PROTEIN ENRICHMENT OF CASSAVA WITH YEAST FOR CASSAVA FLOUR PRODUCTION

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ABSTRACT

Cassava flour (lafun), a processed product from cassava consumed in Nigeria as a staple food, is a major source of carbohydrate, though it is nutritionally inferior in terms of protein contents. This study investigated the use of yeast strains isolated from palm wine for the enrichment of cassava flour. A total of nine yeast strains were isolated on Potatoe Dextrose Agar (PDA) using pour plate method while four were selected based on their potentials to grow in cassava medium using ultra violet spectroscopy to determine the turbidity level at 600nm. The isolates were thereafter characterized using biochemical and molecular methods. The proximate composition, microbiological safety as well as sensory evaluation of the cassava flour samples were determined using standard methods. Four experimental setup of cassava were produced using the four organisms as monoculture which were inoculated with PDA broth containing 3.5×10^2 - 4.5×10^2 cfu/mL of the yeast cells while the fifth setup without organism served as control and were allowed to ferment for 3 days. Selected potential yeast isolates were identified using 16rRNA as *Candida ethanolica* (NR077165.1), (Pw2b), *Candida ethanolica* (NR077165.1) (Pw3b), *Candida ethanolica* (NR077165.1) (Pw4) and *Sacchromyces cerevissiae* (LC413771.1) (Pw6). Results of proximate composition revealed that cassava flour enriched with *Candida ethanolica* (No26) has the highest crude protein content of 2.54% compared with the crude protein of 1.74 % for commercial control. Uneriched cassava flour has the highest moisture and fat content of 15.07 % and 1.03 % while enriched cassava flour with *Candida ethanolica* (NR077165.1) (Pw3b) and *Saccharomyces cerevisiae* have the least values of 8.20 % and 0.56 % respectively. The crude fibre and ash content obtained for all the cassava flour samples ranged from 0.07 – 0.16 % and 0. 14 - 0.53 % respectively. The carbohydrate content of the commercial control (88. 41 %) was higher than all the values obtained for enriched (83.94 – 88.36 %) and unenriched (81.65 %) cassava flour. The microbial load for bacteria and fungi of the enriched and unenriched cassava flour ranged from 4×10^4cfu/g - 9×10^4cfu/g and 2×10^3 cfu/g - 8×10^3 cfu/g while the commercial control have bacterial and fungal load of 1.1×10^5 cfu/g and 9×10^3 cfu/g respectively. Microorganisms identified on all the cassava flour included *Bacillus cereus, Bacillus mycoides, Staphylococcus aureus, Micrococcus luteus, Streptococcus* sp, *Aspergillus niger, Candida* spp and *Saccharomyces cerevisiae*. Generally, there was good acceptability and organoleptic qualities (taste, aroma, colour, texture) in the protein enriched cassava flour when compared to the unenriched and commercial control cassava flour samples analyzed. This study suggests that cassava flour can be enriched with yeast strains to enhance nutritional quality.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Food is a basic human need, and everyone requires it for survival. Nigeria's agricultural sector provides food for the country's expanding population as well as money for millions of small holder farmers. It employs around 65 percent of the labour force for industrial development and provides raw materials to industries. In underdeveloped countries, cassava (*Manihot esculanta, Crantz*) is one of the most significant tropical root crops. For more than 500 million people, its starchy roots constitute an important source of dietary energy. In Nigeria, over 95 % of cassava produced is used for human food. It is a major staple food in Nigeria. A staple, as defined by the International Institute of Tropical Agriculture (IITA) (2009), is a food that is consumed on a regular basis and provides a significant amount of calories and/or nutrients to the population. Cassava, whether eaten raw or cooked, fulfills this function. Cassava has become a significant element of the diet of more than 70 million Nigerians as a result of increased urbanization (Food and Agriculture Organization (FAO) (2005).

Among staple crops, it is regarded to be the most carbohydrate-producing crop. Cassava, on the other hand, is high in carbohydrates but poor in protein, thus those who consume exclusively cassava are malnourished. During the twentieth century, the crop became more widespread and attained its current importance as a food source. According to Azogu (2010) the amount of cassava grown in Nigeria increased by 10 million tonnes in just four years. Cassava and its products have also seen an increase in demand on both the domestic and foreign markets. This is due to the crop's rising use as a human and livestock food source as

well as an industrial raw material. Demand has risen as a result of ongoing government programme aimed at increasing the production, use, and export of value-added cassava products, as well as significant population growth, which has necessitated the need for more convenience food. As a result, cassava's commercialization in Nigeria has the potential to be boosted (IITA, 2009).

Fermented cassava flour, as it's known in Nigeria, is a fermented cassava product that's popular in the country's south west (Adebayo-Oyetoro *et al*., 2017). It is made by submerging cassava roots in water for 3–5 days to allow the fermentation process to take place, then sun-drying the product before milling the dried fermented roots to powder. Various biochemical changes occur throughout the fermentation process, including the breakdown of cyanogenic compounds and the synthesis of flavour molecules (Adebayo-Oyetoro *et al*., 2013). The most essential indicator for determining the end of "lafun" fermentation is the degree of root softening. Pectinolytic enzymes such as pectin methyl esterase and pectate lyase have been reported to be active in this regard.

Cassava flour, like other traditional fermented foodstuffs, is spontaneously fermented and has a diverse microbiological composition. Microorganisms (algae, fungi, bacteria, and yeasts) can be used as a protein supplement since they contain a large amount of protein, grow quickly, and can be cultured on a variety of substrates (Bala *et al*., 2012). These microorganisms have a faster rate of growth than higher plants, making them particularly appealing as higher protein crops. While only one or two grain crops are cultivated each year, yeast or mold crops can be harvested weekly, and bacteria can be harvested on a daily basis. Yeast, on the other hand, has been the most extensively accepted and employed microbe for

improving human and animal food and feeds. It has the best protein composition, as it contains a wide range of amino acids and is the richest source of lysine (Ijah *et al*., 2004). Yeasts can be used to produce a variety of proteins. Their biomass has been employed as a direct protein supplement or as a by-product of fermentation (Day and Morawicki, 2018). According to Oseni and Akindahunsi (2011), fermentation can boost dietary protein content by secreting microbial proteins, accumulating other nitrogenous microbial components such as chitin and hydrolyzing peptides. When protein in the substrate is concentrated and carbohydrates are digested by microbes, the crude protein level of a food product may rise (Day and Morawicki, 2018). The introduction of yeast strains that boost the nutritional content and improve the quality of "lafun" could raise consumer acceptance, ensuring its survival in the food chain (Fawole *et al*., 2021).

1.2 Statement of the Research Problem

Energy, protein, and essential amino acids deficiency are all key issues in human nutrition in emerging and underdeveloped countries like Nigeria. Cassava products, such as cassava flour, are in high demand due to their high energy content, and they serve as a staple food for low-income earners in developing countries like Nigeria. However, indigenous spontaneous fermentation is characterized by a longer acidification time, inconsistencies in nutritional composition and poor quality of the final products. Protein-rich foods are generally expensive and out of reach for the majority of the population. Its scarcity has a greater impact on youngsters, whose physical and mental growth necessitates a nutritious diet (Uche *et al*., 2018). Due to the obvious poor protein content, low minerals, vitamins, and the presence of cyanide toxicity in cassava flour and other cassava products, its consumption has been linked

to malnutrition. However, efforts have been undertaken to enhance the poor protein content in cassava products, such as cassava flour by enriching it with microorganisms (Akoja and Mohammad, 2011).

1.3. Aim and Objectives of the Study

The aim of this study was to enrich the protein content of cassava with yeast for cassava flour production.

The objectives of this study were to:

i isolate and identify yeast strains from palmwine

ii screen yeast isolates for ability to grow in cassava medium

iii determine the proximate composition of enriched and unenriched cassava flour produced using yeast strains.

iv determine the microbiological quality of cassava flour produced using yeast strains (enriched and unenriched).

