CHARACTERISATION AND FUNCTIONAL PROPERTIES OF YEAST ISOLATED FROM TRADITIONAL FERMENTED ALCOHOLIC BEVERAGES AND CEREAL GRUEL FOR APPLE CIDER PRODUCTION

BY

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A Thesis in the Department of Microbiology

Submitted to the Faculty of Science

In partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN.

JUNE, 2019.

ABSTRACT

Various groups of microorganisms are associated with traditional apple cider production. These include molds, yeasts and bacteria whose incidental occurrence often leads to inconsistency in the products' quality. The unpredictability of the complex microflora in the fermentation and inadequate taxonomic knowledge often hinder optimisation of the apple cider fermentation process. Therefore, this study was designed to characterise yeasts from selected fermented foods and determine their functional properties for apple cider fermentation.

Yeast species were isolated from palmwine, *burukutu* and *ogi-baba* samples collected within Ibadan metropolis and laboratory-prepared *agadagidi*. Isolates were identified phenotypically and by 26S rDNA gene sequencing. Functional properties including acid and bile tolerance, gastric transit test, hydrophobicity, auto-aggregation, bile salts deconjugation and wine traits tests were screened using standard methods. The best yeast strains were selected and used to produce wine from apple (*Malusdomestica*) juiceusing conventional fermentation technique. The wine produced was analysed for physico-chemical properties and the sensory evaluation was done by 20-man panellists using 9-point Hedonic scale. Data were analysed using descriptive statistic and ANOVA at $\alpha_{0.05}$.

One hundred and twenty-six yeast strains were obtained. Forty-two were identified as *Saccharomyces cerevisiae* (14), *Pichiafabianii* (7), *Pichia guilliermondii* (1), *Pichia kudriavzevii* (1), *Galactomycesgeotrichum* (1), *Trichosporon*species (1) *Candida tropicalis* (5), *C. parapsilosis* (2) and *C. glabrata* (10). All the strains grew in 0.1-1.0% ox-bile. In 3.0% bile salt, the viable count ranged from 4.81to 5.35 $\log_{10}$cfu/mL. For the gastric transit test, the viable count ranged from 6.80 to 9.50$\log_{10}$cfu/mL and 7.40 to 8.40$\log_{10}$cfu/mL for pH 2 and pH 3, respectively. Selected *Saccharomyces cerevisiae* strains showed viable count which ranged from 5.4 to 7.5 $\log_{10}$cfu/mL and 6.2 to 7.7 $\log_{10}$cfu/mL at pH 2 and pH 3, respectively. Hydrophobicity of the selected strains ranged from 35.7 to 83.5% while auto-aggregation ranged from 89.8 to 99.9% indicating the ability of yeast strains to survive gastrointestinal tract stress.All the selected strains deconjugated the sodium bile salts.*Saccharomyces cerevisiae* PW02 and *S. cerevisiae* AG08 produced alcohol of 9.4.0% and 9.2% respectively at initial ^{o}Brixof 28.0% and had the highest attenuation activity (percentage of sugar converted to alcohol) of 52.5% and 41.6% respectively. The highest fermentation efficiency of 45% and 27% were observed in *S. cerevisiae* PW02 and *S. cerevisiae*AG08. The pH of the apple cider produced ranged from 3.57 to 3.63 in samples fermented with *S. cerevisiae* PW02 and *S. cerevisiae* AG08 with corresponding total titratable acidity of 38.40 mg/mL and 40.80 mg/mL.*Saccharomyces cerevisiae* PW02 produced 10.2% alcohol and had the highest overall acceptability value of 7.82 which was significantly different from others.

Saccharomycescerevisiae PW02was identified and exhibited optimal functional properties for apple cider production.

Keywords: *Saccharomycescerevisiae,* Apple cider optimisation, Functional properties, Wine quality, Fermentation efficiency.

Word count: 451

ACKNOWLEDGEMENTS

First, I will like to thank God for giving me the opportunity to carry out this study. I really appreciate His loving kindness over me and my entire family, I thank the omnipresent, omniscience and omnipotent God, and may His name be glorified forever and ever, amen. Moreover, I will like to appreciate sincerely from the bottom of my heart, my supervisor Prof. A.I Sanni for his fatherly support, help, encouragement and positive criticism throughout the period of this project. If not for your support this project might not be a reality. I will like to thank all my lecturers in the Department of Microbiology. May God continue to uphold you and be with you all in your life endeavor. Also, I will like to appreciate Dr.S. T. Ogunbanwo, Dr. Kola Banwo and Dr (Mrs) Onipede for their support and encouragement during the course of this program. I must state that without your support the dream of this project will not become a reality.

At this junction I will like also to appreciate my children David and Dorcas for their encouragement and support. May God be with them and protect them from all evil, Amen. I will also like to appreciate my elder sister MsAdesokan Mary Abosede and her husband for their support, encouragement and prayers towards the success of this project. I must also express my gratitude to my late uncle Revd (Professor) E.O Oyelade for his kindness and support for my academic success right from First degree to my Master degree. May his gentle soul rest in perfect peace. I will also like to express my deepest gratitude to my late mother Madam Esther Alake Adesokan for her kindness and support since the time I was born till she passed away. May her gentle soul rest in perfect peace (Amen).

I will like to thank the Canadian Commonwealth Scholarship program for an award to carry out some aspects of this project in University of Western Ontario, London, Canada. Also, I am grateful to Education Trust Fund (ETF) Nigeria for financial assistance to carry out some aspects of this project in CSK Himachal Pradesh Agricultural University, Palampur, India.

CERTIFICATION

This is to certify that this work was carried out by Adesokan, Isaac Ayanniran (Matric No: 120162) under my supervision in the Department of Microbiology, University of Ibadan, Oyo State.

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DEDICATION

This thesis is dedicated to God who have been with me since the beginning of this study and to my late mother.

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CHAPTER ONE
1.0 INTRODUCTION

1.1 Background of the Study

Yeasts are simple unicellular eukaryote that can grow on in-expensive growth media. The advantage of utilization of yeasts is their use in heterologous genes expression. The yeasts have the ability of growing to ultra high cell densities and produce protein extra-cellularly. They also grow under non-selective conditions and carry out post translation modifications and this includesdisulphide bond formation, glucosylation, endoproteolytic cleavage and multimeric assembly (Oslan*et al*., 2012).

The physiological attributes of yeasts that are very useful have resulted in their application in the area of biotechnology, and fermentation ofsugar is thelargest and oldest use of this technological application (Chatterjee *et al*., 2011). Different types of yeasts are used in food production and these include brewer's yeast in beer manufacturing, baker's yeast in bread production, and yeast in wine fermentation (Chatterjee *et al*., 2011).Historically yeasts have played veryvital role in the growth of industries and different yeast species have been used in different applications in the food industry; expression system and as models for the study of higher eukaryotic organisms (Oslan*et al*., 2012). Additionally, to being used in brewing, baking and production of ethanol; yeasts have also been employed as one of the most powerful expression system (Oslan*et al*., 2012).

Baker's yeast, *Saccharomyces cerevisiae* have been employed in different human endeavour and most importantly as a host for external production of protein. The protein produced by yeasts have Generally Recognised As Safe (GRAS) Status and therefore safe for human. In the present time, people are interested in the use of other yeasts as expression system for manufacture of protein (Oslan*et al*., 2012). The other expression systems that are widely usedare *Hansenulapolymorpha, Pichia pastoris, Yarrowialipolytica* and *Arxulaadeninivorans* (Oslan*et al*., 2012).

Yeasts are very valuable microorganisms since they are sources of enzymes, antibioticsand other bioactive compounds which are used in biotechnological applications and medicine.
From the above, it is clear that yeast is in great demand and therefore identification and characterization of yeasts should be carried out to meet industrial and food needs.

In the past, identification of yeasts was based on looking only at sexual state, hyphae and pseudohyphae formation; assimilation of carbon and nitrogen compounds and sugar fermentation. In accordance to tradition yeast identification to species and strain level was based on phenotypic properties (Husin*et al*., 2004). The phenotypic characteristics of yeast are strongly influenced by the condition of culture whichare unreliable. Moreover, it is necessary to carry out 50 – 100 tests inorder to identity yeast to species level. The phenotypic characterization of yeasts is arduous, cumbersome and time consuming.On the other hand, molecular biology technique is a very rapid method that is now used for identification and classification of microorganisms. This method of identification allows some amount of phylogenetic classification, moving from the genus, species, subspecies, biovar to the strain levels (Husin*et al*., 2004). In the present time yeasts classification depends on sequences of the small subunit rRNA gene (Deak and Peter, 2013).

Yeast and bacteria have been implicated in the uncontrolled fermentation of indigenousfood products in sub-Saharan African countries like Nigeria, Benin Republic and Niger. There are different indigenous fermented food products in Nigeria, and these include*ogi* (fermented maize and sorghum gruel), *gari* and *fufu* (cassava products), *nunu* (fermented milk product), palm wine and *burukutu* (sorghum beer). To select yeasts as starter for food and industrial production, reliable identification methods that are not cumbersome and time consuming are needed. Consequently, the aims and objectives of this study are to isolate yeasts from selected Nigerian indigenous fermented foods using polyphasic methods and to determine some of their technological properties for possible industrial applications (Antai and Nkwelang, 1997; Akabanda*etal*., 2010).

1.2 Statement of Problem

Most yeast in the production of indigenous fermented food materials in Nigeria were inadequately characterized leading to inconsistency in the quality and composition of

product derived from these organisms due to incorrect identification. Hence to make these products to be of high quality, there is the need to ensure valid taxonomy and also determine the technological properties of the yeast isolates for development of startercultures that can be employed for cottage or semi- industrial applications. Such properties include probiotic application and production of apple cider wine.

1.3Justification of the Study

Identification by phenotypic methods of yeasts is cumbersome, consuming a lot of time and leading to inconsistent identification of associated microorganisms of traditional fermented foods in Africa including Nigeria. However, the use of complementary molecular techniques will solve the problem of mis-identification of the yeast isolates from the fermented foods. It is then thatstarter cultures elaborating desirable technological properties can be developed and employed for industrial applications that will yield products of high quality, wholesomeness and consistency.

1.4Aim and Objectives

To accurately identify and determine the technological properties(acid and bile tolerance, gastric transit test, hydrophobicity, auto-aggregation, bile salts deconjugation) of yeasts isolated fromfermented foods with a view to selecting starter culturesfor industrial applications.

Specific Objectives

The precise objectives of the study are to:

i. isolate yeasts from Nigerian traditional fermented foods and alcoholic beverages
ii. identify the yeasts using phenotypic and genomicmethods
iii. screen the yeasts for their technological properties.
iv. determine wine traits in the yeast isolates
v. produce wine from apple using selected yeast strains
vi. analyse the physico-chemical properties of wine produced

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Yeasts

Yeasts may be defined as microorganisms that belong to eukaryotic grouped in the kingdom fungi with more than 1,500 species presently studied (Kurtzman and Fell, 2006) and this form only about 1/100 of all fungal species (Kurtzman and Piskur, 2006). Yeasts occur as single cell organisms but some species could form a chain of connected cells after division by budding to form what is known as pseudohyphae and become multicellular. There are many differences between yeasts species and their method of reproduction is mitosis by the process called budding.

2.2Yeast Fermentation

Through the process of fermentation the yeast *Saccharomyces cerevisiae* is able to ferment carbohydrates to carbondioxide and alcohol. The carbondioxide produced by yeasts have been used in baking while alcohol is used in alcoholic beverages for many years (Legras*et al*., 2007). As a result of phylogenetic diversity yeasts are grouped in two different phyla: the Ascomycota and the Basidiomycota. The order Saccharomycetales contains the budding yeasts. The word "yeast" comes from the Indo-European roots and Old English which means boil, foam or bubble and yeast is likely to be the earliest domesticated organisms. Historically, it has been documented that yeasts are used for fermentation and baking. It was Dutch scientist named Anton Van Leeuwenhoek who first observed yeast under the microscope in 1680; but did not regard them to be living organisms at that time but rather globular structure. It was Louis Pasteur, the French microbiologist who showed that production of alcohol by fermentation was carried out by yeasts which are living things (Barnett, 2003). Yeasts obtain their energy from organic compounds and therefore they are chemoorganotrophs and do not require sunlight to grow. The yeasts carbon sources are glucose and fructose (monosaccharides), sucrose and maltose (disaccharides), ribose (pentose sugar) sugar alcohols and organic acids (Barnett, 1975). Yeasts could be obligate aerobes (i.e require oxygen for aerobic respiration or anaerobes (i.e do not require oxygen) but could also be

facultative anaerobes. Yeasts are different from bacteria in that there are no yeast species that grow completely in the absence of oxygen (obligate anaerobes). The yeasts have different optimum range of temperature and grow best at neutral or slightly acidic pH condition. For instance, *Candida slooffi, Leucosporidiumfrigidum* and *Saccharomyces telluris* grow best between 28 to 45^{o}C, - 2 to 20^{o}C and 5 to 35^{o}c respectively (Arthur and Watso, 1976; Balasubramania*et al*., 2001;Basso *et al*.,2008).

2.3Distribution of Yeasts in the Environment

Yeasts are commonly found in the surrounding especially in materials rich in sugars. For example yeasts are located on the surface of fruits and berries and exudates from plants like saps or cacti. They could be found inside the soil and on the body surface of insects (Suh *et al*., 2005). The biodiversity and ecological function of yeasts are not well known when compared to other microorganisms (Herrera and Pozo, 2010).Some yeasts are found in the toes of people living there as normal flora (Oyeka and Ugwu, 2002).Also,yeasts are found in deep-ocean environments and in the gut flora of insects and mammals(Martini, 1992; Bass *et al*., 2007).

2.4Reproduction in Yeasts

2.4.1Reproduction by Buding and Fission

The common reproduction in yeasts is by budding but they undergo asexual and sexual reproduction cycle like other fungi.During budding, small part of parent cell form an outgrowth called daughter cell, and the nucleus of the mother cell divide into two and one migrate into the daughter cell.The bud continue to grow and eventually separate from parent cell and is usually smaller than the mothercell.The fission yeasts divide into two equal halves to produce identical daughter cells and a typical example is *Schizosaccharomyces pombe* (Bischoff and Crowe,2005;Bilgicli*et al*.,2006).

2.4.2Sexual Reproduction in Yeasts

Under unfavourable environmental conditions like nutrient starvation yeasts can enter sexual cycle. In such hash condition, haploid cells will die but diploid cell will undergo sporulation, where sexual reproduction commences.Diploid spores are produced by mating of different haploid spores (Neiman, 2005).

2.5Respiration in Yeast

Yeasts are said to be facultative anaerobes as they grow when oxygen is present or absent.Yeasts convert sugar to carbondioxide, energy and biomass when oxygen is present.In the absence of oxygen yeast convert sugars to bye products like ethanol, glycerol and carbondioxide (Feldmann, 2005).Therefore, when yeast biomass production is the target, there is necessity for adequate provision of air. Glucose is the major supplier of carbon and energy for most yeast.

2.6Classification of Yeasts

Yeast classification is based on the method of production of energy from pyruvate either through respiration and/or fermentation. Environmental factors such as glucose and oxygen concentration regulates the processes. During respiration, carbon atom is removed from pyruvate in the mitochondrion to acetyl CoA which is then completely oxidized in the citric acid cycle (CAC) to carbondioxide, energy and intermediates to promote growth of yeast (Bowling *et al*., 1993;Verstrepeh*et al*., 2004).

2.7Metabolism in Yeast

Yeasts are able to utilize different carbon compounds particularly sugars such as glucose, sucrose and maltose. The extracellular enzyme, invertase first hydrolyses sucrose and then metabolises it into glucose and fructose (Feldmann, 2005). For instance, there are certain yeasts which can utilize lactose by having enzyme β-galactosidase. There are certain yeasts of genera *Candida* and *Kluyveromyces* which can be cultivated in whey and producing biomass in certain situation, with great food production application. Certain biological polymer likepectin, starch, cellulose and hemicellulose can be metabolized by certain yeasts directly or after non-yeast enzymatic hydrolysis(Gellisen and Hollenberg, 1997; Caridi,2007).

2.8Recombinant DNA Technology

Technically, the problem of inability to utilize some sugars can be overcome by recombinant DNA technology.This is usually achieved by bringing genes of the related enzymes from related species.Elements including nitrogen, phosphorus, sulfur, ferrous, copper, zinc and manganese are very important to all yeasts. These are added to the growth media as a matter of necessity.Only a few yeast species possess the ability to use nitrates as nitrogen source but most yeasts are able to assimilate directly ammonium

ions and urea directly.Sulfur and phosphorus are commonly utilized inform of sulphates and phosphorus respectively (Bekatorou *et al*., 2006). Yeasts have been used extensively in the field of biotechnology because of the importance of their physiological properties.There are many applications of yeasts in food production such as bread baking and alcoholic fermentation. Xylitol production is another area of yeast application. In Genetics and Cell Biology, yeasts are used as model organisms for proper understanding ofhigher eukaryotes (Ringbom*et al*., 1996; Ross, 1997;Rao *et al*., 2004).

2.9 Fermentation and Fermented Food

Fermentation is defined as a form of energy-productionby microorganisms during metabolic process in which an organic substrate, usually a carbohydrate, is partially oxidized and an organic carbohydrate acts as the electron acceptor (Steinkraus, 1997). The processes of fermentation are developed for many years so as to supply food through out the year to incorporate good flavour and to reduce toxicity (Achi, 2005). Fermented foods are food substances in which good microorganisms have been incorporated and whose enzymes such as proteases, amylases and lipases act on proteins,polysaccharides and lipids respectively, with flavour,texturesand aromas that are pleasant and acceptable to man (Sandhu and Waraich, 1985; Steinkraus, 1997; Rubio-Texeira, 2006).

2.10 Food Preservation

The most ancient method offood processing and preservation is food fermentation. The knowledge of man in the usage of microorganisms in the preparation of food products is second to none. For many years, all over the world fermented food productsadded so much to the diets of many people (Achi, 2005). In the production of indigenous fermented food products microbes are used in the production and safe- keeping of food products with improved nutritional value, flavour and other attributes associated with edibility (Achi, 2005). Microbial fermentation is categorized by their restricted requirement for energy input which makes fermentations togo on without external heat sources.Fermentation, drying and salting are the oldest methods of food preservation which have been incorporated in indigenous community life style. Historically fermentation processes are developed by women to keep food for period of lack, to incorporate good flavour and to reduce toxicity (Holzapfel, 2002). Nowadays,

fermentation is normally carried out as a small scale or village community-level technology in numerous countries and only few processes are done by industries (Sefa-Dedeh*etal*., 1999; Holzapfel, 2002; Schneider *etal*., 2005).

2.11 History of food Preservation

The technology of food fermentation is more than 6000 years old and must have likely developed from interaction of microorganisms with positive impact. Our forefathers in tropical region have been able to live on periods when there is lack of foods and those in the cold areas to live on in cold period by enhancing the shelf-life and safety of foods and beverages through fermentation (Marshal and Mejia, 2011).

2.12Classification of Fermentation

There are different ways by which food fermentation can be classified: By categories, classes and commodity (Steinkraus, 1997). Achi (2005) classified traditional fermented foods based on the raw materials or substrates used in the processing. They include fermented starchy foodstuffssuch asgari and fufu; fermented cereals (ogi); fermented alcoholic beverages (pito, burukutu, obiolo); fermented legumes and oil seeds includingdawadawa, iru, ogiri, okpiye; and fermented animal proteins such asnono, kilishi and wara.

2.13Principle of Food Fermentation

The preservation of traditional processed foods dependson oxidation of carbohydrates and related substrate to produce alcohol, acids, and carbon dioxide as end products (Caplice, 1999). Ogi is a common example of fermented food whose fermentation is mediated by Lactic Acid Bacteria (LAB) and yeasts. The growth of spoilage and pathogenic microbes is inhibited by lactic acid and other organic acids produced whereby the shelf-life of the product is enhanced (Olasupo*et al*., 2010).

2.14Effects of Food Fermentation

The nutritional property of fermented food is enhanced by increasing digestibility such as in fermentation of locust bean to iru (Olasupo*et al*., 2010). The toxic components of food may also be removed or reduced to minimum level in fermentation of cassava to fufu, gari,pupuruand abacha (Caplice, 1999). In Nigeria different foods and alcoholic beverages are produced from various agricultural products (Sanni and Lonner, 1993).

The shelf-life of plant materials is enhanced by natural fermentation method that requires low level technology and energy input. Infact, fermentation introduces unique sensory properties to the end product (Braumane*t al*., 1996).

2.15 Microorganisms Involved in Fermentation

Different types of microbes are found in fermented foods. Examples are yeasts, bacteria and molds. The route by which microbes enter fermented food are plant or animal materials, utensils, containers and surrounding and are appointed through adaptation to the substrate (Tamang, 2010). Bacteria play many important parts in many fermented foods. The most significant are Lactic Acid Bacteria (LAB), Bacilli and members of the Micrococcaceae (Tamang, 2010). The species commonly associated with fermented food condiments are *Bacillus natto*, *B. licheniformis, B. subtilis, B. megaterium, B. thuringiensis* and *B. coagulans* (Tamang, 2010). Members of the family Micrococcaceae are aerobic, non-spore-forming, non-motile, catalase and Gram positive with irregular clusters or packet in Shape (Tamang, 2010). There are four genera in the family micrococcaceae and only two such as *Staphylococcus* and *Micrococcus* are found in fermented meat and fish products (Tamang, 2010).The other genera that are found in indigenous fermented food products include *Klebsiella pneumonia, Halococcus spp., Halobacterium spp., Enterobacter cloacae, Haloan-aerobiumspp, Pseudomonas spp* and so on (Tamang, 2010).

Different yeast species are found in various indigenous fermented foods and beverages of the world. These include *Debaryomyces*, *Cryptococcus*, *Candida*, *Brettanomyces*, *Geotrichum*,*Galactomyces*,*Hansenula*, *Hanseniaspora*, *Metschnikowia*, *Pichia*, *Hyphopichia*,*Kluyveromyces*, *Saccharomycodes*, and *Saccharomyces*. Others are *Rodotorula*, *Schizosaccharomyces*, *Saccharomycopsis*, *Zygosaccharomyces*, *Yarrowia* and *Trichosporon* (Tamang, 2010). The mycelia fungi are relatively involved in fermented foods. The genera that are frequentlyencountered are *Aspergillus*, *Rhizopus*,*Penicillium*, *Neurospora*, *Monoascus*, *Amylomyces,Actinomucor* and *Ustilago* (Tamang, 2010).

Several yeast species have been reported in many indigenous fermented food products in different areas of Africa.Fermentation of food is in most cases carried out by complex group of microorganisms whereby bacteria, yeasts and moulds participate in the

fermentation. Traditionally the microorganisms associated with fermented foods have been studied by using phenotypic methods (Cocolin and Ercolini, 2008). However, this phenotypic method is not suitable to identify complex group of microorganisms. Therefore, new approaches which depend on the utilization of 26S rRNA methods have been created (Deak*etal*., 2000; Dahan*et al*., 2003; Cocolin and Ercolini, 2008).

2.16Types of Fermented Foods from Africa

2.16.1Fermented Cassava:*Agbelima*

Agbelima, an indigenous fermented cassava product in Ghana, contained *Geotrichumcandidum, Candida krusei, C. tropicalis,* and *Zygosaccharomyces* spp. The yeasts *C. tropicalis* and some strains of *Zygosaccharomyces* spp. exhibited cellulase activity and contributed significantly to the modification of cassava texture during fermentation. The yeast species also showed linamarase (β-glucosidase) activity and were therefore able to break down the cyanogenic glucoside found in cassava tissue (Amao-Awua, 1997).

2.16.2 Fermented Cassava: *Foofoo*

Foo-foo is a fermented cassava product in Congo. The yeasts isolated during the submerged fermentation were *Candida* spp. They only appeared after 48 hours and their numbers increased to highest of 10^2 to 10^5 Colony Forming Unit per gram (dry weight) when the process is completed (Braumanet *al*., 1996).

2.16.3 Fermented Cassava: *Fufu*

Fufu is an indigenous cassava product in West Africa which is usually produced by submerged fermentation method. The involvement of yeasts in the fermentation of cassava for *fufu* production was reported by Oyewole (2001). Six different species of yeast, namely *Candida krusei, C. tropicalis*, *Pichia saitoi, Sacchromyces cerevisiae, P. anomala*and*Zygosacchromycesbaillii*were isolated from fermenting *fufu*. All the yeast isolates showed polygalacturonase activity except*Zygosacchromyces*spp. Only *Candidakrusei*was able to exhibit linamarase activity. The growth of *C. krusei*was not enhanced by *L. plantarum*, but the growth of *Lactobacillus plantarum* was encouraged by *C. krusei*(Oyewole, 2001).

2.16.4 Fermented Cassava: *Gari*

The occurrence of *Candida* species obtained from cassava during low level production of*gari* in Nigeria has been evaluated by Oguntoyinbo (2008). The species reported were *Candida tropicalis*, *C. rugopellicosa*, *C. inconspicua, C. krusei, C. guilliermondii, C. glabrata*, and *C. maris*. Yeasts were isolated throughout the fermentation and the yeast count was $6.62\log_{10}$CFU/g at 96 hours. There was a general decrease in pH with appropriate increment in titratable acidity (TTA), which may have favoured the rapid proliferation of the yeasts in the fermenting cassava.

2.16.5 Fermented Cassava: *Lafun*

*Lafun*is another cassava-based African traditional food product in which yeasts have been isolated. The species isolated were *Saccharomyces cerevisiae*, *Pichia scutulata, Kluyveromycesmarxianus, Hanseniasporaguilliermondii, Pichia rhodanensis,* and *C. glabrata*. Forty nine yeast isolates were identified. The yeasts grew throughout the fermentation, increasing in numbers from $2.7\log_{10}$CFU/g or lower at the beginning to $5.1\text{-}5.9\log_{10}$CFU/g at the end of the process (Padanou*et al*., 2009).

