyl	MALDI-TOF Score	Probable Identity
YS01	-	-	+	+	-	+	+	+	+	+	-	1.738	*Saccharomyces cerevisiae*
YS02	-	-	+	+	-	+	+	+	+	+	-	1.551	*Saccharomyces cerevisiae*
YS03	-	-	+	+	-	+	-	-	-	+	+	1.559	*Candida tropicalis*
YS04	-	+	+	+	+	-	-	+	+	-	+	1.680	*Candida kefyr*
YS05	-	-	+	+	-	+	+	+	+	+	-	1.757	*Saccharomyces cerevisiae*
YS06	-	-	+	+	-	+	-	-	-	+	+	1.791	*Candida tropicalis*
YS07	-	-	+	+	-	+	+	+	+	+	-	1.465	*Saccharomyces cerevisiae*
YS08	-	-	+	+	-	+	-	-	-	+	+	1.851	*Candida tropicalis*
YS09	-	-	+	+	-	+	+	+	+	+	-	1.803	*Saccharomyces cerevisiae*
YS10	-	-	+	+	-	+	+	+	+	+	-	1.426	*Saccharomyces cerevisiae*
YS11	-	+	+	+	+	-	-	+	+	-	+	1.634	*Candida kefyr*
YS12	-	+	+	+	+	-	-	+	+	-	+	1.707	*Candida kefyr*
YS13	-	-	+	+	-	+	-	-	-	+	+	1.654	*Candida tropicalis*
YS14	-	+	+	+	+	-	-	+	+	-	+	1.731	*Candida kefyr*
YS15	-	-	-	+	-	-	-	-	-	+	+	1.883	*Candida glabrata*
YS16	-	-	-	+	-	-	-	-	-	+	+	2.014	*Candida glabrata*
YS17	-	-	-	+	-	-	-	-	-	+	+	1.751	*Candida glabrata*
YS18	-	+	+	+	+	-	-	+	+	-	+	1.703	*Candida kefyr*
YS19	-	-	-	+	-	-	-	-	-	+	+	1.610	*Candida glabrata*
YS20	-	-	-	+	-	-	-	-	-	+	+	2.050	*Candida glabrata*
YS21	-	+	+	+	+	-	-	+	+	-	+	1.558	*Candida kefyr*
YS22	-	-	+	+	-	+	+	+	+	+	-	1.409	*Saccharomyces cerevisiae*
YS23	-	-	-	+	-	-	-	-	-	+	+	2.069	*Candida glabrata*
YS24	-	-	-	+	-	-	-	-	-	+	+	2.059	*Candida glabrata*
YS25	-	-	+	+	-	+	+	+	+	+	-	1.409	*Saccharomyces cerevisiae*
YS26	-	-	+	+	-	+	-	-	-	+	+	1.771	*Candida tropicalis*
YS27	-	-	-	+	-	-	-	-	-	+	+	2.017	*Candida glabrata*
YS28	-	-	+	+	-	+	-	-	-	+	+	1.654	*Candida tropicalis*
YS29	-	+	+	+	+	-	-	+	+	-	+	1.505	*Candida kefyr*
YS30	-	+	+	+	+	-	-	+	+	-	+	1.632	*Candida kefyr*
YS31	-	+	+	+	+	-	-	+	+	-	+	1.690	*Candida kefyr*
YS32	-	-	+	+	-	+	-	-	-	+	+	1.744	*Candida tropicalis*
YS33	-	-	+	+	-	+	-	-	-	+	+	1.726	*Candida tropicalis*
YS34	-	-	+	+	-	+	+	+	+	+	-	1.703	*Saccharomyces cerevisiae*
YS35	-	+	+	+	+	-	-	+	+	-	+	1.700	*Candida kefyr*
YS36	-	-	-	+	-	-	-	-	-	+	+	2.104	*Candida glabrata*
YS37	-	+	+	+	+	-	-	+	+	-	+	1.632	*Candida kefyr*
YS38	-	+	+	+	+	-	-	+	+	-	+	1.534	*Candida kefyr*
YS39	-	-	-	+	-	-	-	-	-	+	+	2.078	*Candida glabrata*
YS40	-	-	-	+	-	-	-	-	-	+	+	2.032	*Candida glabrata*

Key:Ara= Arabinose; Cel= Celiobiose; Gala= Galactose; Gluc= Glucose; Lact=Lactose; Mal= Maltose; Mel= Melezitose; Rafn= Raffinose; Sucr= Sucrose; Tre= Trehalose; Xyl= Xylose; - = Negative; + = Positive

Tables 4.5 and 4.6 showed the morphological, biochemical, physiological sugar fermentation test and biotyping using the MALDI TOF MS of LAB isolated from fermented cassava. The entire LAB isolates tested positive to gram staining, had rod and cocci shapes, do not produce endospore and indicted negative to catalase test, most of the LAB were heterofermentative and a few were homofermentative. The LAB isolates grew at 15^{o}C; a few however were not able to grow at 45^{o}C. Positive growth was observed in all the LAB isolates at 4% NaCl, 6% NaCl, pH 3.9 while there were variations in the growth of most of the LAB at 8% NaCl and at pH 9.4.

The sugar fermentation test on the LAB isolated from fermentedcassava showed that the entire LAB could breakdown fructose, galactose, lactose and maltose while some of the isolate were not capable of fermenting raffinose, sucrose, arabinose, sucrose and ribose. The MALDI score of the LAB isolates ranged from 2.269 to 1.699 while some of the isolates had lower MALDI score (Table 4.6).

The yeast strains obtained from fermented cassava appeared medium-sized, creamy to creamy-white in colony and some exhibited budding characteristics when viewed microscopically. The yeast strains grew at 25^{o}C, 30^{o}C, 37^{o}C and at 50% and 60% glucose while negative results were observed in urease test, starch hydrolysis and utilization of nitrogen sources, potassium nitrate and sodium nitrite; varied results were obtained in the growth of the yeasts at 0.1% and 0.01% cyclohexamide.MALDI TOF MS was employed for further identification ofthe yeast isolates furthermore;majority of the yeast isolates had a MALDI score of 1.669 above (Tables 4.7 and 4.8).

Table 4.9 shows the screening pattern of LAB isolated from traditionally produced *Ogi*subjected to acid and oxidative stress. The entire LAB grew at the various stress conditions but showed varying degrees of responses to the stress conditions. The growth of the LAB at pH 1, 2, 3 also 4 (acid stress) indicated that the LAB had weak growth (1-19%) at pH 1 and a profuse growth (80-100%) at pH 4. The growth of the LAB at different concentrations of hydrogen peroxide (H_2O_2) (oxidative stress) showed different variations in

their growth responses, theLAB were able to grow better in 1mM H_2O_2 (20-79%) than growth in 3mM H_2O_2 and 5mM H_2O_2 (1-20%).

The screening pattern of yeasts isolated from traditionally produced *Ogi*subjected to acid and oxidative stress is shown in Table 4.10. Most of the yeasts were able to grow strongly at pH 4 (80-100%), moderately at pH 3 (50-89%) and survived with weak growth at pH 1 (1-20%). The variations in the growth responses of the yeasts at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 was observed to range from 50-79%, 80-89% and 90-100% respectively.

Tables 4.11 shows the screening pattern of LAB isolated from fermented cassava subjected to acid and oxidative stress. The growth of the LAB at different pH concentration (acid stress) indicated that LAB could grow strongly at pH 4 (80-89%) while stay viable with weak growth observed at pH 1 (1-20%). The LAB were all able to grow at 1 mM H_2O_2 (50-79%) while weak growth was observed in most of the LAB at 3 mM H_2O_2 and 5 mM H_2O_2.

The screening pattern of yeasts isolated from fermented cassava subjected to acid and oxidative stress is shown in Table 4.12. Most of the yeasts recorded weak growth (1-20%) at pH 1 while variations in the growth was observed at pH 2, 3 and 4 ranged from 1-20%, 20-49% and 50-100% respectively. All the yeasts grew at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2.

Figure 4.5 shows the physiological response of LAB isolated from traditionally produced *Ogi*subjected to acid stress. The acid stress response of LAB physiologically indicated that most of the LAB grew at pH 1, 2, 3 and 4; increased growth rate of the LAB was noticed as the pH increases. *Lactobacillus amylovorus* recorded the highest growth at pH 1 (0.235), 2 (0.253), 3 (0.389) and 4 (0.413) compared to unstressed cells (control) (1.056) while the lowest growth of the LAB at pH 1, 2, 3 and 4 was observed in *Pediococcus pentasaceus* (0.2, 0.226, 0.232, 0.285) compared to unstressed cell (0894).

Table 4.5: Morphological, biochemical and physiological test on LAB isolated from fermented cassava

Isolate code	Colony Morphology	Cell Morphology	Gram stain	Catalase test	Spore stain	HM/HE test	15°C	45°C	4% NaCl	6% NaCl	8% NaCl	pH 3.9	pH 9.4
LC01	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC02	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC03	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC04	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC05	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC06	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC07	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC08	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC09	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC10	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC11	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC12	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC13	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC14	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC15	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC16	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC17	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC18	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC19	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC20	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC21	Small, smooth, round creamy white	Rods arranged singly, in chains	+	-	-	HE	+	-	+	+	W	+	W
LC22	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC23	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	+	+	W	W	+	W
LC24	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC25	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC26	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC27	Small, smooth spherical, creamy-white	Cocci arranged singly and in pairs	+	-	-	HE	+	-	+	+	+	-	+
LC28	Small, smooth, round creamy white	Cocci arranged singly and in pairs	+	-	-	HM	+	+	+	+	+	+	+
LC29	Medium-sized, smooth round creamy	Short rods	+	-	-	HE	+	-	+	+	+	+	-
LC30	Small, tiny round, creamy-white	Rods	+	-	-	HE	W	-	+	+	-	+	-
LC31	Small, tiny round, creamy-white	Rods	+	-	-	HE	W	-	+	+	-	+	-
LC32	Small, smooth spherical, creamy-white	Cocci arranged in pairs and chains	+	-	-	HE	+	-	+	+	+	-	+

Key: - = negative; + = positive; W = weak growth; HM = Homofermentative; HE = Heterofermentative

Table 4.6:Sugar fermentation test and MALDI-TOF scoring on LAB isolated from fermentedcassava

Isolate code	Ara	Fruc	Gala	Gluc	Lact	Mal	Rafn	Rib	Sucr	Xyl	MALDI-TOF Score	Probable Identity
LC01	+	+	+	+	+	+	+	+	+	-	2.080	*Lactobacillus plantarum*
LC02	+	+	+	+	+	+	+	+	+	-	1.985	*Lactobacillus plantarum*
LC03	+	+	+	+	+	+	+	+	+	-	2.078	*Lactobacillus plantarum*
LC04	+	+	+	+	+	+	+	+	+	-	2.058	*Lactobacillus plantarum*
LC05	+	+	+	+	+	+	+	+	+	-	2.219	*Lactobacillus plantarum*
LC06	-	+	+	+	+	+	-	+	+	-	2.135	*Enterococcus faaecalis*
LC07	-	+	+	+	+	+	-	+	+	-	2.224	*Enterococcus faaecalis*
LC08	-	+	+	+	+	+	-	+	+	-	2.253	*Enterococcus faaecalis*
LC09	-	+	+	+	+	+	-	+	+	-	2.145	*Enterococcus faaecalis*
LC10	-	+	+	+	+	+	-	+	+	-	2.269	*Enterococcus faaecalis*
LC11	-	+	+	+	+	+	-	+	+	-	2.260	*Enterococcus faaecalis*
LC12	-	+	+	+	+	+	-	+	+	-	1.428	*Enterococcus faaecalis*
LC13	+	+	+	+	+	+	-	-	+	-	1.319	*Lactobacillus paracasei paracasei*
LC14	+	+	+	+	+	+	+	+	+	-	1.519	*Lactobacillus plantarum*
LC15	+	+	+	+	+	+	-	-	+	-	1.588	*Lactobacillus paracasei paracasei*
LC16	+	+	+	+	+	+	+	+	+	-	1.424	*Lactobacillus plantarum*
LC17	+	+	+	+	+	+	-	-	+	-	1.699	*Lactobacillus paracasei paracasei*
LC18	+	+	+	+	+	+	-	-	+	-	1.786	*Lactobacillus paracasei paracasei*
LC19	+	+	+	+	+	+	-	-	+	-	1.583	*Lactobacillus paracasei paracasei*
LC20	+	+	+	+	+	+	-	-	+	-	1.309	*Lactobacillus paracasei paracasei*
LC21	+	+	+	+	+	+	-	-	+	-	1.670	*Lactobacillus paracasei paracasei*
LC22	-	+	+	+	+	+	-	+	+	-	2.173	*Enterococcus faaecalis*
LC23	-	+	+	+	+	+	-	+	+	-	1.790	*Lactobacillus plantarum plantarum*
LC24	+	+	+	+	+	+	+	+	+	-	1.763	*Lactobacillus plantarum*
LC25	+	+	+	+	+	+	+	+	+	-	1.653	*Lactobacillus plantarum*
LC26	-	+	+	+	+	+	-	+	+	-	2.351	*Enterococcus faaecalis*
LC27	+	+	+	+	+	+	+	+	+	-	1.825	*Leuconostoc pseudomenseteriods*
LC28	-	+	+	+	+	+	-	+	+	-	1.766	*Enterococcus faaecalis*
LC29	+	+	+	+	+	+	+	+	+	-	1.675	*Lactobacillus plantarum*
LC30	+	+	+	+	+	+	-	+	-	+	2.155	*Lactobacillus brevis*
LC31	+	+	+	+	+	+	-	+	-	+	2.221	*Lactobacillus brevis*
LC32	+	+	+	+	+	+	+	+	+	-	1.832	*Leuconostoc pseudomenseteriods*

Key:

Ara= Arabinose; Fruc= Fructose; Gala= Galactose; Gluc= Glucose; Lact=Lactose; Mal= Maltose; Rafn= Raffinose; Rib= Ribose; Sucr= Sucrose; Xyl= Xylose;

- = Negative; + = Positive

Table 4.7: Morphological, biochemical and physiological test on yeasts isolated from fermentedcassava

Isolate code	Colony Morphology	Cell Morphology	25°C	30°C	37°C	50% Glucose	60% Glucose	Urease test	Potassium nitrate	Sodium nitrite	1% acetic acid	0.01% cyclo hexamide	0.1% cyclohexamide	Starch hydrolysis
YC01	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC02	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC03	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YC04	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC05	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC06	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC07	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC08	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC09	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC10	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC11	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC12	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YC13	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC14	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC15	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC16	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC17	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC18	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC19	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC20	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YC21	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC22	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC23	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC24	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC25	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC26	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC27	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC28	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC29	Medium-sized, creamy white, smooth, raised	Oval shaped	+	+	+	+	+	-	-	-	-	W	W	-
YC30	Medium sized, Creamy, smooth, lobate edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC31	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-
YC32	Medium-sized, creamy, smooth edges	Oval shaped	+	+	+	+	+	-	-	-	-	-	-	-

Key: - = Negative; + = Positive; w = weak

Table 4.8:Sugar fermentation test and MALDI-TOF scoring on yeasts isolated from fermentedcassava

Isolate code	Ara	Cel	Gala	Gluc	Lact	Malt	Mel	Raf	Suc	Tre	Xyl	MALDI-TOF Score	Probable Identity
YC01	-	-	+	+	-	+	+	+	+	+	-	1.865	*Saccharomyces cerevisiae*
YC02	-	-	-	+	-	-	-	-	-	+	+	2.213	*Candida glabrata*
YC03	-	-	+	+	-	+	-	-	-	+	+	1.864	*Candida tropicalis*
YC04	-	-	+	+	-	+	+	+	+	+	-	1.669	*Saccharomyces cerevisiae*
YC05	-	-	+	+	-	+	+	+	+	+	-	1.910	*Saccharomyces cerevisiae*
YC06	-	-	+	+	-	+	+	+	+	+	-	2.020	*Saccharomyces cerevisiae*
YC07	-	-	+	+	-	+	+	+	+	+	-	1.542	*Saccharomyces cerevisiae*
YC08	-	-	+	+	-	+	+	+	+	+	-	1.720	*Saccharomyces cerevisiae*
YC09	-	-	+	+	-	+	+	+	+	+	-	1.671	*Saccharomyces cerevisiae*
YC10	-	-	+	+	-	+	+	+	+	+	-	1.716	*Saccharomyces cerevisiae*
YC11	-	-	+	+	-	+	+	+	+	+	-	1.516	*Saccharomyces cerevisiae*
YC12	-	-	+	+	-	+	-	-	-	+	+	1.463	*Candida tropicalis*
YC13	-	-	+	+	-	+	+	+	+	+	-	1.628	*Saccharomyces cerevisiae*
YC14	-	-	+	+	-	+	+	+	+	+	-	1.471	*Saccharomyces cerevisiae*
YC15	-	-	+	+	-	+	+	+	+	+	-	1.644	*Saccharomyces cerevisiae*
YC16	-	-	-	+	-	-	-	-	-	+	+	1.600	*Candida glabrata*
YC17	-	-	+	+	-	+	+	+	+	+	-	1.743	*Saccharomyces cerevisiae*
YC18	-	-	+	+	-	+	+	+	+	+	-	1.451	*Saccharomyces cerevisiae*
YC19	-	-	+	+	-	+	+	+	+	+	-	1.564	*Saccharomyces cerevisiae*
YC20	-	-	+	+	-	+	-	-	-	+	+	1.654	*Candida tropicalis*
YC21	-	-	+	+	-	+	+	+	+	+	-	1.748	*Saccharomyces cerevisiae*
YC22	-	-	+	+	-	+	+	+	+	+	-	1.761	*Saccharomyces cerevisiae*
YC23	-	-	-	+	-	-	-	-	-	+	+	1.700	*Candida glabrata*
YC24	-	-	+	+	-	+	+	+	+	+	-	1.812	*Saccharomyces cerevisiae*
YC25	-	-	-	+	-	-	-	-	-	+	+	1.839	*Candida glabrata*
YC26	-	-	-	+	-	-	-	-	-	+	+	1.703	*Candida glabrata*
YC27	-	-	+	+	-	+	+	+	+	+	-	1.493	*Saccharomyces cerevisiae*
YC28	-	-	-	+	-	-	-	-	-	+	+	1.857	*Candida glabrata*
YC29	-	-	+	+	-	+	-	-	-	+	+	1.947	*Candida tropicalis*
YC30	-	-	+	+	-	+	+	+	+	+	-	1.335	*Saccharomyces cerevisiae*
YC31	-	-	-	+	-	-	-	-	-	+	+	1.752	*Candida glabrata*
YC32	-	-	-	+	-	-	-	-	-	+	+	1.680	*Candida glabrata*

Key:

Ara= Arabinose; Cel= Celiobiose; Gala= Galactose; Gluc= Glucose; Lact=Lactose; Malt= Maltose; Mel= Melezitose; Raf= Raffinose; Suc= Sucrose; Tre= Trehalose; Xyl= Xylose; - = Negative; + = Positive

Table 4.9: Screening of LAB isolated from traditionally produced *Ogi* subjected to acid and oxidative stress

Isolate code	Probable Identity	Acid Stress				Oxidative stress		
		pH 1	pH 2	pH 3	pH 4	1 mM H_2O_2	3mM H_2O_2	5mM H_2O_2
LS01	*Pediococcus pentasaceus*	W	w	++	+++	+	W	W
LS02	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS03	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS04	*Pediococcus pentasaceus*	W	w	++	+++	+	W	W
LS05	*Pediococcus pentasaceus*	W	w	++	+++	+	W	W
LS06	*Lactobacillus fermentum*	W	w	++	+++	+	W	+
LS07	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS08	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS09	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS10	*Pediococcus pentasaceus*	W	w	++	+++	+	W	W
LS11	*Lactobacillus fermentum*	W	w	++	+++	+	W	W
LS12	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS13	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS14	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS15	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS16	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS17	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS18	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS19	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS20	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS21	*Pediococcus pentasaceus*	W	w	++	+++	+	W	W
LS22	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS23	*Lactobacillus fermentum*	W	w	++	+++	+	W	W
LS24	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS25	*Lactobacillus fermentum*	W	w	++	+++	+	W	W
LS26	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS27	*Lactobacillus acidophilus*	W	w	++	+++	++	+	W
LS28	*Lactobacillus fermentum*	W	w	++	+++	+	W	W
LS29	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS30	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+
LS31	*Pediococcus pentasaceus*	W	w	++	+++	+	W	W
LS32	*Lactobacillus amylovorus*	W	+	++	+++	++	++	+

Key:
W = 1 – 19%; + = 20 – 49%; ++ = 50 – 79%; +++ = 80 – 89%; ++++ = 90 – 100%

Table 4.10:Screening of yeasts isolated from traditionally produced *Ogi* subjected to acid and oxidative stress