1.4. Justification for the Study

The ever-increasing use of cassava and its products in Nigeria, such as cassava flour, has necessitated this study due to its ease of cultivation, processing, and, as a result, accessibility to all. Protein content in carbohydrate-based foods can be increased to improve nutritional quality (Etsuyankpa *et al*., 2015). It can be deduced that yeast, a low-cost, non-pathogenic saprophytic aerobe, has been proven to improve the nutritional potential of cassava products by boosting nutrient content (protein). The use of microorganisms such as *Saccharomyces cerevisiae* and *Lactobacillus plantarum* for the enrichment of cassava products such as 'garri' and 'fufu' has brought some enhancement on the nutritional composition especially its

protein content (Bala *et al*., 2012; Rosales-Soto *et al*., 2016). Also, yeast such as *Trichoderma viride* have been used to enrich cassava by product such as peel which is used as animal feed (Olufunke *et al*. 2010). Scanty information has been reported on the potential of yeast for the enrichment of cassava flour. In addition, to the best of our knowledge, much work has not been reported for the enrichment of cassava flour with yeast but, work has been done for other cassava product like garri and fufu.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cassava Plant

Cassava (*Manihot esculenta Crantz*) is a woody plant native to South America that belongs to the spurge family, *Euphorbiaceae* (Venturini *et al*., 2016). Classification of cassava is shown in Table 2.1. Cassava is a widely cultivated annual crop in tropical and subtropical regions of the world for its edible starchy tuberous root, which provides energy to approximately 500 million people because it is a very cheap source of carbohydrate and is the main carbohydrate source in the diet of the teeming population of the third world countries where it is primarily grown. It consists of an ariel section (2-4 m) and a clandestine component (edible root) concentrated around the plant's lowermost half and extending 60 cm on all sides (Oyinlola *et al*., 2016). A mature root is made up of an innermost vascular core, cortex (flesh), and phelloderm (peels). Under ideal conditions, a single root can weigh up to four kilograms. Apart from being the supplementary staple food of many Africans, Asia, South America and India countries, it was however considered as a substandard food crop (Oyinlola, *et al*., 2016), poor people′s crop (*Venturini et al., 2016*) and a hazardous crop because of its major limitations like low protein content, short post-harvest shelf life and the presence of toxic cyanogenic glucosides. Garri, lafun, and fufu are traditional African foods made from cassava by a number of processes including jarring, dewatering, fermenting, and roasting. Cassava is also utilized as feed for monogastric animals (Banjoko *et al*., 2008).

Table 2.1 Classification of Cassava

Taxonomic group	Plant
Kingdom	Plantae
Pylum	Tracheophytes
Class	Angiosperms
Order	Malpighiales
Family	Euphorbiaceae
Genus	*Manihot*
Specie	*Esculenta*

Source: (Fawole *et al*., 2019)

2.1.1 Origin of cassava

Cassava has become a security and industrial crop in tropical Africa, alleviating the food crisis in many war-torn and famine-ravaged areas of the continent since it can be grown year-round, regardless of the seasons. It has a high yield and requires little water to grow (Oyinlola *et al*., 2016). Although it was thought to have originated in Venezuela, South America, around 2700 B.C., Pope *et al*. (2001) discovered that the Portuguese were the first to introduce it to Nigeria and other regions of West Africa. According to FAO estimates, African countries contributed 37 percent of the 13.4 million ton global production. Nigeria, Benin, Kenya, Zambia, Tanzania, Uganda, Ghana, Zimbabwe, the Democratic Republic of Congo, and Mozambique are the top African cassava producers (Oyinlola *et al*., 2016). In 2018, global cassava root production totaled 278 million tonnes, with Nigeria accounting for 21% of global production. Thailand and the Democratic Republic of Congo were also major producers (Food and Agriculture Organization Corporate Statistical Database) (FAOSTAT, 2019). Although it has now become one of Nigeria's most important staple food crops, it is known by several names among the various cultural groups of the country. The Yoruba name it gbaguda or ege, the Hausa call it rogo, karaza or doyar kudu, the Igbo call it akpu, abacha or jigbo, igari by the Benin’s, the Efik call it iwa unene, and imidaka by the Urhobo’s.

2.1.2 Importance and consumption of cassava

Because of its efficient production of food energy, year-round accessibility, tolerance to extreme weather conditions, and compatibility to current farming and food systems in Africa, cassava plays a crucial role in alleviating food crisis in African (Oyinlola, *et al*., 2016). Cassava's value has been widely recognized, such

as its usage as a source of income and raw materials in the production of processed foods, animal feed, and industrial items (Gupta, et al., 2003). Cassava products can also be used to boost revenue for producers, processors, and traders in rural areas (Salvador *et al.,* 2014). Using the International Food Policy Research Institute baseline data, it was projected that total world cassava use would increase from 172.7 million to 275 million tons over a 27-year period (1993-2020), whereas a higher prediction of demand and production growth puts 2020 production at 291 million tons (Scott *et al*., 2000). Cassava consumption in Africa accounts for 62 percent of global production, with an average of 102 kilograms per person per year (Etsuyankpa *et al*., 2015). Almost all of the seven species of cassava grown around the world are used for human use, either as flour or in fermented forms and other products, with barely 5% being utilized industrially (Ajao and Adegun, 2009; FAOSTAT, 2019).

Figure 2.1: Matured Cassava Root

Source : (Fawole *et al*., 2019)

2.1.3 Cassava varieties

Natural cassava varieties (also known as cultivars) are classed based on morphological characteristics such as taste, cyanide content, average yield, disease performance, and pubescence (Gbadegesin *et al*., 2013). However, a number of regional programs have been established to develop cassava cultivars in order to increase productivity and disease resistance. Physicochemical, functional, and other quality parameters of cassava products are heavily influenced by varietal chemical composition, such as dry matter, starch content, and quality, according to several studies (Oyinlola and Onilude, 2018). More than 5,000 cassava cultivars have been identified worldwide (Salvador *et al*., 2014). In Nigeria, numerous improved cassava varieties have been suggested and distributed (IITA, 2009). Cassava comes in two flavors: sweet and bitter (Salvador *et al*., 2014). The flavor of the root is determined by the amount of cyanogenic glycoside present in it. Cyanogenic glycosides have cyanide equivalents ranging from 10-500mg/kg dry matter (Salvador *et al*., 2014). Bitter variants have more than 100 mg of hydrogen cyanide per kilogram of fresh weight (Mckey *et al*., 2010). Bitter cassava variants account for over 90% of cassava production due to increased yields and pest and disease resistance. It can be stored in the soil for more than a year. Sweet cultivars have less than 100 mg of hydrogen cyanide per kilogram of fresh weight and can be eaten raw or fresh (Donovan *et al*., 2011).

2.1.4. Nutritional composition of cassava

Cassava's nutritional profile varies depending on the plant portion (root or leaves), geographical region, variety, age, and environmental circumstances (Salvador *et al*., 2014). Cassava roots are primarily made up of carbohydrates, and while they are low in protein (less than 1.5 % of fresh weight), they are high in carbohydrates (31

percent of fresh weight), the majority of which is present as starch, which accounts for 32-35 percent of the mass of fresh roots and 80-90 % of the mass of dried roots (Blagbrough *et al*., 2010; Salvador *et al*., 2014). According to Salvador *et al*. (2014) the carbohydrate composition of cassava roots is primarily made up of amylopectin (83%) while amylase comprises just about 17 %. Water is a key element in cassava storage, with low levels resulting in a more desired and longer shelf life (Padonou *et al*., 2010; Harris and Koomson, 2011). Cassava has a protein concentration of roughly 1-2 %, making it mostly a carbohydrate meal (Charles *et al*., 2005). On a dry matter basis, the protein content is low (1–3 %) and between 0.4 and 1.5 percent per 100 g fresh weight (Fadahunsi *et al*., 2011). However, total protein accounts for roughly half of the crude protein in the roots, with the remaining half consisting of free amino acids (mainly glutamic and aspartic acids) and non-protein components such as nitrite, nitrate, and cyanogenic chemicals. According to Charles *et al*. (2005) the lipid content of cassava roots varies between 0.1 and 0.3 percent on a fresh weight basis and between 0.1 and 0.4 percent on a dry weight basis. When compared to maize and sorghum, this concentration is modest, but it is higher than potato and comparable to rice. The root also contains a lot of dietary fiber, however according to Salvador *et al*. (2014) the amount of fiber varies depending on the variety and stage of growth of the cassava root. It is less than 1.7 percent in fresh root, but 4 percent in cassava flour. Cassava also has low levels of minerals like magnesium, salt, zinc, and iron compared to other carbohydrate foods. It is high in vitamin c between 15-45mg/100g for edible portion but low in all other vitamins (Charles *et al*., 2005). Even though the cassava tuber has been chastised for its low protein content, it produces more carbohydrate per unit area than other staple food crops

grown under similar agro-climatic conditions, making it an energy-dense food with a high energy value of 250 x 10^3 cal/ha/day, compared to 176 x 10^3 for rice, 110 x 10^3 for wheat, 200 x 10^3 for maize, and 114 x 10^3 for sorghum (Jisha *et al*., 2010). Raw cassava root however, has more carbohydrate than potatoes and less carbohydrate than wheat, rice, yellow corn, and sorghum on a 100g basis (Montagnac, 2009). Palmitate and oleate are the most common fatty acids found to be present in cassava (Oyinlola *et al*., 2016).