2.16.6 Fermented Cassava: *Attieke*

*Attieke*is an indigenous cassava product eaten only in Cote d'Ivoire. The yeasts associated with its fermentation are *Candida tropicalis*, *C. krusei* and *Saccharomyces cerevisiae*. *Candidatropicalis* is the main yeast found in its fermentation and its mean count stood at about 10^5 CFU/g after the fermentation (N'guessan*et al*., 2011).

2.16.7 Fermented Sorghum:*Ogi-Baba*

Odunfa and Adeyele (1985) investigated microbiological changes during processingof sorghum for the production of *ogi-baba* which is consumed in West Africa. They reported the presence of some yeast species that included*Rhodotorulagraminis, Candidakrusei, C. tropicalis, Geotrichumcandidum*and*G. fermentans*. The yeasts all exhibited lipase and esterase activities. All but *G. fermentans*and *R. graminis*exhibited phytase activity and amylase activity was present only in *S. cerevisiae* (2.60%) and *C. krusei* (7.41%) were reported to show amylase activities. Furthermore, *Candida* sp. and *S. cerevisiae* were reported to show very strong lipase and esterase activities respectively compared to other yeasts(Omemu*et al*., 2007). The difference in the

diversity of yeasts isolated during fermentation of *ogi* may be due in part to different substrate used for its production

2.16.8 Nigerian Traditional Alcoholic Beverages

The yeasts associated with some Nigerian traditional alcoholic beverages were studied, namely palm wine, *sekete, burukutu, pito*and *agadagidi*. The yeasts were *Saccharomyces cerevisiae, Schizosaccharomyces pombe, S. japonicas, Candida castellii, C. frustus, C. intermedia, Geotrichumcandidum*, and *Kluveromyces africanus*, among others.The viable count (CFU/g) of yeasts in the alcoholic beverages ranged between 2.8 x 10^5 and 8.4 x 10^4. The pH of the alcoholic beverage ranged between 3.4 and 5.4 and the alcoholic content ranged between 2.0 and 3.2% (Sanni and Lonner, 1993).

2.16.9 Fermented Cereal: *Boza*

Boza is an alcoholic beverage with low level of alcohol made in South Africa. It is produced from the fermentation ofmillet or maize or barley or oats or wheat or rice. The yeasts isolated from its fermentation were *Candida diversa, Candida inconspicua, Candida pararugosa, Issatchenkiaorientalis, Pichia fermentans, P. guilliermondii, P. norvegensis, Rhodorulamucilaginosa* and *Torulasporadelbrueckii*. Some of these yeasts, for example *C. inconspicua*, are human opportunistic pathogens. *Saccharomyces cerevisiae* was not detected during fermentation of *boza* (Botes*et al.,* 2006).

2.16.10 Fermented Tchukutu

Tchukutu is a refreshing beverage produced from sorghum which is an alcoholic in Togo. The yeasts isolated during its fermentation are *S. cerevisiae, Saccharomyces* sp., *Candida species* and*Geotrichumcandidum* (Campbell-Platt, 1987). Thetaste is acidic and has a yeast flavour, and it is produced by mixing wheat flour,spices,salt, yoghurt, yeast, and vegetables. An important increase in niacin, riboflavin, ascorbic acid, and pantothenic acid was observed during its fermentation(Aidoo and Nout, 2010).

2.16.11 Fermented Cereal:*Togwa*

*Togwa*is a fermented soft porridge produced from flour made from cereals likefinger millet, maize and sorghum, but the root of cassava is also used for its production (Hellstrom*et al.,* 2010). The yeasts isolated from *togwa* fermentation were

*Issatchenkiaorientalis, Pichia anomala, P. guilliermondii,P. norvegenesis*and *P. burtonii.* When the yeast was used alone it had little activity within 12 hours, but reduced the pH to 3.57-4.81 and there was an increase in the acidity to 0.11-0.21%. As fermentation progressed the numbers of yeasts and LAB increased while that of coliforms decreased(Mugula*et al*., 2001).

2.16.12 Fermented Fruit Juice:*Masau*

In Zimbabwe,*Ziziphusmauritiana* fruit, which is traditionally known as*masau* fruit, is one of the fruits that are locally fermented into beverages through uncontrolled fermentation. The yeasts isolated from *masau* fruits and the fermented fruit pulps were*Aureobasidiumpullulans,Pichia fabianii, Candida glabrata,Sacchromyces cerevisiae,Issatchenkiaorientalis,* and *S. fibuligera*. The isolates were divided into two groups, red/pink and cream/white, based on their pigmentation. The yeasts counts (log_{10}CFU/g) for the red/pink group ranged between 1.23 ± 0.04 and 3.74 ± 0.09, and 3.30 ± 0.1 and 9.26 ± 0.4 for the cream/white group (Nyanga*et al.*, 2007).

2.16.13 Fermented Maize:*Mawe*

Mawe is acidic dough produced from maize in Republic of Benin. The yeasts isolated during its production are*S. cerevisiae,Issatchenkiaorientalis, Candida kefyr, C. glabrata* and*Kluyveromycesmarxianus*. The yeasts derived benefit from the presence of other organism like LAB which results in better growth and acid production. The yeasts also showed phytase activity which results into availability of micronutrients for consumers (Aidoo and Nout, 2010).

2.16.14 Fermented Dairy Products

In a recent study by El-Sharoud*etal*. (2009) the yeast isolated from Egyptian traditional fermented dairy products have been reported. The dairy products investigated include *miati* cheese, *kariesh* cheese and *matared* cream; and the yeasts are *Issatchenkiaorientalis* (13), *C. albicans* (4), *Clavisporalusitaniae* (9), *Kodamaeaohmeri* (1), *Kluyveromycesmarxianus* (6) and *C. catenulate* (7).

2.16.15 Agadagidi

Plantain (*Musasapientum*) is among the most important staple food crops for millions of people in different parts of the world. It is a food crop commonly consumed in the

North America, Mexico, the Caribbean and West Africa sub-region. It is consumed by both the poor and the rich. Plantain contains high quantity of nutrient and therefore can be processed into alcoholic beverage due to high sugar content. Agadagidi is a tradition alcoholic beverage produced from over ripe plantain in South West Nigeria. It is produced by soaking over ripe plantain in water for two day. *Bacillussubtilis*, *Escherichiacoli*, *Enterococcus* species, *Pediococussacidilactic*, *Lactobacillusplantarum*, *L. fermentum*, *Saccharomycescerevisiae* and *Geotrichum* species were among the organisms isolated during its production (Oriola*etal*., 2017).

2.16.16 Palm wine

Palm wine is the total name given to a group of alcoholic beverages made by spontaneous fermentation of the sap got from different palm trees. It is an alcoholic beverage that is made and consumed in various parts of the world (Santigo-Urbina and Ruiz-Teran, 2014). Palm wine is called by different names in various parts of the world. It is called Emu in South West Nigeria and Toddy in India. The sap of the palm trees which is sweet originally serves as a rich medium for the growth of different kinds of microbes. The sap is fermented spontaneously which led to multiplication of yeasts and bacteria. This resulted into conversion of the sweet substrate into many metabolites majorly ethanol, lactic acid and acetic acid. The organisms involved in palm wine fermentation are yeasts, lactic acid bacteria and acetic acid bacteria.

The process by which palm sap is obtained is known as tapping. The process involved perforation of the trunk, insertion of a tube in the hole and collection of the sap in a container. There are different methods of obtaining palm sap. The first method is by climbing the tall palm trees and perforates the trunk in the top of the tree. The second method involves felling or cutting down the palm trees before tapping of the palm sap. The palm sap continues to flow for a period of 2 to 8 weeks depending on the species of the palm trees. Palm wine is normally collected in the morning and in the evening. It can be consumed immediately or stored for sale later. Palm wine has an important role in many religious, social, nutritional and medical uses such as traditional festivals, traditional wedding ceremonies, prayers and in treatment of malaria. The sap obtained from palm trees is very rich in nutrient capable of supporting the growth of different groups of microorganisms. The groups of microorganisms include coliform bacteria, lactic acid bacteria, acetic acid bacteria and yeasts.

2.17Yeast Fermentation

A different variety of products are derived from yeasts. Yeasts perform very important role during manufacturing of indigenous fermented food products like wines , bread and beer (Demain*et al*., 2000). Many secondary metabolites like antibiotics, enzymes and vitamins are also manufactured by yeasts (Arroye-Lopez *et al*., 2008). Various yeasts were identified from many Nigerian indigenous processed foods where they contribute to alcoholic content, aroma and/or flavor of such food products (Olasupo*et al*., 2010). Most of the previous studies on the occurrence of yeasts in Nigerian traditional fermented food products solely relied on biochemical, morphological and physiological criteria for identification (Sanni and Lonner, 1993; Brauman*et al*., 1996; Omemu*et al*., 2007). For this reason, molecular system has been adopted for yeast strains identification (Hierro*et al*., 2004; Schuller *et al*., 2004; Fadda*et al*., 2004).

2.18Yeast Identification

2.18.1Phenotypic Identification

After isolation in pure culture yeasts are usually identified using a battery of standardized phenotypic tests (Kurtzman and Fell, 1998). The phenotypic tests which consist of cultural, biochemical and physiological tests are used for naming of new species which is then Latinized in a publication according to the rules of the International Code of Botanical Nomenclature (Kurtzman and Fell, 1998; Barnett, 2004). The phenotypic method of identification is regarded as artificial since a combination of characters is used for species allocation.

2.18.2 Yeast Morphology

The growth of yeasts may be studied on malt or yeast extract in liquid or solid media. Microscopic study reveals if the cells exist in pairs, clusters or scattered and the presence or absence of hyphae. The duration of this examination is three to five days and may be followed by cultural examinations like surface texture or colour. There is variation in incubation time and temperature of strains and it is species specific. The growth of *Eremothecium* at 22^{o}C for ten days is typical example (Kurtzman and Fell, 1998).

2.18.3Hyphae Formation

Hyphae formation can be promoted on Dalmau plate technique. Corn meal or potato dextrose agars are the media usually used for this technique (Kurtzman and Fell, 1998). Pseudo or true hyphae formation is determined within a week or two at 25^{o}C. The cultures are streaked as dots and lines and then covered with sterile cover slips (Kurtzman and Fell, 1998). Incase the strain of interest is known or suspected to have a sexual state, induction of ascospores is carried out by combining together cultures that is actively growing of different strains and incubated at 15 and 25^{o}C (Kurtzman and Fell, 1998). Sporulation is then examined microscopically after three weeks (Yarrow, 1998). Examination of ascospore may proceed within the first week as it may develop at this period (Glushakova*et al*., 2010). The morphological characteristics of ascospores such as size, number and ascus ornamentation may be recorded.

2.18.4Assimilation Tests

During assimilation test a carbon compound is added to a basal medium like yeast Nitrogen Base and it acts as the only source of energy. Glucose, sucrose, raffinose, starch, xylose, methanol, adonitol and erythritol are examples of common laboratory carbon containing compounds. Other are succinic acid, citric acid, glucoside, arbutin and salicin are also used in assimilation tests. Nitrogen assimilation test is also carried out by using nitrogen containing compounds like glucosamine, nitrate and nitrite with liquid yeast carbon Base as the basal medium (Vander Wattand Yarrow, 1984).

2.18.5Sugar Fermentation and other Tests

Yeasts can ferment sugar weakly or vigorously (Van der Walt, 1984). On the other hand, there are certain yeasts such as *Lipomyces* and *Cryptococcus* which do not carry out fermentation. Carbon dioxide is liberated during fermentation of carbon compounds when the conditions are favourable. The carbon dioxide produced is collected in Durham tubes as bubble. Other tests that are performed are production of Starch-like (amyloid) compounds, Osmo-tolerant on high sugar and salt concentration and growth in vitamin-free medium (Yarrow, 1998). Starch test is performed by flooding with iodine solution. Additionally, urea hydrolysis is performed on 20% urea and gelatin liquefaction is carried out. The classification into ascomycetes and basidiomycetes is carried out using the Diazonium Blue B (DBB) test. Basidiomycetes develop a dark red

or purple colour while ascomycetes produce no colour after flooding with DBB solution (Ebabhi*etal*., 2013).

2.18.6Molecular Characterization

Molecular identification is very crucial to accurately allocate strains, species and to certain levelgenera according to their phylogenetic relationship. The internal transcribed spacers (ITS1 and ITS11), D1/D2 domain and 18S small subunit are the important regions of the ribosomal RNA (or rRNA) employed in the molecular identification of basidiomycetes and ascomycetes taxa (Kurtzman,1994;Kurtzman and Fell, 1998kurtzman and Robnett, 1998). The above mentioned regions mutate very slowly and are considered as a collection molecular barcodes. As a result, they contribute extensively in the natural characterization system or molecular identification of yeasts.

The ribosomal RNA (rRNA) consists of the 18s rRNA small sub-unit (SSU), the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA between these spacers as well as the 26SrRNA large sub-unit (LSU). The D1/D2 domain is found on the 5' side of the 26S rRNA (Sugita and Nishikawa, 2003) and is about 600 bases long. The tandem repeats of the ribosomal RNA (rRNA) are separated by the non transcribed (or intergenic) spacers (NTS or IGS1 and IGS2 separated by the 5S) but the 3' and 5' sides are flanked by the external transcribed spacers (ETS) (Kurtzman and Fell, 1998;Kurtzman and Fell,2005;Keshani*et al*., 2015).

2.18.7 Polymerase Chain Reaction (PCR)

The D1/D2 domain is amplified using polymerase chain reaction (PCR) and sequenced with universal primers, NL-1 and NL-4 after DNA has been isolated from the yeast strains. The ITS-4 and ITS-5 were developed to amplify the internal transcribed spacers which are about 600 base pairs for most basidiomycetes (Kurtzman and Fell, 1998).

2.18.8Sequencing of PCR Products

PCR products are sequenced after DNA amplification using appropriate molecular analysis tools. The sequences obtained are compared with available yeast sequences on the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.govl). The D1/D2 domain and ITS regions are usually used in

association with the 18S small subunit (SSU) r RNA in most recent studies (Kurtzman and Robnetti, 1998).

2.18.9 Fermentation Products of Yeast

Different groups of aroma-active compounds are produced by the yeast *Saccharomyces cerevisiae* during fermentation. These aroma-active compounds are very important for the complex flavor of processed food products like bread, beer, wine and sake. There are six categories of flavour-active compounds produced by fermenting yeasts and these are carbonyl compounds, organic acids, higher alcohol, volatile esters, sulphur-containing molecules and phenolic compounds (Rao *et al*., 2004). Volatile esters are the most important set of yeast – derived aroma-active compounds even though they are produced in trace amount in fermented beverages (Bekatorou*et al*., 2006; Kutty and Philip,2008).

2.19Alcoholic Beverages

Alcoholic drinks could be defined as beverages containing ethanol (C_2H_5OH). The ethanol content of these beverages is almost always produced by yeasts under anaerobic or low oxygen situation. All alcoholic beverages utilize yeasts at certain stage of their production. The ethanol content of a distilled beverage has been purified by distillation. A dilute solution of alcohol is produced from carbohydrate rich plant material by yeasts. Distillation is carried out on dilute solutions of ethanol to produce spirit like whiskey and rum (Bekatorou*et al*., 2006).

2.20Single Cell Protein

Worldwide different microbes are used for human consumption and as part of traditional food starters such organisms include algae, bacteria and fungi. *Saccharomyces cerevisiae* and *Candida utilis* are the major yeasts that are eaten by humans, but very few yeastspeciesareavailable commercially(Jay, 1996). Baker's yeast or brewer's yeast *S. cerevisiae* is the most common food grade yeast used in bread baking and beer brewing. The most widely used yeast species is *S. cerevisiae* which has been selected for use in breweries, wineries and distilleries for the production of beer, wine and distillates respectively. Presently, pure yeasts are produced industrially to meet the need of brewing, baking and winery industries(Goldammer, 2000).

2.21 Alcoholic Wine Beverage

During wine making yeast converts sugars that are found in the grape juice (must) into ethanol. Yeasts are naturally found on the grape skins and fermentation can be performed using these wild yeast, but the result of the fermentation is unpredictable (Ross, 1997). Therefore, yeast starter culture is commonly added to the must and quickly takes over the fermentation process. The natural yeasts are inhibited and thereby giving expected and predictable fermentation result (Techera*et al*., 2001). The yeast starter culture is usually selected based on parameters like geographical area, climate, type of grape and the necessary sensory attribute. Yeast starter culture is also used to perform secondary fermentation of Champagne and Sparkling wine or to solve the problem of stuck or sluggish fermentations (Bekatorou*et al*., 2006).

2.22 Distilled Alcoholic Beverages

Alcohol and spirits like brandy, whiskey, rum and tequila are manufactured using distiller's yeasts and these are obtained from large scale fermentation. They are selected based on the desired quality like alcohol yield, productivity, flavour, and other technological properties (Techera*et al*., 2001). Properties of distiller's yeasts include high stress-tolerance, low foam formation and high alcohol production. The secondary metabolites are ethyl esters, aldehydes, fatty acids and higher alcohols which are essentials for quality distillation products (Techera*et al*., 2001). The substrates that distiller's yeast commonly utilized are wheat, corn, barley and potato after the initial enzymes hydrolysis (Ibragimova *et al*., 1995).

2.23 Bioethanol Production

As a result of depletion of energy supply of the world, there is a great effort towards the production of alternative energy resources such as production of bio-ethanol from renewable energy resources (Lin and Tanaka, 2006). There is supplementation of bioethanol up to 15% in gasoline by many European Countries (Mojovic*et al*., 2006). Sugar cane is the main bioenergy crops used for fuel ethanol production in tropical countries like India and Brazil (Arapoglou*et al*., 2010). The complex sugars are broken down into simple sugars by addition of glucoamylase. Fermentation of sugars is carried out to obtain up to 96% ethanol concentration (Arapoglou*et al*., 2010). Xylose one of the major fermentable sugars is found in agriculture residue, paper wastes and wood chips and the yeast *Saccharomyces* have been engineered to ferment it. This means that

bioethanol could be produced efficiently from cheap cellulosic waste (Arapoglou*et al*., 2010).

2.24Non-Alcoholic Fermented Beverages

Root beer and other carbonated drinks are examples of non-alcoholic beverage produced as beer, but the fermentation is stopped earlier. Trace quantity of ethanol and carbon dioxide is left in drink with significant quantity of sugar. Kvass is an example of fermented beverage in Eastern Europe produced from rye and it has small amount of alcoholic content.*Kombucha* is fermented tea produce by yeasts in symbiotic relationship with acetic acid bacteria. The yeast species associated with the tea fermentation are *Candida stellata, Zygosaccharomyces bailii, Schizosaccharomyces pombe, Torulassporadelbrueckii* and *Brettanomyces bruxellensis* (Tofighi*et al*., 2014).

2.25Nutritional Brewer's Yeast

The inactive yeast that remains after the brewing process is the commercial nutritional brewer's yeast. In the process of production of brewer's yeast, *S. cerevisiae* is cultivated on malted barley, selected after wort fermentation, debittered and dried. It is employed as a nutrient supplement rich in vitamin B and a good source of protein. Brewer's yeast is normally supply in the form of tablets, flakes, powders and liquid form. Enzymatically digested yeasts are supplied in form of liquid for better break down, absorption and utilization (Bonciu*et al*., 2010). Nutritional brewer's yeast has been investigated because of it medicinal value and it is a good source of manganese, cromium, B vitamins,carcium, phosphorus, potassium, magnesium, copper, ironand zinc,. It is commonly used in the treatment of conditions like chronic ache, loss of appetite, diabetes and diarrhea (Bonciu*et al*., 2010). Brewer's yeast is a good supplement for healthy hair and nails. On the other hand yeast might cause some health problems like memory disorders, chronic fatigue, irritable bowel syndrome, allergies, immunodeficiency and so on because of yeast antigens and large amount of salicylates (Tofighi*et al*., 2014).

2.26Composition of Baker's Yeasts

Baker's yeast is made up approximately 35.0 – 45.0% of carbohydrates,4.0 – 6.0% of lipids, the nitrogen content ranges between6.5 – 9.3%, of, protein, 40.6 – 58.0%, dry materials 30-33%,minerals 5.0 - 7.5% and different vitamins quantity whichdepends on

growth conditions. Fresh marketable baker's yeasts are produced in diverse form in the vein ofcompressed or creamy, active dry and liquid forms. Compacted baker's yeast consists of only one *Saccharomyces cerevisiae* and it is the most commonly used product. Instant dry yeast and active dry yeast are usually produced from special strains of *S. cerevisiae*. Instant dry yeast is available in small particles that require no rehydration before use, but active dry yeast is made up of grains of living dried yeast having leavening power. Inactive dry yeast is a commodity having no leavening properties and it is used for conditioning dough properties during baking and for developing the usual flavour (Bekaloroue*t al*., 2006).

2.27Probiotics Organisms

Probiotic organisms could be described as living microbes preparation which when administered in correct propotions provide good condition of health for the host (FAO/WHO, 2001). We may consume probiotic preparation either as part of food or as food poducts. Many lactic acid bacteria have been commercialise as probiotic and there is an expanding research interest in the search for yeast strains with great probiotic capability (Moslehi-Jenabia, 2010). Many yeast species have been demonstrated to have some probiotic potentials and such yeasts included *Torulasporadelbrueckii, Kluyveromyces lactis, K. marxianus*and *Debaromyceshansenii* (Kumura*et al*., 2004).The reported probiotic yeasts demonstrated survivalin the gastrointestinal tract and have ability to inhibit GI pathogens which include *Escherichia coli*, *Salmonella* and *Shigella*. *Saccharomyces boulardii* is athermophilic yeast that is not pathogenic and have been employed in the livestock industry as feed probiotic supplement for more than 50 years. It is also used as treatment agent in the control of gut diseases like diarrhea. There is no known danger in its consumption, and it is resistant to antibiotics and, also multiplies to a very high number in the intestine. However, production of polyamines which has strong effect on the growth of cells and differentiation denote probiotic properties as reported by Moslehi-Jenabia(2010).*S. boulardii* have been demonstrated to have antigenotoxicity as shown by SOS chromotest when it was incubated along with genotoxins mentioned above (Toma *et al*., 2005).Rats liver fibrosis has been induced by Dimethy nitrosamine (DMN) and protective effect (probiotic effect) of yeast against the disease was studied.

In an experiment, 6 groups of rats which have been treated with DMN representing the positive and negative control were sacrificed after 35 or 60 days respectively. The yeast showed no side effect on glutamate–oxalo-acetate-transaminase (GOT), alkaline phosphatase and glutamate pyruvate-transaminase (GPT) enzymes activities. Feeding the rats with yeast for higher number of days (60) had significant improvement (Zoheir and Amara, 2012).

2.28Yeast Immune Modulation

In a study where *S. boulardii* and *S. cerevisiae* were exposed to CaCO-2 cell lines, it was observed that there was increase in trans-epithelial electrical resistance (TER) (Klingberg*et al*., 2008). Another study showed that *S. boulardii* had protective effect on T84cells infection induced by enteropathogenic *E. coli*. When bacteria infections occurs there is phosphorylation of myosin light chain protein (MLC) and *S. boulardii* have been demonstrated to prevent phosphorylation of MLC and this way eliminated trans-epithelial electrical resistant (Lee *et al*., 2005).

2.29. Immune Stimulation by Probiotic Yeasts

Many researches have demonstrated that probiotic yeasts are capable of activating both innate and adaptive immunity after pathogenic infections. In a study where healthy volunteers were orally given *S. boulardii,*there were many cellular and humonal alterationin peripheral blood which is a sign of activation of the complement and reticuloendothelial system.Given oral administration of *S. boulardii* has led to the secretion of adaptive immune system which has been demonstrated by Buts *et al.* (1990).

2.29.1Effects of *S. boulardii*Homeostasis

It has been demonstrated that *S. boularadii* possess trophic effects restoring the intestinal balance. It was also shown that orally giving of yeast to volunteers who are humans or rats increased the activity of brush border membrane enzymes which have a positive influence on nutrient degradation and absorption (Buts *et al*., 1986; Jahn *et al*., 1996). Giving the probiotic yeast through oral administration after partial resection of the small bowels, led to increase disaccharide activities and improved the absorption of D-glucose (But *et al*., 1999). *S. boulardii* has been shown to contain high level of polyamine which could lead to endoluminal release of polyamines which could be

beneficial to the release of intestinal enzymes (Buts *et al*., 1990; Gadaga*et al*.,2000; Foligne*et al*., 2010).

2.29.2Effect of Probiotic Yeasts on Short Chain Fatty Acid (SCFAs)

SCFAs are involved in water and electrolyte absorption and are produced by the anaerobic bacteria in the colon. It has been shown that patients on long term enteral nutrition have low level of fecal anaerobic bacteria and fecal SCFAs. Probiotic yeasts have been shown to increase the level of total fecal SCFAs up to 9 days after termination of the treatment in these patients. The modification of fecal flora was not done by yeast, but increase in SCFAs may explain the preventive effects of the yeasts in enteral nutrition-induced diarrheal (Schneider *et al*., 2000).

2.30Production ofYeast Extracts

In the production of yeast extract, yeast cell constituent is digested by endogenous and exogenous enzymes. It is normallyuse as additive in culture media because it is rich invitamins, nucleotides, peptides and amino acids. It is employed in the pharmaceutical industry and as flavour and taste enhancer in different canned foods (taking the place of glutamates and nucleotides). The residual yeasts obtained from breweries are destined for production of commercial food grade yeast extract inspite of the presence residual beer flavour compounds(Chao and Joo, 2001).

2.31Torula Yeasts

Candida utilis, which is called *Torula or Candida* yeasts have been used in nutrition supplements in animal feeds for over 60 years. *Candida* yeast is commonly produced by cultivation in molasses, brewing bye-products and cellulosic wastes containing sugars and minerals. The yeasts are harvested, washed, thermolyzed and dried after cultivation. The yeasts are rendered in active by thermolysis, then losing their fermentation capacity; and they are turned into fine powder by spray-drying having yeasty and meaty flavour. *Torula*yeast is very rich with over 50% protein, mineral and vitamins (lysine, threonine, valine, glutamic acid, niacin, panthothenic acid and B vitamins respectively) (Kuzela*et al.,* 1976; Lezano, 2005).