Isolate code	Probable Identity	Acid Stress				Oxidative stress		
		pH 1	pH 2	pH 3	pH 4	1mM H_2O_2	3mM H_2O_2	5mM H_2O_2
YS01	*Saccharomyces cerevisiae*	W	++	++	+++	+++	+++	+++
YS02	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS03	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS04	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS05	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS06	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS07	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS08	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS09	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS10	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS11	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS12	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS13	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS14	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS15	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS16	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS17	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS18	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS19	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS20	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS21	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS22	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS23	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS24	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS25	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS26	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS27	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS28	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS29	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS30	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS31	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS32	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS33	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YS34	*Saccharomyces cerevisiae*	w	++	++	+++	+++	+++	+++
YS35	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS36	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS37	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS38	*Candida kefyr*	+	++	+++	++++	++++	++++	+++
YS39	*Candida glabrata*	+	++	++	+++	+++	+++	++
YS40	*Candida glabrata*	+	++	++	+++	+++	+++	++

Key:

W= 1 – 19%; + = 20 – 49%; ++ = 50 – 79%; +++ = 80 – 89%; ++++ = 90 – 100%

Table 4.11: Screening of LAB isolated from fermentedcassava subjected to acid and oxidative stress

Isolate code	Probable Identity	Acid Stress				Oxidative stress		
		pH 1	pH 2	pH 3	pH 4	1mM H_2O_2	3mM H_2O_2	5mM H_2O_2
LC01	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC02	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC03	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC04	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC05	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC06	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC07	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC08	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC09	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC10	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC11	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC12	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC13	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC14	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC15	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC16	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC17	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC18	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC19	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC20	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC21	*Lactobacillus paracasei paracasei*	w	w	+	+++	++	W	W
LC22	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC23	*Lactobacillus plantarum plantarum*	w	w	++	+++	++	+	W
LC24	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC25	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC26	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC27	*Leuconostoc pseudomenseteriods*	w	w	+	+++	++	+	W
LC28	*Enterococcus faaecalis*	w	w	+	+++	++	W	W
LC29	*Lactobacillus plantarum*	w	+	++	+++	++	+	W
LC30	*Lactobacillus brevis*	w	w	+	+++	+	+	W
LC31	*Lactobacillus brevis*	w	w	+	+++	+	+	W
LC32	*Leuconostoc pseudomenseteriods*	w	w	+	+++	++	+	W

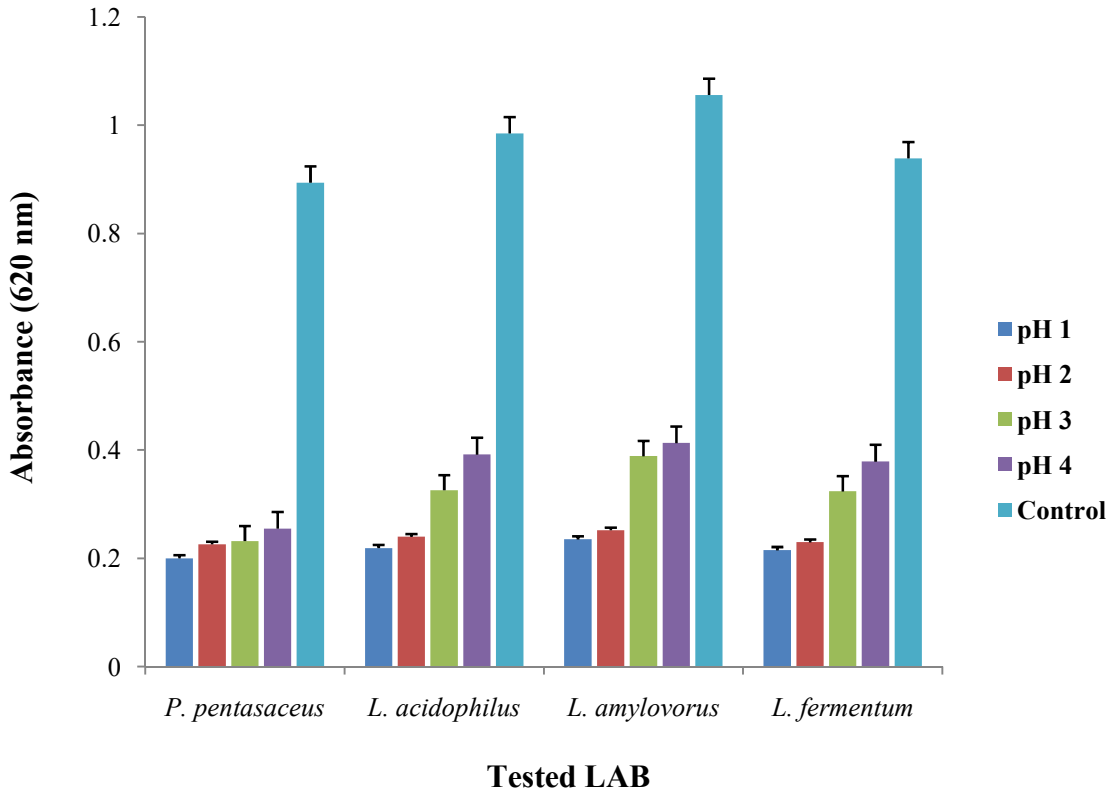
Key:
W= 1 – 19%; + = 20 – 49%; ++ = 50 – 79%; +++ = 80 – 89%; ++++ = 90 – 100%

Table 4.12:Screening of yeasts isolated from fermentedcassava subjected to acid and oxidative stress

Isolate code	Probable Identity	Acid Stress				Oxidative stress		
		pH 1	pH 2	pH 3	pH 4	1mM H_2O_2	3mM H_2O_2	5mM H_2O_2
YC01	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC02	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC03	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YC04	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC05	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC06	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC07	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC08	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC09	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC10	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC11	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC12	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YC13	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC14	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC15	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC16	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC17	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC18	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC19	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC20	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YC21	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC22	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC23	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC24	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC25	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC26	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC27	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC28	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC29	*Candida tropicalis*	w	+	++	+++	+++	+++	++
YC30	*Saccharomyces cerevisiae*	w	+	++	+++	+++	+++	+++
YC31	*Candida glabrata*	+	++	+++	++++	++++	++++	+++
YC32	*Candida glabrata*	+	++	+++	++++	++++	++++	+++

Key:

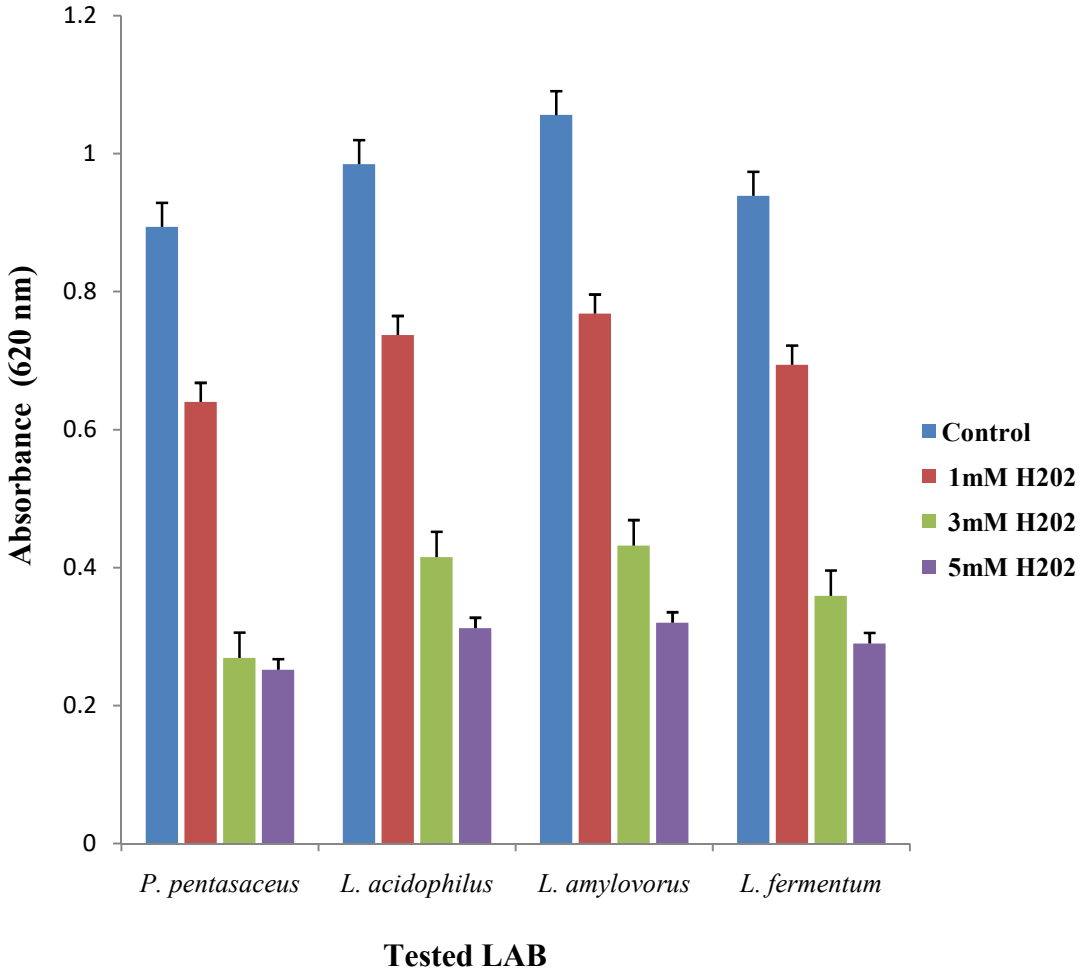
w = 1 – 19%; + = 20 – 49%; ++ = 50 – 79%; +++ = 80 – 89%; ++++ = 90 – 100%

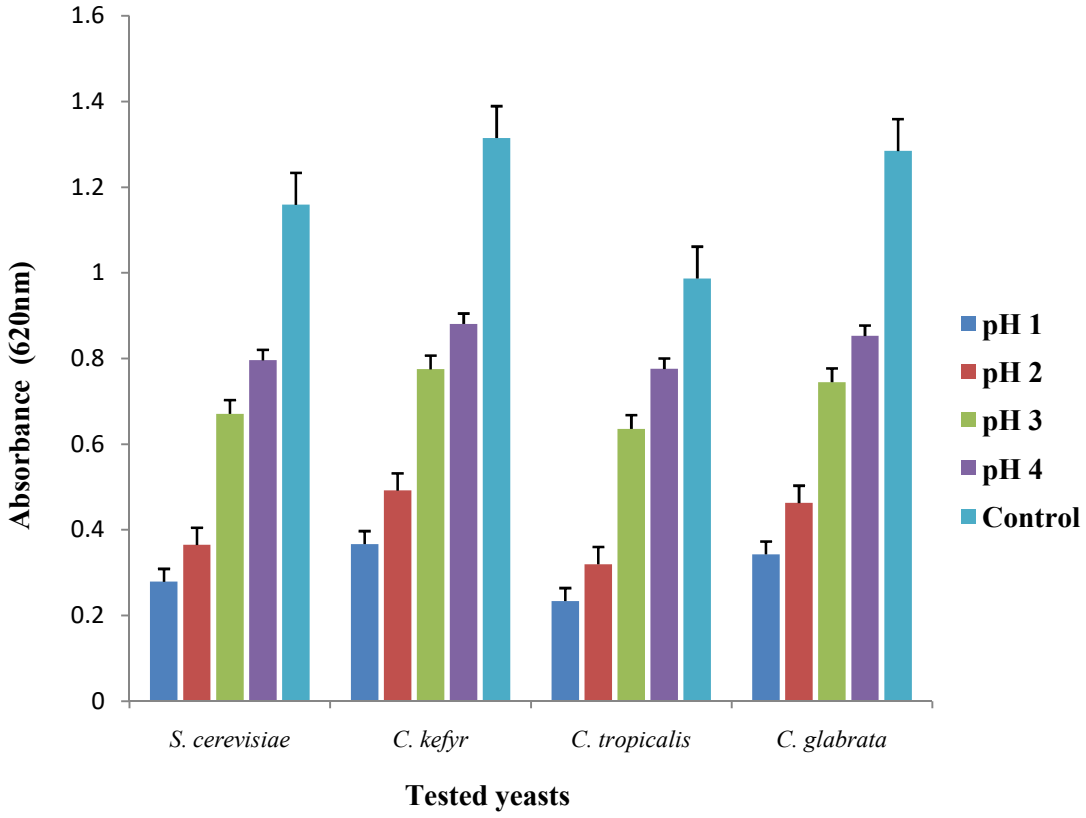

1.2
1
0.8
0.6
0.4
0.2
0
Absorbance (620 nm)
P. pentasaceus
L. acidophilus
L. amylovorus
L. fermentum
Tested LAB
pH 1
pH 2
pH 3
pH 4
Control

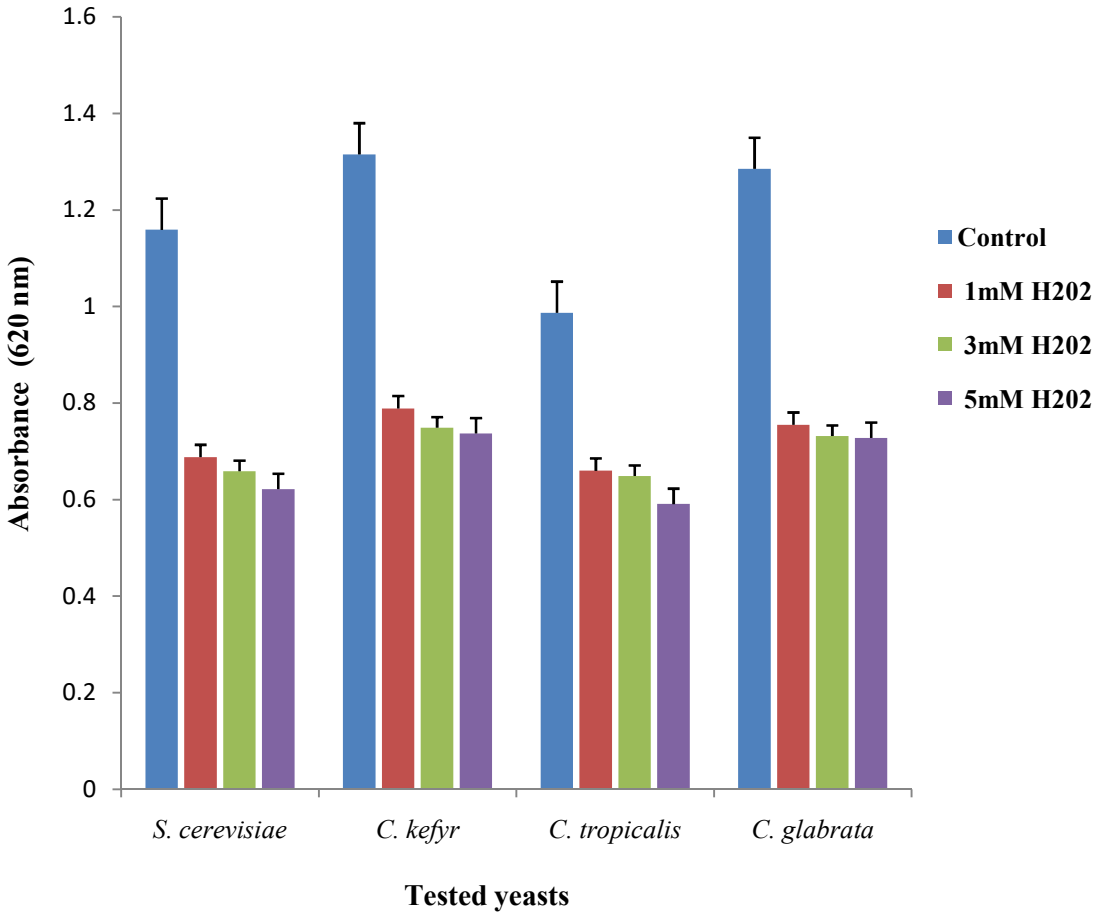
The physiological response of the entire LAB isolated from traditionally produced *Ogi* subjected to oxidative stress at different concentrations of hydrogen peroxide (1mM H_2O_2, 3mM H_2O_2, 5mM H_2O_2) showed a significant decline in their growth of LAB when exposed to 3mM H_2O_2 and 5mM H_2O_2 than at 1mM H_2O_2. The lowest growth at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 wereobserved in *Pediococcus pentasaceus* (0.640, 0.269 and 0.252 respectively) and *Lactobacillus fermentum* (0.694, 0.359 and 0.290 respectively), while the highest growth at 1mM H_2O_2, 3mM H_2O_2, 5mM H_2O_2 wereobserved in *Lactobacillus amylovorus* (0.768, 0.432 and 0.320 respectively) and *Lactobacillus acidophilus* (0.737, 0.415 and 0.312) (Figure 4.6).

The physiological response of yeasts isolated from traditionally produced *Ogi*subjected to acid and oxidative stress is shown in Figures 4.7 and 4.8. The physiological response of the yeasts to acid stress at pH 1, 2, 3 and 4 showed an increase in the growth of the yeasts from pH 1 to pH 4. The highest growth at pH 1, 2, 3 and 4 was observed in *Candida kefyr* (0.367, 0.492, 0.775 and 0.881 respectively) compared to the unstressed cells (1.315) and the lowest growth at pH 1, 2, 3 and 4 was observed in *Candida tropicalis* (0.234, 0.320, 0.636 and 0.776 respectively) compared to the unstressed cells (0.987) (Figure 4.7)

Figure 4.8 represents the physiological response of yeasts isolated from traditionally produced *Ogi* subjected to oxidative stress at 1mM H_2O_2, 3mM H_2O_2, and 5mM H_2O_2. All the yeasts were able to grow moderately at 1mM H_2O_2, 3mM H_2O_2, and 5mM H_2O_2, however, the lowest growth were observed in *Candida tropicalis* (0.660, 0.649 and 0.591 respectively) and *Saccharomyces cerevisiae* (0.688, 0.659 and 0.622 respectively) while the highest growth was observed in *Candidakefyr* (0.789, 0.749 and 0.737 respectively) and *Candida glabrata* (0.755, 0.732 and 0.728 respectively).