2.2 Cassava Fermentation

Cassava fermentation has been reported to be the most important and commonly utilized method of cassava processing. The historical traditional cassava processing method is still in use, but it is associated with a number of issues because it relies on random inoculation from the environment (Oyinlola *et al*., 2016). As a result, the fermentation process takes longer time and product quality varies from one processor to the other, as well as from one production batch to the next even within the same processor and from one season to the other (Onyinlola and Onilude, 2018). improvements in cassava processing, which have been employed over the years has helped to reduce the duration of processing to economically viable limits, maximize the detoxification process in order to improve the final products' physical and nutritional properties. Fermentation techniques vary from one region to another such as grated root fermentation, underwater/soaking fermentation and mold fermentation. (Etsuyakpa *et al.,* 2015). Cassava root fermentation is performed in numerous parts of Africa for a variety of reasons. The main reasons are to obtain a desirable sour product, such as gari, agbelima, and fufu, to remove a significant

proportion of cyanide from high cyanide cassava types, and to change the texture of the final product (Oyinlola *et al*., 2016). The first stage of cassava fermentation is the retting or breakdown of cell membranes with the help of microbes, followed by microbial fermentation. Microbial fermentation has been used for many years in all regions of the world to improve palatability and textural quality, as well as to increase nutritional value by adding protein and lowering harmful components (Alloys & Ming, 2006).

2.2.1 Submerged fermentation

Underwater fermentation is the cultivation of microorganisms in liquid nutrient medium in which the bioactive compounds are secreted into the fermentation medium (Subramaniyam and Vimala, 2012). It involves the soaking of cassava roots under water for 3-5 days, causing the root to relax and expand, allowing linamarase and linamarin to mix while also allowing cyanogens to be leached (Oyinlola *et al*., 2016). The softened roots can be readily broken into bits by hand, sieved to remove the fibers and leave a smooth paste. In the initial phase, a mixed microflora was reported to be involved, which was later dominated by lactic acid bacteria (Oyinlola *et al*., 2016). The size to which the roots were cut prior to soaking was also found to affect the pace of fermentation and the quality of the product (Oyinlola *et al*., 2016).

2.3 Role and Function of Fermentation on Cassava Based Food

Fermentation is defined as the activity of microbes and/or enzymes on animal or plant tissues to produce desired biochemical changes and significant food quality adjustment thus, qualifies cassava and its by-products products as fermented foodstuffs (Niguse and Chinthapalli, 2020). The general reasons for such fermentation are as listed below:

2.3.1 Aroma and flavor change

Fermentation changes raw food in a variety of ways, giving the fermented product new sensory attributes. The balance of unstable molecules formed during fermentation (aldehyde, organic acids, alcohol, alkanes, terpenes, ketones, nitrogen compounds) determines the aroma and flavor of food (Tefera *et al*., 2014). *Saccharomyces cerevisiae* and several lactic acid bacteria enhanced unstable chemicals in fermented rice, according to Hasan *et al*. (2014). According to Niguse *et al*. (2019) the number of unstable chemicals such as organic acid, aldehyde, alcohols, alkanes, and ketone created during fermentation is dependent on the microorganisms involved in the process.

2.3.2 Food preservation

Some biochemical compounds produced during the cassava fermentation process, such as hydrogen peroxide, can be inhibitory to some microorganisms (Aloys and Zhou, 2006), whereas carbon dioxide produced during fermentation can directly produce an anaerobic environment that is toxic to some aerobic food microorganisms due to its action on cell membranes. The optimal pH conditions for *Lactobacillus plantarum* and *Saccharomyces cerevisiae,* according to Gunawan *et al*. (2015), were 3.5-4.5 and 3.5-6.0, respectively. The growth of pathogenic

microbes is hindered when the pH is reduced below 4, which aids in the synthesis of organic acid and so extends the shelf life of foods (Taiwo, 2009).

2.3.3 Anti-nutrient reduction

Anti-nutrient chemicals such phytates are prevalent in cassava (624 mg/100 g in roots) and can bind cations including magnesium, calcium, iron, zinc, and molybdenum, preventing mineral absorption and utilization (Hambridge *et al*., 2008). Phytic acid can also bind proteins, preventing them from being fully digested by enzymes. According to Taiwo (2009), fermenting cassava lowered phytic acid levels in ikivunde from 6.12 to 0.66 g/kg and inyanga from 6.12 to 1.44g/kg. The enzymes phytase and phosphatase hydrolyze phytate into inositol and orthophosphate, resulting in phytate decrease during fermentation. According to Guira *et al*. (2016) phytate loss is greatest after 24 to 48 hours of fermentation and declines after 48 hours. After 48 hours, the pH drops, slowing the breakdown of phytate. Tannin is another anti-nutrient found in cassava root. It reduces the nutritional content of food by building a compound with protein, which prevents digestion and absorption. Taiwo (2009) found that 4.2 g/kg of tannin before fermentation was reduced to 0.63 and 2.3 g/kg at the conclusion of the fermentation period in ikuvunde and inyanga respectively.

2.3.4 Cyanide reduction

In cassava, cyanogenic glucosides are produced in the form of linamarin (93 %) and lotaustralin (7 %). (Guira *et al*., 2016). When compared to the FAO/WHO (2005) recommendation of less than 10mg cyanide equivalents/kg DM to prevent acute poisoning in humans, this number is higher. The remaining cyanogen content in

processed cassava, such as glucoside, cyanohydrin, or free cyanide, is just as dangerous as the parent chemicals in fresh cassava (Taiwo, 2009). Fermentation has been shown to lower the cyanide concentration of cassava (Niguse *et al*., 2019). After 24 hours of fermentation with *L. plantarum* at a 0.5 mL inoculum level, the free cyanide level in unfermented cassava reduced from 197.19 mg/g to 4.09 mg/g. Niguse and Chinthapalli (2020) observed a drop in cyanide level in fermenting cassava from 1158 to 339.6 mg/kg after 48 hours of fermentation, a reduction of 70.67 %. According to Niguse *et al*. (2019) *Lactobacillus plantarum* and *Lactobacillus coryneformis* play a crucial role in cyanide detoxification.

2.3.5 Protein enrichment

Since cassava root is a primary source of carbohydrate and has a low protein level, post-harvest processing procedures may be used to improve its protein content (Montagnac *et al*., 2009; Tefera *et al*., 2014). Nwabueze *et al*. (2007) discovered that after 48 hours of fermentation, the protein content of fermented cassava increased from 0.74 percent to 4.58 percent. Similarly, the crude protein content of cassava-teff flour fermented with 1.5 mL *Saccharomyces cerevisiae* inoculums (13.31±0.02 %) was higher than *Lactobacillus plantarum* and *Lactobacillus coryneformis* (Niguse *et al*., 2019). Boonnop *et al*. (2009) discovered that fermenting cassava chips with *Saccharomyces cerevisiae* can boost protein content from 2 % to 32.4 %. The increase in crude protein content could be attributed to extracellular enzyme production and the effect of microbial cell expansion (Bala *et al*., 2012).