2.32Utilization of Whey by Yeast

Whey is the main by-product of milk processing industry and it is used by a variety of microorganisms but only a few of them have GRAS by USFDA for production (Flores *et al*., 2000). Lactose utilizing*Kluyveromyces*yeasts (*K. lactis,* and *K. marxianus*) are commonly used and studied at industrial scale for yeast biomass production from whey. It has been reported that *Kluyveromyces*yeast could utilize lactose as sole carbon sources and under aerobic conditions it produces intermediate metabolite like alcohols, aldehydes, esters and low yield of biomas (Flores *et al*., 2000;Nanson and Roje,2001; Harta*et al*.,2004).

2.33Lactose Fermentation in Kefir

Among the natural mixed cultures found in the white people's milk drink are the lactose fermenting yeasts. It contain a mixed group of microorganisms comprising of lactose fermenting yeast like*Saccharomyces, Debaryomyces,Kluyveromyces, Candida, Zygosaccharomyces,* LAB and once in a while acetic acid bacteria (Simova*et al*., 2002). Yeasts of Kefir origin have been applied at sub-industrial level for utilization of lactose and production of ethanol, biomass, lactic acid and alcoholic beverages.

2.34Sourdough Fermentation

Sour dough consists of a mixture of water,flour, yeast *Saccharomyces cerevisiae* and LAB used as starters. Sour dough has a lot of advantages over ordinary bread like production flavour and texture, an extended shelf life and production of antimicrobial compound like bacteriocin (Hansen and Schleberle, 2005). By using a combination of yeast and LAB, sour dough is produced on a large scale giving a product with improved baking properties and special sensory properties (Plessas*et al*., 2005).

2.35Production of Single Cell Protein

Single cell proteins are considered good source of protein for man and animals as well. Nutrient like carbohydrates, proteins, vitamins, minerals and essential amino acids could be obtained from food grade yeasts (Rincon and Benitez, 2001). The nucleic acid found in yeasts is lower than that of bacteria and the amount of lysine is higher than bacteria or algae.*Saccharomyces cerevisiae* is the only organism that is fully accepted as food for humans. Food intolerances may be caused by yeast in human although in lower

frequency than other food stuffs likemeat, milk, nuts, fish and so on. Human may react to allergic reactions caused by foreign protein in yeast(Thannonke*et al*., 2011).

2.36Production of Brewer's Yeasts

The demand of brewing industry is met by production of pure brewer's yeast culture. Two different species of *Saccharomyces* are used in production of beer. *Saccharomyces uvarum* is a bottom fermenter while *Saccharomyces cerevisiae* carry out top fermentation (Goldammer, 2000). Beer is produced using different strains of yeasts with specific characteristics. The type of yeast selected will depend on brewing equipment and beer style. The desired yeasts characteristics include rapid fermentation, rate of attenuation, stress tolerance, flocculation, beer flavour and yeast stability on long term basis (Goldammer, 2000). Yeast must be able to conduct fermentation rapidly with little biomass production and adequate amount of ethanol based on overall flavour of the product. The growth and ethanol production of four *Zymomonasmobilis* strains isolated in Thailand from Jerusalem artichoke juice have been investigated. It was strain TISTR 548 that produced the highest ethanol concentration at 30 to 35^{o}C when compared to the others (Thannonke*et al*., 2011).

2.37Condition Necessary for Brewer's Yeast Production

There are many factors that affect brewer's yeast during fermentation; therefore the yeast must be tolerant to alcohol and osmotic pressure (Goldammer, 2000). Ethanol tolerant ability of *Saccharomyces cerevisiae* is due to composition of plasma membrane. The membrane is made of ergosterol instead of cholesterol and phospholipid which is made up of unsaturated fatty acyl residues (Basso *et al*., 2008). *Saccharomyces cerevisiae* were evaluated for alcohol tolerance and a limit of 3 – 12% was reported based on the method reported by Keo (1967). There is ever increasing demand for ethanol as a result of biotechnological applications. To meet those demands, it is necessary to develop potential *S. cerevisiae* isolates for production of ethanol from cheap and readily available raw materials like molasses (Keo, 1967). Large percentage of molasses produced as a bye-product of sugar mills in Bangladesh remains unutilized every year (Cimpeanu*et al*., 2010; Kayode *et al*., 2011).

2.38Bioethanol Production from Molasses

It is necessary to remove inhibitory substance from molasses by undergoing processes like clarification and pretreatment (Cimpeanu*et al*., 2010). Yeast genera like *Saccharomyces, Kluyveromyces* and *Zygosaccharomyces* were evaluated for ethanol production from molasses and maximum ethanol production at 15% sugar concentration have been reported (Kiran *et al*., 2000). The initial concentrations of sugar have been reported to affect alcoholic fermentation by yeast. When the initial concentration is too high, it leads to increasing lag phase, reducing growth rate and reduction in ethanol tolerance (Edgardo *et al*., 2008). The appropriate sugar concentration in molasses have been reported to be 11-15% as higher concentration of sugar might lead to unnecessary osmotic pressure on the yeast which leads to reduce ethanol production (Edgardo *et al*., 2008). In alcoholic fermentation the percentage of sugars transformedto alcohol and carbon dioxide is called attenuation which is measured by specific gravity. The common sugars usually fermented by yeasts are glucose, sucrose, maltose and fructose. In order to select yeasts that are good attenuator, they must completely utilize the sugars. There are significant difference between the rate and extent of sugar utilization by yeasts (Goldammer, 2000). There is an inverse relationship between attenuation and flocculation. The characteristic of high attenuation yeast is usually dry clean and fully finish product, but it a longer time to clear; whereas low attenuation yeasts produce fuller bodied and more complex beer and quicklysettle down. It is therefore necessary to carefully select suitable yeast with appropriate attenuation to match the style of the beer.

2.39Fermentation Efficiency

Fermentation efficiency could be described as the actual amount of ethanol produced as compared to the amount produce theoretically. There are many factors that affect fermentation efficiency and this include sugar concentration, temperature, pH and nitrogen supplement. Increase in fermentation efficiency has been reported at high concentrationof sugar and with supplementarynitrogen source. Also, it has been shown that yeasts with low nitrogen requirement are more effective fermenters (Balakumar*et al*., 2001).

2.40Famous varieties of apple

2.4.1Red delicious apple

The red delicious apple was discovered at an orchard in 1880 and was initially called the "Hawkeye" before Stark Brother Nurseries bought the rights and changed the name. Eating it fresh is good and in making salads, but has poor baking properties. It is deeply red in colour and is occasionally streaked with yellow. They have sweet taste and juicy and is available between August and November (Chaudhary *etal*., 2014).

2.4.2Fuji apple

It has its origin from Japan and was developed by Tohoku Research Station since 1962. It has a sweet taste and it is reddish-pink in colour. It is good to be eating as snack and in making salads, pies, sauces and baking. It has a poor freezing property and its season is from October to August (Chaudhary *etal*., 2014).

2.4.3 Cortland apple

This apple was developed by New York State Experimental Station in 1915. It has a red colour over a greenish-yellow background. It is sweet in taste with a trace of tartness. The apple is good as snacks, salads and pies. It is also good for baking and for freezing. Its season is from September to April (Chaudhary *etal*., 2014).

2.4.4 Grammy Smith apple

This apple was developed by a chance seed by Mrs Thomas Grammy Smith in Australia. It has a red and yellow colour and it is a recently introduced variety (Chaudhary *etal*., 2014).

2.4.5 Mcintosh apple

This variety of apple was developed by John Mcintosh who was a farmer in Ontario Canada in the early 19^{th} Century. It is a brilliant green variety of apple with sweet taste and just a trace of tartness. It could be used for baking or freezing but is not a good choice for making pies and salads (Chaudhary *etal*., 2014).

2.4.6 Jonathan apple

This variety of apple is an old American apple discovered in 1864. It is spicy in taste with fragrant, juicy and sweet tart and is good for cooking. It is an excellent snack and could be stored in the refrigerator for a good 120 days (Chaudhary *etal*., 2014).

2.4.7 Gala apple

The origin of this apple is in New Zealand. Its colour is pinkish stripes with a yellow background. The apple has an excellent sweet taste and is good for salads, sauces and snacks. It is also good for making pies and baking but not good as freezing apple. The season of this apple is from September to May (Chaudhary *etal*., 2014).

2.4.8 Golden delicious

This variety was developed by Anderson Mullins in 1890 in West Virginia, United State of America. It has a regular sweet taste with a silky texture and crisp, thin skin. It is yellow in colour with a bright pink blush if cool nights come before harvest. It is good for making pies, baking and sauce. It has a great taste when eating fresh (Chaudhary *etal*., 2014).

2.4.9 Jonagold apple

This is a great quality apple of American origin developed by New York State Agricultural Experimental Station in 1940 and was introduced to the market in 1943. It has a big size with sweet juicy crisp cream coloured flesh and a refreshing taste. It has a bright red skin over a yellow base. Its season is between mid March and August in Australia (Chaudhary *etal*., 2014).

2.4.10 Rome Beauty apple

This popular apple variety has its origin in Ohio, United States of America. It was introduced into the market in1816. It is a mildly tart red apple and it is good as baking apple. It has good properties as pies or sauces and for making salad or eating as snacks. It is one of the best apple varieties that are available (Chaudhary *etal*., 2014).

2.4.11 Ginger gold apple

This variety was developed by Ginger Harvey in United States of America in 1960. It is made up of 5 distinct crowns on the base and is deep red to crimson in colour. It is average in sweetness with juicy creamy white flesh and a highly aromatic flavor. It has a soft texture than other varieties which makes it suitable for young children (Chaudhary *etal*., 2014).

2.4.12 Honey crisp apple

This variety of apple is modern day apple developed by the University of Minnesota in 1960 and was made available in the market in 1990. It has a freckled green skin and it is crisp and juicy with a sweetly tart flesh. It is the most suitable for eating and cooking. It has a maximum shelf life of 240 days under refrigeration (Chaudhary *etal*., 2014).

2.5 Examples of products of apple

2.5.1 Applecandy

This is a confectionery made from concentrated sugar solution. Apple flavor is usually added to make yummy, mouth watering apple candy. It slowly melts in the mouth with a rich taste of apple and it is a good source of energy (Chaudhary *etal*., 2014).

2.5.2 Applejam

This product consists of real apple flavor. Apple jams are normally made from the pulp and juice of the fruit. Jam is made from apple juice and pieces of the fruit. It is soft with even consistency and a good fruit flavor, bright colour and a semi jellied texture. It is easy to spread over but has no free liquid. Apple jam function as alternative medicine supplements and vitamins. It enhances immune function and promoting well-being (Chaudhary *etal*., 2014).

2.5.3 Applejelly

This product is a semi solid made from juice, sugar and pectin. It is a clear or translucent fruit spread, made from sweetened fruit juice and set using naturally occurring pectin present in the fruit or by addition of pectin if it is not sufficient. Colouring and flavouring agents are added so as to enhance the quality and acceptability of the product. The products are produced using the clarified concentrate of apple. It has a good apple flavor which is soft and smooth (Chaudhary *etal*., 2014).

2.5.4 Applejuice

Apple juice is produced by crushing and pressing of apple. The juice that is obtained is further processed by centrifugal and enzymatic clarification for removal of pectin and starch. Apple juice is good for lung health and it is a source of dietary fibre, vitamins A and C (Chaudhary *etal*., 2014).

2.5.5 Applepulp

Apple pulp is produced from fresh, clean and sound and fully matured apple. It is employ in the production of cookies, cakes, muffins and some other bakery products. Apple possesses anti-oxidant functions. Raw apple pulp or apple juice can be applied on the skin daily (Chaudhary *etal*., 2014).

2.5.6 Applesauce

Apple sauce is a puree made of apples. It is an inexpensive and readily available food. Apple sauce contains fibre and vitamin C. Apple sauce normally contains no fat and few calories (Chaudhary *etal*., 2014).

2.5.7 Applebutter

This apple product is a highly concentrated form of apple sauce produced by long, slow cooking of apple. It can be used as a fat substitute in reduced fat or fat-free cooking. The copper in apple butter helps body properly and metabolize iron, and it plays a critical role in the function of nervous and immune system (Chaudhary *etal*., 2014).

2.5.8 Applepectin

Pectin is found in all plant cell walls and tissue and it is a type of fibre. The amount of pectin found in apple is very high. Pectin is primarily used in the treatment of digestive disorders. It is also an antioxidant which protects the body like lowering of cholesterol, and management of diabatics and reduced the risks of some cancers (Chaudhary *etal*., 2014).

2.6 Health benefits of apple

2.6.1 Apples boost gum health

Apples have been referred to as natural tooth brush. Although eating an apple does not really cleanse the teeth, but biting and chewing apple stimulate the gum. Also the sweetness of apple causes an increased flow of saliva which prevents tooth decay by reducing the amount of bacteria in the mouth (Chaudhary *etal*., 2014).

2.6.2 Beat diarrhea and constipation

Apple can counter both diseases even though they are two different stomach ailments. In the case of constipation apple fibre pull out excess water from the colon and absorb excess water in the case of diarrhea (Chaudhary *etal*., 2014).

2.6.3 Glowingskin

The least known benefits of eating apple is that it is great for the skin. It contains a lot of vitamin C and antioxidants which prevent signs of aging, wrinkle and keeps skin glowing (Chaudhary *etal*., 2014).

2.6.4 Anticholesterol

Apple is very high in fibre. When apple is eaten the soluble fibres compete with fats in the intestine. This competition leads to reduction in the level of bad cholesterol and increase the level of good cholesterol (Chaudhary *etal*., 2014).

2.6.5 Improvedmemory

Eating one apple per day prevents the decline of a neurotransmitter known as acetycholine thus improving your memory. A study also reported that apples can slow the mental decline affecting people with Alzheimer's disease. In man taking high quantity of vegetable and fruits such as apples reduce the risk of degenerate conditions such as Alzheimer's disease (Dai *etal*., 2006).

2.6.6 Apple boosts immunity

The red delicious apples contain an antioxidant known as quercetin. This compound helps boost immune system to build the natural defences of the body. Recent studies have found that quercetin can help boost and fortify your immune system, especially when you are stressed out (Jeanelle and Rui, 2004).

2.6.7 Apple helps weight loss

Apples satisfy hunger for few calories so it can be part of healthy diet that promotes weight loss. Apple and pear intake has also been associated with weight loss in middle aged overweight women in Brazil (Moura, 2003).

2.6.8 Apple heals asthma

The healing properties of apple have been attributed to the presence of phyto-chemical and polyphenols. It helps in recovering from asthma, breathing problems and improve the general functioning of the lungs. It has been reported that chidren of mothers who eat apples during pregnancy are much less likely to exhibit symptoms of asthma including wheezing (Willer*etal*, 2007).

2.6.9 Apple heals diabetes

Apple contains high quantity of soluble fibre which helps to blunting blood sugar swings.The highly soluble pectin in apples helps in controlling blood sugar levels in the body by transporting the sugar into the blood stream at a slower rate. It has been reported that women who eat at least one apple a day are 28 per cent less likely to develop type 2 diabetes than those who don't eat apple (Moura, 2003).

2.6.10 Apple has anti cancer properties

Apples have very strong anti cancer abilities that protect the body from the life-sucking cancerous cells. This is the most important apple fruit benefit of all. In a study in Finland involving 10,000 men and women and a 24-year follow –up, a strong inverse association was seen between flavonoid in take and lung cancer development (Knekt*etal*., 1997).

2.7 Apple phytochemicals

Apples have a high concentration of flavonoids, and other variety of phytochemicals. The concentration of these phytochemicals may depend on many factors such as cultivar of the apple, harvest and storage of the apples and processing of the apples. The concentration of phytochemical found in apple peels and flesh differs greatly. Some of the most well studied antioxidant compounds in apples include quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyaniding-3-galactoside, coumaric acid, chorogenic acid, gallic acid and phloridzin. In a recent study, the average phenolic concentrations among six cultivars were: quercetin glycosides, 13.2 mg/100 g fruit; vitamin C, 12.8 mg/100 g fruit; procyanidin B, 9.35 mg/100 fruit; chlorogenic acid, 9.02 mg/100 g fruit; epicatechin 8.65 mg/100 g fruit and phloretin glycosides, 5.59 mg/100 g fruit (Ferretti *et al*., 2014).

Different types of fruit with health benefits are produced throughout the world. Fruits are very delicious with diverse kinds of nutrients like fibers, vitamins, minerals, antioxidant, water and vitamin (Jamil *et al*., 2015). Tropical countries have different kinds of fruits which can be exploited commercially. Some of them are considered rare and exotic (Chakraborty *et al*., 2014). Africans are reported to consume fruits on regular basis as snacks and relishes. These fruits are very rich in vitamins most especially vitamin C, minerals, fat and sugars (Enidiok and Attah, 2010).The apple

genus is *Malus* and species is*domestica* (*Malusdomestica*) (Hussain *et al*., 2014). Apple is very rich in nutrient such as vitamin C, potassium and fiber. Apple contains appropriate food elements like sugar (11%), protein (0.3%), carbohydrate (14%), vitamin and minerals (4%) and remaining portion is 80% water (Hussain *et al*., 2014).

Theapple sizesvary in calories of energy. When compared to other fruits, apple has the third highest anticancer activity (Hussain *et al*., 2014). Apple is very good in maintaining good health (Hussain *et al*., 2014), and it is reported to possess good healing power, and therefore used in the treatment of arthritis and rheumatism (Hussain *et al*., 2014).

Wine is commonly produced from grapes but recently it is now produced from other fruits like apple (Satavi*et al*., 2017). Man has been producing wine from time immemorial and wine making is one of the man's most prosperous biotechnological applications (Jagtap and Bapat, 2015). The processing of apple juice into a refreshing alcoholic beverage has been carried out in Eastern Mediterranean areas more than 2000 years ago (Alberti *et al*., 2011). Nowadays, fermentation of apple juice is carried out to obtain cider, which is aflavoured drink consumed in many parts of the world (Alberti *et al*., 2011).

Apple is made up of all needed nutrients for the growth of yeast. It contains soluble sugars as main source of carbon and energy at a concentration of 120g/l and this is enough to support the growth of yeasts to reach 6.0 x 10^7 cell/ml (Alberti *et al*., 2011). There are two different stages of fermentation. Yeast fermentation is the first stage where sugar is converted to ethanol and higher alcohol by inoculation of starter culture or by spontaneous fermentation (Bozoglu*et al*., 2015). Malolactic fermentation is the second phase in which malic acid is changed to lactic acid and carbondioxide through the action of LAB. Most important alcohol in wine is ethanol and could be up to 10 – 13% (Bozoglu*et al*., 2015). Yeasts are very important in the process of producing alcoholic beverages. It is therefore important to select appropriate yeasts so as to maximize alcoholic yield and to produce alcoholic beverages of acceptable sensory quality (Walker and Stewart, 2016).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Isolation of Yeasts

Palm wine samples (PAW),*burukutu* (BKT) and *ogi-baba* (OBB)were collected from local producers within Ibadan metropolis immediately after production and transported in sterile containers to the laboratory in the Department of Microbiology,University of Ibadan for immediate studies. Samples of laboratory-prepared *agadagidi*(AGG) were also taken for isolation of yeasts.*Agadagidi* was prepared by soaking ripe plantain in tap water for two days following the method of Sanni and Lonner (1993).The samples were serially diluted and appropriate dilutions were cultured on potato dextrose agar(PDA)supplemented with3µg/mL of streptomycin and chloramphenicol. The yeast isolates were subcultured and purified by repeated streaking on potato dextrose agar(Kurtzman *etal*., 2011). Pure cultures obtained were stored under refrigeration until needed for further studies.

3.2 Phenotypic Characterization

3.2.1 Microscopy

The pure cultures of yeasts were streaked onto thin sterile agar on slide, covered with coverslip and the cells were studied with a LeitzOrtholux phase-contrast microscope(Lachance *etal*., 1999) and the images were recorded with a Sony XC-75CCD video cameraelectronically.

3.2.2 Biochemical Tests

3.2.2.1 Gelatin Liquefaction

The gelating liquefaction test was employed to examine the capacity of the yeasts isolates to liquefied gelatin. Ten gram of gelatin and malt extract was dissolved in 95ml distilled water and 1.5g of agar agar was added to the medium and the mixture was autoclaved at 121^{o}C for 15 minutes. The medium was allowed to cool and solidify and then inoculated with the pure isolates of the yeasts. The Petridishes containing the yeasts were incubated at 28^{o}C for 48 hours (Lourens-Hattingh and Viljoen, 2001).

3.2.2.2 Casein Hydrolysis

This experiment was done to verify the capacity of the yeasts to hydrolyze casein. Exactly 0.3g beef extract, 0.5g Tryptone, 0.1g glucose and 1.5g agar agar was dissolved in 50mL distilled water. Then 2.4g skim milk was separately dissolved in 50mL distilled water. The solutions were sterilized separately at 121^{o}C for 15 minutes. After cooling to 50^{o}C the solutions were aseptically added together and then poured into culture plates. The Petri dishes containing the media were then inoculated with the wholesome cultures of the yeast isolates and incubated at 28^{o}C for 48 hours (Lugwisha*et al*., 2016).

3.2.2.3 Lipid Hydrolysis

This experiment was to determine the capacity of the yeasts to hydrolyze lipids. Peptone (1.0 g), 0.1g yeast extract, 0.5g NaCl, 0.02g $CaCl_2$, 1.5 g agar and 0.44g Tween 80 were dissolved in one hundred mL distilled water. The combination was homogenized and then autoclave at 121^{o}C for 15 minutes. It was allowed to solidify and inoculation was done with pure culture of the yeast isolates. The cultures were incubated at 28^{o}C for 48 hours (Meignen*et al*., 2001).

3.2.2.4 Acid Production from Glucose

This experiment was to examine the capacity of the yeasts to produce acid from glucose. Glucose (5.0 g), 0.5g of yeast extract, 1.0g of $CaCO_3$ and 1.5g of agar were allowed to dissolve in 100mL of distilled water. The mixture was homogenised and then sterilized at 121^{o}C for 15 minutes. After solidification in a slanting position and inoculated with pure cultures of yeast isolates and incubation was done at 28^{o}C for 48 hours (Olowonibi, 2017).

3.2.3 Physiological Tests

3.2.3.1 Tolerance to Different Concentration of Sodium Chloride (NaCl)

This test was carried out to determine the influence of osmotic pressure on the growth of yeast isolates. 5.0g of glucose, 0.8g of Yeast Nitrogen Base (YNB), and 1.5g of agar agar were dissolved in 50ml distilled water. Different concentration (5%, 10% and 15%) of NaCl was added separately with the basal medium and the medium was sterilized at 121^{o}C for 15 minutes. The medium was allowed to solidify and then

inoculated with pure cultures. Incubation was done at 28^{o}C for 48 hours (Shetty *et al*., 2007).

3.2.3.2 Influence of Inhibitors on Growth ofIsolates

This experiment was performed to investigate the influence of acid/base inhibitors on the increase of the yeast isolates. The basal medium contained 4.1g of YM agar dissolved in 100mL distilled water.Inhibitors including deoxycholate (0.5%), cinnamic acid, 5-fluorocytosine, canavanine SO_4, 10ppm, 100ppm and1000ppm of cycloheximide (prepared by adding 40.0µL, 400.0µL and 4.0mL of cycloheximide respectively), 10ppm and 50ppm CTAB, 1% and 0.5% acetic acid, and 1.5%, 2.0% and 2.5% phosphoric acid were also prepared (Kanwar and Keshani, 2016). Sterilization by autoclaving was carriedout and the medium waspermitted to cool to 50^{o}C and then dispensed into Petri dishes. The plates containing the medium were inoculated with the pure cultures of the isolates.

3.2.3.3 Growth on Phenylalanine Agar

This experiment was carried out to investigate the effect of phenylalanine on the growth of the yeast isolates. The basal medium consists of YNB without nitrogen and 2.0g agar agar dissolved in 50ml distilled water. 1.0g phenylalanine was dissolved in 50ml distilled water. The solutions were separately sterilized at 121^{o}C for 15 minutes and then mixed together and dispensed into Petri-dishes and allowed to solidify. After solidification of the medium, inoculation with the pure cultures of the yeast isolates was done and incubation carried out at 28^{o}C for 48 hours (Ravindra, 2000).

3.2.3.4 Growth at Different Temperatures

This test was performed to detect the influence of various incubation temperatures on the growth of the yeast isolates. YM agar plates were produced by mixing 4.1g of YM in 100mL distilled water and the medium was sterilized at 121^{o}C for 15 minutes. After allowing the medium to cool to 50^{o}C it was dispensed into sterile Petri dishes. Inoculation was done with pure culture of the yeast isolates and incubation at different temperatures was performed (4^{o}C, 30^{o}C and 37^{o}C) for 48 hours and appearance of viable colonies on the plateindicates growth which is positive result (Simova*et al*., 2002).

3.2.4 Assimilation of Nitrogen and Carbon Compounds

3.2.4.1 Assimilation of Carbon Compounds

This experiment was performed to test the capability of the yeasts to assimilate various carbon sources. 2.7g Yeast nitrogen base (YNB) was dissolved in 400mL distilled water and 150 - 500µl of Glucose and other sugars such as Glycerol, Glucitol, Starch, Inulin and other sugars were added separately in l litre flasks. Finally 6.0g agar was added and the medium sterilized by autoclavingat 121^{o}C and 15 lb per square inch above atmospheric pressure. The medium was allowed to cool down to 45^{o}C and then poured into sterile Petri dishes tosolidify. Inoculation with the pure cultures of the yeast isolates was done and incubation at 28^{o}C for 48 hours was carried out (Soares and Mota, 1997).Appearance of viable colonies on the plate indicates assimilation of carbon which is positive result.