1.2
1
0.8
0.6
0.4
0.2
0
Absorbance (620 nm)
Control
1mM H202
3mM H202
5mM H202
P. pentasaceus
L. acidophilus
L. amylovorus
L. fermentum
Tested LAB

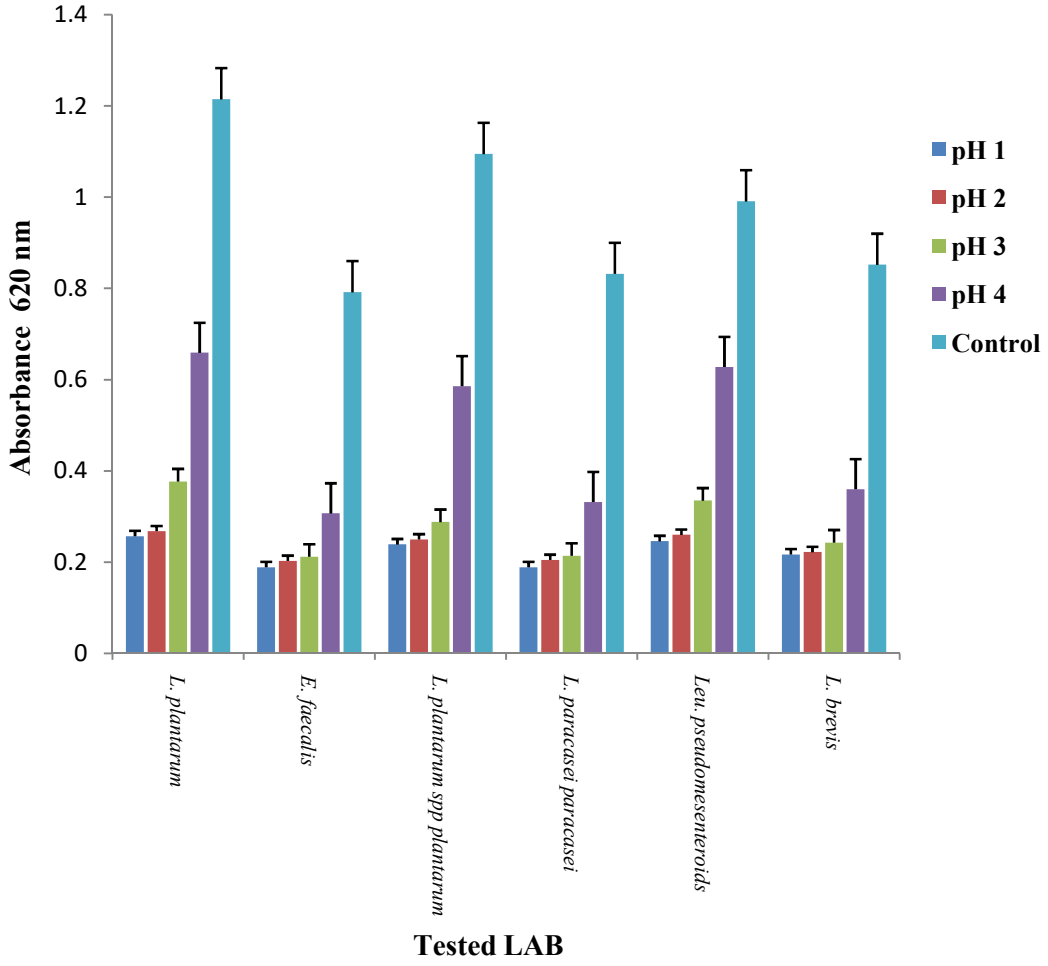

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance (620nm)
S. cerevisiae
C. kefyr
C. tropicalis
C. glabrata
Tested yeasts
pH 1
pH 2
pH 3
pH 4
Control

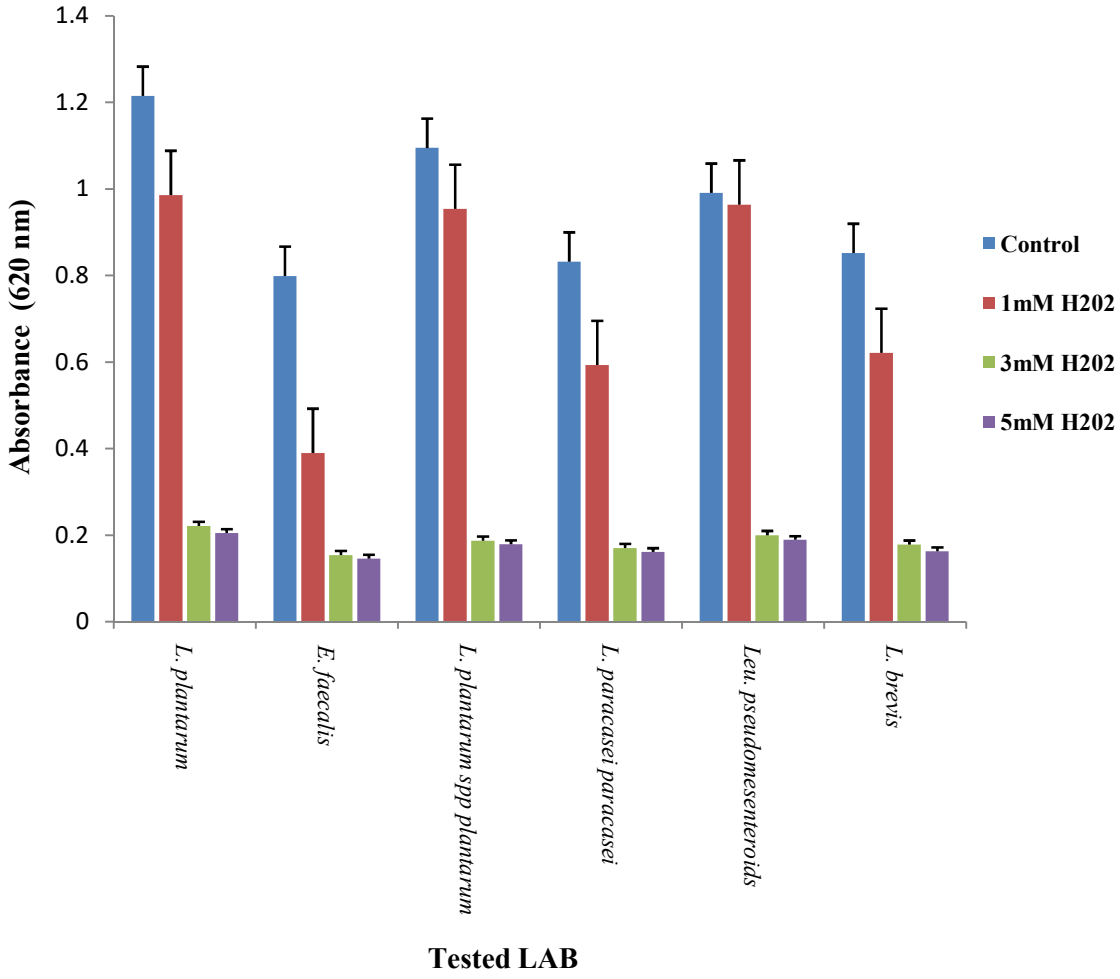

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance (620 nm)
Control
1mM H202
3mM H202
5mM H202
S. cerevisiae
C. kefyr
C. tropicalis
C. glabrata
Tested yeasts

Figures 4:9 and 4.10 show the physiological response of LAB isolated fromfermented cassavasubjectedto acid and oxidative stress. The growth of the LAB at different concentration of pH is shown in Figure 4.10. It was observed that there was an increase in the growth of the LAB as the pH increases. *Lactobacillus plantarum* recorded the highest growth at pH 1 (0.257), 2 (0.268), 3 (0.377) and 4 (0.659) while lowest growth at pH 1, 2, 3 and 4 was observed in *Enterococcus facaelis* (0.189, 0.208, 0.212 and 0.307 respectively). Moderate growth of LAB at pH 1, 2, 3 and 4 was observed in *Lactobacillus plantarum* spp *plantarum, Lactobacillus paracasei paracasei, Leuconostoc pseudomesenteroids* and *Lactobacillus brevis.*

The physiological response of the LAB isolated from fermented cassava subjectedto oxidative stress is presented in Figure 4.10. Growth of the LAB at diverse hydrogen peroxide concentrationsindicated that the LAB could grow moderately at 1mM H_2O_2 while at 3mM H_2O_2 and 5mM H_2O_2, weak development of LAB was observed. The development of LAB at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 were observed to be highest in*Lactobacillus plantarum* (0.986, 0.221 and 0.205 respectively), *Leuconostoc pseudomesenteroids* (0.964, 0.200 and 0.189 respectively) *and Lactobacillus plantarum* spp *plantarum* (0.954, 0.187 and 0.179respectively)while the lowest growth of the LAB at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 was observed in *Lactobacillus brevis* (0.621, 0.178 and 0.189 respectively), *Lactobacillus paracasei* (0.593, 0.170 and 0.161 respectively),and*Enterococcus facaelis* (0.390, 0.154 and 0.146 respectively).

The physiological response of yeasts isolated from fermented cassava subjectedto acid stress is shown in Figures 4.11. The results showed that *Candida glabrata* had the highest growth of 0.399, 0.811, 0.875 and 0.967 at pH 1, 2, 3 and 4 respectively while the lowest growth at pH 1, 2, 3 and 4 was recorded in *Candida tropicalis* (0.313, 0.636, 0.732 and 0,798 respectively).


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance 620 nm
pH 1
pH 2
pH 3
pH 4
Control
L. plantarum
E. faecalis
L. plantarum spp plantarum
L. paracasei paracasei
Leu. pseudomesenteroids
L. brevis
Tested LAB


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance (620 nm)
Control
1mM H202
3mM H202
5mM H202
L. plantarum
E. faecalis
L. plantarum spp plantarum
L. paracasei paracasei
Leu. pseudomesenteroids
L. brevis
Tested LAB

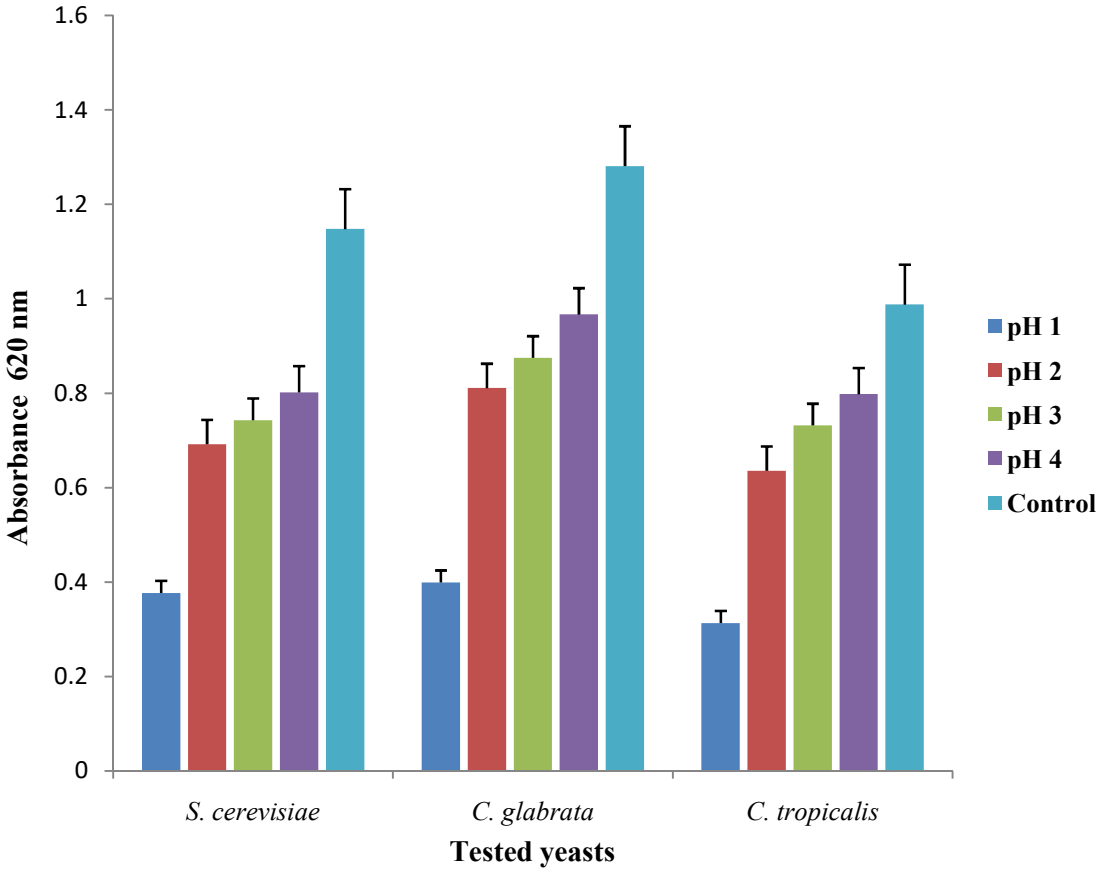

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance 620 nm
S. cerevisiae
C. glabrata
C. tropicalis
Tested yeasts
pH 1
pH 2
pH 3
pH 4
Control

Figure 4.12 represents the growth of the yeasts isolated from fermented cassava subjected to oxidative stress at different concentration of hydrogen peroxide. All the yeasts were able to grow at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2. The highest growth of yeast at 1mM H_2O_2, 3mM H_2O_2 and5mM H_2O_2 was recorded in *Candida glabrata* (0.868, 0.832, and 0.791 respectively) while the lowest growth of the yeast at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 was observed in *Saccharomyces cerevisiae* (0.688, 0.67 and 0.659 respectively) and *Candida tropicalis* (0.675, 0.667 and 0.654 respectively).

The quantity of protein extracted from *Lactobacillus amylovorus* and *Candida kefyr* isolated from traditionally produced *Ogi* at acid and oxidative stress is presented in Table 4.13. The outcome of the experiment revealed that the amount of protein extracted from *Lactobacillus amylovorus* was within the range of 683.5-2483.5 µg/µl. The highest amount of protein extracted from *Lactobacillus amylovorus* was recorded in the control (without stress conditions) (2483.5 µg/µl) whereas the leastquantity of protein was noted at pH 3 and 3mM H_2O_2 (683.5 µg/µl). Similarly, the highest amount of protein extracted from *Candida kefyr* was recorded at the stress condition 5mM H_2O_2 (3883.5 µg/µl) whereas the least quantity of protein was noted at pH 1 and pH 2 (1633.5 µg/µl). The amount of protein recorded in Table 4.13 was used to calculate the correct volume to load in order to get 10mg of protein in each SDS PAGE lane and in the rehydration of strips for 2 - dimensional electrophoresis.

Table 4.14 shows the quantity of protein extracted from *Lactobacillus plantarum* and *Candida glabrata* isolated from fermentedcassava at acid and oxidative stress. The quantity of protein extracted from *L. plantarum* revealed that the maximum amount of protein was recorded at pH 3 (1083.5 µg/µl) whereas the least amount of protein extracted was observed in the unstressed cells (33.5 µg/µl). In the same manner, the quantity of protein extracted from *Candida glabrata* showed that the amount of protein extracted was within the range 633.5- 2733.5µg/µl. The highest amount of protein extracted from *Candida glabrata* was recorded at1mM H_2O_2 (2733.5 µg/µl) while the lowest was recorded at pH 1 (633.5 µg/µl). The amount of protein recorded in Table 4.14 was used to calculate the correct volume to

load in order to get 10mg of protein in each SDS PAGE lane and in the rehydration of strips for 2 - dimensional electrophoresis.

Plate 4.1 presents the sodium dodecyl sulphate polyacrylamide gel electrophoresis of *Lactobacillus amylovorus* isolated from traditionally produced *Ogi* at acid and oxidative stress. About 11 and more gels bands were visible on each lane of the gels. Three gel bands P01, P02 and P03 of an approximate molecular weight of 97.9kDa, 30.0kDa and 20.1kDa respectively were cut out from the gels and processed for identification of protein.

The SDS PAGE of *Lactobacillus amylovorus* isolated from traditionally produced *Ogi* at acid stress is presented in Plate 4.1 A. It was noted that the band P01 at pH 4 showed increased gel intensity compared to the gel band P01 at pH 1, 2 and 3 compared to the control. In the same vein, reduced gel intensity was observed on the gel band P02 at pH 2, 3 and 4 when compared to the control.

The SDS PAGE of *Lactobacillus amylovorus* isolated from traditionally produced *Ogi* at oxidative stress is presented in Plate 4.1 B. Reduced intensity on gel bands, P01 and P02 was noted at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 compared to the gel band P01 and P02 on lane C (control).

The SDS PAGE of *Candida kefyr* isolated from traditionally produced *Ogi* at acid and oxidative stress is shown in Plate 4.2. One gel band P04 of an approximate molecular weight of 53.6kDa was excised from the SDS PAGE of *Candida kefyr* isolated from traditionally produced *Ogi* and digested for protein identification.

Plate 4.2 (A) shows the SDS PAGE of *Candida kefyr* isolated from traditionally produced *Ogi* at acid stress. Increased intensity of the gel band P04 was observed at pH 4 compared to gel bands at pH 1, 2 and 3 and control.

Plate 4.2 (B) represents the SDS PAGE of *Candida kefyr* isolated from traditionally produced *Ogi* at oxidative stress. Reduced gel band P04 was observed at 3mM H_2O_2 and 5mM H_2O_2compared to the gel band P04 at 3mM H_2O_2 and control (lane C).

Plate 4.3 represents the Two-Dimensional electrophoresis of *Candida kefyr* isolated from traditionally produced *Ogi* at no stress condition and at acid (pH 3) and oxidative stress (5mM H_2O_2). A total of over 50 spots were visible on the gels at the stress conditions and the control. Four spots, P05, P06, P07 and P08 which were visualized on the 2 DE gels were cut out and processed for identification of protein. The spots had an approximate molecular weights and theoretical isoelectric point namely; P05 (46.8kDa; 6.8), P06 (21.9kDa; 9.6), P07 (18.4kDa; 7.5) and P08 (17.4kDa; 5.6).

Plate 4.3 (B) shows the 2 DE of *Candida kefyr* isolated from traditionally produced *Ogi* at pH 3. It was observed that there was a decrease in the spot intensity of P05, P06, P07 and P08 in comparism to the control as presented in Plate 4.3 (A).

The 2 DE of *Candida kefyr* isolated from traditionally produced *Ogi* at 5mM H_2O_2 is represented in Plate 4.3 (C). It was observed that the spots P05 and P08 did not show any increase or decrease in their spot intensity while reduced spot intensity was seen in spots P06 and P07 in comparism to the control as presented in Plate 4.3 (A).

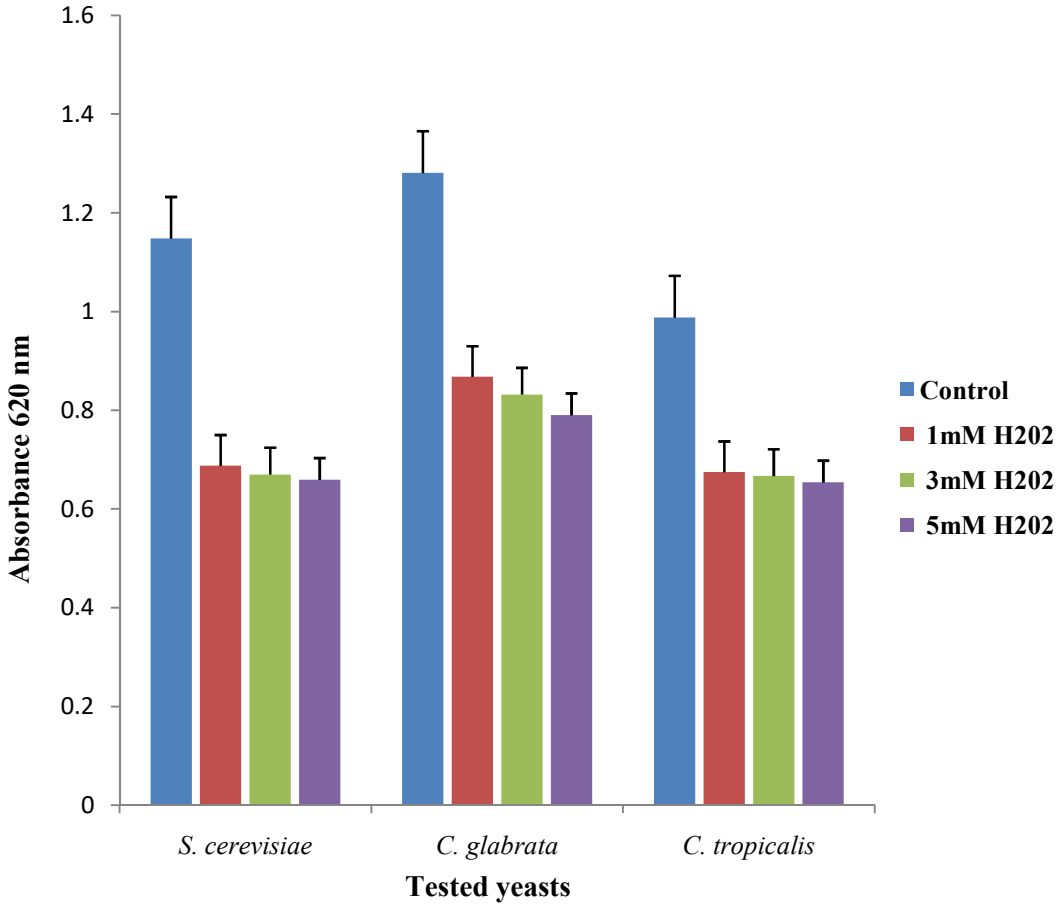
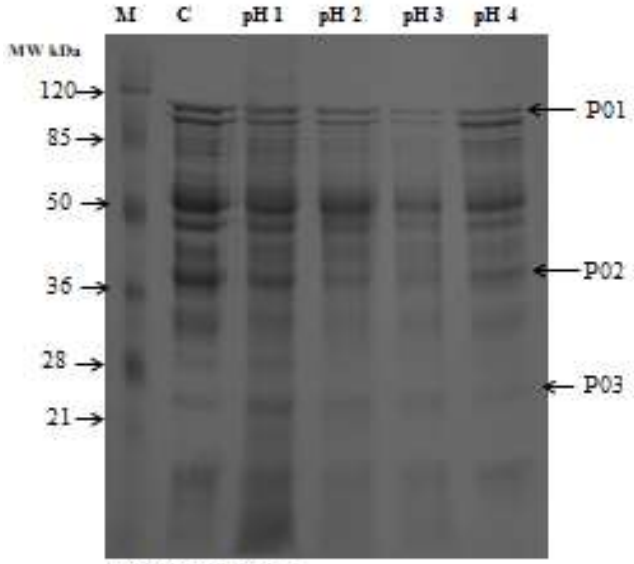

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance 620 nm
S. cerevisiae
C. glabrata
C. tropicalis
Tested yeasts
Control
1mM H202
3mM H202
5mM H202

Table 4.13: Quantity of protein (µg/µl)extracted from *Lactobacillus amylovorus* and *Candida kefyr* isolated from traditionally produced *Ogi* at acid and oxidative stress