2.4 Microorganisms Associated with Cassava Fermentation

Starch is a complex carbohydrate that can be degraded either by microorganisms that produce α-amylase or an inducible/constitutive amylase to produce simple sugars which can then be readily metabolized by many microorganisms. It has been claimed that indigenous natural fermentation involves a mixed colony of microorganisms such as molds, bacteria, and yeasts (Ekundayo and Okoroafor, 2012; Guira *et al*., 2016). Despite the fact that microbial size in food is often small, they have a significant impact on the nature of the meal, particularly in terms of flavor and other organoleptic properties. Because the type of bacterial flora created in each fermented food varies depending on water activity, pH, salt concentration, temperature, and substrate composition, fermentation products are determined by the microorganisms involved in the fermentation process (Oyinlola *et al*., 2016). These bacteria are non-toxic to humans and produce enzymes that hydrolyze food, such as proteases, amylases and lipases which break down food into simple, non-toxic compounds with a pleasant texture and aroma (Oyinlola *et al*., 2016). A wide spectrum of microorganisms have been linked to cassava fermentation by numerous researchers and these includes *Bacillus*, *Leuconostoc*, *Klebsiella*, *Corynebacterium*, *Lactobacillus*, *Aspergillus*, *Candida*, *Geotrichum*, *Streptococcus*, *Enterococcus*, *Aerococcus* and *Pediococcus* species (Guira *et al*., 2016). However, Omemu *et al*. (2011) also reported that *Saccharomyces*, *Candida*, *Kluyeromyces*, *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium* and *Debaryomyces* are involved in the fermentation of cassava. Lactic acid bacteria are mainly responsible for the rapid acidification that characterizes cassava fermentation. *Bacillus* sp. seems to be responsible for inducing the retting of cassava root tissues during the submerged fermentation of whole roots. Additionally, root fermentation has been attributed to lactic acid

bacteria and it is essential for the development of the sensory attributes and the preservation of cassava foods.

2.5 Products Made from Cassava

Cassava roots are huge and damp, as previously mentioned, making transportation of the tubers the markets laborious and expensive. Because they contain cyanide, which is fatal to humans and animals, raw roots and uncooked leaves are not palatable. As a result, it must be processed into a variety of forms in order to increase shelf life, facilitate shipping and marketing, lower cyanide levels, and improve palatability. Cassava roots have traditionally been processed into a number of products and used in a variety of ways, depending on regional customs and preferences. Cassava roots and leaves are utilized in a number of traditional cuisines, and the leaves are consumed as vegetables in some locations. Cassava can also be fortified with other protein-rich crops to increase its nutritional profile (Oyinlola *et al*., 2016). Garri, abacha, cassava flour, edible starch (usi), fufu, tapioca cakes, and other traditional cassava processing methods are known, and some of the outputs of these processes in Nigeria include garri, abacha, cassava flour (lafun), edible starch (usi*),* fufu, and tapioca cakes.

2.5.1 Cassava flour

Cassava flour is traditionally made and sold as a fermented dry powder that may be made into porridge and eaten with boiling water. 'Cossette' is the name given to it in Zaire and Rwanda; "lafun*"* by the Yorubas; and "garin rogo" by the Hausas in Nigeria. It is primarily consumed in Nigeria's western regions, as well as sections of West and Central Africa. Whole or peeled roots are soaked in water for 3-4 days

for fermentation and tissue softening. The fermenting roots are removed and crushed into little crumbs, which are then dried in the sun on mats, racks, flat rocks, cement floors, or roofs. Depending on the weather, the fermented roots must be dried for 1-3 days. The dried crumbs are then crushed into flour (Guira *et al.*, 2016).

2.6 Methods of Improving Nutrient Contents of Cassava Products

There are two main strategies for boosting the nutritious content and amino acids of fermented cassava products (Oyinlola *et al*., 2016). First, microorganisms could be coaxed to develop in huge quantities in the mash before fermentation through controlled fermentation. Palm wine have been shown to contain yeasts which are predominantly *Saccharomyces sp* as well as a variety of bacteria, including lactic acid and acetic acid bacteria, *Bacillus*, *Corynebacterium*, and *Staphylococcus*. The microorganisms in palm wine descend to the bottom of the storage vessels after a while. This section of the drink is unappealing due to its high density, which can account for up to 20% of the entire volume of the drink. After the supernatant is drunk, this precious protein-rich biomass is discarded (Oyinlola *et al*., 2016). Because of the high concentration of these organisms in the dregs, palm wine could be utilized as an inoculum for protein and amino acid enrichment of cassava flour and other fermented starchy foods. The second technique comprises supplementing the insufficient diet with protein from external sources while preserving the organoleptic qualities of the original food. This technology has an advantage over the first because of the vast range of protein sources available, its simplicity for groups with limited technological capabilities and its comparatively low cost (Uche *et al*., 2018). Other researchers have found that cassava products supplemented with

adequate vitamins, minerals, and protein offer the poor and malnourished with a full day's worth of nourishment in a single meal. Given the low protein content of cassava, this is consistent with the fact that the majority of people who eat cassava products as a main staple food are poor and often cannot afford the protein component of fish or meat required to balance cassava meals (1 - 2 %).

2.6.1 Nutritional enhancement of cassava products through microbial fermentation

Oboh *et al. (*2002) evaluated gari and cassava flour fermented with pure strain of *A. niger*. The results of the proximate analysis revealed that there was a significant increase ($P < 0.05$) in the protein content (flour (12.2±0.2 %) gari (7.3±0.1)). Conclusively, the results of the study showed that *Aspergillus niger*, a cheap, non-pathogenic saprophyte, has the capability to increase the nutritional potential of cassava products by increasing the protein and fat contents present in them. Oyinlola *et al*. (2016) carried out analysis on flour and gari processed from cassava pulp inoculated with *Saccharomyces cerevisiae* which revealed that there were significant increases in the protein content (flour (10.9 %), gari (6.3 %)). Gari was prepared from cassava mash inoculated with 0, 1, 5 and 10 % (v/w) of palm wine dregs just before dewatering and fermentation and analyzed for protein content and other characteristics by Bala *et al.* (2012). Inoculation with palm wine dregs increased microbial activity in cassava mash, particularly the activity of lactic acid bacteria. The results showed that protein composition of gari was improved. Mohammed-Lawal *et al.* (2013) carried out comparative studies on the ability of pure strain of *Rhizopus oryzae* and *Saccharomyces cerevisae* to alter the nutritional quality of cassava flour produced from low- and medium-cyanide variety of cassava

tubers. The proximate, mineral and the anti-nutrient composition of the cassava flour were determined. The results of the study revealed that the unfermented flour from low-cyanide cassava variety had higher protein, fibre, ash, fat, Ca, Na and K contents, while those produced from medium-cyanide variety, had higher anti-nutrients (tannin, cyanide and phytate), Zn, Mg and Fe contents. However, solid substrate fermentation of the cassava mash using *R. oryzae* and *S. cerevisiae*, independently, caused a significant ($P < 0.05$) increase in the protein and fat content. The nutrient enrichment was significantly higher ($P < 0.05$) in flour produced from low-cyanide cassava variety. In addition, *S. cerevisiae* fermentation brought about a higher increase in the nutrient content than *R. oryzae* fermentation. Conversely, fermentation of the cassava caused a significant decrease ($P < 0.05$) in the anti-nutrient content of the flour; although, the level of decrease was more in the flour produced from low-cyanide variety than medium-cyanide variety (Oboh and Elusiyan, 2007). Krisada *et al*. (2009) conducted a study on cassava root fermentation by yeasts in order to enhance the nutritive value of their products (fresh pulp and chips). Products were analyzed for proximate composition, mineral composition, essential amino acids and anti-nutrient content. The result of the analysis showed that there were increases ($P < 0.01$) in protein (30.4 % in cassava chip (CC) and 13.5 % in fermented cassava root (FCR) and fat contents. *Candida utilis* strain BKT4 and *Saccharomyces cerevisiae* strain BKT7 isolated from burukutu (a local wine brewed from sorghum) were used to enrich fufu as evaluated by Fagbemi and Ijah, (2006). Fufu (prepared by crushing and sieving fermenting cassava roots) enriched with 0.5 g of *C. utilis* strain BKT4, *S. cerevisiae* BKT7 and a mixed culture of the two organisms revealed a crude protein of 7.90, 6.34 and

10.0% respectively as compared to 2 % protein content of the enriched fufu. There was a corresponding increase in protein content of the product as the quantity of the enrichment yeast was increased from 0.5 to 3.0 g (Fagbemi and Ijah, 2006). Yeasts are famously known for their roles in food production. They are the critical component in the fermentation process that converts sugar into alcohol, an ingredient shared in beer, wine and distilled beverages.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Niger State, a predominantly rural, and among the least industrialized states in the federation, belongs to the second generation of states. The State is located between latitude 8º20´ N and 11º30´N and between longitude 3º30´ E and 7º20´ E thus giving the geographical location of the State within the middle belt or what is now referred to as the North Central part of Nigeria. The map of Niger State is shown in Figure 3.1. At its creation, Niger State was made up of seven local government Areas (LGAs) but through subsequent administrative reforms, this number rose to ten in 1987 and nineteen in 1991.