3.2.4.2 Assimilation of Nitrogen Compounds

This test was carried out to screen the yeasts isolates for their capacity to utilize diverse ompounds containing nitrogen. A solution of Yeast Carbon Base (YCB) was produced by mixing 1.1gin 100ml distilled water in six separate 250ml conical flasks. Then 0.06g ofcadaverine, sodium nitrate, lysine, sodium nitrite and ethylamine were separately dissolved in YCB solution and 1.5g of agar was added into each conical flask. The mixture was homogenised and then sterilized by autoclaving. After pouring into Petri dishes and solidification of the medium the plates were inoculated with the pure cultures of the yeast isolates and incubated at 28^{o}C for 48 hours (Su*et al*., 1999).Appearance of viable colonies on the plate indicates assimilation of nitrogen which is positive result.

3.4 Molecular Characterization

3.4.1 Selection of Yeast Isolates

The organisms were grouped together based on their phenotypic properties and representative members were selected for molecular characterisation.

3.4.2 Amplification of D1 and D2 Domains of the 26S rDNA by Polymerase Chain Reaction

Whole cells grown on Yeast extract-Malt extract agar (0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 1.0% glucose, 2.0% agar) for 1-3 days were mixed to a density of 1+ in water. 20µl of the mixture was incorporated into 50 ml amplification

reaction. The resultant cell concentration produced the highest yield of amplified DNA compared with other concentrations. The D1 and D2 domains of the large subunit ribosomal DNA were amplified using primers NL1 (52-GCATATCAATAAGCGGAGGAAAAG) and NL4 (52-GGTCCGTGTTTCAAGACGG). Amplification by the polymerase chain reaction was conducted following the directions provided by the supplier of heat-activated *Taq* polymerase, in the presence of 1.5mM $MgCl_2$ in a Perkin-Elmer System 2400 cycler. The mixture was held for 12 min at 95^{o}C and then subjected to 35 cycles at 94^{o}C for 15 seconds, varying annealing temperatures for 10 seconds, and 72^{o}C for 20 seconds, with final extension for 5 minutes at 72^{o}C. The annealing temperature was varied from 55.7 to 53.7^{o}C during the first 20 cycles and kept constant for the remainder.

3.4.3 DNA Sequence Analysis

The amplified DNAs were centrifuged to get rid of cell debris, concentrated and cleaned by ultrafiltration in MicroCon 100 concentrators (Amicon), and sequenced with ABI sequencer at the John P Robarts Research Institute, London, Ontario, Canada. The sequences were edited and aligned with the program MEGA version 7.0 and evaluated with published sequences (Lachance *etal*., 1999).

3.5Determination of Functional Properties of the Yeast Isolates

3.5.1Survival Tolerance Studies

The selected test organisms obtained from various indigenous fermented food products were used in this experiment. *Saccharomycescerevisiae*SC10obtained from the laboratory in Palampur, India was incorporated as a positive control.

3.5.2Acid Tolerance Test

Potato dextrose broth (PDB) was adjusted to pH 2 and 3 using 5.0M HCL. Inoculation of approximately 10^{10}cfu/ml of each yeast strain was done into the modified PDB and incubation was carried out at 37^{o}C for 3 hours. Samples were withdrawn at one hour interval and then spread plated on PDA plates. The initial plates count at 0 hours was compared with that of 3 hours to determine acid tolerance (Sourabh *etal*., 2011).

3.5.3Bile Tolerance Tests

3.5.3.1 Minimum Inhibitory Concentration Method

Twenty microlitre of each culture of *S. cerevisiae* were spotted in Potato dextrose agar containing 0.1 – 1% (w/v) ox bile. The cultures were incubated aerobically for 48 hours and minimum inhibitory concentration(MIC) was taken as the smallest concentration completely inhibiting the development of spots (Sourabh *etal*., 2011).

3.5.3.2 Plate Count Method

Inorder to study bile tolerance of the yeast isolates, ox bile was used using plate count method. Potato dextrose broth (PDB) containing 3% (w/v) of ox bile was attuned to pH 5.8 by 5.0M HCl. The PDB containing bile salts were inoculated with 10^{10}cfu/ml and incubated at 28^{o}C for 8 hours. Viable count (cfu/mL) was measured at 0, 4 and 8 hours interval on PDA and was incubated at 28^{o}C aerobically for 48 hours.

3.5.4 Effects of Simulated Gastric and Intestinal Juices on Survival of Yeast Isolates

3.5.4.1 Production of replicatedGastric and Intestinal Juices

In order to prepare gastric juice, pepsin was dissolved in sterilized normal saline (0.85% w/v) to a finishing concentration of 3g/l. In order to prepare intestinal juice pancrease was dissolve to a finishing concentration of 1g/l and its pH attuned to pH 8. Millipore filters were used for the sterilization of the juices obtained. Then the yeast cultures were inoculated into the juices separately and incubation was done at 37^{o}C for 4 hours(Vitali *etal*., 2012).

3.5.4.2Production of Washed Yeast Cell preparation

The yeasts were cultured in PDB for 48hours at 28^{o}C. Then centrifugation at 4000 xg at 4^{o}C for 10 minutes was done. The yeasts harvested were mixed with phosphate buffered saline(PBS) and washed thrice at pH7. The pellets obtained were mixed with normal saline and number of cells that are viable was determined by spread plating method (Vitali *etal*., 2012).

3.5.4.3Assay of Upper Gastrointestinal Transit Tolerance

The yeast cultures were inoculated (10^{9}cfu/ml) into simulated gastric juice (pH2.0 and 3.0). Same volume of 0.2ml cleanedcell preparation was added to 0.3ml of sodium

chloride (0.5% w/v) and 1.0ml of tested gastricjuice (pH 3 or pH 2). 0.1mL of sample was taken at 30minutes interval up to 240 minutes and spread plated on PDA and viable count determined (Chen *etal*., 2014).The intestinal transit tolerance was determined by taking 0.1mL of each isolate and added to 1mL ofreplicated intestinal fluid (pH 8.0) and later mixed with 0.3ml of sodium chloride (0.5% w/v). The cultures were later incubated aerobically at 28^{o}C and viable counts determined after 0, 1, 4 and 8hours of incubation.The yeast isolates were studied for growth in potato dextrose broth (PDB). Fresh cultures of yeast isolates (1%) were incubated in the PDB and incubated at 28^{o}C and monitored for growth at 620nm with a spectrophotometer on an hourly basis for 9hours or until absorbance increase by 0.3 unit (Vitali *etal*., 2012).

3.5.5Hydrophobicity of the Yeast Isolates

In order to determine cell exterior hydrophobicity, connectionof microbes to hydrocarbons which are n-hexadecane, xylene and toluene was investigated. The yeast cultures were grown in PDB for 48hours at 28^{o}C. The cells were harvested by centrifugation and then washed two times with phosphate buffer with pH 6.5 and remixed again in the same phosphate buffer. 3ml of yeast cells was mixed with 0.6ml of n-hexadecane, xylene and toluene separately and vortexed for 120s. The separation of the two phases was carried out at 28^{o}C for 1hour. The water containing phase was removed cautiously and absorbance was measured at 250nm. Percentage hydrophobicity was measured according to the following equation/formula:

$\% H = [(A_o - A]/A_o] \times 100$

where $A_o$was the absorbance value before extraction and A was absorbance value after extraction with hydrocarbons (Syal and Vohra, 2013).

3.5.6AutoaggregationAbility of Yeast Isolates

Yeast isolates were cultured in PDB and incubated at 28^{o}C for 48hours. Potato dextrose broth (PDB) containing the yeast cells was centrifuged and then re-suspended inphosphate buffered saline(PBS).5ml of yeast cells was obtained and incubation was done at 28^{o}C for 20hours. After the culture had settled down, 3mL of the upper part of suspension was carefully obtained into sterile tubes and theoptical density (O.D) read at 600nm. Autoaggregation strength of the yeast isolates was estimated as percentage: (upper-suspensionO.D/ total yeast-suspensionO.D)X100 (Syal and Vohra, 2013).

3.5.7Bile Salt Deconjugation Activity of the Yeast Isolates

Potato dextrose agar (PDA) containing 0.5% (w/v) of different sodium salts was prepared (sodium glycodeoxycholate, sodium taurodeoxycholate hydrate, sodium taurocholate, sodium glycolate and sodium glycolate hydrate). The plates were spot inoculated and incubated at 28^{o}C for 3 days.Positive result is taken as the appearance of precipitate around the yeast colonies(Garcia-Hermandez*etal*., 2012).

3.5.8 β-Galactosidase Enzyme Production by the Yeast Isolates

Approximately 2.383mg ofisopropylβ-D-l-thiogalactopyranoside(IPTG) was dissolved in 100ml of sterile double distilled water to prepare 100mM solution of IPTG. Accordingly, 50mg/mL solution of x-gal was also prepared in N, N-dimethyl formamide. The two solutions were sterilized byfilteration through 0.2µm (Millipore) filters. To detect the cultures producing β-Galactosidase, 100µL of IPTG and 20µL of x-gal solutions, were poured on the surface of PDA plated aseptically. Then the yeast cultures were inoculated on the Petridishes and incubation done at 28^{o}C for 48hours. The colonies producing this enzyme are blue in colour while non-producers are white in colour (Sourabh *etal*., 2011).

3.6Determination of Wine-making Traitsamongthe Yeast Isolates

3.6.1Determination of Alcohol Production by the Yeast Isolates

The concentration of molasses was diluted to 15^{o} Brix in order to investigate the highest amount of alcohol produced by yeasts. Various amount of sucrose was added to give concentration ranging from 22 to 28^{o} Brix.Yeast isolates were then inoculated (1%) and fermentation was carried out at 28^{o}C for 48 hours(Sourabh *etal*., 2011).

3.6.2Determination of Alcohol Tolerance

Molasses concentration was diluted to 22^{o}Brix and poured into five different conical flasks. Different concentration of alcohol was added to give the initial alcohol concentration to be 1%, 2% 3%, 4% and 5%. The mixture was then inoculated with the yeast isolates and incubation was performed for 72 hours at 28^{o}C. The amount of ethanol produced was calculated usingthe technique described by Keshani*et al*.(2015).Original specific gravity (OSG) and final specific gravity (FSG) were

measured. The percentage alcohol by volume was determined by the following equation:

OSG-FSGX100Equation 3.1.

7.36.

Where 7.36 is the alcohol factor.

3.6.3Influence of Molasses Concentration on Fermentation of Yeasts

The inluence of molasses concentration on the fermentation capacity of yeasts was investigated by varying the initial concentration between 22^{o}Brix and 35^{o}Brix. Molasses was inoculated with 1% yeast cultures and incubated at 30^{o}C for 3 days. The amount of ethanol produced was evaluated using the technique described byKeshani*et al*. (2015).

3.7 Evaluation of Attenuation and Fermentation Efficiency of Yeast Isolates

Attenuation could be defined as the percentage of sugars converted to carbondioxide and alcohol measured by specific gravity method.

Attenuation = (OG- FG) (OG- 1) x 100 Equation 3.2.

OG means original gravity (specific gravity before pitching).

FG means final gravity (Specific gravity at the end of fermentation)

Fermentation efficiency (%) = Actual ethanol recovery/Theoretical ethanol recovery x 100% (Keshani*et al*., 2015).

3.8 Analysis of Killer Activityof the Yeasts

The yeast isolates were cultured in potato dextrose broth (PDB) (in a 500ml conical flask with shaking (120rpm). The cultures were centrifuged (10,000xg, at 5^{o}C for 10 minutes) and the supernatant obtained was filtered using 0.45µm.The filterate obtained was incubated at 4^{o}C for four hours and centrifuged (10,000 x g for 20min) and precipitate obtained was mixed with 0.1M citrate–phosphate buffer (pH 4.2). The mixture obtained was stored at 4^{o}C before use. The solution was introduced into wells (10mm) cut into the sensitive lawns of selected test organismand incubatedat 30^{o}C for 3–7 days (Lim and Tay, 2011).

3.9 Production of Apple Cider Wine

3.9.1 Extraction of Apple Juice

Royal delicious apple variety was obtained from local market in Palampur, India and was moved to the laboratory for immediate use. The apple was washed twice with sterilized distilled water and was allowed to dry under hygenic condition. The apple juice was extracted using a Binatone juice extractor;filteration was carried out using a cleaned muslin cloth and then pasteurized at 90^{o}C for 30 minutes(Kanwar and Keshani,2016).

3.9.2 Fermentation of Apple Juice

Apple juice that has been pasteurized was distributed into six different sterilized conical glass wares and inoculation was done with 2% seed inoculum of selected yeast isolates where *Saccharomyces cerevisiae* SC10was used as control and incubation was done at 28^{o}C for 24 hours with shaking using a rotary shaker (model MRC TOS-4030P Japan). Apple juice that has been pasteurized was later inoculated with 1% inoculums, supplemented withdi-Ammonium hydrogen phosphate(DAHP) (300mg w/v)and fermentation was carried out at 30 ±2^{o}C. Samples were taken on 24 hours basis for physical and chemical analysis and yeast counts (Torija*et al*., 2003; Umeh*et al*., 2015).

3.9.3Physico-chemical Analysis of Apple Cider Wine

3.9.3.1 Determination of pH of Apple CiderWine

Sample of fermenting apple wine was taken aseptically and pH was measured using a digital pH meter that had been standardized.

3.9.3.2 Determination of Total Soluble Solids

A hand refractometer was used to measure total soluble solids (N-1a ATAGO, Tokyo).

3.9.3.3 Total Titratable Acidity of AppleCider Wine

In order to determine totaltitratable acidity, sample (10ml) was titrated using 0.001mLof phenolphthalein with 0.1N NaOH. The equationEquation3.3 was used to determine the titratable acidity by taking into consideration the amount of 0.1N NaOH consumed at the end of the reaction.

Titratable Acidity (%) =

$$\frac{((\text{mL of 0.1 N NaOH}) \times \text{N NaOH}) \times 0.067 \text{ (malic acid coefficient)} \times 100}{\text{mL sample}}$$ Equation 3.3.

3.9.3.4 Brix-Acid Ratio of the Apple Wine

Brix–acid ratio was determined by dividing total soluble solid values with acidity (Jayasena and Cameron, 2008).

3.9.3.5 Ascorbic Acid Contentof the AppleCider Wine

Mixed 25 ml of metaphosphoric acid (5%) solution andfive ml of applecider wine was filtered using a filter paper. Centrifugation was carried out on the filterate obtained at 2000 rpm for 15 minutes. The filtrate was then transferred into a conical flask and titrated alongside with 2,6-dichlorophenol-indophenol (0.025%) reagent. The experiment was carried out in duplicates and average titer values determined (Weatherholtz and Holsing, 1975).

3.9.3.6Reducing Sugarsof the AppleCider Wine

Five ml of apple wine obtainedwas mixed with 95 ml distilled water to make 100ml and filled into a burette. 5ml of Fehling A and B solutions with 10ml of distilled water transfered into a conical flask and boiled. After boiling, it was titrateduntil redcolour was obtained. The solution was tested with Methylene blue untilthe red colour persisted. The amount of sample consumed was recorded and percent reducing sugar was calculated by using the following formula:

x ml of sample contains 0.05g of reducing sugar-100ml of sample solution contains 100 x 0.05 =Y g of reducing sugar X ml (Yeong, 2005).

3.9.3.7 Total Sugarsof the Apple CiderWine

Ten ml of apple wine was transfered into a conical flask and distilled water was used todilute upto 100 mL. 10 mL of 1 N HCl was added to 20 mL of this solution in a flask andsugars wereinverted by gently boiled in a water bath. The mixture was chilled before neutralizing using 10 mL 1N NaOH and the volume adjusted upto 150 mLusing distilled water. The solutions were then distributed into burrette. 5 ml of the solutions of Fehling A and B with 10 mL distilled water was introduced into a conical flask and till the colour become red. This was then tested with methylene blue till red colour perseveres. The total sugar was decided using the formula:

Total sugar=x mL of sample solutionX mL of total sugarsolution Equation 3.4.

Where x mL of sample solution=0.05

Total sugar solution=250 mL

Total sugar =250 x 0.05

3.9.4Alcoholic Content of Apple CiderWine

The alcoholic content of the fermenting apple wine was evaluated using the technique ofCaputi*et al*. (1968). Original specific gravity (OSG) and final specific gravity (FSG) were measured. The percentage alcohol by volume was determined by the following equation:

Percentage alcohol = OSG-FSGX100Equation 3.5.

7.36.

3.9.5Changes in Yeast Population during Fermentation of AppleCider Wine

The yeast population was evaluated by spreading 0.1 ml of apple wine on potato dextrose agar (PDA) at 24 hours interval and the plates incubated at 28^{o}C for 48 hours. The colony forming unit was expressed as logCFU/ml.

3.9.6Sensory Evaluation of AppleCider Wine

The sensory quality of the apple cider wine was evaluated after six days of fermentation by 20 man panelist who is familiar with the product.The panelists were supplied with 30 mL apple ciderwine samples and water to rinse their mouths in between samples. The samples were rated on 9 points hedonic scale ranging from 1-disliked extremely and 9-liked extremely. Parameterswere assessed forflavour,taste,appearance, mouthfeel and overall acceptability (Kanwar and Keshani, 2016).

Statistical Analysis

All the experiments were carried out in duplicate and the data obtained were analyzed using one way analysis of variance (ANOVA) and significance was accepted at the level of $\alpha_{0.05}$. Means were separated using Duncan multiple range test.

CHAPTER FOUR

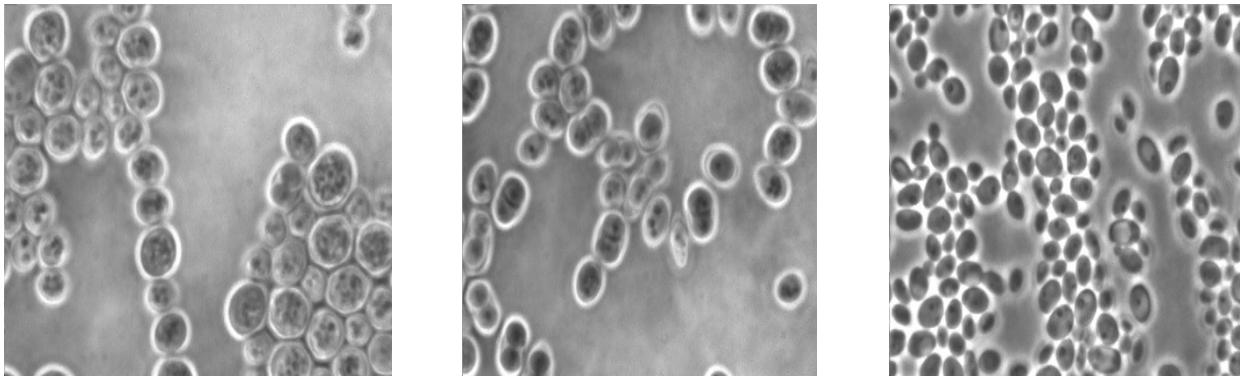
RESULTS

One hundred and twenty-six yeast isolates were characterized into six species belonging to five groups: *Pichiagulliermondii*, *Saccharomyces cerevisiae*, *Pichia fabianii*, *Candida glabrata*, *Candida tropicalis* and *Galatomycesgeotrichum*based on their distinct cellular and cultural characteristics, biochemical, physiological and morphologicalproperties. The colour of the tested isolates showed whitish to cream on yeast extract agar plates. The cells have circular or oval shapes, opaque with entire edges. The colonial morphology revealed smooth, convex and cream colonies on potato dextrose agar plates.

The phase contrast microscopy revealed that the yeast isolates are of diferrent shapes (Plate 4.1-4.5).*Saccharomycescerevisiae* PAW02 exhibited oval shape and the cells were jointed together after cell division. Most of the remaining yeast isolates also exhibited oval shapes withvisible scars of budding on some of the yeast isolates. Cylindricalshape was shown by *Galactomycesgeotrichum* 0BB02A (Plate 4.5).

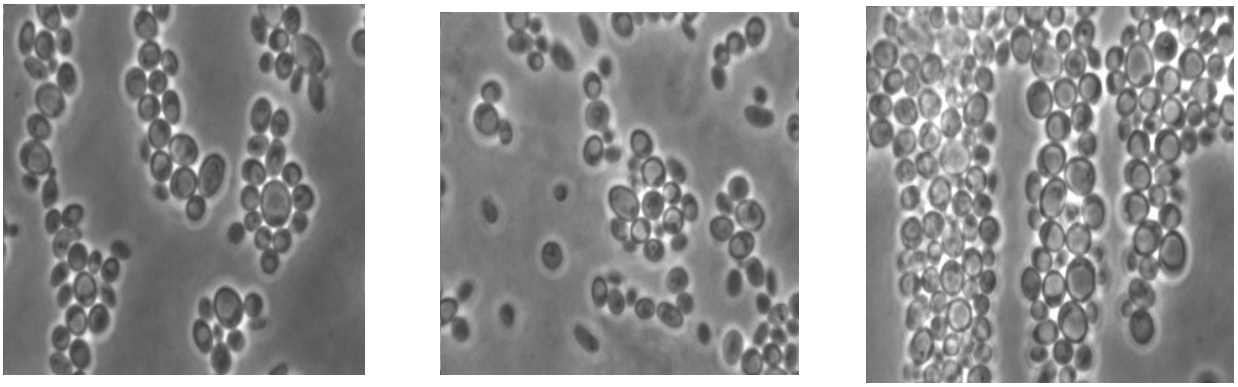
The morphologicalandbiochemicalproperties of the tested isolates from thefour substrates are presented. All the isolates were elliptical shaped except isolates OBB02A and OBB02B isolated from *Ogi-baba* (Table 4.1). All the yeasts isolated from palm wine did not liquefy gelatin and they did not produce acid from glucose. The isolates vary in their ability to hydrolyse casein and lipid (Table 4.2). Similarly, none of the isolates obtained from *Agadagidi* liquefied gelatin. The isolates did not hydrolyse casein but some of them were able to hydrolyse lipids and produced acid from glucose (Table 4.3).

Table 4.4 shows the morphology and biochemical characteristics of yeasts isolated from *Burukutu*. All the isolates did not liquefy gelatin. Only one isolate (BKT12A) hydrolysed lipid while others did not. Some of the isolates hydrolysed casein and were able to produce acid from glucose. All the isolates obtained from *ogi-baba* did not



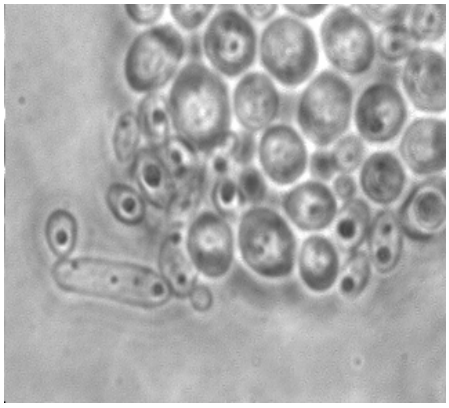
S. cerevisiae PAW02 *S. cerevisiae* PAW03A *S. cerevisiae* PAW08

Plate 4.1: Phase contrast microscopy image of strains of *Saccharomyces cerevisiae* isolated from Palm wine

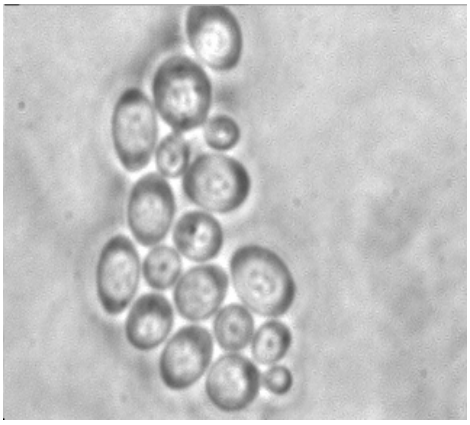


Candida glabrata OBB02C *C. glabrata* OBB06 *C. glabrata* OBBO8

Plate 4.2: Phase contrast microscopy image of strains of *Candida glabrata* isolated from *Ogi-baba*

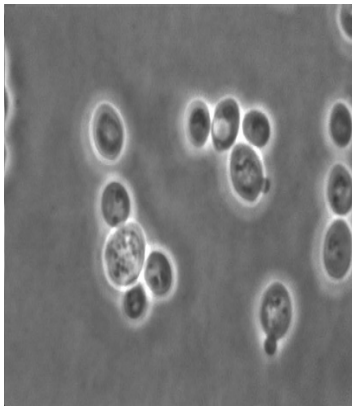


Candida tropicalis OBB01

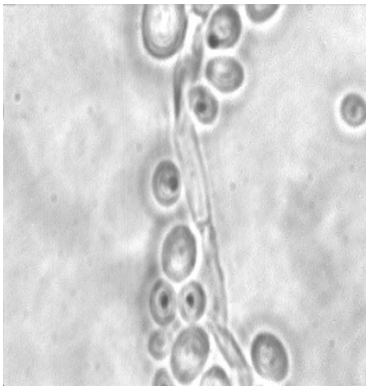


C. tropicalis OBB05A

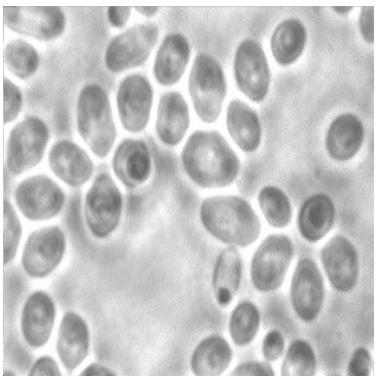
Plate 4.3: Phase contrast microscopy image of strains of *Candida tropicalis* isolated from *Ogi-baba*