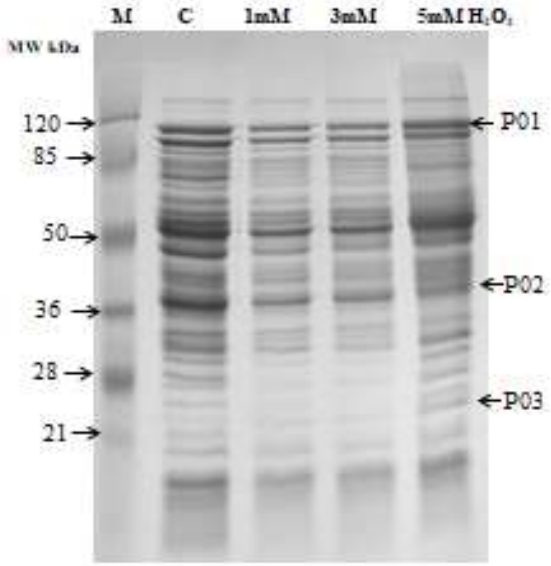
Conditions		*Lactobacillus amylovorus*	*Candida kefyr*
Control (No stress)		2483.5	3183.5
Acid stress	pH 1	1533.5	1633.5
	pH 2	983.5	1633.5
	pH 3	683.5	3183.5
	pH 4	933.5	3333.5
Oxidative stress	1mM H_2O_2	733.5	3433.5
	3mM H_2O_2	683.5	3233.5
	5mM H_2O_2	983.5	3383.5

Table 4.14: Quantity of protein (µg/µl)extracted from *Lactobacillus plantarum* and *Candida glabrata* isolated from fermented cassava at acid and oxidative stress

Conditions		*Lactobacillus plantarum*	*Candida glabrata*
Control (No stress)		33.5	1383.5
Acid stress	pH 1	333.5	633.5
	pH 2	883.5	2033.5
	pH 3	1083.5	1833.3
	pH 4	833.5	2083.5
Oxidative stress	1mM H_2O_2	333.5	2733.5
	3mM H_2O_2	483.5	2483.5
	5mM H_2O_2	783.5	1783.5



(A) Acid stress



(B) Oxidative stress

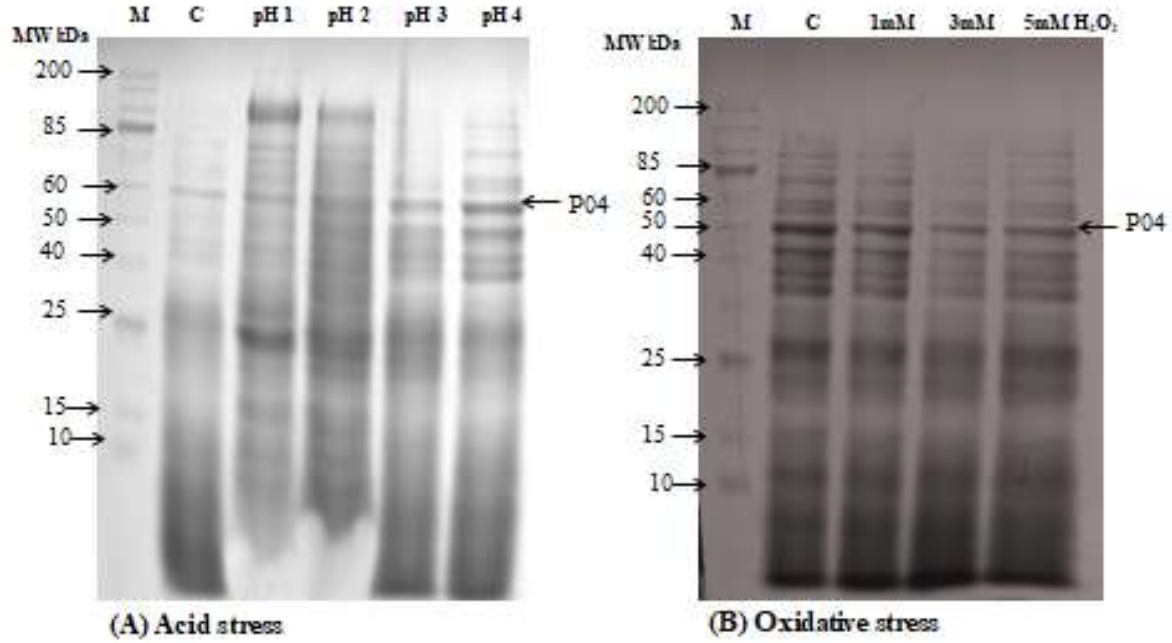


Plate 4.2: SDS PAGE of *Candida kefyr*isolated from traditionally produced *Ogi* at (A) acid and (B) oxidative stress.

Lanes M, Crepresents molecular marker, control respectively**.**

MW- Molecular weight, kDa- kilo dalton

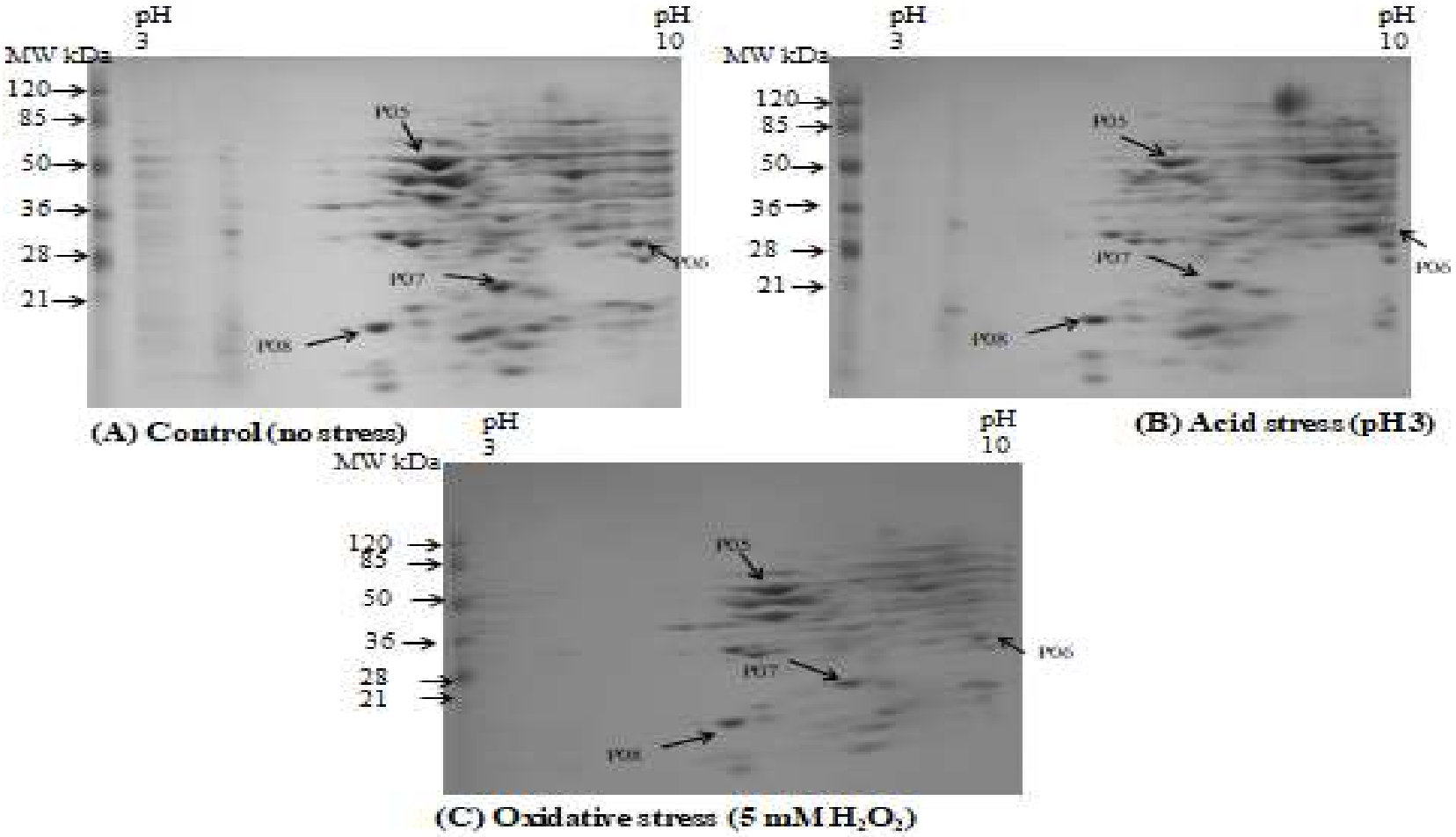


Plate 4.3: Two dimensional electrophoresis of *Candida kefyr* isolated from traditionally produced *Ogi* at (A) control (no stress), (B) acid (pH 3) and (C) oxidative stress (5 mM H_2O_2)

MW- Molecular weight, kDa- kilo dalton

The SDS PAGE of *L. plantarum* isolated from fermented cassava at acid stress and oxidative stress is shown in Plate 4.4. Three gel bands were excised and analysed for protein identification. The gel band and their approximate molecular weights were namely P09 (36.6kDa), P10 (43.3kDa) and P11 (22.3kDa).

Plate 4.4 (A) shows the SDS PAGE of *Lactobacillus plantarum* isolated from fermented cassava to acid stress. It was observed that gel band P09 showed more intensity at pH 2 and 3than at pH 1, 4and control (Lane C). In the same vein, gel band P10 showed more intensity at pH 1 and 2 compared to acid stress at pH 3 and 4 and control (Lane C). More gel band intensity was observed in P11 at pH 2 compared to pH 1 and 3. Low gel band intensity was observed at pH 4 and Control (Lane C).

The SDS PAGE of *Lactobacillus plantarum* isolated from fermented cassava at oxidative stress was observed in Plate 4.4 (B). The gel bands P09 and P10 showed more gel intensity at 5mM H_2O_2 than at 1mM H_2O_2 and3mM H_2O_2. There was no observed gel band P11 at 1mM H_2O_2, 3 mM H_2O_2, 5 mM H_2O_2 and the control (Lane C) under oxidative stress (Plate 4.4 B).

The SDS PAGE of *Candida glabrata* isolated from fermented cassava at acid stress and oxidative stress is shown in Plate 4.5. Two gel bands, P12 and P13 with an approximate molecular weight of 46.3kDa and 27.5kDa respectively were excised and analysed for protein identification.

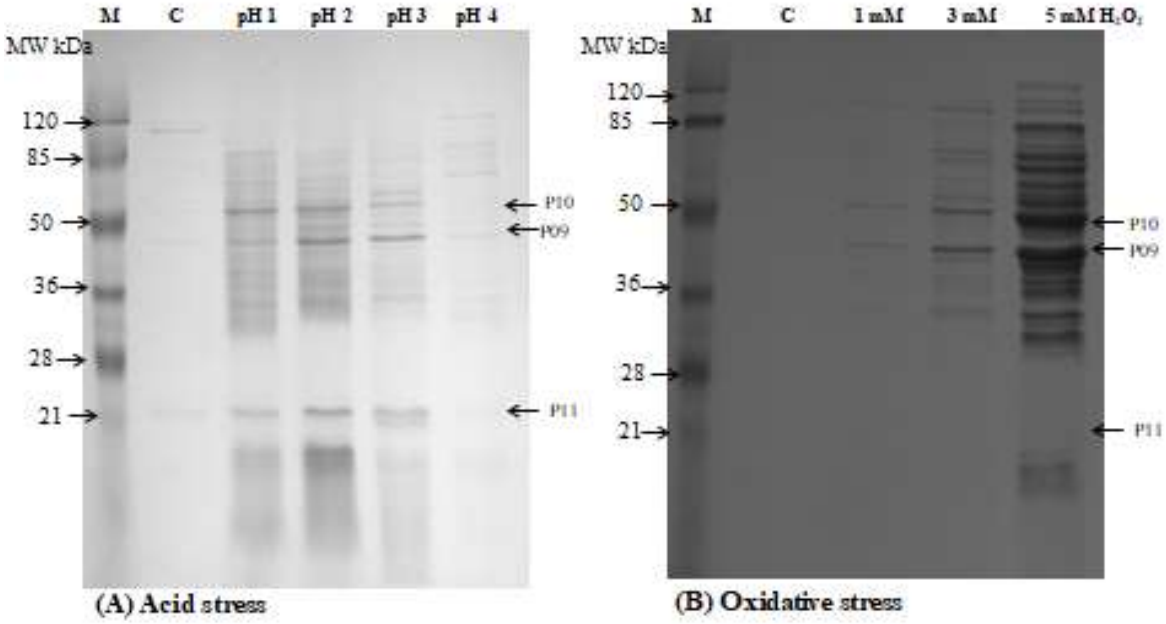
The SDS PAGE of *Candida glabrata* isolated from fermented cassava at acid stress is presented in Plate 4.5 (A). It was noted that the gel band P12 showed an increase in the band intensity at pH 2, 3 and 4 in constrast to the control. Observation revealed that the gel band P13 showed an increase in the band intensity at pH 2. No observable increased or decreased intensity of band P13 was noted at pH 2 and 3.

The SDS PAGE of *Candida glabrata* isolated from fermented cassava at oxidative stress is presented in Plate 4.5 (B). There was a notable increase in the gel band intensity of P12 at 1

mM H_2O_2 and 3 mM H_2O_2 while a decrease in the intensity of P12 was observed at 5 mM H_2O_2. Similarly, the gel band P13 showed an increase in intensity at 1 mM H_2O_2 and 3 mM H_2O_2 while a decrease in the intensity of P13 was observed at 5 mM H_2O_2.

Plate 4.6 shows the two-dimensional electrophoresis of *Candida glabrata* isolated from fermented cassava at no stress condition, acid (pH 3) and oxidative stress (5 mM H_2O_2). Two spots, P14 and P15 were visualized on the 2 DE gels and had an approximate molecular weights and theoretical isoelectric point of 46.3kDa; 6.8 and 22.6kDa; 8.3 correspondingly. Increased spot intensity of P14 and P15 was seen at acid stress of pH 3 (Plate 4.6 B) while a decrease in the spot intensity of same spots (P14 and P15) was noted at oxidative stress of 5mM H_2O_2 (Plate 4.6 C) compared to the control (Plate 4.6 A)

Table 4.15 shows the identification of protein using the LC ESI MS/MS analysis of gel bands and spots of *Lactobacillus amylovorus* and *Candida kefyr* isolated from traditionally produced *Ogi*. The bands P01, P02 and P03 obtained from *Lactobacillus amylovorus* recorded 58, 73 and 16 kDa respectively as the number of peptide matches and 0.61, 2.50, 0.86 as the Exponential Modified Protein Abundance Index (emPAI). From the LC-ESI-MS analysis, the protein identified from gel bands P01, P02 and P03 were within the score range of 1217 and 3726 and are namely bifunctional acetaldehyde- CoA/alcohol dehydrogenase, 30S ribosomal protein S2 and 50S ribosomal protein L5 respectively. Band P04 and spots P05, P06, P07 and P08 obtained from *Candida kefyr* were identified as 6- phosphogluconate dehydrogenase, enolase, heat shock protein 26, peroxiredoxin type 2 and peptyl-prolyl cis-trans isomerase respectively with the organism reference to *Kluyveromyces maxianus* DMKU31042. The protein identified from *Candida kefyr* had apparent number of peptide matches between 88 and 785 and an emPAI between 17.21 and 112.95.


M
C
pH 1
pH 2
pH 3
pH 4
MW kDa
120
85
50
36
28
21
P10
P09
P11
(A) Acid stress
M
C
1 mM
3 mM
5 mM H2O2
MW kDa
120
85
50
36
28
21
P10
P09
P11
(B) Oxidative stress

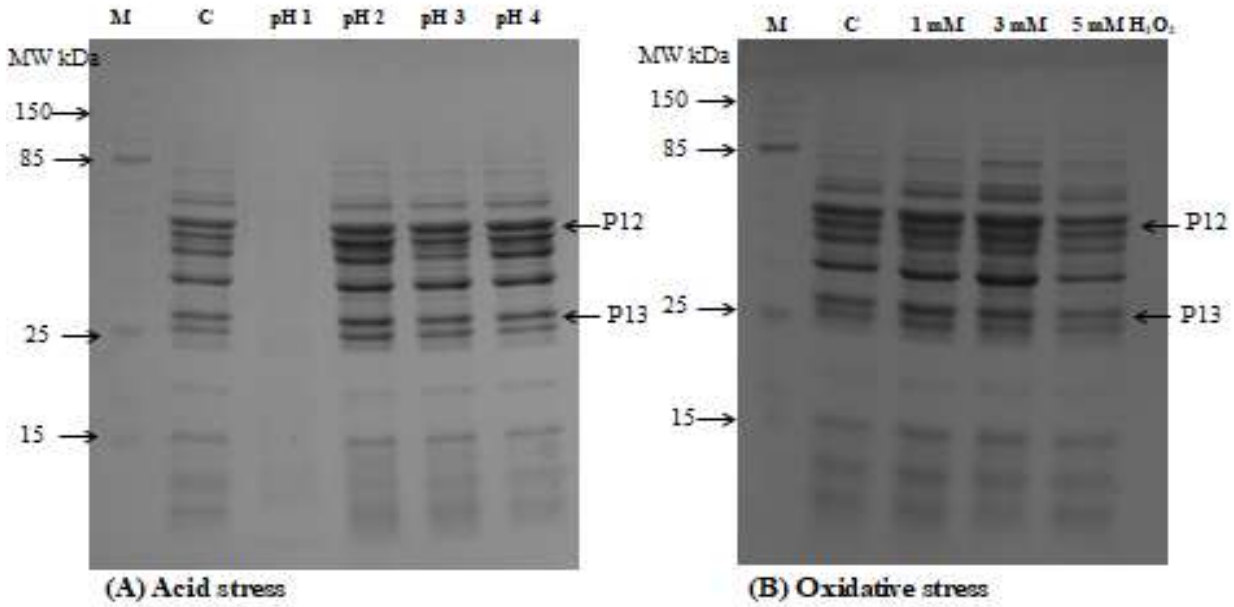


Plate 4.5: SDS PAGE of *Candida glabrata* isolated from fermentedcassava at (A) acid and (B) oxidative stress

Lanes M, C represents molecular marker, control
MW- Molecular weight, kDa- kilo dalton

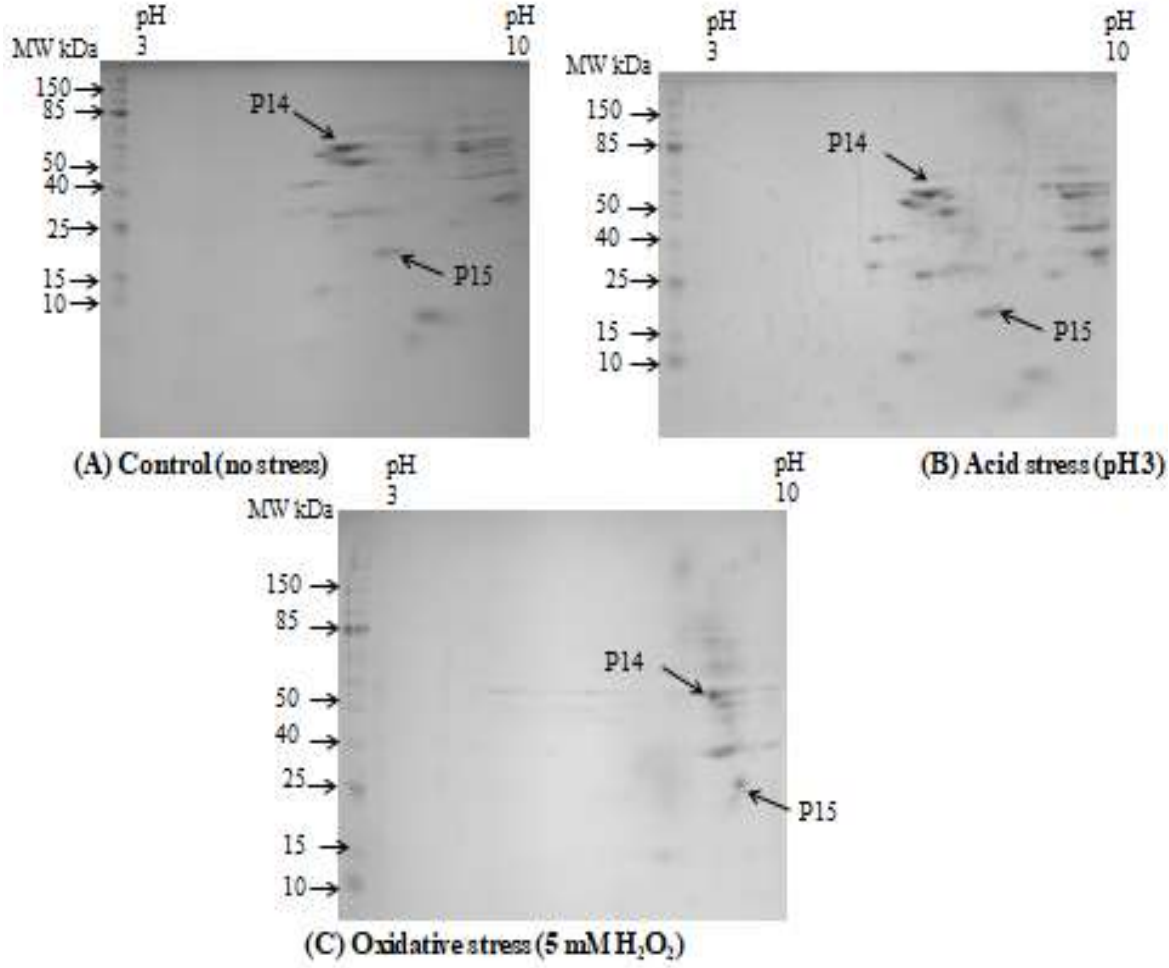

pH 3
pH 10
MW kDa
150
85
50
40
25
15
10
P14
P15
(A) Control (no stress)
(B) Acid stress (pH3)
(C) Oxidative stress (5 mM H_2O_2)