The State has the highest land area in Nigeria accounting for about 9% (about 76,000 km2) of the country's total land area (8.44 × 105 km2) due to the inclusion of Borgu and Agwara as part of its local government. Niger State experiences the hottest period of the year in March and April just before the onset of the first rains and throughout the year, the daily maximum temperature is as high as 32 °C. According to the 1991 national head count, Niger State had a total population of 2,482,367 people and 52 % of this figure (1,290,720) was males while 48 % (1,191,647) were females.

The average population density of the state is very low about 33 persons per square kilometres) in large Local Government areas like Borgu, Shiroro and Wushishi. This suggests that the state is endowed with expanses of large land resources waiting to be developed by agricultural activities and industrialization.

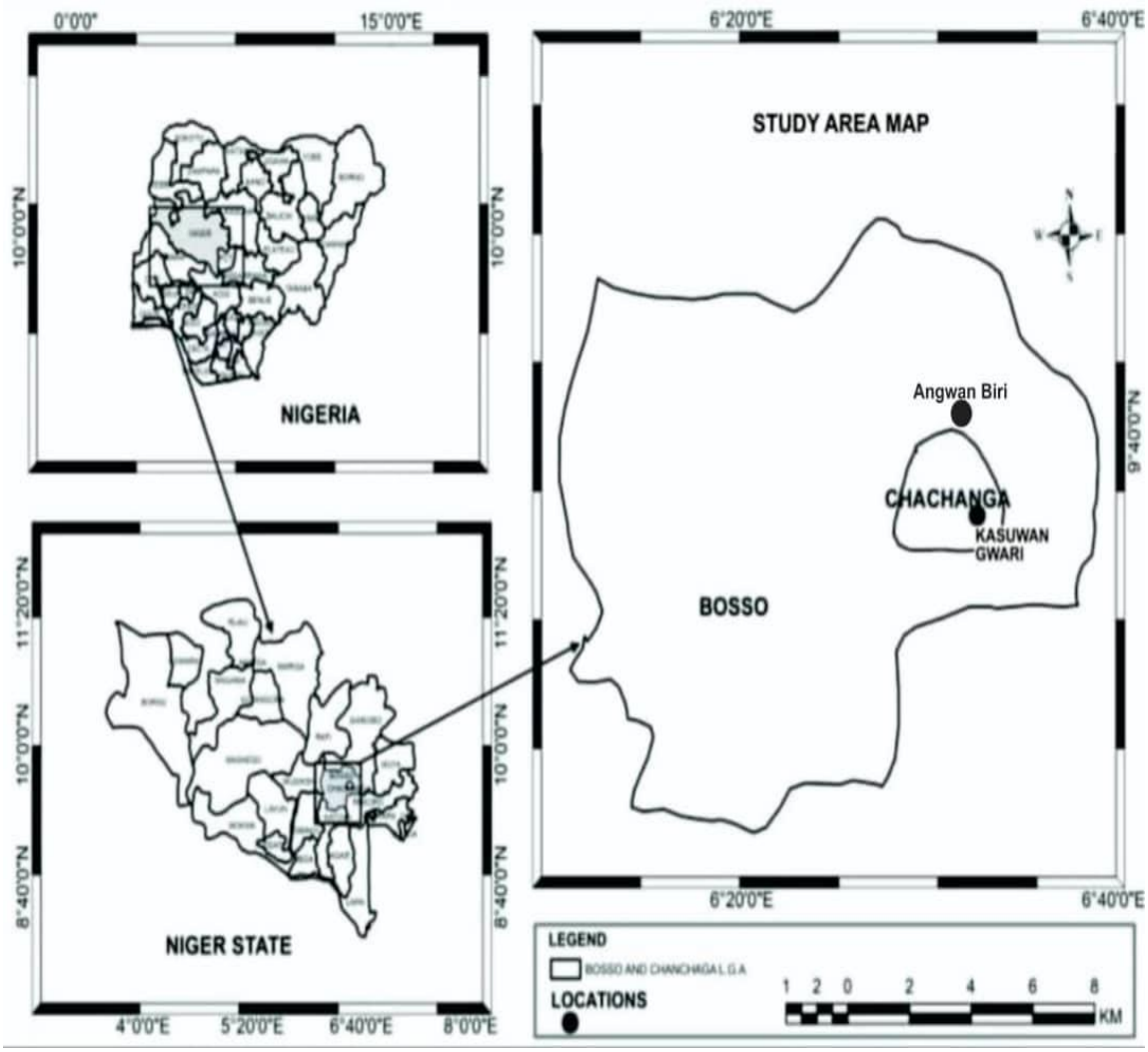

Figure 3.1: Map of Niger State

Source: (Geography Dept, Federal University of Technology Minna).

3.2 Experimental Design

A completely randomized design (CRD) was used in this study. Five experimental units (200g of chopped cassava root) designated A-E was set up. Each chopped cassava (A-D) was inoculated with yeast isolated at random. Each treatment was duplicated. The fifth experimental unit which was designated E served as control without inoculation with yeast. No restriction was imposed on the randomization.

3.3 Collection of Samples

Samples of palm wine were collected in sterile bottles from sales point at Angwan Biri, Bosso, Minna, Nigeria and transported in ice box to the laboratory for the isolation of yeasts. Fresh tubers of cassava (*Manihot esculenta crantz)* were obtained from Gwari market, Minna, Nigeria in polythene bags and transported to the laboratory.

3.4 Isolation of Yeasts

The pour plate method described by Bala *et al.* (2012) was employed for the isolation of yeast species. A milliliter of each of the palmwine sample was transferred into 10 mL of sterile distilled water to obtain a stock solution.10^{-1} dilution was obtained by transferring 1 mL of the stock solution into 9 mL of sterile distilled water. Further serial dilutions were made up to 10^{-4} Yeast strains were isolated by plating out the 3rd diluents on Sabouraud dextrose agar (SDA) and incubated at room temperature (28± 2^{o}C) for 48 hours. Different isolated colonies were sub-cultured repeatedly on fresh media to obtain pure cultures of the isolates which were then stored on slant bottles for further use.

3.5 Identification of Yeasts

Identification of yeast was done by standard morphological and physiological tests as described by Kurtzman *et al.* (2011). Yeast morphology under magnification by microscope was noted and colony appearances on agar plates were observed for surface appearance, margin, colour and shape. They were also characterized using cellular characteristics, ascospore formation, vegetative reproduction and utilization of sugars. The organisms were identified by comparing their characteristics with those of known taxa using the scheme of Cheesebrough, (2012).