Pichia fabianii AGG01A

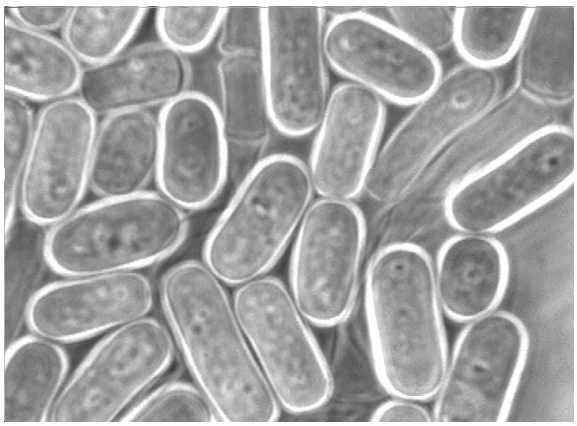


*P. fabianii*AGG07



P. fabianii AGG12

Plate 4.4: Phase contrast microscopy image of strains of *Pichia fabianii*isolated from*Agadagidi*



Galactomycesgeotrichum OBB02A

Plate 4.5: Phase contrast microscopy image of *Galactomycesgeotrichum*0B02A isolated from *Ogi-baba*

Table 4.1: Morphology and Some Biochemical Characteristics of Yeasts Isolated from *Ogi-baba*

Isolate Code	Morphology	Biochemical Characteristics			
		Gelatin liquefaction	Casein hydrolysis	Lipid hydrolysis	Acid production from glucose
OBB01	Elliptical	-	++	++	-
OBB02A	Cylindrical	-	++	-	-
OBB02B	Cylindrical	-	-	-	-
OBB02C	Elliptical	-	-	-	-
OBB03A	Elliptical	-	-	-	-
OBB03B	Elliptical	-	W	-	-
OBB04A	Elliptical	-	W	++	-
OBB04B	Elliptical	-	-	-	-
OBB05A	Elliptical	-	W	++	-
OBB05B	Elliptical	-	W	++	-
OBB06	Elliptical	-	-	-	-
OBB07	Elliptical	-	W	-	W
OBB08	Elliptical	-	-	-	-
OBB09	Elliptical	-	W	++	-
OBB10A	Elliptical	-	W	++	-
OBB10B	Elliptical	-	W	++	-
OBB11	Elliptical	-	-	-	-
OBB12A	Elliptical	-	W	-	-
OBB12B	Elliptical	-	W	-	-
OBB13	Elliptical	-	W	++	-
OBB14	Elliptical	-	W	++	-
OBB15A	Elliptical	-	W	++	-
OBB15B	Elliptical	-	-	++	-
OBB16	Elliptical	-	-	++	-
OBB17	Elliptical	-	-	-	-
OBB18	Elliptical	-	-	-	-
OBB19	Elliptical	-	-	-	-
OBB20	Elliptical	-	-	++	-
OBB21	Elliptical	-	-	-	-
OBB22	Elliptical	-	-	++	-
OBB23A	Elliptical	-	-	++	-
OBB23B	Elliptical	-	-	-	-
OBB24	Elliptical	-	-	-	-
OBB25	Elliptical	-	-	-	-

KEY:

+ = Hydrolysis
++ = Strong hydrolysis
- = No liquifacton, hydrolysis,nor acid production from glucose
W = Weak hydrolysis or weak acid production from glucose

Table 4.2: Morphology and Some Biochemical Characteristics of Yeasts Isolated FromPalm Wine

		Biochemical Characteristics			
Isolate Code	Morphology	Gelatin liquifaction	Casein hydrolysis	Lipids hydrolysis	Acid production from glucose
PAW01	Elliptical	-	-	++	-
PAW02	Elliptical	-	W	-	-
PAW03A	Elliptical	-	W	-	-
PAW03B	Elliptical	-	W	-	-
PAW04	Elliptical	-	-	-	-
PAW05	Elliptical	-	++	++	-
PAW06A	Elliptical	-	-	++	-
PAW06B	Elliptical	-	-	++	-
PAW07	Elliptical	-	++	++	-
PAW08	Elliptical	-	-	-	-
PAW09A	Elliptical	-	-	++	-
PAW09B	Elliptical	-	-	++	-
PAW10	Elliptical	-	W	-	-
PAW11	Elliptical	-	-	-	-
PAW12	Elliptical	-	-	-	-
PAW13	Elliptical	-	++	++	-
PAW14	Elliptical	-	-	-	-
PAW15	Elliptical	-	++	++	-
PAW16	Elliptical	-	++	++	-
PAW17A	Elliptical	-	-	-	-
PAW17B	Elliptical	-	-	-	-
PAW18	Elliptical	-	-	-	-
PAW19	Elliptical	-	-	++	-
PAW20	Elliptical	-	-	++	-
PAW21	Elliptical	-	-	-	-
PAW22	Elliptical	-	-	-	-
PAW23A	Elliptical	-	-	++	-
PAW23B	Elliptical	-	-	++	-
PAW24	Elliptical	-	-	-	-
PAW25A	Elliptical	-	-	++	-
PAW25B	Elliptical	-	-	++	-

KEY:

+ = Hydrolysis
++ = Strong hydrolysis
- = No liquifacton, hydrolysis,nor acid production from glucose
W = Weak hydrolysis or weak acid production from glucose

Table 4.3: Morphology and SomeBiochemical Characteristics of Yeasts Isolated from *Agadagidi*

Isolate Code	Morphology	Biochemical Characteristics			
		Gelatin liquefaction	Casein hydrolysis	Lipids hydrolysis	Acid production from glucose
AGG01A	Elliptical	-	-	-	W
AGG01B	Elliptical	-	-	-	W
AGG02	Elliptical	-	-	-	W
AGG03	Elliptical	-	-	-	W
AGG04	Elliptical	-	-	-	W
AGG05	Elliptical	-	-	-	W
AGG06	Elliptical	-	-	-	W
AGG07	Elliptical	-	-	-	W
AGG08	Elliptical	-	-	-	-
AGG09	Elliptical	-	-	-	W
AGG10A	Elliptical	-	-	-	W
AGG10B	Elliptical	-	-	-	W
AGG11	Elliptical	-	-	++	-
AGG12	Elliptical	-	-	++	-
AGG13	Elliptical	-	-	++	-
AGG14	Elliptical	-	-	++	-
AGG15	Elliptical	-	-	++	-
AGG16	Elliptical	-	-	++	-
AGG17	Elliptical	-	-	++	-
AGG18A	Elliptical	-	-	-	-
AGG18B	Elliptical	-	-	-	-
AGG19A	Elliptical	-	-	-	-
AGG19B	Elliptical	-	-	-	-
AGG20	Elliptical	-	-	++	W
AG21A	Elliptical	-	-	++	-
AGG21B	Elliptical	-	-	++	-
AGG22	Elliptical	-	-	++	-
AGG23A	Elliptical	-	-	-	-
AGG23B	Elliptical	-	-	-	-
AGG24	Elliptical	-	-	++	-

KEY:

+ = Hydrolysis
++ = Strong hydrolysis
- = No liquifacton, hydrolysis,nor acid production from glucose
W = Weak hydrolysis or weak acid production from glucose

Table 4.4: Morphology and SomeBiochemical Characteristics of Yeasts Isolated from *Burukutu*

Isolate Code	Morphology	Biochemical Characteristics			
		Gelatin liquefaction	Casein hydrolysis	Lipids hydrolysis	Acid production from glucose
BKT01A	Elliptical	-	W	-	-
BKT01B	Elliptical	-	-	-	W
BKT02A	Elliptical	-	-	-	-
BKT02B	Elliptical	-	W	-	W
BKT03	Elliptical	-	W	-	W
BKT04	Elliptical	-	-	-	-
BKT05	Elliptical	-	-	-	-
BKT06A	Elliptical	-	-	-	-
BKT06B	Elliptical	-	-	-	W
BKT07	Elliptical	-	-	-	-
BKT08	Elliptical	-	-	-	-
BKT09	Elliptical	-	-	-	-
BKT11	Elliptical	-	-	-	-
BKT12A	Elliptical	-	-	W	-
BKT12B	Elliptical	-	-	-	W
BKT13A	Elliptical	-	-	-	-
BKT13B	Elliptical	-	W	-	W
BKT14A	Elliptical	-	W	-	-
BKT14B	Elliptical	-	W	-	W
BKT15A	Elliptical	-	W	-	W
BKT15B	Elliptical	-	W	-	-
BKT16	Elliptical	-	-	-	-
BKT17	Elliptical	-	-	-	-
BKT18	Elliptical	-	-	-	-
BKT19	Elliptical	-	-	-	-
BKT20	Elliptical	-	-	-	-
BKT21	Elliptical	-	-	-	-
BKT22	Elliptical	-	-	-	-
BKT23	Elliptical	-	-	-	-
BKT24	Elliptical	-	-	-	-
BKT25	Elliptical	-	-	-	-

KEY:

+ = Hydrolysis
++ = Strong hydrolysis
- = No liquifacton, hydrolysis,nor acid production from glucose
W = Weak hydrolysis or weak acid production from glucose

liquify gelatin and they did not produce acid from glucose except OBB07 which showed weak producton of acid. Most of the isolates weakly hydrolysed casein and lipid (Table 4.4).

Tables 4.5 – 4.8 represent the physiological properties of the yeasts obtained from *Agadagidi*, *Burukutu*, palm wine, and *ogi-baba* respectively. Ten isolates from palm wine (PAW03A, PAW03B, PAW04, PAW11, PAW12, PAW14, PAW18, PAW21, PAW22 and PAW24) showed no growth at the 3 concentations of NaCl while the remaining 21 isolates varied in their ability to grow at these concentrations (Table 4.5). All the isolates grew in 0.5% deoxycholate and 2.0% phosphoric acid but varied in their ability to grow in the other inhibitors. Only three of the isolates (PAW01, PAW06A and PAW06B) were able to grow at 4^{o}C but all of them grew at 30^{o}C and 37^{o}C (Table 4.5).

The physiological properties of yeasts obtained from *Agadagidi*are shown in Table 4.6. All the isolates except AGG23A and AGG03B grew at 5% and 10% NaCl concentrations but none was able to grow at 15%. The isolates all grew in 10ppm CTAB but varied in their ability to grow in 0.5% deoxycholate, 10ppm canavanine SO_4, 10ppm cycloheximide, 50ppm CTAB and 0.5% acetic acid. None of the isolates grew in 200ppm cinnamic acid, 5-fluorocytosine, 100ppm cycloheximide, 1.0% and 1.5% acetic acid and on phenylalanine. The isolates did not grow at 4^{o}C, their growth varied at 30^{o}C but they all grew at 37^{o}C (Table 4.6).

Table 4.7 describes the physiological characteristics of yeasts isolated from *Burukutu*. The isolates varied in their growth at 5% and 10% NaCl concentrations but were unable to grow at 15%. Most of the isolates grew in 0.5% deoxycholate, 10ppm CTAB and 0.5% acetic acid. All the isolates grew in 2.0% and 2.5% phosphoric acid but none grew at 200ppm cinnamic acid, 5-fluorocytosine (10ppm), 100ppm and 1000ppm cycloheximide, 1.0% and 1.5% acetic acid, and on phenylalanine. All the isolates grew at 30^{o}C and 37^{o}C but were unable to grow at 4^{o}C. The growth of the yeasts isolated from *ogi-baba* varied in 5% and 10% NaCl while none of them grew at 15% concentration (Table 2d). The isolates were unable to grow in most of the inhibitors except in 2.5% phosphoric acid where all were able to grow. No growth was observed in the isolates at 4^{o}C but they all grew at 30^{o}C and 37^{o}C (Table 4.8).

Table 4.5: Physiological Characteristics of Yeasts Isolated from Palm Wine

Isolate code	Tolerance to NaCl			Effects of inhibitors														Growth on phenylalanine	Growth at different temperature		
	5%	10%	15%	Deoxycholate 0.5%	Cinnamic acid 200ppm	5-Fluorocytosine 10ppm	Canavanine SO_4 10ppm	Cycloheximide (10ppm)	Cycloheximide (100ppm)	Cycloheximide (1000ppm)	CTAB (10ppm)	CTAB (50ppm)	1.0% Acetic acid	0.5% Acetic acid	1.5% Phosphoric acid	2.0% Phosphoric acid	2.5% Phosphoric acid		4°C	30°C	37°C
PAW01	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+
PAW02	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+
PAW03A	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW03B	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW04	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW05	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
PAW06A	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+
PAW06B	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+
PAW07	+	+	-	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW08	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW09A	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+
PAW09B	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+
PAW10	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW11	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW12	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW13	+	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
PAW14	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW15	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
PAW16	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
PAW17A	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW17B	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW18	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
PAW19	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
PAW20	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
PAW21	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+	+
PAW22	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	+
PAW23A	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+
PAW23B	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	+	+
PAW24	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+	+
PAW25A	+	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+	+
PAW25B	+	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+	+

Table 4.6: Physiological Characteristics of Yeasts Isolated from *Agadagidi* (AG)

Isolate code	Tolerance to NaCl			Effects of inhibitors														Growth on phenylalanine	Growth at different temperature		
	5%	10%	15%	Deoxycholate 0.5%	Cinnamic acid 200ppm	5-Fluorocytosine 10ppm	Canavanine SO_4 10ppm	Cycloheximide (10ppm)	Cycloheximide (100ppm)	Cycloheximide (1000ppm)	CTAB (10ppm)	CTAB (50ppm)	1.0% Acetic acid	0.5% Acetic acid	1.5% Phosphoric acid	2.0% Phosphoric acid	2.5% Phosphoric acid		4°C	30°C	37°C
AGG01A	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG01B	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG02	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG03	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG04	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG05	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG06	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG07	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG08	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG09	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG10A	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AG10B	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG11	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+
AGG12	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG13	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG14	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+
AGG15	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG16	+	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+
AGG17	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG18A	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG18B	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG19A	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG19B	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+
AGG20	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	-	-	+	+
AGG21A	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG21B	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG22	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+
AGG23A	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+	+
AGG23B	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+	+
AGG24	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+

Table 4.7: Physiological Characteristics of Yeasts Isolated from *Burukutu* (BK)

Isolate code	Tolerance to NaCl			Effects of inhibitors														Growth on phenylalanine	Growth at different temperature		
	5%	10%	15%	Deoxycholate 0.5%	Cinnamic acid 200ppm	5-Fluorocytosine 10ppm	Canavanine SO_4 10ppm	Cycloheximide (10ppm)	Cycloheximide (100ppm)	Cycloheximide (1000ppm)	CTAB (10ppm)	CTAB (50ppm)	1.0% Acetic acid	0.5% Acetic acid	1.5% Phosphoric acid	2.0% Phosphoric acid	2.5% Phosphoric acid		4°C	30°C	37°C
BKT01A	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT01B	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT02A	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT02B	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT03	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT04	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT05	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT06A	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT06B	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT07	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT08	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT09	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT11	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT12A	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT12B	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT13A	+		-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT13B	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT14A	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT14B	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT15A	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+
BKT15B	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT16	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT17	+	+	-	+	-	-	-	+	-	-	+	-	-	-	-	+	+	-	-	+	+
BKT18	+	-	-	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+
BKT19	+	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT20	+	+	-	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+
BKT21	+	-	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+	+
BKT22	+	+	-	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+
BKT23	+	-	-	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+
BKT24	+	+	-	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	+	+
BKT25	+	+	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+

Table 4.8: Physiological Characteristics of Yeasts Isolated from *Ogi-baba* (OBB)

Isolate code	Tolerance to NaCl			Effects of inhibitors															Growth on phenylalanine	Growth at different temperature		
	5%	10%	15%	Deoxycholate 0.5%	Cinnamic acid 200ppm	5-Fluorocytosine 10ppm	Canavanine SO_4 10ppm	Cycloheximide (10ppm)	Cycloheximide (100ppm)	Cycloheximide (1000ppm)	CTAB (10ppm)	CTAB (50ppm)	1.0% Acetic acid	0.5% Acetic acid	1.5% Phosphoric acid	2.0% Phosphoric acid	2.5% Phosphoric acid		4ºC	30ºC	37ºC
OBB01	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB02A	+	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+
OBB02B	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+
OBB02C	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB03A	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB03B	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	+
OBB04A	+	+	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	+
OBB04B	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB05A	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	+
OBB05B	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
OBB06	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+
OBB07	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB08	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB09	+	+	-	+	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	+
OBB10A	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
OBB10B	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
OBB11	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	+
OBB12A	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB12B	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB13	+	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	+
OBB14	+	+	-	+	-	-	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+
OBB15A	+	+	-	+	-	-	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+
OBB15B	+	+	-	+	-	-	-	+	+	+	+	-	-	-	-	+	+	-	-	+	+
OBB16	+	+	-	+	-	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	+
OBB17	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	+
OBB18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
OBB19	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	+
OBB20	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+
OBB21	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	+
OBB22	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	+
OBB23A	+	+	-	+	-	-	-	+	+	-	+	+	-	-	-	+	+	-	-	+	+
OBB23B	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	+
OBB24	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	+
OBB25	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	+

The ability of the yeasts isolated from the four substrates toassimilate various carbon and nitrogen compounds is as shown in Table 4.9 – Table 4.12. The pattern of assimilation of these compounds was used to assign probable identities to the isolates. Isolates from palm wine were recognized to be *Saccharomyces cerevisiae,Pichia gulliermondii*, and *P. fabianii* (Table 4.9). Yeasts isolated form *Agadagidi*were identified as *Pichia fabianii* and *S. cerevisiae* (Table 4.10), those isolated from *Burukutu*were *Candida glabrata* and *S cerevisiae* (Table 4.11) while those from *ogi-baba* were *Candida tropicalis*, *Galactomycesgeotrichum*, *Candida glabrata*, *S. cerevisiae* and *Pichia fabianii* (Table 4.12).

The sequence analysis of the D1/D2 domain of the 26S rDNA was used to assign the yeasts into five genera: *Saccharomyces* sp., *Candida* sp., *Pichia* sp., *Galactomyces* sp., and *Trichosporon* sp.(Figure 4.1).The species identified based on nucleotide sequences were *Saccharomyces cerevisiae, Candida glabrata, Candida parapsilosis, Candida tropicalis, Pichia fabianii, Pichia kudriavzevii, Trichosporon*sp.and*Galactomycesgeotrichum.* The yeasts were clustered into eight groups based on their phylogenetic relatedness. The first cluster consists of *Saccharomyces cerevisae* and it is the most dominant species. This is followed by second cluster which is made up of *Candida glabrata*. The third cluster consists of *Pichia fabianii* and the fourth and the fifth clusters consist of *Candida parapsilosis* and *C. tropicalis* respectively. The sixth, the seventh and eighth clusters consist of *Pichia kudriavzevi*, *Trichosporon* sp. and *Galactomycesgeotrichum*.

The yeasts grew to different extent in the acidic medium. The viable count recorded for the yeasts in the medium where the pH has beenadjusted to 2 ranged between 5.4 and 7.5$\log_{10}$CFU/ml. The highest viable count recorded was 6.2$\log_{10}$CFU/ml for isolates AGG23A and OBB03A after three hours of incubation (Table 4.13). The yeasts also grew to different extent in the acidic medium when the pH was adjusted to 3 and incubation was done for 3 hours. Two *Saccharomycescerevisiae*strains BKT07 and OBB17 had the highest growth of 7.2$\log_{10}$CFU/ml while *Saccharomycescerevisiae* SC01 had the lowest growth of 6.1$\log_{10}$CFU/ml at the end of 3 hours incubation (Table 4.14).

Table 4.9: Carbon and Nitrogen Assimilation Pattern of Yeast Isolates from Palm wine and their Probable Identity

	Assimilation of Carbon Compounds																																				
	Simple Sugars																			Alcohols											CHO		Assimilation of Nitrogen compounds				
Isolate code	Glucose	Sucrose	Raffinose	Melibiose	Galactose	Lactose	Trehalose	Maltose	Melezitose	Salicin	Sorbose	Rhamnose	Xylose	L-arabinose	D-arabinose	Ribose	Methanol	Ethanol	2-propanol	1-Butanol	Glycerol	Erythritol	Ribitol	Xylitol	Galactitol	Mannitol	Glucitol	Inositol	Starch	Inulin	Sodium nitrate	Sodium nitrite	Ethylamine	Lysine	Cadaverine	Probable Identity
PAW01	+	+	+	+	+	-	+	+	+	W	S	S	+	S	S	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	+	S	+	*Pichia gulliermondii*
PAW02	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW03A	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW03B	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW04	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW05	+	+	-	-	-	-	S	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	S	-	S	+	-	S	-	S	+	+	S	+	*Pichia fabianii*
PAW06A	+	+	+	+	+	-	+	+	+	W	S	S	+	S	S	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	+	S	+	*Pichiagulliermondii*
PAW06B	+	+	+	+	+	-	+	+	+	W	S	S	+	S	S	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	+	S	+	*Pichiagulliermondii*
PAW07	+	+	-	-	-	-	S	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	S	-	S	+	-	S	-	S	+	+	S	+	*Pichia fabianii*
PAW08	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW09A	+	+	+	+	+	-	+	+	+	W	S	S	+	S	S	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	+	S	+	*Pichiagulliermondii*
PAW09B	+	+	+	+	+	-	+	+	+	W	S	S	+	S	S	-	-	+	-	-	+	-	+	+	+	+	+	-	-	+	-	-	+	S	+	*Pichiagulliermondii*
PAW10	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW11	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW12	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW13	+	+	-	-	-	-	S	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	S	-	+	+	-	S	-	S	+	+	S	+	*Pichia fabianii*
PAW14	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW15	+	+	-	-	-	-	S	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	S	-	S	+	-	S	-	S	+	+	S	+	*Pichia fabianii*
PAW16	+	+	-	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	S	-	+	+	-	S	-	S	+	+	S	+	*Pichiafabianii*
PAW17A	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW17B	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW18	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW19	+	+	-	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	S	-	+	+	-	S	-	S	+	+	S	S	*Pichia fabianii*
PAW20	+	+	+	+	-	-	W	W	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Saccharomyces cerevisiae*
PAW21	S	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW22	S	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW23A	+	+	S	+	+	-	+	+	+	W	W	S	+	S	S	-	-	+	-	-	-	-	+	S	S	+	+	-	-	S	-	-	+	+	+	*Pichiagulliermondii*
PAW23B	+	+	S	+	+	-	+	+	+	W	W	S	+	S	S	-	-	+	-	-	+	-	+	S	S	+	+	-	-	S	-	-	+	+	+	*Pichiagulliermondii*
PAW24	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
PAW25A	+	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	+	-	S	S	S	S	+	*Pichiafabianii*
PAW25B	+	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	S	S	S	S	+	*Pichiafabianii*

CHO: Carbohydrate

Table 4.10: Carbon and Nitrogen Assimilation Pattern of Yeast Isolates from *Agadagidi* and their Probable Identity

	Assimilation of Carbon Compounds																													Assimilation of Nitrogen compounds					
	Simple Sugars																			Alcohols									CHO							
Isolate code	Glucose	Sucrose	Raffinose	Melibiose	Galactose	Lactose	Trehalose	Maltose	Melezitose	Salicin	Sorbose	Rhamnose	Xylose	L-arabinose	D-arabinose	Ribose	Methanol	Ethanol	2-Propanol	1-Butanol	Glycerol	Erythritol	Ribitol	Xylitol	Galactitol	Mannitol	Glucitol	Inositol	Starch	Inulin	Sodium nitrate	Sodium nitrite	Ethylamine	Lysine	Cadaverine	Probable Identity
AGG01A	+	S	S	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	-	+	*Pichiafabianii*
AGG01B	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG02	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+	*Pichia fabianii*
AGG03	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG04	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG05	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG06	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichia fabianii*
AGG07	+	S	W	-	-	-	W	+	+	+	+	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichia fabianii*
AGG08	+	S	W	-	S	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
AGG09	+	S	+	-	-	-	+	S	S	+	+	-	+	-	-	-	-	W	-	-	+	-	-	+	-	+	+	-	+	-	S	S	S	S	+	*Pichiafabianii*
AGG10A	+	S	S	-	-	-	W	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
AGG10B	+	S	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
AGG11	+	+	+	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	S	-	-	-	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG12	+	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	S	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG13	+	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	S	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG14	+	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	S	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG15	+	+	+	-	-	-	+	+	+	+	-	-	+	-	-	-	-	+	-	-	S	-	-	+	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG16	+	+	+	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	S	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG17	+	+	+	-	-	-	+	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG18A	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
AGG18B	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
AGG19A	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
AGG19B	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
AGG20	S	S	S	-	+	-	+	S	S	+	-	-	+	-	-	-	-	+	-	-	S	-	-	S	-	+	+	-	+	W	+	S	S	S	+	*Pichiafabianii*
AGG21A	S	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG21B	S	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG22	S	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabianii*
AGG23A	S	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
AGG23B	S	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
AGG24	S	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Saccharomyces cerevisiae*

Table 4.11: Carbon and Nitrogen Assimilation Pattern of Yeast Isolates from *Burukutu* and their Probable Identity

Isolate code	Assimilation of Carbon Compounds																															Assimilation of Nitrogen compounds					Probable Identity
	Simple Sugars																		Alcohols										CHO									
	Glucose	Sucrose	Raffinose	Melibiose	Galactose	Lactose	Trehalose	Maltose	Melezitose	Salicin	Sorbose	Rhamnose	Xylose	L-arabinose	D-arabinose	Ribose	Methanol	Ethanol	2-propanol	1-Butanol	Glycerol	Erythritol	Ribitol	Xylitol	Galactitol	Mannitol	Glucitol	Inositol	Starch	Inulin	Sodium nitrate	Sodium nitrite	Ethylamine	Lysine	Cadaverine	
BKT01A	+	+	W	-	-	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT01B	+	+	+	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candidaglabrata*
BKT02A	+	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT02B	+	+	W	-	W	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT03	+	+	W	-	W	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT04	+	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	*Candida glabrata*
BKT05	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	W	-	-	*Candida glabrata*
BKT06A	+	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	*Candida glabrata*
BKT06B	+	+	W	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT07	+	+	+	-	+	-	W	W	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT08	+	+	+	-	+	-	W	W	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT09	+	+	+	-	+	-	W	W	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT11	+	+	+	-	+	-	W	W	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT12A	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	W	-	-	*Candidaglabrata*
BKT12B	+	+	W	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candidaglabrata*
BKT13A	+	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	W	-	-	*Candidaglabrata*
BKT13B	+	+	W	-	W	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candidaglabrata*
BKT14A	+	+	W	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT14B	+	+	W	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candidaglabrata*
BKT15A	+	+	W	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candidaglabrata*
BKT15B	+	+	W	-	W	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT16	+	+	S	-	W	-	-	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT17	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT18	+	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT19	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT20	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT21	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
BKT22	+	W	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT23	+	W	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT24	+	W	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
BKT25	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	-	*Candida glabrata*