Table 4.15: Identification of protein characterized by LC ESI MS analysis from*Lactobacillus amylovorus* and *Candida kefyr* from traditionally produced *Ogi*to acid and oxidative stress

Band/spot code	Organism origin	Database	Accession number	Mass (kDa)	Pl	Number of peptide matches	Number of sequences	emPAI	Score	Protein identity	Organism/Reference
Band-P01	*Lactobacillus amylovorus*	NCBIprot	WP_050611443.1	97.944	-	58	10	0.61	1966	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	*Lactobacillus agilis*
Band-P02	*Lactobacillus amylovorus*	NCBIprot	WP_086201376.1	30.039	-	73	6	2.50	3726	30S ribosomal protein S2	*Lactobacillus salivarius*
Band-P03	*Lactobacillus amylovorus*	NCBIprot	WP_035452965.1	20.161	-	16	2	0.86	1217	50S ribosomal protein L5	*Lactobacillus composti*
Band-P04	*Candida kefyr*	NCBIprot	XP_022673986.1	53.682	-	88	33	17.21	5723	6-phosphogluconate dehydrogenase	*Kluyveromyces marxianus DMKU3-1042*
Spot-P05	*Candida kefyr*	NCBIprot	XP_022673980.1	46.830	6.8	785	44	88.34	57671	Enolase	*Kluyveromyces marxianus DMKU3-1042*
Spot-P06	*Candida kefyr*	NCBIprot	XP_022675979.1	21.932	9.6	151	19	35.76	8814	Heat shock protein 26	*Kluyveromyces marxianus* DMKU3-1042
Spot-P07	*Candida kefyr*	NCBIprot	XP_022676023.1	18.418	7.5	255	21	112.95	19166	Peroxiredoxin type-2	*Kluyveormyces marxianus* DMKU3-1042
Spot-P08	*Candida kefyr*	NCBIprot	XP_022676959.1	17.408	5.6	469	15	71.73	29159	Peptidyl-prolyl cis-trans isomerase	*Kluyveromyces marxianus* DMKU3-1042

Key:

NCBI- National center for Biotechnology information

NCBIprot- database for protein, is a pool of sequences from some resources comprising translation from noted coding segment in GenBank and TPA, along with SwissProt, PIR, PRF and PDB

Accession number: chains of figures that are allocated successively to every single sequence data sorted by NCBI.

Mass (kDa): theoretic mass expected from the amino acid series of the known protein.

pI: theoretic isoelectric point expected from amino acid series of the known protein.

Number of sequence: analysis of the amino acid series of the known protein.

Number of Peptide matches: total of corresponding peptides centered on MS/MS data searching, without the replica corresponds.

emPAI is the Exponentially Modified Protein Abundance Index

Organism reference: organism discovered after alignment of the known protein series.

The identification of protein using theLC ESI MSanalysis from gel bands and spots of *Lactobacillus plantarum* and *Candida glabrata* isolated from fermented cassava is shown in Table 4.16. Gel bands P09, P10 and P11 obtained from *Lactobacillus plantarum* recorded the number of peptide matches as 652, 542 and 99 respectively and a corresponding emPAI of 77.07, 23.48 and 33.27 respectively with a score range of 4992 and 41098. The protein identity obtained from gel bands P09, P10 and P11 were namely type 1 glyceraldehyde 3 phosphate dehydrogenase, elongation factor Tu and chitin-binding protein with a reference organism from *Lactobacillus plantarum.* The protein obtained from gel band P12 and P13 and spots P14 and P15 from *Candida glabrata* were named as enolase 2, uncharacterized protein, enolase 2 and peptdyl-prolyl cis-trans isomerase with reference organism, *Candida glabrata*. The number of peptide matches obtained from P12, P13, P14 and P15 using the LC-ESI-MS analysis was between 47 and 834 and an emPAI between 9.96 and 81.10 and the highest score recorded was 30.974 (P14) while the lowest score recorded was 3732 (Spot P15).

Table 4.17 represents the changes in the pH of laboratory prepared *Ogi* and *Gari* using starter cultures. *Lactobacillus amylovorus, Candida kefyr* and the combination of *Lactobacillus amylovorus* and *Candida kefyr* were used as starters in producing *Ogi*. Decreased pH were recordedin*Ogi* produced using the starter cultures and the control (spontaneous fermentation).The lowest pH was found in *Ogi* made with the single starters of *Lactobacillus amylovorus* (3.44^{c})which were significantly different corresponding to the outcome of the analysis of variance in constrast to *Ogi* made with the single starters of *Candida kefyr*(3.69^{a}) and the combination of *Lactobacillus amylovorus* and *Candida kefyr*(3.66^{a}) and the control (spontaneous fermentation) (3.52^{b}).

Single starters,*Lactobacillus plantarum, Candida glabrata* and the combination of *Lactobacillus plantarum* and *Candida glabrata w*ere utilized as starters in the course of fermenting cassava. Decreased pH were recorded infermented cassava prepared using the starter cultures and the control (spontaneous fermentation). The changes observed in the pH of fermented cassava made using the single starter culture *Lactobacillus plantarum* recorded the lowest pH (3.81^{d}) compared tofermented cassava produced using the single starter

culture*Candida glabrata* (4.01^{b}) and the combination of *Lactobacillus plantarum* and *Candida glabrata* (3.96^{c}) and the control (spontaneous fermentation) (4.46^{a}) (Table 4.17).

The proximate composition of laboratory prepared*Ogi*and *Gari*using starter cultures is represented in Table 4.18. *Ogi* made with the combined starters of *Lactobacillus amylovorus* as well as *Candida kefyr* recorded the highest result in the protein content (10.94^{b} %), fat content (3.45^{a} %), acid detergent fibre (8.51^{b}%) and neutral detergent fibre (37.54^{a} %) showed significant difference from *Ogi* produced using the single starter culture is in accordance to the outcome of the analysis of variance. In this study, no remarkable substantial change in the nitrogen free extract (NFE) in *Ogi* made with the combined starters of *Lactobacillus amylovorus* as well as *Candida kefyr* and the single starters of *Lactobacillus amylovorus*and *Candida kefyr.*

The results obtained from the proximate composition of laboratory prepared*Gari*showed substantial variations as regards the protein content, ash content ADF and NDF *Gari* produced using the combined starters of *Lactobacillus plantarum* and *Candida glabrata* compared to *Gari* produced with single starter cultures of *Lactobacillus plantarum* and *Candida glabrata.* The highest value of protein content (0.94^{c} %) and ash content (2.80^{a} %) was observed in fermented cassava made with combined starters of *Lactobacillus plantarum* and *Candida glabrata* compared to the single starters of *Candida glabrata* (0.85^{b} % and 2.56^{c} % respectively) and *Lactobacillus plantarum* (0.90^{a} % and 2.64^{b} %). *Gari* produced with single starter *Candida glabrata* recorded the highest moisture content (8.55^{a} %), while *Gari* produced with combined use of the starter cultures*Lactobacillus plantarum* and *Candida glabrata* recorded the lowest moisture content (6.08^{b} %).It was observed that no significantvariation was noted in the fat content and NFE of *Gari* produced with single starter *Lactobacillus plantarum* and the combined use of *Lactobacillus plantarum* and *Candida glabrata*(Table 4.18).

Table 4.16: Identification of protein characterized by LC ESI MS analysis from*Lactobacillus plantarum* and *Candida glabrata*isolated from fermented cassava to acid and oxidative stress.

Band/spot code	Organism origin	Database	Accession number	Mass (kDa)	pl	Number of peptide matches	Number of sequences	emPAI	Score	Protein identity	Organism/Reference
Band-P09	*Lactobacillus plantarum*	NCBIprot	WP_024521321.1	36.641	-	652	36	77.07	41098	Type I glyceraldehyde-3-phosphate dehydrogenase	*Lactobacillus plantarum*
Band-P10	*Lactobacillus plantarum*	NCBIprot	WP_044431076.1	43.364	-	542	25	23.48	29526	Elongation factor Tu	*Lactobacillus plantarum*
Band-P11	*Lactobacillus plantarum*	NCBIprot	WP_076633771.1	22.368	-	99	15	33.27	4992	Chitin-binding protein	*Lactobacillus plantarum*
Band-P12	*Candida glagrata*	NCBIprot	SCV15549.1	46.362	-	834	42	76.77	27747	Enolase 2	*Candida glabrata*
Band-P13	*Candida glabrata*	NCBIprot	XP_445966.1	27.574	-	393	28	81.10	22619	Uncharacterized protein	*Candida glabrata*
Spot-P14	*Candida glabrata*	NCBIprot	SCV15549.1	46.362	6.8	507	41	58.24	30974	Enolase 2	*Candida glabrata*
Spot-P15	*Candida glabrata*	NCBIprot	KTB16110.1	22.675	8.3	47	10	9.97	3732	Peptidyl-prolyl cis-trans isomerase D	*Candida glabrata*

Key:

NCBI- National center for biotechnology information

NCBIprot- database for protein, a pool of sequences from some resources comprising translation from noted coding segment in GenBank and TPA, along with SwissProt, PIR, PRF and PDB

Accession number: chains of figures that are allocated successively to every single sequence data sorted by NCBI.

Mass (kDa): theoretic mass expected from the amino acid series of the known protein.

pI: theoretic isoelectric point expected from the amino acid series of the known protein

Number of sequence: analysis of the amino acid series of the known protein

Number of peptide matches: total of corresponding peptides centered on MS/MS data searching, without the replica corresponds.

emPAI is the Exponentially Modified Protein Abundance Index

Organism reference: organism discovered after alignment of the known protein series.

Table 4.17: Changes in pH of laboratory prepared*Ogi* and *Gari* using starter cultures

	Ogi						***Gari***					
pH	Ctrl	*C. kef*	*L. amy*	*C. kef + L.amy*	P VALUE	SEM	Ctrl	*C. gla*	*L. pla*	*C. gla + L. pla*	P VALUE	SEM
0 h	6.49^{a}	6.40^{b}	6.36^{c}	6.38^{c}	0.001	0.03	6.74^{a}	6.67^{b}	6.41^{d}	6.59^{c}	0.001	0.03
72 h	3.52^{b}	3.69^{a}	3.44^{c}	3.66^{a}	0.001	0.09	4.46^{a}	4.01^{b}	3.81^{d}	3.96^{c}	0.001	0.09

Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05.}$

Key:

Ctrl-Control (spontaneous fermentation), *C.kef- Candida kefyr, L. amy- Lactobacillus amylovorus, C. kef +L. amy- Candida kefyr +Lactobacillus amylovorus, C. gla- Candida glabrata, L. pla- Lactobacillus plantarum, C. gla + L. pla- Candida glabrata +Lactobacillus plantarum*

P value- Probability value, SEM-Standard Error Mean

Table 4.18: Proximate composition of laboratory prepared*Ogi* and *Gari* using starter cultures

Proximate composition (%)	*Ogi*						*Gari*					
	Ctrl	*C. kef*	*L. amy*	*C. kef + L.amy*	P VALUE	SEM	Ctrl	*C. gla*	*L. pla*	*C. gla + L. pla*	P VALUE	SEM
Moisture	11.50^{a}	9.75^{b}	8.16^{c}	6.35^{d}	0.0001	0.721	6.32^{d}	8.55^{a}	6.45^{c}	6.08^{b}	0.0001	0.362
Protein	10.72^{d}	10.74^{a}	10.76^{c}	10.94^{b}	0.0001	0.452	0.91^{a}	0.85^{b}	0.90^{a}	0.94^{c}	0.0001	0.019
Ash	0.18^{c}	0.63^{b}	0.68^{a}	0.69^{a}	0.0001	0.267	2.31^{d}	2.56^{c}	2.64^{b}	2.80^{a}	0.0001	0.267
Fat	3.36^{b}	3.14^{c}	3.15^{c}	3.45^{a}	0.0001	0.360	0.39^{c}	0.41^{c}	0.66^{a}	0.54^{b}	0.0001	0.360
NFE	1.72^{b}	1.76^{a}	1.72^{b}	1.76^{a}	0.0001	0.007	0.15^{a}	0.14^{a}	0.63^{a}	0.13^{a}	0.055	0.122
ADF	8.66^{b}	9.04^{a}	8.31^{d}	8.51^{b}	0.001	0.101	5.87^{d}	6.77^{b}	7.47^{a}	7.66^{c}	0.001	0.218
NDF	19.79^{c}	24.11^{b}	11.07^{d}	37.54^{a}	0.001	3.612	7.64^{d}	11.15^{b}	12.90^{a}	13.84^{c}	0.001	0.840

Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05.}$

Key:

Ctrl-Control (spontaneous fermentation), *C.kef- Candida kefyr, L. amy- Lactobacillus amylovorus, C. kef +L. amy- Candida kefyr + Lactobacillus amylovorus, C. gla- Candida glabrata, L. pla- Lactobacillus plantarum, C. gla + L. pla- Candida glabrata +Lactobacillus plantarum*

P value- Probability value, SEM-Standard Error Mean

The mineral composition of laboratory prepared*Ogi*and *Gari*using starter cultures is presented in Table 4.19. *Ogi* made using the combined starters of *Lactobacillus amylovorus* and *Candida kefyr* recorded the highest mineral content compared to the *Ogi* produced using the single starter cultures. However, there was no remarkable change observed in the mineral composition, calcium, zinc, magnesium, copper, potassium, manganese, sodium and phosphorus in *Ogi* made using combined starters of *Lactobacillus amylovorus* and *Candida kefyr* and *Ogi* madeusing single starters in accordance to the outcome of the analysis of variance. However, *Ogi* made using the combined use of starters *Lactobacillus amylovorus* as well as *Candida kefyr* recorded significant difference in iron (0.00855^{b}%) in comparism to *Ogi* made with the single starters of *Lactobacillus amylovorus* (0.0067^{c}%) and *Candida kefyr* (0.00765^{a}%) and the control (0.0076^{b}%).

Gari produced using joined starters of *Lactobacillus plantarum* as well as *Candida glabrata* recorded the utmost mineral content in calcium (2.06^{a}%) and zinc (0.00075^{a}%) which showed significant differences compared to the *Gari* produced using single starter cultures *Lactobacillus plantarum;* calcium (0.08^{b}%) and zinc (0.0004^{b}%) and *Candida glabrata;* calcium (0.09^{b} %) and zinc (0.00035^{b}%) following the results of the analysis of variance. The highest potassium content was observed in *Gari* produced using single starter culture of *Lactobacillus plantarum* (1.05^{a}%) which showed no significant difference compared to *Gari*made with single starter of *Candida glabrata*(0.97^{b}%) and joined starters of *Lactobacillus plantarum* as well as *Candida glabrata* (0.98^{b}%) and control (0.95^{b} %)(Table 4.19).

Table 4.20 represents the anti-nutrient composition of laboratory prepared*Ogi*and *Gari*using starters. The utilization of the joined starters of *Lactobacillus amylovorus* in addition to *Candida kefyr* in the production of *Ogi* recorded the lowest anti-nutrients components (tannin, phytate, alkaloids and cyanide) in contrast to *Ogi* made using the single starters of *Lactobacillus amylovorus* otherwise *Candida kefyr*. According to the outcome of the analysis of variance, *Ogi* produced with combined staters of *Lactobacillus amylovorus*and *Candida kefyr*recorded the lowest cyanide content (3.20^{b}mg/kg) which showed significant difference to

Ogi produced with single starter of *Lactobacillus amylovorus*(3.27[b]mg/kg) and single starter of *Candida kefyr*(3.23[c]mg/kg) and the control (3.36[a]mg/kg).

In correspondence to the outcome of the analysis of variance, the anti-nutrient composition of *Gari* produced without starter culture (control) recorded the highest anti-nutrient components which showed significant differences to anti-nutrients composition of *Gari* produced with starter cultures. The lowest tannin (0.0007[d] %), phytate (0.0078[c] %), alkaloids (0.14[c] %) and cyanide (6.49[d]mg/kg) was observed in *Gari* made using joined starters of *Lactobacillus plantarum* and *Candida glabrata* compared to *Gari* made using single starters of *Lactobacillus plantarum* or *Candida glabrata*(Table 4.20).

The organoleptic properties of laboratory prepared*Ogi* produced using starters are presented in Figure 4.21. The highest average scores of first choice (flavour 7.02[a], texture (7.84[a]) appearance (6.60[a]) and general overall acceptability (7.92[a]) was obtained in *Ogi* made using joined starters of *Lactobacillus amylovorus* and *Candida kefyr* which revealed remarkable changes in accordance to the outcome of analysis of variance compared to *Ogi* made using the single starter. Moreover, *Ogi* made using the single starter of *Candida kefyr* (7.82[b]) recorded a higher mean score of preference in comparism to *Ogi* made using single starters of *Lactobacillus amylovorus* (7.53[c]).

Table 4.22 shows the organoleptic properties of laboratory prepared *Gari* produced using starters. The outcome of the variance analysis disclosed remarkable changes of mean scores of preference (flavour, texture, appearance and general overall acceptability) in *Gari* made using joined starters of *Lactobacillus plantarum* and *Candida glabrata.* The least mean score of preference (flavour 6.94[c], texture 7.63[c], appearance 6.29[d], general overall acceptability 7.13[d]) was observed in *Gari*produced with no starter culture (control) compared to *Gari* produced with combined and single starter cultures.