3.5 1 Sugar fermentation potential

Sugar fermentative ability of the yeast isolates as described by Jimoh *et al.* (2012) was determined using the following sugars: glucose, sucrose, lactose, galactose, maltose and raffinose. The basal medium contained 0.5 % (w/v) yeast extract, 0.75 % (w/v) peptone and phenolphthalein red indicator, 2 g of each of the sugars and chloramphenicol. The medium was dispensed into test tubes containing inverted durham tubes, cotton-plugged, sterilized at 121 ^{o}C for 15 minutes and allowed to cool at room temperature (28±2 ^{o}C). The test tubes were inoculated with the yeast isolates. The un-inoculated tube was used as control for each sugar substrate and the growth medium tubes were incubated at room temperature (28 ^{o}C) for 48 hours. Thus, gas production and medium colour change indicated the fermentative activity of the yeasts.

3.6 Screening of Yeasts Isolates for Ability to Grow in Cassava Medium

The method described by Bala *et al*. (2012) was employed in the screening of the yeast isolates for their ability to grow on cassava medium. Mineral salt broth (MSB) containing 0.2 g $MgSO_4$, 1.2g KH_2PO_4, 1.2g NaCl, 1.0g NH_4Cl, 1.0 g yeast extract, 0.5 g peptone water, 5.0 g cassava flour (carbon source) and chloramphenicol was prepared in 250 mL

of water. The medium was dispensed into test tubes, sterilized at 121 ^{o}C for 15 minutes and allowed to cool at room temperature (28±2 ^{o}C). The test tubes were inoculated with the yeast isolates. The un-inoculated tube was used as control and the tubes were incubated at room temperature (28±2 ^{o}C) for 48 hours under stationary condition. At the end of the incubation, the medium was observed for turbidity using Uv-Spectrophotometer at wave length 600nm as index utilization of the incorporated carbon source.

3.7 Molecular Identification of Yeast Isolates

3.7.1 DNA extraction

Genomic DNA extraction was carried out using the protocol stated by Campbell-Platt (1994) with solution Preparation Kit following manufacturer's instructions. Fungi cells were harvested from 1000 µL aliquot of broth culture using a micro-centrifuge at 15,000 g for 1min. The residual pellet was resuspended in 300 µL of resuspension Buffer and 1.5 µL of proteinase K Solution. The mixture was homogenized by inverting several times thereafter incubated at 55 °C for 1 hour. Resuspended cells were recovered by centrifugation and lysed by adding 300 µL of Lysis solution. The mixture was vortexed vigorously and centrifuged at 15,000 g for 3 mins after adding 100 µL of protein precipitation solution to precipitate the protein. The supernatant was transferred to a clean 2 µL micro-mixed gently by inverting for 1min to precipitate the DNA. DNA was pelleted by centrifugation at 15,000 g for 1 min, washed with 500 µL washing buffer and washing buffer was discarded. 50 µL hydration solution and 1.5 µL RNase A was added to the air-dried DNA pellet. The sample was subjected to initial incubation at 37 °C for 1 hour followed by a final incubation at 65 °C for 1 hour to completely hydrate the dried DNA pellet.

3.7.2 Polymerase chain reaction (PCR)

Each PCR reaction mixture consisted of 12.5 µL mastermix (2x JENA Ruby hot start mastermix), 1 µl (10pmol) each of forward primer ITS1-TCCGTAGGTGAACCTGCGG and reverse primer ITS4-TCCTCCGCTTATTGATATGC, 1 µl DNA template and 9.5µl sterile nuclease free water to make up a total reaction volume of 25 µL. PCR amplification was carried out in an Applied Biosystem 2720 thermocycler. The mixture was subjected to an initial denaturation at 95 °C for 5 mins; followed by 35cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 45 s; and a final extension at 72 °C for 5 mins

3.7.3 Gel electrophoresis:

PCR products were visualized on a 2 % agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0) using blue led transilluminator (Figure 1).

3.7.4 Sequencing of genes

PCR products were purified and sequenced by Sanger sequencing method using AB1 3730XL sequencer and done by Inqaba biotec, Pretoria, South Africa.

3.8 Production of Biomass

The method described by Fawole *et al*. (2021) was employed in the production of biomass for the enrichment process with little modification. 24 hours old cultures of the yeast isolates were grown in 10 mL sterile potatoe dextrose broth for 24 hours. Aliquot (1 mL) of broth cultures were introduced into another batch of 9 mL sterile broth and incubated at 28^{o}C for 5 hours in order to reduce the yeast population. An aliquot (1 mL) was plated out on potatoe

dextrose agar in order to determine the inoculum size being used in the enrichment process.

3.8 Processing of Cassava Flour

Cassava roots was processed by peeling and washing cassava roots that are manually cut into different sizes by using a hand knife and soaking in water for 3 to 5 days for fermentation and softening of the tissues. The fermenting roots were then removed and crused into small crumbs, sun-dried on flat rocks, cement floors or roofs of houses. Figure 3.2 shows the processing procedures for cassava flour. Drying of the fermented roots takes 1-3 days, depending on the prevailing weather. The dried crumbs were then milled into flour.

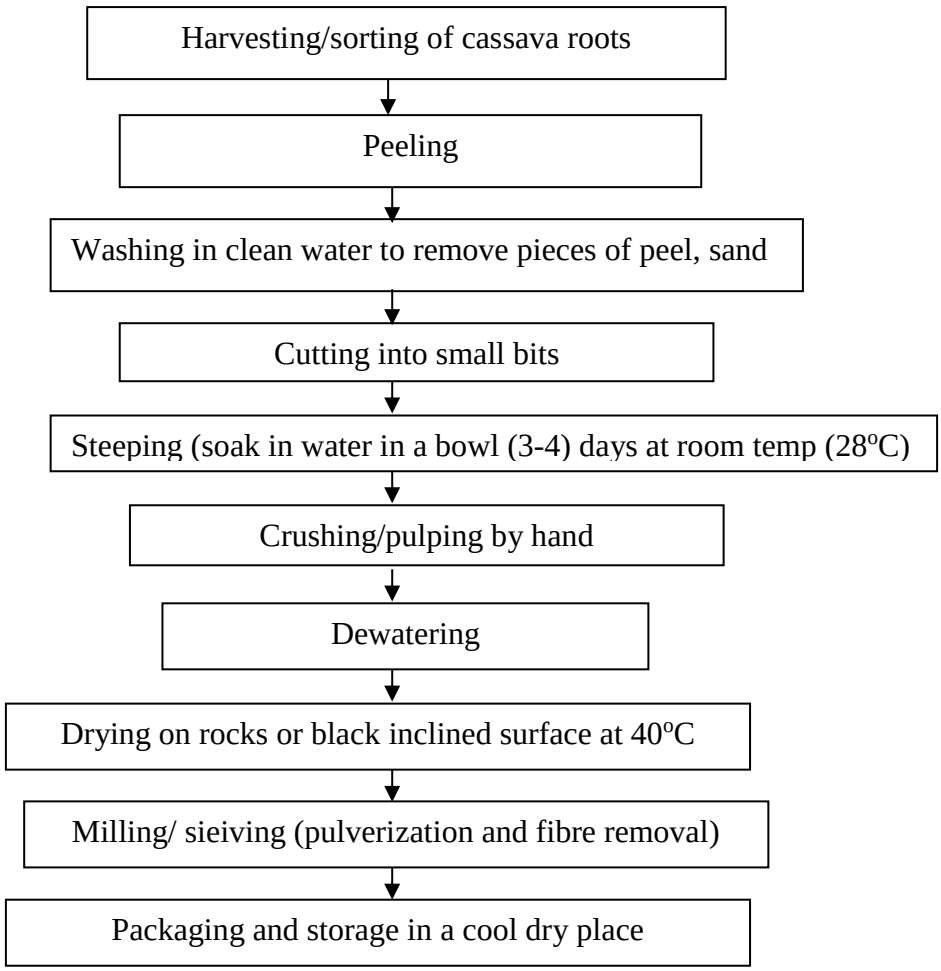

Figure 3.2: Cassava flour processing

3.9 Enrichment of Cassava Flour (Lafun) through Yeast Fermentation

Four cassava flour samples were produced using the selected strains in duplicate.