Table 4.12: Carbon and Nitrogen Assimilation Pattern of Yeast Isolates from *Ogi-baba* and their Probable Identity

	Assimilation of Carbon Compounds																																						
	Simple Sugars															Alcohols																CHO		Assimilation of Nitrogen compounds					
Isolate code	Glucose	Sucrose	Raffinose	Melibiose	Galactose	Lactose	Trehalose	Maltose	Melezitose	Salicin	Sorbose	Rhamnose	Xylose	L-arabinose	D-arabinose	Ribose	Methanol	Ethanol	2-propanol	1-Butanol	Glycerol	Erythritol	Ribitol	Xylitol	Galactitol	Mannitol	Glucitol	Inositol	Starch	Inulin	Sodium nitrate	Sodium nitrite	Ethylamine	Lysine	Cadaverine	Probable Identity
OBB01	+	+	W	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	+	-	+	S	-	+	+	-	-	-	-	-	+	+	+	*Candida tropicalis*
OBB02A	+	W	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+	S	+	+	-	-	-	-	S	W	-	-	-	-	-	+	+	W	*Galactomycesgeotrichum*
OBB02B	+	W	-	-	+	-	-	W	-	-	+	-	+	-	-	-	-	+	S	-	-	-	-	-	-	+	W	-	-	-	-	-	+	+	W	*Galactomycesgeotrichum*
OBB02C	+	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
OBB03A	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB03B	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB04A	+	+	+	-	-	-	S	+	+	+	-	-	W	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-	W	-	+	+	+	+	+	*Pichiafabianii*
OBB04B	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pichiafabianii*
OBB05A	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB05B	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB06	+	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
OBB07	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB08	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Candida glabrata*
OBB09	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB10A	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB10B	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB11	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB12A	+	+	+	+	+	-	S	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB12B	+	+	+	+	+	-	S	+	-	-	-	-	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB13	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB14	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB15A	+	+	-	-	+	-	+	+	+	-	S	-	+	W	-	-	-	+	-	-	-	-	+	S	-	+	+	-	W	-	-	-	+	+	+	*Candida tropicalis*
OBB15B	+	+	+	-	+	-	+	+	+	W	S	-	+	W	-	-	-	+	-	W	-	-	+	W	-	+	+	-	+	-	-	-	+	+		*Candida tropicalis*
OBB16	+	+	+	-	+	-	+	+	+	W	S	-	+	W	-	-	-	+	-	W	-	-	+	W	-	+	+	-	+	-	-	-	+	+	+	*Candida tropicalis*
OBB17	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB18	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB19	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB20	+	+	+	-	+	-	+	+	+	W	S	-	+	W	-	-	-	+	-	W	-	-	+	W	-	+	+	-	+	-	-	-	+	+	+	*Candida tropicalis*
OBB21	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB22	+	+	+	-	+	-	+	+	+	W	S	-	+	W	-	-	-	+	-	W	-	-	+	W	-	+	+	-	+	-	-	-	+	+	+	*Candida tropicalis*
OBB23A	+	+	+	-	-	-	W	+	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	W	-	+	+	-	+	-	+	+	+	+	+	*Pichiafabiani*
OBB23B	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB24	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*
OBB25	+	+	+	+	+	-	W	+	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Saccharomyces cerevisiae*

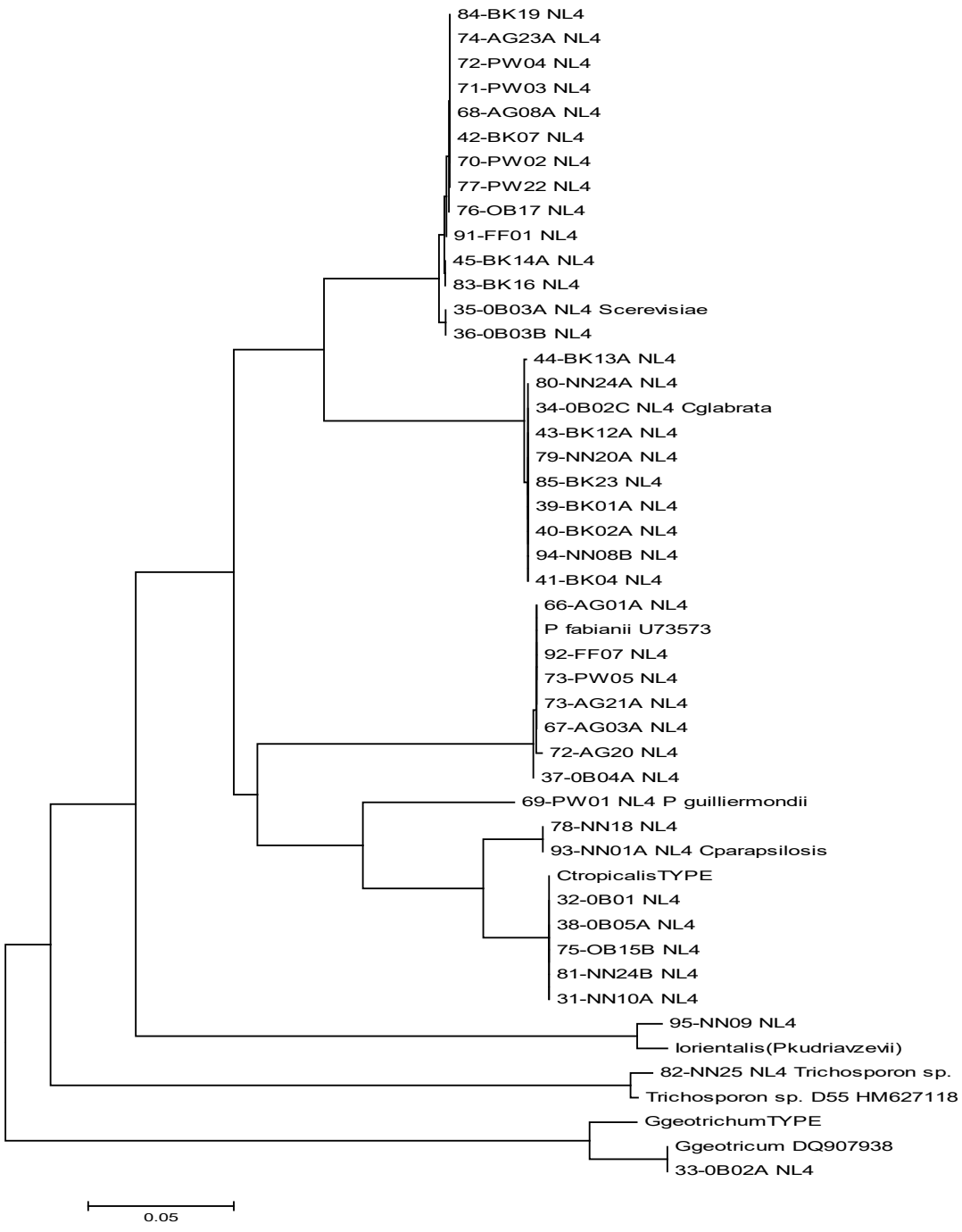


Figure 4.1: Dendogram Showing Phylogenetic Relationship Among the Yeast Isolates

Table 4.13: Survival Toleranceof the Indigenous *Saccharomyces cerevisiae* (log_{10} CFU/ml) (pH 2) Isolated from Fermented Food Products.

S/N	Isolates	Time (hours)			
		0	1	2	3
1	*Saccharomyces cerevisiae*PAW02	7.5 ± 0.02^{a}	6.6 ± 0.03^{a}	6.2 ± 0.06^{b}	6.1 ± 0.05^{a}
2	*Saccharomyces cerevisiae*PAW24	7.4 ± 0.05^{ab}	6.5 ± 0.06^{b}	6.4 ± 0.06^{a}	6.0 ± 0.09^{bc}
3	*Saccharomyces cerevisiae*BKT07	6.7 ± 0.04^{b}	6.5 ± 0.03^{bc}	6.2 ± 0.03^{ab}	6.0 ± 0.12^{b}
4	*Saccharomyces cerevisiae*BKT19	6.7 ± 0.05^{c}	6.5 ± 0.04^{c}	5.4 ± 0.03^{ac}	6.0 ± 0.05^{c}
5	*Saccharomyces cerevisiae*AGG23A	7.6 ± 0.06^{d}	6.5 ± 0.00^{d}	6.1 ± 0.05^{ab}	6.2 ± 0.09^{bc}
6	*Saccharomyces cerevisiae*AGG08	7.5 ± 0.04^{cd}	6.6 ± 0.03^{cd}	6.2 ± 0.04^{de}	6.1 ± 0.03^{ab}
7	*Saccharomyces cerevisiae*OBB17	7.3 ± 0.05^{a}	6.4 ± 0.05^{a}	6.3 ± 0.05^{d}	6.1 ± 0.04^{ac}
8	*Saccharomyces cerevisiae*OBB03A	7.4 ± 0.06^{c}	5.9 ± 0.01^{ab}	6.4 ± 0.12^{ab}	6.2 ± 0.05^{de}
9	*Saccharomyces cerevisiae*SC01	7.5 ± 0.05^{b}	6.5 ± 0.02^{ac}	6.2 ± 0.21^{bc}	6.0 ± 0.06^{d}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.14: Acid Tolerance of the Indigenous*Saccharomyces cerevisiae* (log_{10}CFU/ml) (pH 3)Isolated from Fermented Food Products

S/N	Isolates	Time (hours)			
		0	1	2	3
1	*Saccharomyces cerevisiae*PAW02	6.9 ± 0.05^{a}	7.3 ± 0.04^{a}	6.8 ± 0.15^{a}	7.0 ± 0.01^{c}
2	*Saccharomyces cerevisiae*PAW24	6.5 ± 0.06^{b}	7.7 ± 0.05^{a}	7.1 ± 0.19^{bc}	6.7 ± 0.05^{b}
3	*Saccharomyces cerevisiae*BKT07	6.7 ± 0.05^{ab}	6.9 ± 0.09^{ab}	7.0 ± 0.05^{ab}	7.2 ± 0.04^{a}
4	*Saccharomyces cerevisiae*BKT19	6.6 ± 0.06^{bc}	6.6 ± 0.07^{c}	6.4 ± 0.04^{a}	6.2 ± 0.04^{cd}
5	*Saccharomyces cerevisiae*AGG23A	6.8 ± 0.05^{c}	7.2 ± 0.04^{de}	6.7 ± 0.06^{b}	7.1 ± 0.02^{de}
6	*Saccharomyces cerevisiae*AGG08	6.6 ± 0.12^{d}	7.8 ± 0.06^{a}	7.2 ± 0.04^{a}	6.8 ± 0.06^{a}
7	*Saccharomyces cerevisiae*OBB17	6.8 ± 0.05^{de}	7.0 ± 0.04^{a}	7.0 ± 0.03^{a}	7.2 ± 0.03^{b}
8	*Saccharomyces cerevisiae*OBB03A	6.7 ± 0.05^{a}	6.7 ± 0.05^{a}	6.5 ± 0.05^{b}	6.2 ± 0.02^{a}
9	*Saccharomyces cerevisiae*SC01	6.6 ± 0.07^{ab}	6.6 ± 0.06^{a}	6.4 ± 0.02^{a}	6.1 ± 0.02^{ab}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

All the yeast isolates were exposed to different concentration of ox bile ranging from 0.1% to 1.0%. All the yeast isolates grew at these various concentrations (Table 4.15). This indicated that all the *Sacharomycescerevisiae* strains were tolerant to ox bile.The yeast isolates demonstrated viable count to different extent in the potato dextrose broth supplemented with 3% ox bile with pH adjusted to 5.8. At the end of 8 hours of incubation, *Saccharomyces cerevisiae*OBB17 had the highest viable count of 5.35$\log_{10}$cfu/ml while*Saccharomyces cerevisiae*BKT19 had least viable count of 4.81$\log_{10}$cfu/ml (Table4.16).

Inorder to evaluate the capacity of the yeast isolates to survive in the stomach, gastric transit and intestinal tests were carried out. The media pHwas attuned to 2 and 3 and length of incubation was 4 hours. At pH 2, zero hour viable count ranged between 8.31 and 9.2$\log_{10}$cfu/ml. At the end of 4 hours of incubation, the highest viable count was 9.50$\log_{10}$cfu/ml. When the pH was adjusted to 3, the viable counts ranged between 7.40 and 8.40$\log_{10}$cfu/ml at the end of four hours of incubation (Table 4.17).For an organism to be designated as probiotic it must survive in the intestine. During the transit in the intestine the yeast must maintain a high viable count. The viable count of these indigenous yeast isolate was very high and it ranged between 7.27 and 8.93$\log_{10}$cfu/ml (Table 4.18).

The adhessive property was measured indirectly as the adhesion of the isolates to hydrocarbons such as xylene, toluene and N-hexadecane. *Saccharomycescerevisiae*OBB17 demonstrated a high percentage of hydrohobicity (83.45%). The yeast that had lowest adhesion against xylene was*Saccharomycescerevisiae*AGG23A with 35.66% (Table 4.19).Autoaggregation is another way of measuring adhesive property of the yeasts. *Saccharomycescerevisiae*OBB03A and *Saccharomycescerevisiae*SC01demonstrated the highest autoaggregation ability (99.91%). *Saccharomyces cerevisiae* BK19 was the only yeast that had 89.80% autoaggregation (Table 4.20).All the indigenous yeast isolates grew on all the bile salts concentration used in this work (Table 4.21) with the exception of SC10 which did not grow on sodium glycolate. Sodium glycodeoxycholate was the only bile salt that was deconjugated by all the isolates.

Table 4.15: Bile Tolerance test for the Indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

Isolates	Concentration of Bile (%)										
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	No bile (Control)
*S. cerevisiae*PAW02	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*PAW24	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*BKT07	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*BKT19	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*AGG23A	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*AGG08	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*OBB17	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*OBB03A	+	+	+	+	+	+	+	+	+	+	+
*S. cerevisiae*SC01	+	+	+	+	+	+	+	+	+	+	+

KEY:
+=growth in the medium

Table 4.16:Tolerance to 3% Bile Concentration by the Indigenous*Saccharomyces cerevisiae* isolated from Fermented Food Products

S/N	Isolates	Time (hours)/log_{10}CFU/ml						
		0	2	4	5	6	7	8
1	*S. cerevisiae*PAW02	6.27±0.02a	5.76±0.05^{a}	5.48±0.02^{a}	5.47±0.04^{a}	5.46±0.04^{a}	5.36±0.04^{a}	5.22±0.15^{a}
2	*S. cerevisiae*PAW24	6.91±0.06^{b}	5.70±0.02^{b}	5.64±0.05^{b}	5.49±0.04^{a}	5.30±0.04^{a}	5.22±0.03^{a}	5.09±0.12^{a}
3	*S. cerevisiae*BKT07	5.95±0.05^{a}	5.81±0.06^{c}	5.70±0.09^{c}	5.58±0.05^{b}	5.12±0.05^{b}	5.12±0.05^{b}	4.91±0.01^{b}
4	*S. cerevisiae*BKT19	5.47±0.02^{b}	5.76±0.05^{d}	5.64±0.06^{b}	5.47±0.04^{a}	5.38±0.03^{a}	5.15±0.06^{c}	4.81±0.05^{b}
5	*S.cerevisiae*AGG23A	7.51±0.05^{c}	6.30±0.02^{e}	5.90±0.09^{c}	5.48±0.04^{a}	5.40±0.04^{a}	5.35±0.05^{a}	5.30±0.06^{b}
6	*S. cerevisiae*AGG08	7.52±0.06^{de}	6.20±0.02^{a}	5.96±0.09^{c}	5.15±0.06^{bc}	5.33±0.04^{a}	4.94±0.06^{a}	4.92±0.03^{c}
7	*S. cerevisiae*OBB17	6.85±0.09^{d}	5.80±0.09^{ab}	5.65±0.05^{b}	5.50±0.05^{a}	5.45±0.03^{ab}	5.38±0.05^{a}	5.35±0.06^{bc}
8	*S. cerevisiae*OBB03A	5.95±0.06^{e}	5.75±0.05^{cd}	5.65±0.06^{b}	5.55±0.06^{bc}	5.50±0.04^{a}	5.39±0.05^{b}	5.34±0.04^{c}
9	*S. cerevisiae*SC01	6.30±0.04^{cd}	5.81±0.09^{cd}	5.65±0.05^{b}	5.59±0.06^{bc}	5.55±0.06^{b}	5.30±0.05^{b}	5.25±0.05^{b}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table4.17:Gastric TransitTestsViable Countfor the Indigenous*Saccharomyces cerevisiae*Isolated from Fermented Food Products

S/N	Isolates	pH 2					pH 3				
		Incubation periods (hour)/ Viable Count (logCFU/ml)									
		0	1	2	3	4	0	1	2	3	4
1	*S. erevisiae*PAW02	8.31±0.05^{a}	9.06±0.05^{a}	9.36±0.04^{a}	9.43±0.04^{a}	9.50±0.05^{a}	8.86±0.05^{a}	8.43±0.04^{a}	8.32±0.02^{a}	8.30±0.04^{a}	7.48±0.04^{a}
2	*S.cerevisiae*PAW24	8.38±0.03^{b}	8.33±0.02^{b}	8.27±0.03^{a}	8.05±0.05^{b}	6.80±0.06^{b}	8.46±0.04^{b}	8.40±0.03^{a}	8.32±0.03^{a}	8.30±0.03^{b}	8.08±0.02^{b}
3	*S.cerevisiae*BKT07	9.15±0.04^{b}	8.02±0.04^{b}	7.68±0.05^{b}	7.63±0.06^{b}	7.49±0.04^{c}	9.76±0.06^{a}	8.95±0.05^{b}	8.52±0.05^{b}	8.50±0.05^{c}	8.35±0.03^{c}
4	*S.cerevisiae*BKT19	8.61±0.06^{a}	8.68±0.05^{a}	8.71±0.06^{b}	8.82±0.09^{b}	8.93±0.09^{a}	8.75±0.06^{a}	8.51±0.06^{b}	8.45±0.06^{b}	8.42±0.04^{a}	8.40±0.04^{a}
5	*S.cerevisiae*AGG23A	8.50±0.05^{a}	8.35±0.04^{b}	8.30±0.02^{c}	8.20±0.02^{c}	8.15±0.02^{c}	8.50±0.05^{a}	8.47±0.04^{a}	8.45±0.06^{b}	8.35±0.03^{b}	8.25±0.02^{b}
6	*S.cerevisiae*AGG08	9.20±0.03^{b}	8.05±0.05^{a}	7.70±0.07^{d}	7.60±0.06^{b}	7.50±0.04^{c}	9.55±0.06^{a}	9.07±0.02^{c}	8.70±0.07^{c}	8.50±0.05^{c}	8.40±0.02^{b}
7	*S. cerevisiae*OBB17	8.40±0.04^{c}	8.30±0.06^{c}	8.25±0.04^{c}	8.20±0.04^{a}	8.00±0.02^{d}	8.55±0.09^{c}	8.50±0.05^{b}	7.80±0.06^{b}	7.50±0.06^{d}	7.40±0.04^{a}
8	*S. erevisiae*OBB03A	8.50±0.05^{d}	8.40±0.05^{a}	8.30±0.03^{c}	8.20±0.04^{a}	8.14±0.04^{bc}	8.49±0.02^{d}	8.40±0.04^{a}	8.35±0.04^{d}	8.28±0.04^{a}	8.0±0.03^{d}
9	*S. cerevisiae*SC01	8.60±0.04^{b}	8.50±0.19^{b}	8.40±0.02^{d}	8.25±0.05^{b}	8.10±0.04^{b}	8.00±0.04^{d}	7.90±0.06^{b}	7.85±0.05^{b}	7.80±0.06^{d}	7.50±0.05^{e}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.18: Intestinal Transit Tests for the Indigenous *Saccharomyces cerevisiae* Isolated from Fermented Food Products

S/N	Isolates	Incubation periods (hours)/Viable Count: (logcfu/ml)			
		0	1	4	8
1	*S. cerevisiae*PAW02	8.90 ± 0.05^{a}	8.88 ± 0.05^{a}	8.63 ± 0.05^{a}	8.32 ± 0.03^{a}
2	*S. cerevisiae*PAW24	7.97 ± 0.06^{b}	7.94 ± 0.06^{b}	7.49 ± 0.04^{b}	7.37 ± 0.04^{b}
3	*S. cerevisiae*BKT07	8.44 ± 0.04^{c}	8.41 ± 0.04^{c}	8.36 ± 0.03^{c}	8.29 ± 0.02^{c}
4	*S. cerevisiae*BKT19	8.96 ± 0.06^{b}	8.93 ± 0.06^{b}	8.18 ± 0.03^{d}	8.04 ± 0.03^{a}
5	*S. cerevisiae*AGG23A	8.45 ± 0.03^{d}	8.40 ± 0.04^{c}	8.37 ± 0.03^{c}	8.30 ± 0.03^{d}
6	*S. cerevisiae*AGG08	8.85 ± 0.06^{b}	8.80 ± 0.05^{a}	8.74 ± 0.06^{e}	8.60 ± 0.05^{a}
7	*S. cerevisiae*OBB17	8.54 ± 0.05^{a}	8.50 ± 0.06^{b}	8.47 ± 0.04^{b}	8.40 ± 0.03^{b}
8	*S. cerevisiae*OBB03A	7.50 ± 0.04^{c}	7.48 ± 0.04^{c}	7.45 ± 0.03^{c}	7.30 ± 0.04^{b}
9	*S. cerevisiae*SC01	8.50 ± 0.05^{a}	8.45 ± 0.04^{c}	8.30 ± 0.03^{c}	8.20 ± 0.02^{c}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.19: Hydrophobicity/Microbial Adhesion to Hydrocarbons by the Indigenous*Saccharomyces cerevisiae*Isolated from Fermented Food Products

S/N	Isolates	Percentage Hydrophobicity (%)		
		Xylene	Toluene	N-hexadecane
1	*S. cerevisiae*PAW02	61.62±0.05^{a}	47.37±0.04^{a}	60.49±0.04^{a}
2	*S. cerevisiae*PAW24	61.80±0.06^{b}	55.85±0.06^{b}	70.55±0.05^{b}
3	*S. cerevisiae*BKT07	42.57±0.05^{a}	57.50±0.05^{c}	57.73±0.06^{c}
4	*S. cerevisiae*BKT19	67.54±0.05^{a}	60.68±0.06^{b}	57.05±0.02^{d}
5	*S. cerevisiae*AGG23A	35.66±0.06^{b}	42.28±0.02^{d}	51.17±0.01^{e}
6	*S. cerevisiae*AGG08	58.00±0.12^{c}	24.21±0.03^{d}	54.48±0.04^{a}
7	*S. cerevisiae*OBB17	76.68±0.06^{b}	64.50±0.05^{c}	83.45±0.04^{a}
8	*S. cerevisiae*OBB03A	63.35±0.05^{a}	76.49±0.04^{a}	66.37±0.05^{b}
9	*S. cerevisiae*SC01	66.82±0.06^{b}	55.50±0.03^{d}	61.08±0.02^{d}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.20: Autoaggregation Ability of the Indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

S/N	Isolates	Optical Density		Autoaggregation (%)
		Upper suspension	Total suspension	
1	*S. cerevisiae*PAW02	0.039±0.01^{a}	0.813±0.01^{a}	95.20±0.04^{a}
2	*S. cerevisiae*PAW24	0.018±0.02^{b}	1.021±0.03^{b}	98.24±0.04^{a}
3	*S. cerevisiae*BKT07	0.023±0.02^{b}	0.957±0.02^{c}	97.60±0.05^{b}
4	*S. cerevisiae*BKT19	0.111±0.01^{a}	1.093±0.03^{b}	89.80±0.05^{b}
5	*S. cerevisiae*AGG23A	0.003±0.00^{c}	0.858±0.02^{c}	99.91±0.06^{c}
6	*S. cerevisiae*AGG08	0.036±0.03^{d}	1.115±0.03^{b}	96.77±0.06^{c}
7	*S. cerevisiae*OBB17	0.004±0.00^{e}	1.093±0.03^{b}	99.63±0.05^{b}
8	*S. cerevisiae*OBB03A	0.001±0.00^{e}	1.127±0.03^{b}	99.91±0.06^{b}
9	*S. cerevisiae*SC01	0.016±0.04^{ab}	0.846±0.02^{c}	99.91±0.06^{b}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.21:Bile salts Deconjugation Activityof theIndigenous Yeasts Isolated from Fermented Food Products

S/N	Isolates	Bile salts				
		Sodium glycodeoxycholate	Sodium Taurodeoxycholate hydrate	Sodium Taurocholate	Sodium glycolate	Sodium Glycolate hydrate
1	*S. cerevisiae*PAW02	+	-	-	-	-
2	*S. cerevisiae*PAW24	+	-	-	-	-
3	*S. cerevisiae*BKT07	+	-	-	-	-
4	*S. cerevisiae*BKT19	+	-	-	-	-
5	*S. cerevisiae*AGG23A	+	-	-	-	-
6	*S. cerevisiae*AGG08	+	-	-	-	-
7	*S. cerevisiae*OBB17	+	-	-	-	-
8	*S. cerevisiae*OBB03A	+	-	-	-	-
9	*S. cerevisiae*SC01	+	-	-	-	-

KEY:

+=growth

-=no growth

For an organism to be designated as probiotics it must produce some enzymes which will help directly during digestion. Among these enzymes one of most important is β-galactosidase as shown in Table 4.22. *Saccharomycescerevisiae*OBB17 had 0.537M while *Saccharomycescerevisiae*AG23A had 48.052M.