Table 4.19: Mineral composition of laboratory prepared*Ogi* and *Gari* using starter cultures

Mineral composition (%)	*Ogi*						*Gari*					
	Ctrl	*C. kef*	*L. amy*	*C. kef* + *L.amy*	P VALUE	SEM	Ctrl	*C. gla*	*L. pla*	*C. gla* + *L. pla*	P VALUE	SEM
Calcium(Ca)	0.015^{b}	0.03^{ab}	0.04^{a}	0.05^{a}	0.0001	0.17	0.06^{b}	0.09^{b}	0.08^{b}	2.06^{a}	0.0001	0.170
Magnesium (Mg)	0.035^{b}	0.04^{b}	0.03^{b}	0.04^{b}	0.513	0.0043	0.06^{a}	0.07^{a}	0.07^{a}	0.07^{a}	0.242	0.043
Potassium (K)	0.09^{b}	0.11^{a}	0.09^{ab}	0.10^{ab}	0.0001	0.11	0.95^{b}	0.97^{b}	1.05^{a}	0.98^{b}	0.0001	0.110
Sodium (Na)	0.01^{a}	0.01^{a}	0.01^{a}	0.02^{a}	0.479	0.01	0.11^{a}	0.11^{a}	0.09^{a}	0.05^{b}	0.0001	0.010
Phosphorous (P)	0.12^{a}	0.12^{a}	0.12^{a}	0.12^{a}	0.121	0.01	0.05^{d}	0.05^{d}	0.05^{d}	0.06^{d}	0.381	0.001
Zinc (Zn)	0.0003^{d}	0.00035^{d}	0.00035^{d}	0.00035^{d}	0.803	0.183	0.0000^{c}	0.00035^{b}	0.0004^{b}	0.00075^{a}	0.0001	1.013
Copper (Cu)	0.0003^{a}	0.00025^{a}	0.00025^{a}	0.00015^{a}	0.242	0.263	0.0002^{a}	0.0003^{a}	0.00025^{a}	0.00025^{a}	0.381	0.189
Manganese (Mn)	0.00045^{b}	0.00075^{a}	0.00075^{a}	0.0007^{a}	0.0001	0.498	0.0011^{b}	0.0017^{a}	0.00125^{b}	0.00165^{a}	0.0001	0.944
Iron (Fe)	0.0076^{b}	0.00765^{a}	0.0067^{c}	0.00855^{b}	0.0001	2.527	0.0017^{d}	0.00155^{d}	0.00145^{d}	0.00165^{d}	0.173	0.441

Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05.}$

Key:

Ctrl-Control (spontaneous fermentation), *C.kef- Candida kefyr, L. amy- Lactobacillus amylovorus, C. kef +L. amy- Candida kefyr + Lactobacillus amylovorus, C. gla- Candida glabrata, L. pla- Lactobacillus plantarum, C. gla + L. pla- Candida glabrata +Lactobacillus plantarum*

P value- Probability value, SEM-Standard Error Mean

Table 4.20:Anti-nutrient composition of laboratory prepared*Ogi*and *Gari*using starter cultures

	Ogi						***Gari***					
Anti-nutrient composition (%)	Ctrl	*C. kef*	*L. amy*	*C. kef* + *L.amy*	P VALUE	SEM	Ctrl	*C. gla*	*L. pla*	*C. gla* + *L. pla*	P VALUE	SEM
Tannin	0.0025^{a}	0.0023^{ab}	0.0023^{ab}	0.0021^{b}	0.005	0.0001	0.0018^{a}	0.0014^{b}	0.0011^{c}	0.0007^{d}	0.005	0.00015
Phytate	0.0081^{a}	0.0072^{b}	0.0074^{ab}	0.0071^{b}	0.006	0.001	0.0093^{a}	0.0089^{b}	0.0086^{b}	0.0078^{c}	0.002	0.00019
Alkaloids	0.16^{a}	0.15^{b}	0.15^{b}	0.15^{b}	0.0014	0.001	0.17^{a}	0.15^{b}	0.14^{c}	0.14^{c}	0.0001	0.00235
Cyanide (mg/kg)	3.36^{a}	3.23^{c}	3.27^{b}	3.20^{b}	0.002	0.018	7.11^{a}	6.59^{b}	6.53^{c}	6.49^{d}	0.0001	0.44015

Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05.}$

Key:

Ctrl-Control (spontaneous fermentation), *C.kef- Candida kefyr, L. amy- Lactobacillus amylovorus, C. kef +L. amy- Candida kefyr + Lactobacillus amylovorus, C. gla- Candida glabrata, L. pla- Lactobacillus plantarum, C. gla + L. pla- Candida glabrata +Lactobacillus plantarum*

P value- Probability value, SEM-Standard Error Mean

Table 4.21: Organoleptic properties of laboratory produced *Ogi* using starter cultures

		Ogi				
Organoleptic properties	Ctrl	*C.kef*	*L. amy*	*C. kef +L. amy*	PVALUE	SEM
Flavour	5.99^{c}	6.94^{a}	6.42^{b}	7.02^{a}	0.001	0.158
Texture	7.30^{c}	7.70^{b}	7.29^{c}	7.84^{a}	0.001	0.091
Appearance	6.50^{c}	6.59^{a}	6.53^{b}	6.60^{a}	0.001	0.016
General overall acceptability	7.23^{d}	7.82^{b}	7.53^{c}	7.92^{a}	0.001	0.103

Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05.}$

Key:

Ctrl-Control (spontaneous fermentation), *C.kef- Candida kefyr, L. amy- Lactobacillus amylovorus, C. kef + L. amy- Candida kefyr +Lactobacillus amylovorus,* P value- Probability value, SEM-Standard Error Mean

Table 4.22:Organoleptic properties of laboratory produced *Gari* using starter cultures

	Gari					
Organoleptic properties	Ctrl	*C. gla*	*L. pla*	*C. gla* +*L. pla*	PVALUE	SEM
Flavour	6.94^{c}	8.18^{b}	7.87^{b}	8.82^{a}	0.001	0.258
Texture	7.63^{c}	7.72^{ab}	7.69^{bc}	7.84^{a}	0.013	0.022
Appearance	6.29^{d}	7.85^{b}	6.90^{c}	7.93^{a}	0.001	0.258
General overall acceptability	7.13^{d}	7.73^{b}	7.63^{c}	7.92^{a}	0.001	0.117

Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05.}$

Key:

Ctrl-Control (spontaneous fermentation), *C. gla- Candida glabrata, L. pla- Lactobacillus plantarum, C. gla + L. pla- Candida glabrata + Lactobacillus plantarum*

P value- Probability value, SEM-Standard Error Mean

CHAPTER FIVE

DISCUSSION

In this study, different species of lactic acid bacteria and yeasts were isolated from sorghum gruel (*Ogi*) and fermented cassava (*Gari*). Lactic acid bacteria and yeasts are frequently found in a broad variety of fermentation of African indigenous food (Adegoke and Babalola, 1988; Steinkraus, 1996; Nayak, 2011; Syal and Vohra, 2013; Angelov*et al.,* 2017; Mishra*et al.,* 2018).Damelin*et al*. (1995) recorded differentvarieties of yeasts as well asLABupon plants and their report presented *Lactobacillus* strains existing as the most prevalent in fare associated environments.

The outcome of the biotyping of LAB and yeasts isolates was doneviamatrix assisted laser desorption/ionization time of flight mass spectrometry analysis. Lactic acid bacteria isolated from traditionally made*Ogi*wereknown as *Pediococcus pentasaceus, Lactobacillus amylovorus, Lactobacillus fermentum* as well as *Lactobacillus acidophilus*. The predominant LAB species isolated was *Lactobacillus amylovorus.* Afolayan *et al.* (2017)however,reported the predominance of *Lactobacillus plantarum* from different varieties of *Ogi*. Most of the LAB isolated from fermenting sorghum for the production of *Ogi*has been known to participate in the fermentation of other food materials. Matthara*et al.* (2004) statedthat*L. plantarum* and *Leuconostoc mesenteroids* were isolated and characterizedfrom kule naoto, the Maasai traditional fermented milk in Kenya. Hounhouigan *et al.* (1993) described the isolation of *Lactobacillus brevis*, *Lactobacillus curatus* and *Pediococcus pentasaceus* during production of Mawe, a maize-based dough from Benin. Uchimura *et al.* (1991)indicated that *P. pentasaceus* participateswhile producing Ragi, an Indonesian Chinese starter. Asmahan and Muna (2009) isolated *Lactobacillus brevis, Lactobacillus fermentum* and *Lactobacillus amylovorus* from fermentation of sorghum dough in the preparation of Sudanese *Kisra.*

The yeasts isolated from traditionally produced *Ogi* were known as *Saccharomyces cerevisiae, Candida kefyr, C. tropicalis* and*C. glabrata, Candida kefyr* was the predominant yeast isolated. The involovement of yeasts have also been described in numerous traditional fermented foods (Blanco *et al*., 1999). Earlier investigation by Omemu *et al.* (2007) revealed the involvement of *C. krusei, S. cerevisiae, R. graminis, C. tropicalis and G. fermentans* in the course of fermenting maize aimed at producing*Ogi*. They bring about distinctive flavour to fermented fares (Annan *et al.,* 2003)as well asenhancing the nutritivecomposition of resultant food produced (Hellstrom *et al*., 2010).

The LAB isolated from fermented cassava wasknown*as L. paracasei paracasei, L. brevis, Leuc.Pseudomesenteroids, L. plantarum* ssp *plantarum,E. faecalis*and *L. plantarum*. The most occurring LAB species was *Lactobacillus plantarum*. Reports from the studies of Moorthy and Mathew (1998) revealed the fermentative action of microbes in the course of fermenting cassava. Also, Kostinek *et al.* (2005) gave an account of the isolation of *L. plantarum,L. fermentum, Leuc.fallax, Leuc.mesenteroides, S. faecium* in *Gari* and observed that *Lactobacillus plantarum*was noted to be most dominant amidst the other LAB isolated. This is in agreement with the findings in this research that *Lactobacillus plantarum* is the most predominant LAB isolated. The outcome of research investigation by Krabi *et al.* (2016) reavealed the most predominant LAB identified as *Lactobacillus plantarum* employing MALDI TOF MS.

During cassava fermentation, LAB aremostlyaccountable for the release and buildup of organic acid, resulting in lowered pH. Reduced pH improves the stability and safety of foods (Caplice and Fitzgerald, 1999) and the sources of carbon for the growth of yeast are majorly organic acids (Walker, 2009).In this study, the yeasts isolated from fermented cassava were identified as *Saccharomyces cerevisiae*, *Candida glabrata* and *Candida tropicalis*. This finding corresponds to the report of Ogunremi *et al. (*2017*)* who described the predominance of *Saccharomyces cerevisiae* in cassava fermentation as well as *Candida krusei, Candida parapsilosis* and *Candida tropicalis*.

Uncontrolled fermentation usuallybrings about the competitive actions of diversemicrobes in a competitive manneras organismsmost tolerable as well asrecording the highest development rate will take overcertainphases of fermentation (Madoroba *et al.,* 2009).LABand yeasts and are associated in producingdiversefermented produces (Mugula *et al*., 2003; Abegaz, 2007, Obinna-Echem *et al.,* 2014). While yeasts are well-known for the accelearation of alcoholic fermentations, LABbrings into being lactic acid as some or most importantresultant product from carbohydrates metabolism (Steinkraus, 2002; Williams and Dennis, 2011).

In this study, the physiological response of LAB and yeasts isolated from *Ogi* and fermentedcassava to acid and oxidative stress was investigated. The physiological response of the tested LAB to acid stress at pH 1, 2, 3 and 4 showed that the growth of the tested LAB gradually reduced as the pH decreased. According to Kandler and Weiss (1986), LAB are neutrophils, which are capable of growing optimally at pH ranging from 5 to 9. Acidity is dangerous to LAB. This is ascribed to the lowering of the concentration of hydrogen ion in the cytoplasm of the cell which is lower than the pH value outside the cell, subsequently inhibiting cellular functions (Kashket, 1987).

Typically, microbescan grow atdifferent pH ranging from highly acidic to alkaline conditions (Padan *et al*., 1981). Regardless ofthe ability of microbes to tolerate broad pH conditions, they are able to retain a balanced cytoplasm. For example, acidophiles with anoptimum pH of 2.0 maintain intracellular pHcloseto 7.0 (Matin, 1990). Species of *Streptococci, Lactobacillus* and*Lactococci* largely grow and continue to existin anenvironment of pH ranging from 4.5 to 7.0 (Kashket, 1987; Kakinuma, 1998).

The pH of the medium usually decreases during fermentation, on account ofincreaseand buildup of acids. Furthermore, the pH in the cytoplasm ofLAB throughout fermentation retains its alkaline conditioncompared to the medium around the cells (Kashket, 1987; Nannen and Hutkins, 1991; Cook and Russell, 1994), owing to the rapid process of cells excreting protonated lactic acid into the cell externally (Konings *et al.,* 1989; Gatje *et al.,* 1991).

During fermentation, the cell membrane is relativelyimpervious to hydrogen ions in addition to lactate molecules formed. Consequently, a difference in pH (ΔpH) of the cytoplasm and the medium created. Development and alsosustenance of change in pH is vital for maintaininga stable pH condition along with theconstituents of chemiosmosis (Mitchell, 1973).

In this study, *Lactobacillus amylovorus* isolated from traditionally produced *Ogi* and *Lactobacillus plantarum* isolated from fermented cassava were more tolerant to low pH (pH 1 and 2) in comparison with other LAB isolated from traditionally produced *Ogi* and fermented cassava (*Gari*). *Lactobacillus plantarum* is recognized to survive acidic environs. For example, two different strains of *Lactobacillus plantarum*couldsurvive acidic pHof 4.5 (Guyot *et al*., 2000). Thesetriats enables strains of*Lactobacillus plantarum*to take part in the latterphase of spontanoeus fermentation of food produce (Daeschel and Nes, 1995). Messens *et al.* (2002) reported the slower development of *L. amylovorus* DCE 471 at pH 3.8 towards the completion of fermentation of sourdough.

The regulation of internal pH homoeostasis is vital for cells to remain viable; however, changes in extracellular pH have negative effect on the yeast life cycle. By utilizing cell buffer systems and consuming H^+ through metabolic pathways (Carlisle *et al.,* 2001), increasing proton extrusion (Serrano *et al.,* 1986; Portillo, 2000), transporting acid between the cystol and organelles (Martinez-Munoz and Kane, 2008), yeast cells maintain an appropriate internal pH.

From this study, the physiological response of yeasts isolated from traditionally produced *Ogi*and fermented cassava (*Gari*) showed optimum growth at pH 4; however growth of the yeasts decreased gradually as the pH decreased from acidic pH 4 to 1. Previously, Chen *et al.* (2009)reported that slightly stressing acidic conditions (for example changeof pH from 6 to 3) does not affect the growth of *Saccharomyces cerevisiae* but instead may be slightly beneficial.

The research on the response of hybrids of *Saccharomyces*species in culture mediumat acidic pH (2.8) and a glucose concentration (250 g/l)has been investigated (Belloch *et al.,* 2008)Investigation outcome from the above stated study points out that yeasts cultured in

complex*Saccharomyces* sensustricto were not influenced by acidic pH or high-containing glucose in the media. Acid stress responses to fragil organic acids in yeast cells have been extensively examined (Kawahata *et al.,* 2006). Results of genomic and functional screening of genes associated with responses to acids suggeststhe likelihood of the acidic conditions affecting the structure of cell wall, genes expression implicated in the breakdown of metal, vacuolar H+-ATPase (V-ATPase), and HOG MAPK protein levels (Kawahata *et al.,* 2006).

The physiological response of the tested LAB to oxidative stress at 1mM H_2O_2, 3mM H_2O_2, and 5mM H_2O_2 showed that the growth of the tested LAB slowed down gradually as the concentration of the hydrogen peroxide increased. Totally, microbes are subjected to ROS for the duration of a typical metabolism with the use of oxygen or subsequently exposed to compounds the produce radical (Halliwell, 2006). Molecular oxygen is more or less not reactive and nontoxic in its initial state, but can go through reduction incompletely to produce some ROS consisting of the O^{-2} and H_2O_2, capable of reacting thereby givingmore substantially responsive hydroxyl radicals. Reactive oxygen species can be considered as harmful catalyst capable of destroying different components of the cells thereby resulting in oxidation proteins, peroxidation of lipid and destruction of the DNA caused by genetic alteration of the DNA. Oxidative stress takes place once the antioxidant and cellular survival methods are not capable of coping with the ROS or the damge caused by them (Morano *et al*., 2012).

Cell viability of the LAB was observed to be lowest at 5mM H_2O_2. The decline in viable cells of LAB with rising concentration of H_2O_2supports the research findings of Marty-Teysset *et al.* (2000)who strongly submitted that H_2O_2 interfere with the early growth of *Lac.delbrueckii*subsp. *bulgaricus.* Likewise, Narendranath *et al.* (2000)stated a reduction in the numbers of LAB studiedusing urea hydrogen peroxide at 30-32mmol/l from approximately 10^7 to 10^2 cfu/ml preincubated for a period of 2 h at 30^{o}C using wheat mash of about 21 g of dissolved solids per ml including the standard amount of suspended grain particles.

Tested yeasts isolates to oxidative stress at 1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2 showed a reduction in cell viability, however, it was observed that *Candida kefyr* isolated from

traditionally produced *Ogi* and *Candidia glabrata* isolated from fermented cassava were able to tolerate the concentrations (1mM H_2O_2, 3mM H_2O_2 and 5mM H_2O_2) of hydrogen peroxide provoked stress condition compared to other yeasts studied in this work. Improved adaptation to H_2O_2-provoked oxidative stress conditions to continuous subjection to the oxidant has been described earlier in *Saccharomyces cerevisiae* (Poljak *et al.,* 2003). Similarly, Grant (1998) and Spencer *et al*. (2014) reported that the yeasts isolates investigated showed inhibition to 5 mM hydrogen peroxide; hence, the use of 5 mM hydrogen peroxide to initiate oxidative stress inhibited the growth of *Saccharomyces crevisiae.*

It is known that H_2O_2 performs a twinfunction in livingorganisms (Afanas'ev, 2010; Ruttkay-Nedecky *et al.,* 2013; Ludovico and Burhans, 2014; Bleier *et al.,* 2015). The consequences of H_2O_2 could be advantageous or injurious. For instance, excessive H_2O_2 concentrations may bring about stress on the cells such that there could be oxidative destruction to the cell structures, whilelow H_2O_2 concentrations is importantin signaling of cellsin organism. Previous report by Semchyshyn and Valishkevych (2016) demonstrated that a rise in the growth of *Saccharomyces cerevisiae* at low concentration of 25mm/l and 50mm/l H_2O_2, however, at higher concentration of 100mm/l H_2O_2, a significantly lower cell growth was observed.

In this work, the analysis of 3 proteins obtained from the sodium dodecyl sulphate polyacrylamide gel electrophoresis of *Lactobacillus amylovorus* isolated from *Ogi* following acid and oxidative stress were identified as bifunctional acetaldehyde CoA/alcohol dehydrogenase, 30S ribosomal protein S2 and 50S ribosomal protein L5. The 97.7 kDa protein (bifunctional acetaldehyde CoA/alcohol dehydrogenase) repressed during acid stress and expressed in oxidative stress in *Lactobacillus amylovorus* has been shown to be involved in cellular alcohol metabolism; where they catalyze the formation of alcohol through the transformation of an acyl-coenzyme A via the aldehyde intermediary bringing about the decomposition of two molecules of NADH with the intention ofsustaining the availability of the coenzyme, nicotinamide adenine dinucleotide throughoutfermentation (Lo *et al*., 2015; van Lis *et al.,* 2017).Various groups of facultative and strict anaerobic bacteria, bifunctional aldehyde/alcohol dehydrogenases can be found (Atteia *et al*., 2013). Previous report from the

study of Echave *et al.* (2003)pointed out that cells whose genome carrying the protein, bifunctional acetaldehyde CoA/alcohol dehydrogenase had been deleted were unable to developin oxgyenon minimummedia;however, cells were exceptionally susceptible to oxidative stress as well asrevealing defects during division.

The repressed expression of 30S ribosomal protein S2 and 50S ribosomal protein L5 during acid and oxidative stress in *Lactobacillus amylovorus* isolated from *Ogi* were clearly detected in this study. Ribosomes which are known as particles that activates the production of messenger RNA-directed protein in all organisms (Maguine and Zimmermam, 2001). The genetic code of messenger RNA iswide-open to permitthe attachment of transfer RNA on the ribosome. This results in amino acids being integrated into the developingchains of polypeptide according to the material evidence present on the gene. Inbound amino acid monomers gain entry into the ribosomal A site as aminoacyl-transportRNAs entangled with elongation factor Tu and guanosine triphosphate (Ramakrishnan and Moore 2001). The increasing chain of polypeptide ismoved to aminoacyl-transportRNA, next, newly-fangled peptidyl-transportRNA is transfered to the P site through the help the elongation factor G and guanosine triphosphate while the deacylated transportRNA is liberated from the ribosome (Ramakrishnan and Moore 2001; Maguine and Zimmermam, 2001). This moreover explains the possible catalysis of mRNA being induced and repressed during stress.

The results obtained from the protein analysis of *Candida kefyr* isolated from traditional produced *Ogi* in this study showed the identification of 5 proteins namely 6-phosphogluconate dehydrogenase, enolase, heat shock protein 26, peroxiredoxin type-2 and peptidyl-prolyl cis-trans isomerase. The presence of 6-phosphogluconate dehydrogenase under acid stress and a disappearance under oxidative stress was observed. 6-phosphogluconate dehydrogenase participates in the pentose phosphate pathway. This pathway functions by providing reduced nicotinamide adenine dinucleotide phosphate used in donating electronsaimed at biogenesissuch asproduction of fatty acid, cocatalyst and aromatic amino acids. The pathway is important for the effective operation of theascorbate-glutathione cycle, avital component of the plant antioxidativedefense system (Corpas *et al.,* 1998). Thus, the intensity and reduction of

6PGDH expression in *Candida kefyr* as noted in this research may indicate an increased or decreased pentose phosphate pathway.

The second protein showing low intensity under acid and oxidative stress in protein analysis of *Candida kefyr* isolated from traditional produced *Ogi*is known as enolase involved in the glycolytic pathway transforming 2-phosphoglyceric acid to phosphoenolpyruvic acid (Petrak *et al.,* 2008). In yeast, glycolytic enzymes may be found attached to the surface of the mitochondria as a multifacetedmolecule. This is validated by the existenceof a number of the protein, enolase-2 firmly attached to the membrane of the mitochondria (Entelis *et al*., 2006). In the proteomic investigation of yeast on comprehensive level,four glycolytic enzymes were discovered aspart of the multifarious cytoplasm (Gavin*et al.,* 2002). This observed organization of glycolytic enzymes possibly will perform an efficientpart in controlling metabolites.