Aseptically, an aliquot (5 mL) of the resultant diluents of yeast cells were used as inoculums to inoculate 200 g of already peeled, chopped and washed cassava that was steeped in 2 L sterile water in 4 L sterile container. The steeped cassava was allowed to ferment under aerobic condition for 4 days at room temperature. A control experiment was set up in which the chopped cassava was not inoculated with the yeast cells and was also allowed to ferment for 4 days. This was also done in duplicate. Plate I shows the fermenting cassava pulp. At the end of the fermentation process, samples were dried on the sun for 3-5 days, milled, packed, and kept at -18 ^{o}C for subsequent analyses.

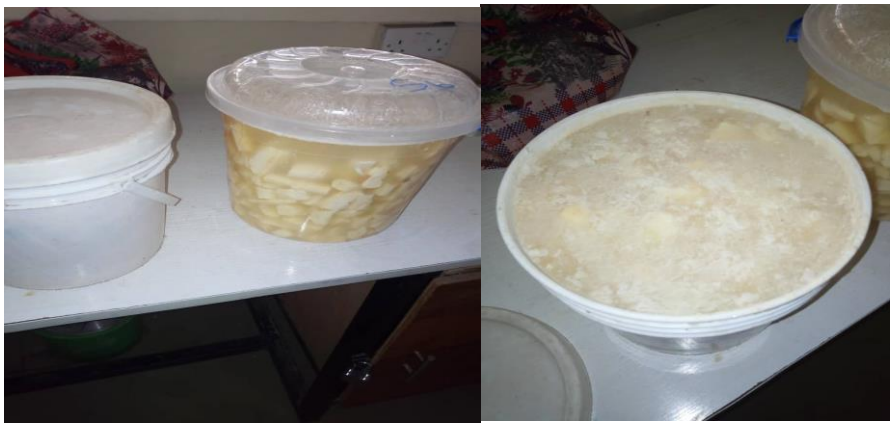

Plate I: Fermenting cassava pulp

3.10. Determination of Proximate Analysis of Enriched and Unenriched Cassava Flour

The method of Association of Analytical Chemistry (AOAC) (2012) was employed in determining the proximate composition of cassava flour to check whether there were significant differences in the nutritional composition of enriched and unenriched product. The cassava flours were analyzed for proximate nutritional components in Biochemistry laboratory, Department of Biochemistry, Federal University of Technology Minna. The crude fibre and protein, lipid, ash and Moisture contents were determined. An external

control obtained from Bosso market, Minna was also analyzed for proximate nutritional components in order to compare with the enriched samples.

3.10.1 Determination of ash content

Ashing of the samples at 400-600 ^{o}C was done in muffle furnace for 4 hours. Crucible was pre-heated in the oven for 30mins at 105 °C, cooled in the desiccator for about 1hr and weighed (W_1). 2 grams of the sample was added into crucible, given a new weight (W_2) It was then being placed in the muffle furnace for ashing at 55 °C for 3 hrs until the content became whitish in color with no black particles, it was removed and cooled in the desiccator, the weight was noted (W_3) and calculated using Equation 1:

$$\% \text{ Ash} = \frac{(W2-W3)}{(W2-W1)} \times 100 \qquad \text{Equation 1}$$

Where;

W1 = weight of crucible

W2 = weight of crucible + sample before ashing

W3 = weight of crucible + sample after ashing

3.10.2 Determination of protein content

Kjedhal Nitrogen method was employed for the determination of protein content. One gram (1.0 g) of cassava flour (lafun) was weighed in a digestion flask. Kjedhal catalyst (0.8 g) was introduced into each flask with 15 mL of concentrated sulphuric acid added. Each flask was heated on pre heated digester for about 30 minutes in fume cupboard. This was digested until a clear homogenous mixture obtained. The flask was removed from the heater, cooled after digestion and the content was diluted with 50 mL of distilled water. The flask was then placed in micro-kjedahl analyzer (distillation unit) where it will receive 5 mL of 40 % NaOH automatically. The mixture was subsequently heated up to

release ammonia which was distilled into a conical flask containing 25 mL of 2 % boric acid for about 15 minutes. During the distillation process, the ammonia was combined with boric acid to form ammonium borate solution which was titrated against 0.1 M HCl until a purplish- grey end point was attained. The percentage protein was calculated using Equation 2:

$$\% \text{ protein} = \frac{A\ X\ 0.0014\ X\ 6.25}{Weight\ of\ sample}\ X\ 100 \qquad \text{Equation 2}$$

3.10.3 Determination of moisture content

Moisture content was determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. The crucibles were weighed (W_1) and 5 grams of the sample was added into the crucible to give a new weight (W_2). It was then placed in the oven at 105 °C for 3 hours, after which it was removed, allowed to cool in the desiccator and oven dried again, this was repeated several times until constant weight was noted (W_2). The percentage moisture content was calculated using Equation 3:

$$\% \text{ Moisture} = \frac{(W2-W3)}{(W2-W1)} \times 100 \qquad \text{Equation 3}$$

Where;

W1 = weight of crucible

W2 = weight of crucible + sample before drying

W3 = weight of crucible + sample after drying

3.10.4 Determination of crude fibre

The organic residue left after sequential extraction of samples with petroleum ether can be used to determine the crude fibre. The fat-free material was then transferred into a flask/beaker and 200 mL of pre-heated 1.25 % H_2SO_4 was added and the solution was gently boiled for about 30 mins, maintaining constant volume of acid by the addition of hot water. The buckner flask funnel fitted with whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was filtered hot through the funnel under sufficient suction. The residue was then washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200 mL of pre-heated 1.25 % Na_2SO_4 was added and boiled for another 30 minutes. It was filtered under suction and wash thoroughly with hot water and twice with ethanol. The residue was dried at 65 ^oC for about 24 hours and weighed. The residue was transferred into a crucible and placed in muffle furnace (400-600 ^oC) and ash for 4 hours. It was cooled in desiccator and weighed. The percentage fibre was calculated using Equation 4:

$$\%\text{Crude fibre}=\frac{\text{Dry weight of residue before ashing} - \text{weight of residue after ashing}}{\text{weight of sample}}x100$$

Equation 4

3.10.5 Determination of crude fat

A powdered moisture free sample was weighed into 3 different pre-weighed fat-free Filter paper and wrapped separately. Filter paper free from fat was weighed (W_1). One gram (1.0 g) of the sample was added into the filter paper, carefully folded and tied to keep the sample intact, the new weight noted (W_2). A 500 mL round bottom flask was filled up to three-quarter with solvent (petroleum-ether).The flask was fitted to Soxhlet extraction with a reflux condenser and placed on an electro-mantle heater. Extraction began as the solvent start refluxing several times. Extraction continued for about 6 hours after which the condenser was detached, the defatted sample removed, and dried to a constant weight

in the oven at 105 °C for 2 hours. The difference between the weight of the defatted sample before and after drying was recorded as the weight of fat (W_3). The % fat was calculated using equation 5:

$$\text{Fat (\%)} = \frac{(W2-W1)}{W} \times 100 \qquad \text{equation 5}$$

3.10.6 Determination of carbohydrate content

The carbohydrate content of each sample was calculated by difference. The total of all the previously determined proximate parameters subtracted from 100 will represent the carbohydrate content. The % carbohydrate was calculated using Equation 6:

% carbohydrate = 100 – (% moisture + % crude fiber + % crude protein + % total ash)

Equation 6

3.11 Enumeration of Microorganisms in Enriched and Unenriched Cassava Flour

The pour plate method described by Cheesbrough (2012) was used for the isolation, enumeration and identification of bacterial and fungal species present in the enriched, unenriched and commercial control of cassava flour. A gram of each of the sample (grounded) was transferred into 10 mL of sterile distilled water to obtain a stock solution. A milliliter (1 mL) of the stock solution was transferred into 9 mL of sterile distilled water to obtain 10^{-1} dilution. Further serial dilutions were made up to 10^{-5}. An aliquot (1 mL) of the 4^{th} and 5^{th} were aseptically inoculated in duplicates on Nutrient agar (for total viable bacterial counts), MacConkey agar (for coliform counts), Mannitol salt agar (for Staphylococci), and the 3^{rd} and 4^{th} diluents were also aseptically inoculated on sabouraud dextrose agar respectively. The inoculated plates were incubated at 37 ^{o}C for 24 hours for bacteria and at room temperature (28± 2 ^{o}C) for 3-5 days for fungi. Colonies which developed on the plates were counted and expressed as colony forming units per gram (cfu/g) of sample. The isolates were purified by repeated sub culturing on fresh media,

stored on Nutrient Agar and Sabouraud agar slants which was then kept at 4 ^{o}C for further characterization and identification. The resulting colonies were counted using coulter counting chamber and calculated using the formula below:

Colony forming unit (cfu) = number of colonies x volume of diluent x reciprocal of dilution.