Alcohol production by the indigenous yeast isolates at different initial sugar concentraction(22 – 28)^{o}Brixis presented in Table 4.23. The highest percentage of alcohol (9.4±0.04%) was produced by*Saccharomycescerevisiae*BKT19 and AGG23A at 26^{o} and 27^{o} Brix respectively. The lowest amount of ethanol (4.4%) was produced by*Saccharomycescerevisiae* BKT07, BKT19, AGG08, AGG23A and SC01 at 22^{o} Brix.

Any yeast that will be employed in fermentation of alcoholic beverages must be ethanol tolerant. In this work, all the *Saccharomyces cerevisiae* tested were able to tolerate alcohol to different extent. The highest amount of alcohol produced (8.4%) was by*Saccharomycescerevisiae* PW02 when the Potato Dextrose Broth (PDB) was adjusted to 5% ethanol.The lowest amount of ethanol (3.8%) was produced by the same organism when the initial ethanol concentration was 2% (Table 4.24).

Molasses are used all over the world for production of many industrial products. The initial concentration of molasses could have an effect on the amount of alcohol produced. When the initial concentration was adjusted to 35^{o}Brix, the highest ethanol (6.2%) was produced by *Saccharomyces cerevisiae* BKT07 (Table 4.25). The lowest quantity of ethanol was manufactured when the initial quantity of molasses was adjusted to 22^{o}Brix. The amount produced was 2.1% by *Saccharomyces cerevisiae* OBB03A.

Attenuation is defined as the percentage of original extract that has been converted by the fermentation process. The highest attenuation of 52.5% was recorded for*Saccharomycescerevisiae*PAW24 while the least (7.5%) was obtained for *Saccharomycescerevisiae*PAW02 (Table 4.26).

The average value obtained for all the yeasts was 41.33%.The fermentation efficiency of 45% was recorded for *Saccharomycescerevisiae*AG08 and AGG23A but the least of 27% was recorded for*Sacharomycescerevisiae* PAW02, BKT07, BKT19 and SC01 (Table 4.27).

Table 4.22: β-galactosidase Activity of theIndigenous Yeasts Isolated from Fermented Food Products

S/N	Isolates	Optical Density			β-glalactosidase Activity (Miller)
		Initial	Final		
		560nm	420nm	560nm	
1	*S. cerevisiae*PAW02	1.256±0.05[a]	0.187±0.05[a]	0.021±0.01[a]	9.037±0.03[a]
2	*S. cerevisiae*PAW24	0.880±0.06[b]	0.185±0.05[a]	0.009±0.02[b]	12.822±0.04[b]
3	*S. cerevisiae*BKT07	0.500±0.05[a]	0.270±0.02[b]	0.092±0.02[b]	14.533±0.05[c]
4	*S. cerevisiae*BKT19	0.954±0.06[b]	0.208±0.02[b]	0.022±0.04[c]	11.845±0.04[b]
5	*S.cerevisiae*AGG23A	0.154±0.01[c]	0.230±0.02[b]	0.068±0.05[d]	48.052±0.06[d]
6	*S. cerevisiae*AGG08	0.858±0.06[b]	0.195±0.05[a]	0.014±0.06[ab]	13.248±0.05[c]
7	*S. cerevisiae*OBB17	0.822±0.06[b]	0.210±0.06[c]	0.018±0.06[ab]	0.537±0.03[a]
8	*S.cerevisiae*OBB03A	1.059±0.05[a]	0.242±0.05[a]	0.043±0.04[c]	10.497±0.04[b]
9	*S. cerevisiae*SC01	1.290±0.05[a]	0.220±0.04[d]	0.048±0.04[c]	7.0284±0.02[d]

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.23: Alcohol Production (%) at Different Initial ^{o}BrixDuring Fermentation by theIndigenous Yeasts Isolated from Fermented Food Products

S/N	Isolates	Initial Total sugar concentration (^{o}Brix)						
		22	23	24	25	26	27	28
1	*S. cerevisiae*PAW02	4.5±0.05^{a}	5.2±0.00^{a}	5.8±0.05^{a}	5.9±0.05^{a}	6.2±0.02^{a}	6.5±0.05^{a}	9.4±0.04^{b}
2	*S. cerevisiae*PAW24	7.0±0.04^{b}	7.6±0.05^{b}	7.8±0.06^{b}	8.0±0.02^{b}	8.4±0.03^{b}	8.6±0.05^{a}	6.8±0.05^{a}
3	*S. cerevisiae*BKT07	4.4±0.04^{b}	5.2±0.04^{c}	7.6±0.06^{b}	8.2±0.02^{b}	8.4±0.03^{b}	8.8±0.06^{b}	8.9±0.05^{a}
4	*S. cerevisiae*BKT19	4.4±0.04^{b}	7.4±0.04^{c}	7.6±0.06^{b}	7.8±0.06^{c}	8.2±0.02^{c}	8.6±0.06^{b}	9.0±0.04^{b}
5	*S. cerevisiae*AGG08	4.4±0.04^{b}	5.4±0.04^{c}	7.4±0.04^{c}	7.8±0.06^{c}	8.0±0.02^{c}	8.5±0.06^{b}	9.2±0.03^{c}
6	*S. cerevisiae*AGG23A	4.4±0.05^{a}	6.8±0.06^{d}	7.8±0.05^{a}	8.0±0.03^{bc}	8.2±0.02^{c}	8.4±0.04^{c}	8.6±0.05^{a}
7	*S. cerevisiae*OBB03A	4.4±0.06c	6.0±0.03^{e}	6.2±0.04^{c}	6.8±0.05^{a}	7.0±0.03^{b}	7.8±0.05^{a}	8.0±0.04^{b}
8	*S. cerevisiae*OBB17	5.6±0.07^{ab}	6.0±0.03^{e}	6.4±0.04^{c}	6.8±0.05^{a}	7.5±0.05^{bc}	7.8±0.05^{a}	8.0±0.04^{b}
9	*S. cerevisiae*SC01	5.0±0.05^{a}	5.6±0.06^{b}	6.2±0.03^{d}	7.0±0.03^{d}	7.8±0.06^{e}	8.0±0.02^{d}	8.6±0.05^{a}

Mean values of two replicates with different superscript down the column are significant differentat $\alpha_{0.05}$

Table 4.24: Alcohol Tolerance of the Indigenous *Saccharomycescerevisiae*Isolated from Fermented Food Products.

S/N	Isolates	Alcohol concentrations (%)				
		2	3	5	7	10
1	*S. cerevisiae*PAW02	3.8±0.05^{a}	6.4±0.04^{a}	8.4±0.03^{a}	6.2±0.04^{a}	6.0±0.04^{a}
2	*S. cerevisiae*PAW24	4.6±0.05^{a}	4.8±0.05^{b}	6.4±0.03^{a}	4.3±0.04^{a}	3.8±0.05^{b}
3	*S. cerevisiae*BKT07	4.2±0.02^{b}	5.0±0.02^{c}	6.6±0.05^{b}	6.2±0.04^{a}	4.2±0.03^{c}
4	*S. cerevisiae*BKT19	4.0±0.03^{c}	6.4±0.03^{d}	6.6±0.05^{b}	6.5±0.05^{b}	4.4±0.03^{c}
5	*S. cerevisiae*AGG08	4.0±0.03^{c}	6.6±0.05^{b}	8.2±0.03^{a}	6.2±0.04^{a}	6.0±0.04^{a}
6	*S.cerevisiae*AGG23A	4.2±0.02^{b}	5.0±0.02^{c}	6.4±0.03^{a}	4.3±0.04^{a}	4.2±0.03^{c}
7	*S.cerevisiae*OBB03A	4.0±0.03^{c}	6.4±0.03^{a}	6.6±0.05^{b}	6.5±0.05^{b}	4.5±0.06^{d}
8	*S. cerevisiae*OBB17	4.2±0.02^{b}	5.0±0.02^{c}	6.6±0.05^{b}	6.2±0.04^{b}	4.2±0.03^{c}
9	*S. cerevisiae*SC01	4.1±0.02^{b}	6.0±0.03^{d}	6.6±0.05^{b}	6.5±0.05^{b}	4.5±0.06^{d}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.25: Effects of Molasses Concentration on Alcohol Production by the Indigenous*Saccharomycescerevisiae*Isolated from Fermented Food Products

S/N	Isolates	Molasses concentration (^{o} Brix)			
		22	25	30	35
1	*S. cerevisiae*PAW02	3.2±0.03^{a}	4.0±0.03^{a}	5.6±0.05^{a}	6.0±0.02^{a}
2	*S. cerevisiae*PAW24	2.6±0.05^{b}	3.8±0.05^{b}	4.0±0.02^{b}	4.4±0.03^{b}
3	*S. cerevisiae*BKT07	2.2±0.03^{a}	3.9±0.06^{b}	4.1±0.03^{c}	6.2±0.04^{c}
4	*S. cerevisiae*BKT19	4.2±0.03^{a}	4.3±0.04^{c}	4.4±0.04^{d}	4.7±0.05^{b}
5	*S. cerevisiae*AGG08	3.1±0.02^{c}	3.8±0.06^{b}	5.1±0.02^{b}	5.2±0.04^{c}
6	*S. cerevisiae*AGG23A	2.6±0.05^{b}	3.9±0.06^{b}	4.1±0.02^{b}	4.4±0.03^{b}
7	*S. cerevisiae*OBB03A	2.1±0.02^{c}	3.9±0.06^{b}	4.0±0.02^{b}	6.0±0.02^{a}
8	*S. cerevisiae*OBB17	4.2±0.03^{a}	4.3±0.03^{a}	4.4±0.04^{d}	5.1±0.02^{a}
9	*S. cerevisiae*SC01	2.6±0.05^{b}	3.2±0.03^{a}	4.3±0.03^{c}	4.6±0.05^{d}

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.26: Attenuation Activity of the Indigenous *Saccharomycescerevisiae* Isolatedfrom Fermented Food Products

S/N	Isolates	Initial Brix	Original Gravity (OG)	Final Brix	Final Gravity (FG)	Attenuation (%)
1	*S. cerevisiae*PAW02	28^{o}	1.120	18^{o}	1.075	52.50
2	*S. cerevisiae*PAW24	28^{o}	1.120	14^{o}	1.057	37.50
3	*S. cerevisiae*BKT07	28^{o}	1.120	16^{o}	1.066	45.00
4	*S. cerevisiae*BKT19	28^{o}	1.120	17^{o}	1.070	41.67
5	*S. cerevisiae*AGG08	28^{o}	1.120	17^{o}	1.070	41.67
6	*S. cerevisiae*AGG23A	28^{o}	1.120	17^{o}	1.070	41.67
7	*S. cerevisiae*OBB03A	28^{o}	1.120	17^{o}	1.070	41.67
8	*S. cerevisiae*OBB17	28^{o}	1.120	17^{o}	1.070	41.67
9	*S. cerevisiae*SC01	28^{o}	1.120	16^{o}	1.066	45.00

Mean values of two replicates with different superscript down the column are significantly differentat $\alpha_{0.05}$

Table4.27: Fermentation Efficiency of the Indigenous *Saccharomycescerevisiae* Isolated from Fermented Food Products

S/N	Isolates	Optical Densityat 600nm	Actual ethanol (%)	Theoretical ethanol (%)	Fermentation efficiency (%)
1	*S. cerevisiae*PAW02	0.237	8	17.92	45
2	*S. cerevisiae*PAW24	0.256	6	17.92	33
3	*S. cerevisiae*BKT07	0.253	5	17.92	27
4	*S. cerevisiae*BKT19	0.244	5	17.92	27
5	*S. cerevisiae*AGG08	0.281	5	17.92	27
6	*S. cerevisiae*AGG23A	0.378	8	17.92	45
7	*S. cerevisiae*OBB03A	0.264	6	17.92	33
8	*S. cerevisiae*OBB17	0.301	7	17.92	39
9	*S. cerevisiae*SC01	0.255	5	17.92	27

Some of the yeast isolates were able to produce certain proteins that kill other sensitive strains while the producer is not affected. In this project all the yeast tested were able to produce toxin as measured by the zone of inhibition (Table 4.28). The zone of inhibition ranged between 24mm and 44mm.

The flunctuation in pH in the fermentation of apple juice are reported in Table 4.29. There was gradual reduction in the pH of the various samples from day 0 to day 6 of fermentation. The pH ranged between3.49and4.05over the six day of fermentation.There was a gradual reduction in total soluble solid from day 0 to day 6 of fermentation. It ranged between 9 and 18mg/ml (Table 4.30).The total titratable acidity increased from day 0 to day 6. At day 0, the total titratable acidity was 18mg/ml but this increased to 40.8mg/ml in samples fermented with *Saccharomycescerevisiae* PAW02, PAW24, BKT07 and AGG08 (Table 4.31).

On the first day of fermentation(Appendix 3), the Brix-acid ratio was 1, but there was gradual reduction from Day 1 to the 6th day of fermentation. The Brix acid ratio ranged between 0.221 and 1 (Table 4.32).There was a steady increase in the ascorbic acid content of the various samples from Day 0 to Day 6. The ascorbic acid content ranged between 4.90 for samples fermented with *Saccharomyces cerevisiae* PAW24 and 74.51 for sample fermented with *Saccharomycescerevisiae* SC01(Table 4.33).

Table 4.34 shows the results of the reducing sugars of apple juice fermented by yeast isolates. There was a steady increase in the reducing sugars from Day 0 to Day 6 of fermentation. The reducing sugars rangedbetween 0.260mg/ml in samplefermented with *Saccharomycescerevisiae* PAW24 and 0.414mg/ml for sample fermented with *Saccharomycescerevisiae*PAW02.

The changes in total sugar are presented in Table 4.35. At Day 0, the total sugar ranged between 0.023 and 0.024mg/g but by 6th day of fermentation it has increased to 0.075mg/g for samples fermented with *Sacharomycescerevisiae* PAW24 and AGG08.

Table 4.28: 'Killer' Activity of the Indigenous *Saccharomyces cerevisiae* Isolated from Fermented Food Products

S/N	Isolates	Zones of inhibition (mm)
1	*Saccharomyces. Cerevisiae*PAW02	24.00 ± 0.02^{a}
2	*Saccharomyces cerevisiae*PAW24	44.00 ± 0.01^{b}
3	*Saccharomyces cerevisiae*BKT07	39.00 ± 0.03^{c}
4	*Saccharomyces cerevisiae*BKT19	34.00 ± 0.04^{d}
5	*Saccharomyces cerevisiae*AGG08	41.00 ± 0.04^{d}
6	*Saccharomyces cerevisiae*AGG23A	34.00 ± 0.01^{b}
7	*Saccharomyces cerevisiae*OBB03A	44.00 ± 0.02^{a}
8	*Saccharomycescerevisiae*OBB17	34.00 ± 0.04^{d}
9	*Saccharomycescerevisiae*SC01	39.00 ± 0.02^{a}

Mean values of two replicateswith different superscript down the column are significantly differentat $\alpha_{0.05}$

Table 4.29: Changes in pH during the Fermentation of Apple Juice by Indigenous *Saccharomyces cerevisiae* Isolated from Fermented Food Products

Sample	Incubation periods (days)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
A	4.01±0.02[a]	3.76±0.05[a]	3.73±0.05[a]	3.69±0.05[a]	3.66±0.05[a]	3.60±0.05[a]	3.57±0.05[a]
B	4.02±0.01[b]	3.78±0.06[b]	3.85±0.06[b]	3.82±0.06[b]	3.79±0.06[b]	3.74±0.06[b]	3.68±0.06[b]
C	3.99±0.05[c]	3.77±0.06[b]	3.86±0.06[b]	3.81±0.06[b]	3.75±0.06[b]	3.68±0.05[a]	3.66±0.06[b]
D	4.05±0.03[d]	3.81±0.07[c]	3.82±0.05[a]	3.80±0.06[b]	3.74±0.06[b]	3.70±0.06[b]	3.67±0.06[b]
E	4.00±0.01[b]	3.74±0.06[b]	3.78±0.05[a]	3.74±0.05[a]	3.70±0.05[a]	3.70±0.06[b]	3.63±0.06[b]
F	4.02±0.01[b]	3.80±0.07[c]	3.62±0.05[a]	3.61±0.05[a]	3.60±0.05[a]	3.59±0.05[a]	3.49±0.05[a]

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.30: Estimation of Total Soluble SolidsDuring Fermentation of Apple Juice by Indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

Sample	Incubation periods (days)/ Total Soluble Solids (mg/g)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
A	18.0±0.01^{a}	17.0±0.03^{a}	15.5±0.05^{a}	15.0±0.02^{a}	14.0±0.02^{a}	12.0±0.02^{a}	10.0±0.01^{b}
B	18.0±0.01^{a}	17.0±0.03^{a}	14.0±0.02^{b}	13.0±0.01^{b}	12.0±0.01^{b}	11.0±0.01^{b}	10.0±0.01^{b}
C	18.0±0.01^{a}	16.0±0.01^{b}	15.0±0.03^{c}	11.5±0.05^{c}	11.0±0.01^{b}	11.0±0.01^{b}	10.0±0.01^{b}
D	18.0±0.01^{a}	16.0±0.01^{b}	14.0±0.02^{b}	12.0±0.02^{a}	12.0±0.03^{c}	10.0±0.02^{a}	11.0±0.02^{a}
E	18.0±0.01^{a}	17.0±0.03^{a}	13.5±0.05^{a}	13.0±0.01^{b}	13.0±0.03^{c}	11.0±0.01^{b}	9.0±0.02^{a}
F	18.0±0.01^{a}	16.0±0.01^{b}	15.0±0.03^{c}	14.0±0.02^{a}	13.5±0.05^{d}	12.0±0.02^{a}	11.0±0.03^{c}

Mean values of two replicateswith different superscript down the column are significantly differentat $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.31: Estimation of Total Titratable Acidicity during Fermentation of Apple Juice by Indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

Sample	Incubation periods (days)\Total Titratable Acidicity (mg/ml)						
	0	1	2	3	4	5	6
A	18.0±0.01^{a}	27.6±0.05^{a}	30.0±0.01^{a}	31.2±0.01^{a}	33.6±0.05^{a}	38.4±0.03^{a}	38.4±0.05^{c}
B	18.0±0.01^{a}	19.2±0.04^{b}	19.2±0.02^{b}	21.6±0.05^{b}	31.2±0.02^{b}	38.4±0.03^{a}	40.8±0.06^{b}
C	18.0±0.01^{a}	18.0±0.01^{c}	21.6±0.05^{c}	22.8±0.06^{c}	28.8±0.05^{c}	38.4±0.03^{a}	40.8±0.06^{b}
D	18.0±0.01^{a}	19.2±0.04^{b}	21.6±0.05^{c}	22.8±0.07^{d}	26.4±0.03^{d}	36.0±0.02^{b}	43.2±0.02^{a}
E	18.0±0.01^{a}	19.2±0.04^{b}	21.6±0.05^{c}	24.0±0.02^{ab}	31.2±0.02^{b}	36.0±0.02^{b}	40.8±0.06^{b}
F	18.0±0.01^{a}	19.2±0.04^{b}	21.6±0.05^{c}	26.4±0.04^{bc}	31.2±0.02^{b}	31.2±0.02^{c}	36.0±0.04^{d}

Mean values of two replicateswith different superscript down the column are significantly differentat $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.32: Estimation of Brix/Acid Ratioduring Fermentation of Apple Juice by the Indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

Sample	Incubation periods (days)/ Brix/Acid Ratio						
	0	1	2	3	4	5	6
A	1	0.620	0.520	0.420	0.360	0.330	0.255
B	1	0.885	0.729	0.601	0.385	0.286	0.245
C	1	0.889	0.694	0.504	0.382	0.286	0.245
D	1	0.833	0.648	0.526	0.455	0.278	0.260
E	1	0.885	0.625	0.542	0.417	0.306	0.221
F	1	0.729	0.694	0.455	0.433	0.385	0.306

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.33: Estimation of ascorbic acid content of apple juice during fermentation by indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

Sample	Incubation periods (days)/ascorbic acid (mg/ml)						
	0	1	2	3	4	5	6
A	5.88±0.06^{a}	7.80±0.05^{a}	21.57±0.05^{a}	39.22±0.06^{a}	41.18±0.05^{a}	58.82±0.06^{a}	62.75±0.06^{a}
B	4.90±0.05^{b}	11.76±0.06^{b}	19.61±0.05^{a}	39.22±0.05^{b}	43.14±0.06^{b}	60.78±0.05^{b}	62.75±0.05^{b}
C	5.88±0.06^{a}	9.80±0.07^{c}	21.57±0.05^{a}	23.53±0.06^{a}	49.02±0.07^{c}	62.75±0.06^{a}	64.71±0.06^{a}
D	6.86±0.06^{a}	11.76±0.06^{b}	23.53±0.06^{b}	25.49±0.06^{a}	43.14±0.06^{b}	58.82±0.06^{a}	66.67±0.06^{a}
E	5.88±0.05^{b}	7.84±0.05^{a}	19.61±0.06^{b}	21.57±0.05^{b}	49.02±0.05^{a}	58.82±0.05^{b}	62.75±0.05^{b}
F	6.27±0.04^{c}	9.80±0.07^{d}	21.57±0.05^{a}	39.22±0.04^{c}	45.10±0.07^{d}	68.63±0.04^{c}	74.51±0.04^{c}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.34: Estimation of reducing sugars during Fermentation of Apple Juice by Indigenous *Saccharomyces cerevisiae* Isolated from Fermented Food Products

S/N	Sample	Incubation periods (days)/ reducing sugars (mg/ml)						
		0	1	2	3	4	5	6
1	A	0.261 ± 0.05^{a}	0.275 ± 0.06^{a}	0.329 ± 0.05^{a}	0.336 ± 0.05^{a}	0.338 ± 0.05^{a}	0.404 ± 0.04^{a}	0.414 ± 0.04^{a}
2	B	0.260 ± 0.05^{a}	0.276 ± 0.05^{b}	0.338 ± 0.06^{b}	0.336 ± 0.06^{b}	0.341 ± 0.04^{b}	0.342 ± 0.04^{a}	0.345 ± 0.05^{b}
3	C	0.261 ± 0.06^{b}	0.277 ± 0.05^{b}	0.329 ± 0.05^{a}	0.332 ± 0.04^{c}	0.335 ± 0.04^{b}	0.340 ± 0.03^{b}	0.347 ± 0.06^{c}
4	D	0.262 ± 0.06^{b}	0.276 ± 0.04^{c}	0.328 ± 0.04^{c}	0.350 ± 0.05^{a}	0.357 ± 0.05^{a}	0.360 ± 0.05^{c}	0.365 ± 0.06^{c}
5	E	0.260 ± 0.04^{c}	0.276 ± 0.04^{c}	0.329 ± 0.05^{a}	0.338 ± 0.06^{b}	0.346 ± 0.04^{d}	0.363 ± 0.05^{c}	0.390 ± 0.07^{d}
6	F	0.263 ± 0.03^{d}	0.278 ± 0.06^{a}	0.330 ± 0.04^{c}	0.340 ± 0.04^{c}	0.361 ± 0.06^{c}	0.366 ± 0.06^{b}	0.374 ± 0.06^{c}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.35: Estimation of Total Sugars during Fermentation ofApple Juice byIndigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

S/N	Sample	Incubation periods (days)/ Total Sugars (mg/ml)						
		0	1	2	3	4	5	6
1	A	0.024 ± 0.005^{a}	0.058 ± 0.001^{c}	0.059 ± 0.002^{b}	0.060 ± 0.001^{c}	0.065 ± 0.000^{d}	0.068 ± 0.003^{a}	0.070 ± 0.003^{c}
2	B	0.023 ± 0.001^{b}	0.058 ± 0.001^{c}	0.061 ± 0.001^{c}	0.065 ± 0.000^{a}	0.069 ± 0.003^{a}	0.070 ± 0.002^{b}	0.075 ± 0.002^{b}
3	C	0.023 ± 0.002^{c}	0.057 ± 0.002^{d}	0.060 ± 0.003^{d}	0.062 ± 0.002^{b}	0.065 ± 0.003^{a}	0.068 ± 0.003^{a}	0.070 ± 0.001^{a}
4	D	0.023 ± 0.001^{a}	0.057 ± 0.003^{e}	0.060 ± 0.004^{ab}	0.065 ± 0.002^{b}	0.068 ± 0.001^{b}	0.070 ± 0.003^{a}	0.072 ± 0.003^{c}
5	E	0.023 ± 0.002^{b}	0.057 ± 0.000^{a}	0.062 ± 0.003^{d}	0.065 ± 0.003^{d}	0.068 ± 0.002^{c}	0.072 ± 0.002^{b}	0.075 ± 0.002^{b}
6	F	0.023 ± 0.001^{a}	0.057 ± 0.003^{e}	0.061 ± 0.002^{b}	0.064 ± 0.001^{c}	0.067 ± 0.001^{c}	0.069 ± 0.003^{a}	0.072 ± 0.003^{c}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

The changes in alcoholic content are presented in Table 4.36. There was an increase in alcoholic content of the fermenting samples. The lowest alcoholic content detected after six day of fermentation was 9.0% for sample fermented with *Sacharomycescerevisiae*BKT07 and AGG08 while the highest was recorded for sample fermented with *Sacharomycescerevisiae* PAW02 (10.2%).The changes in yeast population are presented in Table 4.37. The lowest yeast count was recorded for sample fermented with *Sacharomycescerevisiae* PAW02 (7.40 logcfu/ml) while the highest was recorded for sample fermented with *Sacharomycescerevisiae* BKT07 (12.65 logcfu/ml) on the sixth day of fermentation.

The samples ofapple cider wine were subjected to sensory analysis by 20-man panelists. The samples were analysed for appearance,flavor, mouthfeel, tasteand overall acceptability (Table 4.38).