In this study, notable low intensity of peptidyl-prolyl cis-trans isomerase, peroxiredoxin type-2, and heat shock protein 26 at acid stress at pH 3 and oxidative stress at 5 mM H_2O_2in the protein analysis of *Candida kefyr* in contrast to the control is notable.

Hsf1 and Msn2/Msn4, transcription factors facilitate the response of yeast to heat shock up-regulatingthegenetic material required for heat-shock proteins (Hsps) responsible for trafficking, folding of protein and development as well as breakdown of protein, followed by a down-regulation of the genetic material for the biosynthesis of ribosome (Trott and Morano, 2003). The sHsps family is a stress-inducible group of molecular chaperones that can prevent the polymerization of denatured protein. Assembly mechanisms and the resulting oligomers vary for sHsps from severalorigins (Studer *et al.,* 2002).

Again, the observation of peroxiredoxins type 2 expressed at lower intensity at acid (pH 3) and oxidative stress (5 mM H_2O_2) in *Candida kefyr* in this workare a known as members of the thiol-specific peroxidases seenamong all territories of living entities (Jin and Jeang, 2000; Wood *et al.,* 2003). The entire family of peroxiredoxins maintainsone or more cysteine pattern

at their functional site. A lot of them are well-known to develop oligomers (Wood *et al.,* 2002; Jeon and Ishikawa, 2003).

In most cases, numerous peroxiredoxins are usuallydiscovered in single species. Consequently, six perioxiredoxins are been found in human (Seo *et al*., 2000),three peroxiredoxins were notable in *Esherichia coli* (Zhou *et al.,* 1997)and aboutfive inside *S. cerevisiae* (Park *et al.,* 2000). Four peroxiredoxins amidst the five within the maturing yeast are orthologous to humans.Therefore, yeast may function as anideal example for investigating the naturalroles of peroxiredoxins. A number ofsuggestionshave constantly supported the idea that peroxiredoxins of yeast work together to defend the cells against stress conditions caused by ROS and RNS.

In the same vein, peptidyl-prolyl cis-trans isomerase expressed at low intensity under acid (pH 3) and oxidative stress (5 mM H_2O_2) in *Candida kefyr* was observed. Cyclophilins, Cpr1, exhibits the activity of isomerization of cis-trans peptidyl-prolyl thereby catalysing the configurational isomerism of the dipeptide linksbefore the proline residues (Arevalo-Rodriguez *et al*., 2000; Wang and Heitman, 2005). Previously, the expression of Cpr1 in *Escherichia coli*showed responses to stress condition caused from reactive oxygen species, in addition to acquiring the method to tolerate other stress conditions. Hence, the Cpr1- expressing cells showed an increased stress sensitivity towards menadione, cobalt, and NaCl stress (Kim*et al.,* 2010).

Responses to acid and oxidative stress in *L. plantarum* isolated from fermented cassava revealed a notable expression and repression of Type I glyceraldehyde-3-phosphate dehydrogenase, chitin-binding protein and elongation factor Tu. Expression of these proteins indicates the response of *L. plantarum* to stress. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is important in glycolysis as it results in the oxidative production of 1, 3-diphosphoglycerate from phosphorylation of glyceraldehye 3-phosphate. GAPDH also function in apoptosis, duplication and management of DNA, endocytosis and control of messengerRNA (Bonafe *et al.,* 2005).

The relationship of GAPDH to the cell envelope of*Lactobacilli* has been studied (Ruiz *et al.,* 2009). Remarkably,the connection of the membrane of *L. crispatus*at low pH, while at neutral pHof 7, GAPDH is quickly discharged into the medium which work together with lipoteichoic acid has been noted (Antikianen *et al.,* 2007). Inactivation or reduced activity of GAPDH reduces glycolysis and metabolism in tricarboxylic acid cycle. Hence, there is a redirection of glucose to the pentose phosphate pathway, thereby producing additional NADPH, which is required by antioxidant enzymes (Godon *et al.,* 1998).

The other differentially expressed protein is elongation factor thermo unstable (EF-Tu). The increased intensity of EF-Tu during oxidative stress and the low intensity of EF-Tu during acid stress is noted in this study. In several LAB, EF-Tu was localized to the cell wall and known as a moonlighting protein which is regarded as a protein with several unconnected roles at diverse location of the cell (Siciliano *et al.,* 2008). Riis *et al.* (1990) observed the function of EF Tu inthe synthesis of protein is to transport aminoacyl-transportRNA complex and facilitate its binding to the ribosome (A site)

Secondly, it protects other proteins from aggregating by attaching to the water-repellingareas of the alteredprotein; this shows the chaperone activity of elongation factor Tu (Rao *et al.,* 2004). Thirdly,isomerase activity (protein disulfide) is demonstrated by elongation factor Tu. When compared to other protein disulfide oxidoreductases, EF-Tu activates the establishment of disulfide bond, proteinreduction and isomerization (Richarme, 1998).

The possibility of EF-Tu involvement in protein folding as well as/otherwise defense of *E. coli* from stress plus its vital function in translation and elongationof protein (Caldas *et al.,* 1998). The excessive expression of EF-Tu in the course of adaptation to acid stress has been described in *Propionibacterium freudenreichii* and *Streptococcus mutans* (Leverrier *et al*., 2004) also in adaptation of *Listeria monocytogenes* to osmotic stress (Duche *et al.,* 2002). Therefore, increased intensity of EF-Tu that profer defensive ability on freshly formed protein, in this work may be significant in folding and renaturation of protein which may confer tolerance to stress in*Lactobacillus plantarum* subjected to acid and oxidative stress.

Furthermore, from this study, an increased intensityof chitin-binding protein (CBP) has been noted during acid stress at pH 1, 2 and 3 in *Lactobacillus plantarum*. Previously, three *L. plantarum* strains producedsome proteins extracellularly within the medium with unascertained roles, of which a few of the proteins were correspondinglyfound in the guts (Marco *et al*., 2010).

Furthermore, moreinvestigators have in recent timesspent collossalattempts to discover surface-related proteins with a probablefunctionas regardscoherance of *Lactobacillus plantarum* and itsenvirons (Beck *et al.,* 2009). Chitinization process in*L.lactis* has been reported to be made up ofchitinases andchitin binding proteinsshowing similarity tothose from *Lactobacillus plantarum* (Kleerebezem *et al*., 2003). Chitin binding proteins are termed as complement proteins that do not produce hydrolysis and areimportant for the breakdown of chitin whichattach to *N*-acetylglucosamine existingin varied polymers, in addition to chitin, also mucins (Kirn*et al.,* 2005; Kawada*et al.,* 2008). Hence,the increase in chitin binding protein in *L. plantarum*as seen in this work during acid stress may possiblyparticipate in adapting and tolerating acid stress.

The results obtained from the protein analysis of *Candida glabrata* isolated from fermented cassava in this study showed the identification of 4 proteins namely enolase 2, uncharacterized protein, peptidyl-prolyl cis-trans isomerase and enolase 2. From this study, an increase in the abundance in enolase 2 was observed during acid, osmotic and oxidative stress. This possibly willbring about a more rapidlevel in glycolysis and tricarboxylic acid cycle given as a glycolytic enzyme. Likewise, uncharacterized protein showed a lower level of abundance during acid and oxidative stress compared to the glycolytic enzyme; enolase 2.

Similarly, peptidyl-prolyl cis-trans isomerase was expressed in low abundance under acid (pH 3) and oxidative stress (5mM H_2O_2) in *Candida glabrata* was observed.All organisms have an ability to catalyze the isomerization around proline residues in the polypeptide chains through

the action of peptidyl-prolyl cis/trans isomerase (PPIase). There are several families of enzymes possessing PPIase activity and therefore considered as protein folding catalysts (Galat, 2003).

The identification of peptidyl-prolyl cis-trans isomerase (CyP18) was first carried out by Fischer *et al.* (1984), initiallynamed cytosolic binding protein for cyclosporine A(Handschumacher *et al*.,1984). Besides activatingthe isomerization of cis/trans peptidyl-prolyl bonds existantin oligopeptides, CyP18 catalyzes protein folding (Schonbrunner *et al.,* 1991; Fischer, 1994). The CyP18 homologue in yeasts is stimulated by heat stress (Partaledis and Berlin, 1993). It can therefore be hypothesized that the low intensity of peptidyl-prolyl cis-trans isomerase may be linked to acid and oxidative stress which may decelerate protein folding and development of newly synthesized proteins with defensive roles against stress.

Givingreports from the researches of Sanni (1993); Kimaryo *et al.* (2000) and Franz and Holzapfel (2011), the utilization of the starters could be a suitablemove towards the management and development of the fermentation activity so as toreduce the difficulites associated withdiscrepancies in the sensoryand microbial stabilitynotable in African fermented foods.Starters of LAB are not obtainableat the commercial level especially for small-scale production of indigenous African diets (Holzapfel *et al.,* 2001).

Usuage of LAB and yeasts as starterscorresponds to the study of Steinkraus (1996)who suggested the creation of a conducive environment by LAB by acidifying of the growth mediafor the growth of yeasts while symbiotically, the yeasts make available important nutrients, minerals and other growth factors for the multiplication of LAB.

Fermentation of *Ogi* and grated cassava was characterized by a fall in pH which was noticed during the course of fermentation. The lowering of pH through application of starters has been described in some fermented cereal beverages (Gassem, 1999; Agarry*et al.,* 2010).Reduction in pH arose from rising hydrogen ion content, perhaps owing to the activities of microorganisms in breaking down sugar and more existing food items to yield organic acids (Adeyemi and Umar, 1994).

Fermentation of cereal grains in the course of producing *Ogi* with the participation of *Lactobacillus amylovorus* and production of *Gari* using *Lactobacillus plantarum* is expected to promote and further increase the acidification of the medium for consequent participation of yeasts (Annan *et al.,* 2003). Daeschel (1993) highlighted LAB's ability to yield lactic acid, thus acidifying the medium during fermentation by lowering the pH of the medium,apparently unconducive for the existence of damaging bacteria present in the fermenting substrate during spontaneous fermentation.

During the process of fermentation using *Lactobacillus amylovorus* and *Candida kefyr* in the production of *Ogi* and the fermentation process of grated cassava*,* with the use of *Lactobacillus plantarum and Candida glabrata* may possibly be ascribed towards the joined activity of yeasts and LAB thereby causing further meaningful reduction in the pH as well as a concurrent rise in acidity in the course of fermentation compared to using single starters (Kheterpual and Chaunan, 1990). Previously, Ogunbanwo *et al.* (2013) reported a considerable decline in the pH and a conforming rise in acidity of the medium when fermenting sorghum grains for the production of Burukutu.

From this work, increased protein content of the combined starters of *Lactobacillus amylovorus* and *Candida kefyr* while producing of *Ogi* and the use of *Lactobacillus plantarum and Candida glabrata* during fermentation of grated cassava compared with using single starters and the control (spontaneous fermentation) could be due to an increase in proteolytic actions during the fermentation which might have resulted in production of important amino acids and other simple protein compounds (Saleh*et al.,*2013). Some considerable enhancement in protein content of some fermented cereal products have been previously reported (Mackay and Baldwin, 1990). Food proteins perform an important function in keeping of the body make-up in addition to more materials essential for proper performance of the body system (Hayat *et al.*, 2014).

Results from this work regarding the utilization of the combined starters in producing *Ogi* and fermented cassavarevealed a decrease in the fat content compared to products produced spontaneously. The reduced value in the fat content recorded may possibly be accredited to the actions of enzymes (lipolytic) in the course of fermentation. This is in agreementwith the work conducted by Saleh*et al*. (2013) and Apaliya *et al.* (2017).

Similarly, notable increase in the ash content of the combined starters of *Lactobacillus amylovorus* and *Candida kefyr* in producing *Ogi* and the utilization of *Lactobacillus plantarum and Candida glabrata* in the production of *Gari* compared to the use of the single starters and the control (no introduction of starter cultures) which showed no significant difference may perhaps be as a result dissipation of dry matter brought about by the actions of enzymes and microorganisms in the course of fermentation (Uvere *et al.,* 2010). Generally, high value of ash suggests that the fermented sample is rich in minerals (Iqbal *et al*., 2012).

Increased crude fibre content values (neutral detergent fibre) of traditionally produced *Ogi* and *Gari* using the single starter cultures and the combined starter culture which showed significant differences was noted compared to the control. Some enriching benefits of consumption of dietary fibre include; control of body size by inhibiting intake of diet thereby improving satisfication (Kristensen *et al*., 2010; Rasomanana *et al.,* 2013), decreasing plasma lipid the concentrations, enhancement of glycemic control plus lessened excessive insulin in the blood (Chandalia *et al.,* 2000),boosting abdominal healthiness and to prevent infectious diarrhea (Roberts *et al*., 2013). Furthermore, the physiological benefits such as improving the functional, storage period, textural, and organoleptic attributes of food products are ascribed to dietary fibre (Elleuch *et al.,* 2011).

Increased mineral composition of *Ogi*and *Gari* produced with the starter cultures were noted in the study. For proper regulation and structuring of the living in addition to continuous fight against depression, mineral elements are therefore essential. Calcium is vital for developing the cells of the body and making its activities stabilized, likewise, it results in stimulating a

healthful circulatory set-up which aid in water retention required for keeping existence (Harold and Herbert, 1970).

However,the importance of magnesium in relaxing the muscles aswell as development of a healthy, robust teeth and bones cannot be overemphasized. Magnesium also regulates the pressure on the blood vessels and neurotransmitter. Likewise, iron is an indispensablenutrientnecessary for regulatingthe muscle as well as the red blood cell (Thomas, 2002). Results obtained from this research disclosed that the production *Ogi* with the combined use *Lactobacillus amylovorus* and *Candida kefyr* and *Gari* produced with the use of *Lactobacillus plantarum and Candida glabrata* recorded better and more improved mineral composition compared with *Ogi* made with single starter cultures. Ogunbanwo *et al*. (2013) reported the highest mineral content for Burukutu made using joined starters of *Lactobacillus fermentum* Iand *Saccharomyces cerevisiae.*

From this study, *Ogi* and *Gari* made using single starters and using joined starters showed a decrease in anti-nutrient components (tannin, phytate, total alkaloids and cyanide) compared to the control. Highest reduction in the anti-nutrients was observed in *Ogi* and *Gari* made using combined starters. Anti-nutrients are compounds which can meddle with the assimilation of valuable and importantnatural nutrients and minerals when ingested. They alsoattach to important fibre to avoid them from being assimilated, alter the form of nutrients toproduce compounds that are not digestable or hinderthe digestion of proteins into macro and micro nutrients that can be utilized by the body system (Muller and McAllan, 1992).

Fermentation studies showed decreased anti-nutrients in foods. Decreased tannin composition may be well attained by means of steeping, dehusking, sprouting and fermentation (Ene-Obaong, 1995). A significant reduction of phytate in wild yam beandue to fermentation by *Lactobacillus plantarum* was observed (Azeke *et al.,* 2005). Previously, findings from Ogunbanwo *et al*. (2013) presenteda reduced anti-nutritional components (polyphenols, phytate and tannins) was recorded in Burukutu made using combination of *L. fermentum* I and *S. cerevisiae*.

Although a few measure of anti-nutrients was noted in this work, current studies reported anti-nutrient components including phytate, alkaloids and even tannins, can operate like oxidation inhibitor (Miglio *et al.,* 2008). Also phytate has been stated to aid in hindering the growth of tumor cells through improving the defense network of the body in addition toenhancingprompt action of granular lymphocytes that strike at in order to annihilate cancerous cells (McGee, 2004). In the same vein, tannins improvesthe taste of wine as well as possesses probable anti-viral, anti-bacterialand anti-parasitic potentials (Lu *et al.,* 2004; Kolodziej and Kiderlen, 2005).

Preparation of fermented food is spontaneouslyfermented with varied cultures using yeasts or LAB (Akpinar-Bayizit *et al.,* 2010). In this regard, interactions among fermenters cannot be controlled in the course of fermentation, leading to disparities in theorganolepticqualitiy, shelf life, composition, and fermentation analysis of the fermented foods (Sanni, 1993).

The result from this study showed that *Ogi* made using combined starters of *Lactobacillus amylovorus* and *Candida kefyr* were accepted better as regardsthe appearance, texture and flavour than those produced with the single starter cultures while *Gari*made with the use of joint starters of *Lactobacillus plantarum* and *Candida glabarata* recorded higher acceptability compared to those made using single starters. This supports thefindingsof Mukisa*et al.* (2012) and Muyanja*et al*. (2012). Previous researchers have proposed that the use of staters for fermentation enhances the flavour of the products (Frazier and Westhoff, 1986;Oyewole, 1990; Omemu *et al.,* 2007) and the vaporous compounds produced canaccount for flavour recognized in the products made through fermentation.

CONCLUSIONS AND RECOMMENDATION

This research study presentsa conceptionon the stress responses of LAB and yeasts isolated from Africa fermented foods; *Ogi* and *Gari*. Lactic acid bacteria and yeasts needto surviveseries of ecological stress imposed in the course of food production (fermentation) and storage before it can fulfill its biological role. The most frequently occurring LAB and yeast isolated from *Ogi* include *Lactobacillus amylovorus*LS07 and *Candida kefyr*YS12 respectively while *Lactobacillus plantarum*LC03and *Candida glabrata*YC02had the highest occurrence infermented cassava. The response of identified LAB and yeasts to acid and oxidative stress showed more induced and repressed proteins. The proteins identified may perhaps be implicated in the adaptation ofLAB and yeasts to stress conditions. Furthermore, producing *Ogi* and *Gari* using the single and combined starter cultures revealed that the combined usage of starters brought about an improvement in the mineral content, reduced anti-nutrient content and enhanced organoleptic properties of products. Therefore, *Lactobacillus amylovorus*LS07 and *Candida kefyr*YS12; *Lactobacillus plantarum*LC03and *Candida glabrata*YC02 from sorghum gruel and fermented cassava respectively had increased protein intensity to acid and oxidative stresses. Hence, the stress-adapted organisms as starters are encouraged in food production.

In this study, the starters *Lactobacillus amylovorus*LS07 and *Candida kefyr*YS12 used in the production of *Ogi* and starters *Lactobacillus plantarum*LC03and *Candida glabrata*YC02 used in the production of *Gari*can be employed in producing*Ogi* and *Gari*resulting inreduced anti-nutritent content and an improved nutritional content and organoleptic properties of products. Hence, the data provided in this study will be useful for advance investigation associated with changes and responses of acid and oxidative stress in probiotic LAB and yeasts and possibly beneficial in improving the viability of these microbes in fermented products. Also, the identification of genes responsible for protein expression and repression in response to acid and oxidative stress could be investigated.

CONTRIBUTION TO KNOWLEDGE

The detailed key achievements which were found in this research study are as follows:

1. The incidence of LAB and yeasts in *Ogi*and fermented cassava was confirmed using MALDI TOF MS.
2. *L. amylovorus*LS07 and *C. kefyr*YS12; *L. plantarum*LC03and *C. glabrata*YC02in this study were able to withstand acid stress at pH 2 and oxidative stress at 5mM H_2O_2.
3. An expression of 6- phosphogluconate dehydrogenase was noted at pH 2, 3, 4 and at 1mM H_2O_2 in the SDS PAGE of *C. kefyr*YS12 from *Ogi* compared to the unstressed cells while the 2 DE of *C. kefyr*YS12 revealed a reduced intensity of enolase, perioxiredoxin and peptidyl prolyl cis-trans isomerase at pH 3 and 5mM H_2O_2 compared to the unstressed cells.
4. The SDS PAGE of *L. plantarum*LC03 from fermentedcassava revealed an increased intensityof elongation factor Tu as well as Type 1 glyceraldehyde 3- phosphate dehydrogenase at oxidative stress (5mM H_2O_2) compared to the unstressed cell.
5. An increased intensity of enolase 2 was noted at acid stress at pH 2, 3, 4 and oxidative stress at 1mM H_2O_2 and 3mM H_2O_2 while a reduced intensity of same enolase 2 was noted at 5mM H_2O_2 in the SDS PAGE of *C. glabrata*YC02 from fermented cassava compared to the unstressed cell.
6. Significant differences occurred in the performance of stress-adapted *L. amylovorus*LS07 and *C. kefyr*YS12; *L. plantarum*LC03and *C. glabrata*YC02 used as starters in the production of *Ogi* and *Gari* respectively compared to the uncontrolled fermentation which affected the qualitative value of the products.
7. The combined utilization of starters in producing*Ogi* and *Gari* resulted in an improved mineral content, reduced anti-nutrient content and enhanced organoleptic properties of products.