The sample(B and C) fermented with*Sacharomyces cerevisiae*PAW24 and BKT07 were rated the bestin term of appearance with value of 7.71 while the sample (E) fermented with*Sacharomyces cerevisiae* AGG08 was rated lowest with value of 7.14.

The sample(B) fermented with*Sacharomyces cerevisiae*PAW24 was rated the bestin term of flavor with value of 8.00while the sample (F) fermented with*Sacharomyces cerevisiae*SC01 was rated lowest with value of 6.71.

The sample(A) fermented with*Sacharomyces cerevisiae*PAW02 was rated the bestin term of mouthfeelwith value of 8.00while the sample (E) fermented with*Sacharomyces cerevisiae* AGG08 was rated lowest with value of 7.00.

The sample(B)fermented with*Sacharomyces cerevisiae*PAW24 was rated the bestin term of tastewith value of 8.14while the samples (E and F) fermented with*Sacharomyces cerevisiae* AGG08 and *Sacharomyces cerevisiae*SC01 were rated lowest with value of 7.00.

Table 4.36: Estimation of Alcoholic Contentduring Fermentation of Apple Juice by the Indigenous *Saccharomyces cerevisiae*Isolated from Fermented Food Products

S/N	Sample	Alcoholic content (%) at different incubation periods (days)						
		0	1	2	3	4	5	6
1	A	0.0	2.4±0.02^{a}	8.0±0.02^{b}	8.8±0.05^{b}	8.9±0.06^{a}	9.8±0.07^{b}	10.2±0.02^{a}
2	B	0.0	3.8±0.05^{b}	8.4±0.03^{c}	8.4±0.03^{c}	8.5±0.05^{b}	8.8±0.07^{b}	10.0±0.00^{b}
3	C	0.0	2.6±0.05^{b}	7.0±0.02^{b}	8.4±0.03^{c}	8.5±0.05^{b}	8.9±0.06^{c}	9.0±0.03^{c}
4	D	0.0	5.2±0.02^{a}	8.0±0.01^{a}	8.2±0.02^{d}	8.5±0.05^{b}	8.7±0.05^{d}	9.2±0.01^{d}
5	E	0.0	5.0±0.01^{c}	6.8±0.05^{d}	7.9±0.06^{ab}	8.4±0.03^{c}	8.6±0.05^{d}	9.0±0.01^{d}
6	F	0.0	3.8±0.05^{b}	8.0±0.01^{a}	8.3±0.03^{c}	8.4±0.03^{c}	8.6±0.06^{c}	9.4±0.02^{b}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.37: Changes in yeast Population during Fermentation of Apple Juice by Indigenous *Saccharomyces cerevisiae*

S/N	Sample	Yeast Population (cfu/ml) at different incubation periods (days)						
		0	1	2	3	4	5	6
1	A	7.40 ± 0.02^{a}	8.54 ± 0.06^{b}	10.29 ± 0.02^{a}	10.29 ± 0.01^{a}	10.63 ± 0.05^{a}	11.35 ± 0.03^{a}	11.20 ± 0.01^{a}
2	B	7.74 ± 0.05^{b}	8.01 ± 0.02^{a}	9.93 ± 0.07^{b}	9.96 ± 0.06^{b}	10.68 ± 0.06^{b}	11.15 ± 0.02^{b}	11.22 ± 0.02^{b}
3	C	7.78 ± 0.06^{c}	9.17 ± 0.03^{b}	9.69 ± 0.06^{c}	10.08 ± 0.03^{c}	10.66 ± 0.06^{b}	11.84 ± 0.06^{c}	12.65 ± 0.05^{c}
4	D	7.76 ± 0.05^{d}	8.70 ± 0.07^{c}	10.39 ± 0.03^{d}	10.55 ± 0.05^{bc}	11.37 ± 0.04^{c}	12.69 ± 0.05^{d}	12.61 ± 0.05^{c}
5	E	7.72 ± 0.05^{b}	8.51 ± 0.06^{b}	9.95 ± 0.06^{c}	10.85 ± 0.06^{bc}	11.70 ± 0.05^{d}	11.83 ± 0.06^{c}	11.88 ± 0.06^{d}
6	F	7.72 ± 0.06^{c}	10.16 ± 0.03^{b}	10.30 ± 0.02^{a}	10.36 ± 0.03^{d}	10.74 ± 0.05^{d}	11.74 ± 0.06^{c}	11.99 ± 0.07^{e}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

Table 4.38: Sensory Evaluation of Apple Wine Produced Using Indigenous *Saccharomyces cerevisiae*

S/N	Sample codes	Sensory Parameters				
		Appearance	Flavour	Mouthfeel	Taste	Overall acceptability
1	A	7.43^{b}	7.86^{b}	8.00^{b}	8.00^{b}	7.82^{b}
2	B	7.71^{a}	8.00^{a}	7.86^{a}	8.14^{a}	7.43^{b}
3	C	7.71^{c}	7.14^{c}	7.29^{a}	7.43^{b}	7.19^{a}
4	D	7.43^{b}	7.00^{c}	7.43^{a}	7.43^{b}	7.12^{a}
5	E	7.14^{d}	7.00^{c}	7.00^{b}	7.00^{c}	7.04^{c}
6	F	7.43^{b}	6.71^{b}	7.14^{b}	7.00^{c}	7.07^{c}

Mean values of twenty replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

The sample(E) fermented with*Sacharomyces cerevisiae*AGG08 was rated lowest with overall acceptability of 7.04 while the sample (A) fermented with*Sacharomyces cerevisiae* PAW02 was rated best with overall acceptability of 7.82

CHAPTER FIVE

5.0 DISCUSSION

The predominant yeasts obtained from palm wine were mainly *S. cerevisiae*. Other yeasts obtained from palmwine were *Pichia gulliermondii*and *P. fabianii*. Yabaya*et al*. (2016) reported isolation of *Saccharomyces cerevisiae* from palmwine for fermentation of grape juice. Also, Okagbue (1988) reported the leavening ability of *Saccharomyces cerevisiae* and *Candida* spp. Moreover, *Saccharomyces cerevisiae* has been reportedly isolated from palmwine in a bid to study their bakery potential.

Moreover,Sanni and Lonner (1993) reported a diverse group of yeasts from Nigerian indigenous fermented alcoholic beverages like palmwine, burukutu, pito, sekete and agadagidi. The yeasts reported by these authors are similar to the ones isolated in this present work. Thevariation that exists might be due to the difference in substrate used in production of the beverage and spontaneous nature of their fermentation.The yeasts isolated from agadagidi were*Pichia fabianii* and*Saccharomyces cerevisiae*. Similar yeasts have been reported by other authors (Sanni and Lonner, 1993).

In a recent study by Astudillo-Melgar*etal*. (2019)reported bacterial diversity during the fermentation of palm wine from Mexico. The organisms reported are *Fructobacillus*, *Leuconostoc*, *Glucoacetobacter*, *Sphingomonas* and *Vibrio*.

Burukutu is a type of alcoholic beverage obtained from sorghum and is similar to pito in Ghana and tchoukoutou in Benin Republic. The yeasts isolated in the present study were*Candida glabrata* and *Saccharomyces cerevisiae*. Umeh*et al*. (2015) had reported isolation of *Saccharomyces cerevisiae* from burukutu for production of wine from pawpaw. Isolation ofyeasts from pito in Ghanahad been reported bySefa-Dedeh*et al*. (1999). The yeasts reported were*S. cerevisiae, Candida tropicalis, Kloeckeraapiculata, Hansenulaanomala* among others.

The yeasts isolated from *ogi-baba* were*Candida tropicalis, Saccharomyces cerevisiae, Pichia fabianii*and*Candida glabrata*.Omemu*et al*. (2007) isolated*S.cerevisiaeGeotrichumfermentans,Rhodotorulagraminis,Candida krusei, C. tropicalis* and*Geotrichumcandidum.*

One method that is described for selecting potential probiotic microbes is the *invitro*method which relied on the ability of microbes to stay alive in the tested situation of the digestive tract as this condition must be fulfilled before an organism is designated as probiotic (Sourabh *et al*., 2011). The indigenous yeast isolates tested in the work were able to stay alive in the low acidic pH 2 and pH 3. The results obtained are comparable to those obtained by Sourabh *et al*. (2011) who reported a decrease in viability of 2.710 to 4.120logcfu/ml (pH 2) and 0.88 to 3.06 logcfu/ml (pH 3) after 3 hours of exposure. In another study, five yeast isolates from *Idle* batter and two isolates from *Jalebi* batter demonstrated a high acid tolerance at pH 2 after exposure for up to 3 hours (Syala and Vohrar, 2013). These authors further stated that after mounting the contact time to 5 hours there was no significant reduction in the endurance. This therefore shows that indigenous yeast isolates with proven acid tolerance could be good candidate as probiotics.

In this present study all the nine indigenous yeast isolates were exposed to different concentration of bile. The results obtained showed that every yeast isolate survives at the various concentration tested. The yeast isolates were thereafter subjected to growth in 3% bile concentration with pH adjusted to 5.8. After 8 hours incubation all the yeast isolates were still viable. Garcia-Hermandez*et al*. (2012) reported the capability of avian yeast to survive under stressful condition of high concentration of bile salts.

Bile salts are dangerousto existing cells, and they destroy the constitutionof the cell outer most layer. Studies that have been carried out before showedthat the standard quantity of bile salt in the intestine of human being was around 0.30% (w/v) (Li *et al*., 2012). Consequently, the tolerance of yeast strains to bile salt was thought to be an importantassets for chosennovel strains which can stay alive in the intestine, and the quantity has been used for chosen strains that are bile tolerant (Chen *et al*., 2014).

For any microorganism to be designated as probiotics, it should be capable of surviving the gastrointestinal tract. The capability of the indigenous yeast isolates to survive in these types of environmental conditions was evaluated. Perriocone*et al*. (2014) stated that there was no important change in the cell counts in gastric juice, in bile salts and pancreatic fluid.

Hydrophobicity of potential yeast isolates is an indirect method of measuring the adhesive properties of probiotics attributes. Inorder to evaluate this trait the ability of the yeast isolates to bind to hexadecane, xylene, toluene and tolerance was conducted. The results showed that all the yeast isolates are hydrophobic.Perriocone*et al*. (2014)also reported that only 9 isolates were hydrophobic using hexadecane as the binding agent. Another indirect method of measuring adhesion is the ability to form biofilm (Perriocone*et al*., 2014). But this parameter was not investigated in the study since it had not been investigated by 99% of work recently reported.

The autoaggregation is another indirect way of measuring adhesive properties of probiotic yeasts. Autoaggregation (%) ability of the yeast isolate was evaluated at different time interval. The autoaggregation ability obtained from the present study was between 89.80% for *Saccharomyces cerevisiae* BKT19 and 91.91% in *Saccharomyces cerevisiae* AGG23A, OBB03A and SC01.Syala and Vohrar (2013) stated autoaggregation of above 80% after 3 hours of incubation in their study. They further stated that the autoaggregation ability for all isolates increased to 95-100% after 20 hours of incubation.

One of the significant waysbywhich probiotics beneficially influence the correct condition of the host is by enzymatic actions that advance the nutrients consumptionin the intestine (Sourabh *et al*., 2011). Although these authors could not isolate yeast with β-galactosidase activity, in this present work all the yeast isolates demonstrated β-galactosidase activity. This is a very interesting result because these isolates will be able to hydrolyze lactose.Ammonia production by probiotic has been demonstrated to have antimicrobial activity. It was only one isolate (PAW02) that demonstrated ability to produce this compound (Sourabh *et al*., 2011).Siderophore production was screened for among the yeast isolates and none was found positive for this compound. Siderophore is

known to exert antagonistic effect (Sourabh *et al*., 2011). The authors also reported that out of 23 yeast isolates only one isolate was positive for siderophore production.

Deconjugation is one of the majoractions of intestinal microbes included by theWorld Health Organization (WHO)professionals for selection of probiotic organisms. There is development of white halo or opaque zones surroundingthe colony which resulted from the release of free bile acids upon deconjugation of bile salts that was added and this is regarded as deconjugation activity of an organism (Sourabh *etal*., 2011). All the 9 yeast isolates of this study were able to demonstrate deconjugation activity against sodium glycodeoxycholate but were only able to grow in the presence of all other sodium salts tested. Deshpande *etal*. (2014) reported the ability of *Lactobacilluscasei* to deconjugate bile salts. It was found that isolated *Lactobacilli* cells were able to deconjugate the bile salt which was observed on the surface of colonies in the form of white precipitate. Rasti*etal*. (2011) reported deconjugation activity of *Bifidobacteriumpseudocatenulatum*. It was reported that *B*. *pseudocatenulatum* had ability to deconjugate 100% of the bile salts in 0.25mM of all types at pH 5.7. They further stated that at pH 6.2 the percentage of deconjugation of all types of bile salts (0.25mM) induced by *B*. *pseudocatenulatum* G4 after 24 hours was 100%.

The pH of the various samples fermented with different isolates was measured using a standardize pH meter. There was a general decrease in pH values of the fermenting cider up to sixth day of fermentation. The pH of the samples was in the acidic range throughout fermentation period. Chilaka*et al*., (2010) reported that during fermentation of fruit wines, the changes in pH did not follow any particular trend but was within acidic range. pH obtained for passion fruit wine ranged between 3.1 and 4.6; that of water melon ranged between 3.4 and 4.8 and that of pineapple fruit wines ranged between 3.0 and 4.7 (Chilaka*et al*., 2010).

The total soluble solid was decreasing from 18mg/g to 11mg/g after six day of fermentation in samples PAW02 and SC170. Onwuama (1991) reported a total soluble solid to range between 5.7 and 7.2 % in burukutu fermented with yeasts and bacteria. The variation in values reported could be as a result of the difference in preparatory ingredients used in the production of the alcoholic drinks.

General increase in titratable acidity (TA) was recorded from Day 0 to the sixth day of fermentation. Michodjehoun-Mestres*et al*. (2005) reported that there was an increase in titratable acidity from 4.3 and 3.6mg/g at 0 hour fermentation in the indigenous and the customized*Gowe*to 64.40 and 41.80 mg/g in that order after 72 hours fermentation period.

The Brix measurement is required by many food and beverage processors including producers of wine, sugar, fruit juice and soft drinks. The Brix unit is a degree scale from 1-100.There was a gradual reduction in the Brix-Acid ratio during the fermentation of apple juice for cider wine production as fermentation period increases.

A gradual increase in reducing sugars was recorded from day 0 to sixth day of fermentation. The lowest reducing sugar (0.260mg/g) was recorded for *Saccharomyces cerevisiae* PAW24 and*Saccharomyces cerevisiae* AGG08 while the highest value (0.414mg/g) was recorded for *Saccharomyces cerevisiae* PAW02. Mugula*et al*. (2003) reported that there was anincreasein the sugars from 1.60 to 21.0 mg/g and reached a concentration of 22.0mg/g in cereal gruel after 30 minutes. The observed difference in reducing sugar could be as a result of the difference in substrate and starter culture used for the fermentation. Moreover, Sharma *etal*. (2012) reported a reducing sugar range between 1.48 and 16.42g/l for white wines and 2.83 and 5.86 g/l for red wines.

The ascorbic acid content of the various fermented apple cider wine was determined. For Day 0 the lowest ascorbic acid content was recorded for sample fermented with *Saccharomyces cerevisiae* PAW24 (4.90mg/g). The highest ascorbic content of 74.51mg/g was recorded for sample fermented with *Saccharomyces cerevisiae* SC01 followed by 66.67mg/g for sample fermented with *Saccharomyces cerevisiae* BKT19.As the days of fermentation increase from day 0 to day 6there was a broad decrease in the values of total sugars content of apple cider wine. The values obtained ranged between 0.024 and 0.075 mg/g. Owuama (1991) reported total sugars range of 3.5 and 3.9 mg/ml in *burukutu* samples produced with a mixture of bacteria and yeasts.After 24 hours of fermentation, the lowest alcoholic content was recorded for sample PAW02 (2.4%) but by the sixth day of fermentation, highest alcoholic content recorded was 10.2% for sample PAW24. Al-Judaibi (2011) reported an alcoholic content which ranged between 4.0 and 7.5%.

The change in yeast population during fermentation of apple cider wine was also recorded. The lowest yeast population recorded was 11.20$\log_{10}$cfu/ml for sample fermented with *Saccharomyces cerevisiae* PAW02 while the highest yeast population was 12.6logcfu/ml after six day of fermentation.Diering*et al*. (2013) reported the initial population of fermentative yeast in the apple must made with commercial apples was 4.31 x 10^3cfu/ml. When the must was obtained from unclassified fruits, the yeast population was higher reaching 9.14 x 10^3cfu/ml. Nogueira *et al*. (2007) found higher values between 3.00 x 10^4 and 6.50 x 10^4cfu/ml, in commercial and unclassified apples respectively.

The apple wine produced was tasted by 20-man panelists who rated the alcoholic beverage on 9 points Hedonic scale. The apple cider wine fermented with *Saccharomycescerevisiae* PAW02 received the highest flavor, mouth feel, taste, and overall acceptability. Therefore, this yeast strain could be explored for industrial production of alcoholic beverage from applesubject to some conditions depending on the desired target.

5.1 CONCLUSION AND RECOMMENDATION

Different indigenous locally made food products and alcoholic beverages in Nigeria include *ogi-baba*, palmwine, *agadagidi* and *burukutu*. These fermented food products are usually produced by spontaneous fermentation and therefore, there is inconsistency in the products quality and short self life of the products is not unexpected. Majority of these fermented foods consist of yeasts as the major microbes that carry out the fermentation. Inorder to select yeasts for technological applications it is necessary to use polyphasic approach for identification in order to solve the problems of mis-identification. Therefore, polyphasic approach which include cultural, physiological, biochemical and molecular methods have been used to confirm the yeasts identities. Molecularly the yeasts were identified to be*S. cerevisiae, C. glabrata, C. parapsilosis, C. tropicalis. Pichia fabianii*, *P. kudriavzevi, Trichosporon*sp. *andGalactomycesgeotrichum.* Moreover, some of the *S. cerevisiae* have demonstrated their ability *invitro* as good candidates for probiotic application. In the investigation of wine brewing traits it has been shown that *Saccharomyces cerevisiae*PW02 produced the most acceptable wine from apple. It is therefore recommended that this isolate should be employed for industrial production of wine from apple. The *Saccharomyces cerevisiae* strains that have demonstrated *invitro* probiotic capacity should be evaluated further for their *invivo* probiotic potential using appropriate animal model.

5.2 CONTRIBUTIONS TO KNOWLEDGE

- This work has demonstrated and further confirmed the occurrence of yeasts in fermented food products like *ogi-baba*, *burukutu*, *agadagidi* and palm wine.
- The problems of mis-identification through phenotypic methods alone have been over come by polyphasic approach which includes cultural, physiological, biochemical and molecular methods.
- It has been demonstrated that the indigenous *Saccharomyces cerevisiae* possessed *invitro* potentials as probiotics
- Some of the indigenous *Saccharomyces cerevisiae*possessed capabilities for alcoholic fermentation.
- *Saccharomyces cerevisiae* PAW02 produced wine with the best quality as a result of its highest overall suitability. This could be engaged for the industrial production of wine from apple.

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APPENDIX I

3.18.2 Preparation of Standard Stock Solution of Ethanol

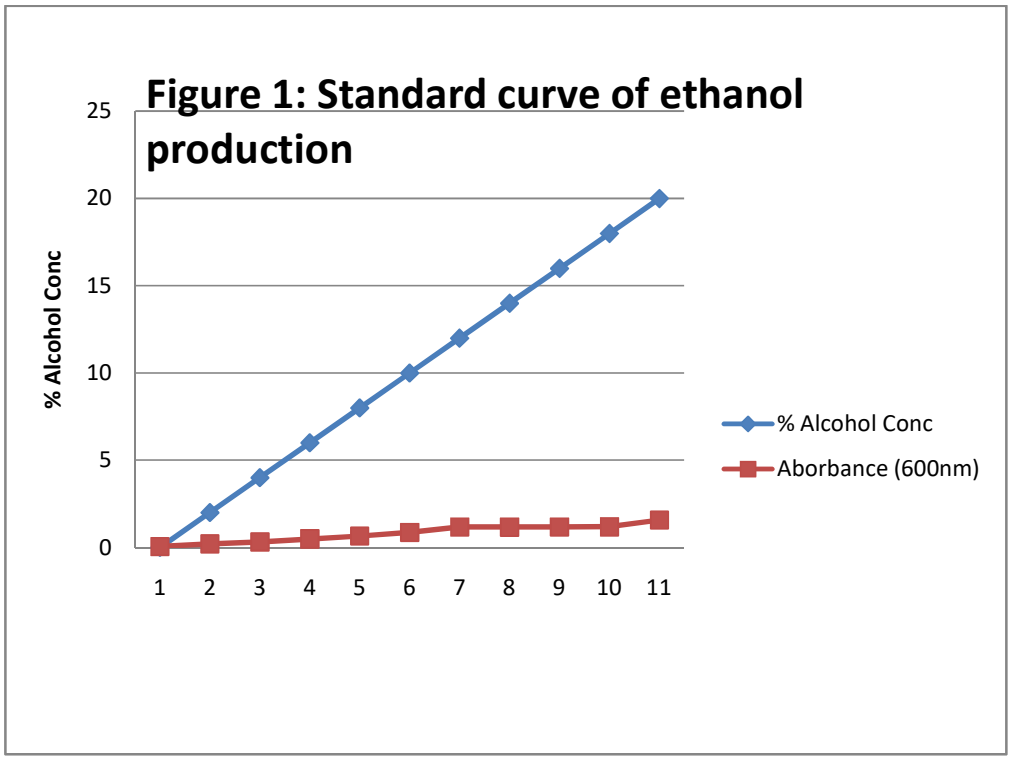
Ethanol – water solution ranging from 0 – 20 percent (v/v) was prepared as standard.

3.18.3 Potassium Dichromate Solution Preparation

The above named solution was prepared by mixing 325ml of concentrated sulphuric acid to 400ml distilled water in a volumentric flask (1 liter). The mixture was allowed to cool to between 80°C and 90°C, then 33.768g potassium dichromate ($K_2Cr_2O_7$) was added and total volume made up to 1litre with distilled water

3.18.4 Preparing Standard Curve for Ethanol Determination

In order to prepare standard curve one ml of different concentration of standard solution was taken in 100ml volumentric flask which contained 25ml of potassium dichromate solution. The mixture was separately heated to 60°C for 20 minutes in a water bath and the absorbance was taken at 600nm after cooling. The standard curve formed is in figure 1.



Appendix 2: Sensory Evaluation questionnaire for laboratory prepared fermented Apple Cider Wine produced with starter cultures

Sensory Evaluation	**Apple Cider Wine**					
	A	B	C	D	E	F
Appearance						
Flavour						
Mouthfeel						
Taste						
Overall acceptability						

Key:

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01

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

APPENDIX 3

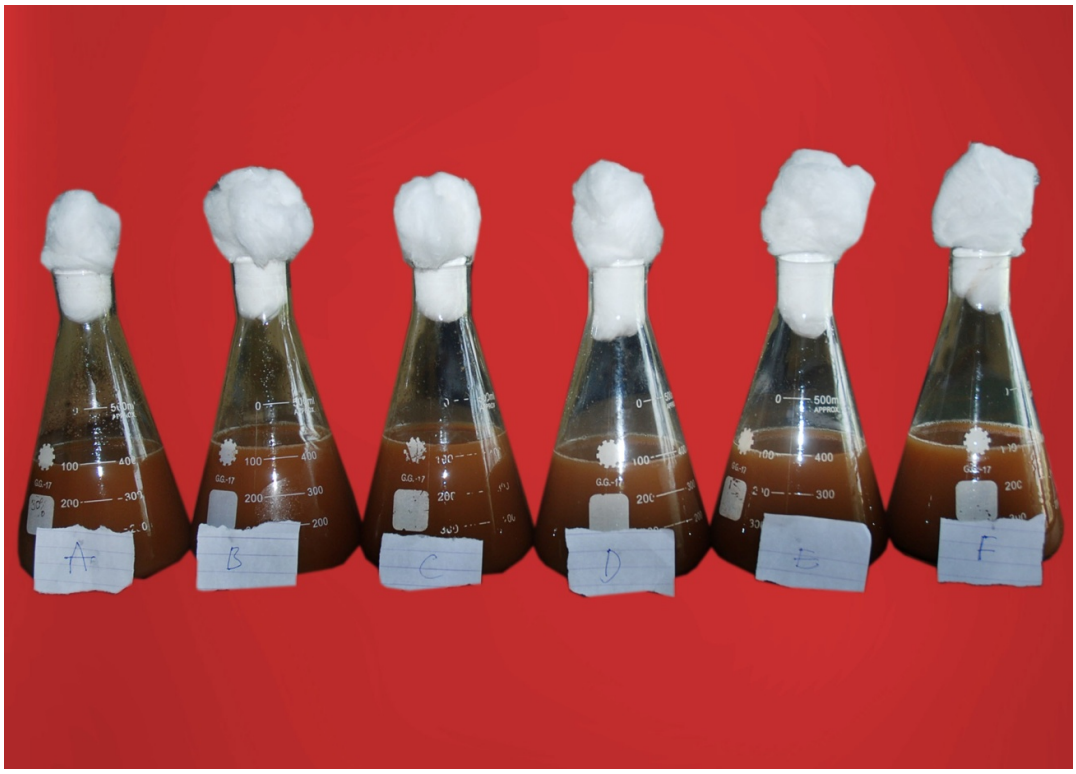


Plate 6: Conical flasks containing apple juice inoculated with starter cultures

Keys:

Sample A: Sample produced with *S.cerevisiae*PAW02

Sample B: Sample produced with *S.cerevisiae*PAW24

Sample C: Sample produced with *S.cerevisiae*BKT07

Sample D: Sample produced with *S.cerevisiae*BKT19

Sample E: Sample produced with *S.cerevisiae*AGG08

Sample F: Sample produced with *S.cerevisiae*SC01