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APPENDIX 1

MEDIA

MRS (de man Rogosa Sharpe Medium) Agar

Mixed peptone	10.0g
Beef extract	10.0g
Yeast extracts	5.0 g
Glucose	20.0g
Tween 80	1ml
Di-potassium phosphate	2.0g
Sodium acetate	5.0g
Di-ammonium citrate	2.0g
Magnesium sulphate	0.2g
Manganese sulphate	0.05g
Distillated water	1000ml

MRS (de man Rogosa Sharpe Medium) Broth

Mixed peptone	10.0g
Lab Lemco	5.0g
Yeast extract	5.0g
Glucose	20.0g
Tween 80	1ml
Di-potassium phosphate	2.0 g
Sodium acetate	5.0 g
Di-ammonium citrate	2.0 g
Magnesium sulphate	0.2 g
Tri ammonium citrate	2.0g
Manganese sulphate	0.05g
Distillated water	1000ml

Yeast Extract Peptone Dextrose (YEP) Agar

Yeast extracts	10.0 g
Peptone	20.0 g
Glucose	20.0 g
Agar	20.0 g
Distilled water	1000 ml

Yeast Extract Peptone Dextrose (YPD) Broth

Yeast extracts	10.0 g
Peptone	20.0 g
Glucose	20.0 g
Distilled water	1000 ml

Rapid Urease Test Broth

Yeast extract	0.100g
Urea	20.0 g
Monopotassium phosphate	0.091g
Disodium phosphate	0.095g
Phenol red	0.010g
Final pH(at 25°C)	6.8±0.2

Bromocresol purple broth base

Peptone	10.0g
Sodium chloride	5.0g
Bromocresol purple powder	0.02g
Distilled water	1000ml
Final pH (at 25°C)	6.8±0.2

Bromothymol blue broth base

Peptone	7.5g
Yeast extract	4.5g
Bromothymol blue powder	0.016g
Distilled water	1000ml
Final pH (at 25°C)	6.8±0.2

Acetic-acid (1%) agar

Glucose	10.0 g
Tryptone	1.0 g
Yeast extract	1.0 g
Agar	2.0 g
Distilled water	100ml
Glacial acetic acid added when medium cooled to between 45^{o}C and 50^{o}C	1ml

Yeast carbon base agar

Glucose	1.0 g
Vitamins	1ml
Amino acids	1ml
Trace elements	1ml
Salts	1ml
Agar	2.0 g
Distilled water	100ml

Yeast carbon base broth

Glucose	1.0 g
Vitamins	1ml

Amino acids	1ml
Trace elements	1ml
Salts	1ml
Distilled water	100ml

Yeast Nitrogen base agar

Ammonium sulfate	0.2g
Vitamins	1ml
Amino acids	1ml
Trace elements	1ml
Salts	1ml
Agar	2.0 g
Distilled water	100ml

APPENDIX 2

REAGENTS

Lugol's iodine solution

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300 ml

Thiourea lysis buffer

2M Thiourea	1.6g
7M Urea	4.4g
1M Tris base	1.2g
4% Chaps	0.4g
1% DTT	0.1g
2% Carrier ampholytes pH 3-10	200µl
10mg Protease inhibitor	0.01g
Distilled water	10ml

Phosphate Saline Buffer

Stock solutions

0.2M monobasic Sodium phosphate $NaHPO_4$ (A)	27.8g
Distilled water	1000ml
0.2M dibasic Sodium phosphate Na_2HPO_4 (B)	53.65g
Distilled water	1000ml

51ml of (A) + 49ml of (B) diluted to a total of 200ml to give pH 6.8

10% Sodium Dodecyl Sulphate (SDS)

SDS	10.0 g
Distilled water	100ml

Acrylamide/Bis concentrate (30%/0.8% w/v)

Acrylamide	29.2g
Bisacrylamide	0.8g
Distilled water	70ml

Add water to final volume of 100ml when completely dissolved

Filter solution under vacuum through a 0.45µm membrane

10% Ammonium persulphate

Ammonium persulphate	10.0 g
Distilled water	100ml

0.5% Bromophenol Blue

Bromophenol Blue	0.5g
Distilled water	100ml

12% resolving gel solution

Water	3.35ml
1.5M Tris-HCl pH 8.8	2.5ml
0.5M Tris-HCl pH 6.8	0ml
Acrylamide/Bis (30%/0.8% w/v)	4ml
10% SDS	100µL
10% Ammonium persulphate	50µL
TEMED	5µL

Stacking gel

Water	2ml
1.5 M Tris-HCl pH 8.8	0 ml
0.5 M Tris-HCl pH 6.8	1.25 ml
Acrylamide/ Bis (30%/0.8% w/v)	0.65 ml

10% SDS	50 µl
10 % Ammonium persulphate	50 µl
TEMED	5 µl

5X Electrophoretic running buffer

Tris-HCl	15.0 g
Glycine	72.0 g
SDS	5.0 g
Distilled water	1000ml

One part of 5X electrophoretic running buffer in four parts gives 1X electrophoretic running buffer

Sample buffer (loading buffer)

Water	4.8ml
0.5M Tris- HCl pH 6.8	1.2ml
10% SDS	2.0ml
Glycerol	1.0ml
0.5% Bromophenol Blue	0.5ml
β-mercartoethanol	500µl

1 Dimensional Electrophoresis Rehydration buffer

2 D protein solubilizer	128µl
Lysate	10-12µl
2M DTT	0.7µl
Carrier ampholotes (v/v)	0.8µl
Bromo phenol Blue	Trace

SDS PAGE Equilibration Buffer I

6M urea	7.2g
2% SDS	0.4g

0.375M Tris HCl	0.908g
30% glycerol	6ml
2% DTT	0.4g
Distilled water	20ml

Equilibration Buffer II

6 M urea	7.2 g
2 % SDS	0.4 g
0.375 M Tris HCl	0.908 g
30% glycerol	6 ml
Iodoacetamide	0.4 ml
Distilled water	20 ml

Overlay Agarose

25mM Tris-HCl	0.3g
192 mM glycine	1.4g
0.1% SDS	0.1g
0.5% agarose	0.5g
Distilled water	100ml

Coomassie Brilliant Blue R-250 staining solution

Coomassie Brillant Blue R-250	0.1 %
Methanol	40 % v/v
Acetic acid	10 % v/v
Distilled water	50 ml

Add weighed amount of coomassie brilliant blue R 250 to the required volume of methanol, followed by the addition of water and acetic acid and filter.

Distaining solution

Methanol	40% v/v
Acetic acid	10% v/v
Distilled water	50ml

Solutions for In- Gel Tryptic digestion of protein

100mM ammonium bicarbonate

100mM ammonium bicarbonate: (1:1 v/v) acetonitrile/water

10mM dithiothreitol (DTT)

55mM iodoacetamide (IAA)

20-25ng/µl trypsin (Promega)

Extraction solution (45% water/50% acetonitrile/5% formic acid)

Desalting of peptide; extraction solution 1 (0.5% Formic Acid in 1:1 (v/v) water: acetonitrile)

Desalting of peptide; extraction solution 11 (100% Acetonitrile)

LC ESI MS Buffers

Buffer A

Acetic acid	0.5 %
Water	99.5 %

Buffer B

Acetonitrile	80 %
Acetic acid	0.5 %
Water	19.5 %

Acid detergent solution

Cetyltrimethylammonium bromide	20.0 g
Sulfuric acid	1L

Neutral detergent solution

Disodium ethylenediaminetetraacetate dihydrogen dehydrate	18.6g
Sodium tetraborate decahydrate	6.8g
Disodium hydrogen phosphate	4.6g
Water	500ml
Sodium n-dodecyl sulfate	30.0 g
Triethylene glycol	10.0 g
Water	up to 1L mark of the flask

Amylase stock solution

Heat-stable α-amylase	1ml
Water	up to10ml mark of the flask

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APPENDIX 3

Appendix 3: Absorbance of protein standard (600nm)

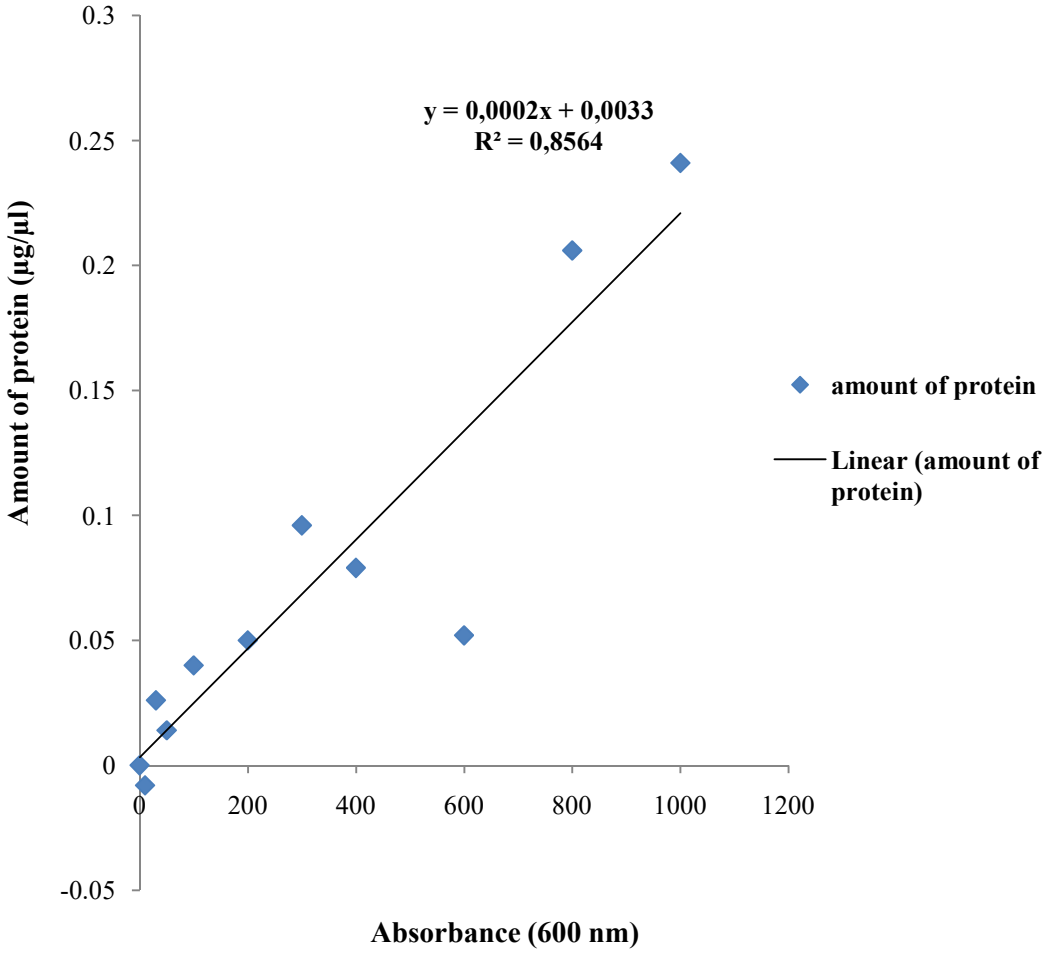
Concentration of protein (µg/ml) x-axis	Absorbance (600nm) y-axis	Amount of Protein
0	0.363457	0
10	0.345705	-0.017752
30	0.362649	0.016944
50	0.357254	-0.005395
100	0.357093	-0.000161
200	0.403453	0.04636
300	0.431093	0.02764
400	0.373515	-0.057578
600	0.484865	0.11135
800	0.678049	0.193184

Appendix 4: Absorbance of protein extracted from *Lactobacillus amylovorus* and *Candida kefyr* isolated from traditionally produced *Ogi*

Stress Conditions	Absorbance (600nm)	
	Lactobacillus amylovorus	***Candida kefyr***
Control (No stress)	0.86147555	1.00278835
pH 1	0.6700263	0.686089
pH 2	0.5591938	0.6913376
pH 3	0.50370175	0.9572977
pH 4	0.5466573	1.02815065
1mM H_2O_2	0.5194908	1.0515076
3mM H_2O_2	0.4930967	1.0892225
5mM H_2O_2	0.5634788	1.0419773

Appendix 5: Absorbance of protein extracted from *Lactobacillus plantarum* and *Candida glabrata* isolated from fermented cassava

Stress Conditions	Absorbance (600nm)	
	Lactobacillus plantarum	***Candida glabrata***
Control (No stress)	0.37096015	0.643645
pH 1	0.4344815	0.487606
pH 2	0.5439965	0.769425
pH 3	0.58120225	0.72589
pH 4	0.52786625	0.781769
1mM H_2O_2	0.42624635	0.908117
3mM H_2O_2	0.4561313	0.864832
5mM H_2O_2	0.51529785	0.718602



Appendix 6: Standard curve of bovine serum albumin (BSA)

Appendix 7: Organoleptic assessment questionnaire for laboratory prepared *Ogi*produced with starter cultures

Organoleptic properties	***Ogi***			
	A	B	C	D
Flavour				
Texture				
Appearance				
General acceptability				

Key:

A- Control (spontaneous fermentation)
B- Fermentation with single starter culture, *Candida kefyr*
C- Fermentation with single starter culture, *Lactobacillus amylovorus*
D- Fermentation with combined starter cultures *Candida kefyr*and *Lactobacillus amylovorus*

Key for accessing products:
9-10: like exceptionally, 6-8: like, 4-5: moderate, 2 – dislike, 1– dislike exceptionally.

Appendix 8: Organoleptic assessment questionnaire for laboratory prepared *Gari* produced with starter cultures

	Gari			
Organoleptic properties	E	F	G	H
Flavour				
Texture				
Appearance				
General acceptability				

Key:

- E- Control (Spontaneous fermentation)
- F- Fermentation with single starter culture, *Candida glabrata*
- G- Fermentation with single starter culture, *Lactobacillus plantarum*
- H- Fermentation with combined starter cultures *Candida glabrata* and *Lactobacillus plantarum*

Key for accessing products:
9-10: like exceptionally, 6-8: like, 4-5: moderate, 2 – dislike, 1– dislike exceptionally

Appendix 9: Frequency of occurrence of LAB isolated from traditionally produced*Ogi*

Isolate Name	Number of occurrence	Percentage (%) of occurrence
Lactobacillus acidophilus	8	25
Lactobacillus amylovorus	13	40
Lactobacillus fermentum	5	16
Pediococcus pentasaceus	6	19
Total	**32**	**100**

Appendix 10: Frequency of occurrence of yeasts isolated from traditionally produced*Ogi*

Isolate Name	Number of occurrence	Percentage (%) of occurrence
Candida kefyr	12	30
Candida glabrata	11	27
Saccharomyces cerevisiae	9	23
Candida tropicalis	8	20
Total	**40**	**100**

Appendix 11:Frequency of occurrence of LAB isolated from fermented cassava

Isolate Name	Number of occurrence	Percentage (%) of occurrence
Lactobacillus plantarum	10	32
Lactobacillus plantarum ssp plantarum	1	3
Enterococcus facaelis	10	31
Lactobacillus paracasei paracasei	7	22
Lactobacillus brevis	2	6
Leuconostoc pseudomesenteroids	2	6
Total	**32**	**100**

Appendix 12:Frequency of occurrence of yeasts isolated from fermented cassava

Isolate Name	Number of occurrence	Percentage (%)of occurrence
Saccharomyces cerevisiae	20	62
Candida glabrata	8	25
Candida tropicalis	4	13
Total	**32**	**100**

Appendix 13:Growth of LAB isolated from traditionally produced *Ogi*subjected to acid stress at different pH concentration

Stress conditions	*P. pentasaceus*	*L. acidophilus*	*L. amylovorus*	*L. fermentum*	Average	Standard deviation	Standard error
pH 1	0.2	0.219	0.235	0.215	0.21725	0.012457428	0.006228714
pH 2	0.226	0.24	0.252	0.23	0.237	0.010049876	0.005024938
pH 3	0.232	0.326	0.389	0.324	0.31775	0.055983815	0.027991907
pH 4	0.255	0.392	0.413	0.379	0.35975	0.061682149	0.030841074
Control	0.894	0.985	1.056	0.939	0.9685	0.059893656	0.029946828

Appendix 14:Growth of LAB isolated from traditionally produced *Ogi*subjectedto oxidative stress at different concentration of H_2O_2

Stress conditions	*P. pentasaceus*	*L. acidophilus*	*L. amylovorus*	*L. fermentum*	Average	Standard deviation	Standard error
Control	0.894	0.985	1.056	0.939	0.9685	0.069159237	0.034579618
1 mM H_2O_2	0.64	0.737	0.768	0.694	0.70975	0.055524019	0.027762009
3 mM H_2O_2	0.269	0.415	0.432	0.359	0.36875	0.073450096	0.036725048
5 mM H_2O_2	0.252	0.312	0.32	0.29	0.2935	0.030435725	0.015217862

Appendix 15: Growth of yeasts isolated from traditionally produced *Ogi*subjectedto acid stress at different pH concentration

Stress conditions	*S. cerevisiae*	*C. kefyr*	*C. tropicalis*	*C. glabrata*	Average	Standard deviation	Standard error
pH 1	0.279	0.367	0.234	0.343	0.30575	0.060560576	0.030280288
pH 2	0.365	0.492	0.32	0.463	0.41	0.080948543	0.040474272
pH 3	0.671	0.775	0.636	0.745	0.70675	0.064303318	0.032151659
pH 4	0.796	0.881	0.776	0.853	0.8265	0.048829636	0.024414818
Control	1.159	1.315	0.987	1.285	1.1865	0.149187801	0.074593901

Appendix 16: Growth of yeasts isolated from traditionally produced *Ogi*subjectedto oxidative stress at different concentration of H_2O_2

Stress conditions	*S. cerevisiae*	*C. kefyr*	*C. tropicalis*	*C. glabrata*	Average	Standard deviation	Standard error
Control	1.159	1.315	0.987	1.285	1.1865	0.129200426	0.064600213
1 mM H_2O_2	0.688	0.789	0.66	0.755	0.723	0.051414978	0.025707489
3 mM H_2O_2	0.659	0.749	0.649	0.732	0.69725	0.043808532	0.021904266
5 mM H_2O_2	0.622	0.737	0.591	0.728	0.6695	0.064025386	0.032012693

Appendix 17: Growth of LAB isolated from fermented cassavasubjectedto acid stress at different pH concentration

Stress conditions	*L. plantarum*	*E. faecalis*	*L. plantarum spp plantarum*	*L. paracasei paracasei*	*Leu. pseudomesenteroids*	*L. brevis*	Average	Standard deviation	Standard error
pH 1	0.257	0.189	0.239	0.189	0.246	0.217	0.222833	0.029288	0.011957
pH 2	0.268	0.203	0.25	0.205	0.26	0.222	0.234667	0.028395	0.011592
pH 3	0.377	0.212	0.288	0.214	0.335	0.243	0.278167	0.067567	0.027584
pH 4	0.659	0.307	0.586	0.332	0.628	0.36	0.478667	0.162113	0.066182
Control	1.215	0.7919	1.095	0.832	0.991	0.852	0.962817	0.167612	0.068427

Appendix 18: Growth of LAB isolated from fermented cassavasubjectedto oxidative stress at different concentration of H_2O_2

Stress conditions	*L. plantarum*	*E. faecalis*	*L. plantarum spp plantarum*	*L. paracasei paracasei*	*Leu. pseudomesenteroids*	*L. brevis*	Average	Standard deviation	Standard error
Control	1.215	0.799	1.095	0.832	0.991	0.852	0.964	0.166183	0.067844
1mM H_2O_2	0.986	0.39	0.954	0.593	0.964	0.621	0.751333	0.250594	0.102305
3mM H_2O_2	0.221	0.154	0.187	0.17	0.2	0.178	0.185	0.023495	0.009592
5mM H_2O_2	0.205	0.146	0.179	0.161	0.189	0.163	0.173833	0.021377	0.008727

Appendix 19: Growth of yeasts isolated from fermented cassavasubjectedto acid stress at different pH concentration

Stress conditions	*S. cerevisiae*	*C. glabrata*	*C. tropicalis*	Average	Standard deviation	Standard error
pH 1	0.377	0.399	0.313	0.363	0.044677	0.025794056
pH 2	0.692	0.811	0.636	0.713	0.08937	0.051597804
pH 3	0.743	0.875	0.732	0.783333333	0.079576	0.045943202
pH 4	0.802	0.967	0.798	0.855666667	0.096438	0.055678641
Control	1.148	1.281	0.988	1.139	0.146707	0.084701436

Appendix 20: Growth of yeasts isolated from fermented cassavasubjectedto oxidative stress at different concentration of H_2O_2

Stress conditions	*S. cerevisiae*	*C. glabrata*	*C. tropicalis*	Average	Standard deviation	Standard error
Control	1.148	1.281	0.988	1.139	0.146707	0.084701436
1 mM H_2O_2	0.688	0.868	0.675	0.74366666	0.107872	0.062279834
3 mM H_2O_2	0.67	0.832	0.667	0.723	0.094409	0.05450688
5 mM H_2O_2	0.659	0.79	0.654	0.701	0.077117	0.044523402