3.12 Identification and Characterization of Organisms isolated from Enriched and Unenriched Cassava Flour

Bacterial isolates were characterized and identified using series of cultural morphological (Gram staining) and biochemical tests such as, coagulase, catalase, carbohydrate fermentation (glucose, lactose and sucrose) citrate utilization, urease production, methyl red, starch hydrolysis, Voges-prokauer and haemolysis production. The bacterial isolates were identified by comparing their characteristics with those of known taxa using the scheme of Buchanan and Gibbons (2008).

3.12.1 Gram staining

Grease-free glass slides were used to prepare smear and the slides were placed on the staining rack. The smear was covered with crystal violet stain and left for 1 minute then washed carefully under running tap water. The smear was flooded with Gram's iodine solution and left for 1 minute. The iodine was drained off the slides and washed in a gentle stream of tap water. The slides were flooded with alcohol for 30 seconds and washed under running tap water then drained completely. The slides were counterstained with safranin for 1 minute and washed in a gentle stream of tap water until no color appears in the effluent then the slides were blotted dry with absorbent paper and observed under the microscope. Gram-positive bacteria appeared dark purple while Gram-negative bacteria appeared pale to dark red (Cheesebrough, 2012).

3.12.2 Motility test

From fresh overnight liquid cultures, a straight wire loop was used to inoculate tubes containing the medium by stabbing straight halfway the tubes. The inoculated tubes were incubated at 37 ^{o}C for 24 hours. The tubes were observed for the presence or absence of growth along the line of stab. Motile bacteria grew along the line of stab and diffused into the medium with turbidity while non-motile bacteria grew only along the line of stab and did not diffuse into the medium without turbidity (Cheesebrough, 2012).

3.12.3 Oxidase test

Colonies from 24 hours cultures were placed on filter papers and a drop of oxidase reagent was added onto each filter paper and examined within 10 seconds. Oxidase-positive bacteria developed bluish-purple colour while oxidase-negative bacteria did not develop blue colour (Cheesebrough, 2012).

3.12.4 Catalase test

A sterile wire loop was used to pick colonies from 24 hours cultures on to dry glass slides. A drop of 3 % hydrogen peroxide (H_2O_2) was placed on each glass slide and observed for the evolution of air bubbles. Catalase-positive bacteria produced copious active bubbles while catalase negative bacteria produced few bubbles or none (Cheesebrough, 2012).

3.12.5 Urease test

The surface of Urea agar slants was streaked with 24 hours broth cultures. The cap of the tubes was left on loosely and incubated at 37 ^{o}C for 48 hours then examined for colour

change. Urease positive-bacteria developed a magenta to bright pink colour in 24 hours while Urease negative-bacteria did not develop colour change (Cheesebrough, 2012).

3.12.6 Citrate utilization test

Inocula picked from the centre of a well isolated colony of 24 hours cultures were streaked on slant tubes containing simmon citrate agar. The slant tubes were incubated at 37 ^{o}C for up to 2 days and observed for colour change along the slants. Bacteria that utilized citrate showed growth with colour change from green to intense blue along the slants while bacteria that did not utilize citrate showed no growth and no colour change (slants remained green).

3.12.8 Methyl red test

Prior to inoculation, the media was allowed to equilibrate to room temperature (28±2 ℃). Inocula from 24 hours bacterial cultures were transferred into tubes containing Methyl red medium. The tubes were incubated at 37 ^{o}C for 24 hours. Following 24 hours of incubation, 1 mL of the broth was transferred into each clean test tube. The remaining broth was re-incubated for an additional 24 hours. Two drops of Methyl red indicator was added to each aliquot and observed for colour change immediately. Red colour indicated positive reaction while yellow colour indicated negative reaction (Cheesebrough, 2012).

3.12.9 Voges-Proskauer test

Prior to inoculation, the Vogues-Proskauer (VP) medium was allowed to equilibrate to room temperature (28±2 ℃). Inocula from 24 hours bacterial cultures were inoculated into the tubes containing VP medium. The tubes were incubated at 37 ^{o}C for 24 hours. Following 24 hours of incubation, 1 mL of the broth was dispensed into each clean test tube. The remaining broths were re-incubated for an additional 24 hours. Six drops of 5

% alpha-naphthol were added to each aliquot and homogenized. Two drops of 40 % potassium hydroxide were added to each aliquot and agitated. The tubes were agitated vigorously for 30 minutes and observed for colour change. A pink-red colour at the surface of the tubes indicated a positive reaction while absence of pink-red colour at the surface of the tubes indicated a negative reaction (Cheesebrough, 2012).

3.12.10 Sugar fermentation test

i. Lactose fermentation

Inocula from 24 hours bacterial cultures were transferred aseptically to sterile tubes of phenol red lactose broth. The inoculated tubes were incubated at 37 ^{o}C for 24 hours and observed for colour change. A colour change from red to yellow indicated a positive reaction while no colour change indicated a negative reaction (Cheesebrough, 2012).

ii. Sucrose fermentation

Inocula from 24 hours bacterial cultures were transferred aseptically to sterile tubes of phenol red sucrose broth. The inoculated tubes were incubated at 37 ^{o}C for 24 hours and observed for colour change. A colour change from red to yellow indicated a positive reaction while no colour change indicated a negative reaction

iii. Glucose fermentation

Tubes of glucose fermentation medium were inoculated with inocula from 24 hours bacterial cultures using a straight wire by stabbing half way to the bottom of the tubes. One tube of each pair was covered with 1 cm layer of ***sterile mineral oil or liquid paraffin*** (creates anaerobic condition in the tube by preventing diffusion of oxygen). The other tubes were left open. All tubes were incubated at 37 ^{o}C for 48 hours, up to 4 days and observed for colour change in the medium. Acid production was detected in the

medium by colour change from green to yellow, which indicated glucose fermentation while no colour change indicated a-non-glucose fermentation (Cheesebrough, 2012).

3.12.11 Haemolysis production

Inocula from 24 hours bacterial cultures were inoculated on blood agar plates. The plates were incubated at 37 ^{o}C for 24 hours and observed for the presence of haemolysis. Beta-haemolysis showed complete lysis of red blood cells surrounding the colonies. Alpha-haemolysis showed greenish discolouration of red blood cells surrounding the colonies while gamma-haemolysis showed slight discolouration in the medium (Cheesebrough, 2012).

3.12.12 Fungal Identification

Fungal isolates were identified macroscopically by examining the colour, shape and appearance of the colonies on cultural plates. Microscopic identification was done by viewing the colonies under the microscope at 10X and 40X objectives. using their morphological and (Abbey, 2007). The structure, shape, spore type, and arrangement of the hyphae were all documented and used to identify the isolates. The features of isolates were compared to those of known taxa using Domsch and Gams methods (Abbey, 2007).

3.13 Determination of Frequency of Occurrence of Microbial Isolates from Cassava Flour

The frequency of occurrence of the isolates was determined by counting the number of occurrence of each organism compared to the total organisms isolated from all the cassava flour (lafun) samples.

Percentage frequency of occurrence was calculated using Equation 7 :

$$\% \text{ frequency of occurrence} = \frac{\text{number of isolate}}{\text{total number of islates}} \times 100 \qquad \text{Equation 7}$$

3.14 Sensory Evaluation

The method of Ogunnaike *et al*. (2014) was used. The enriched cassava flour was prepared by briskly turning 150 g of the flour in freshly boiling water in a pot using wooden stick until a consistently smooth paste was achieved. It was allowed for five minutes and finally stirred and cut inside bowl. The cassava flour (amala) was compared with commercially prepared cassava flour (amala) for the following parameters: taste, colour, texture, aroma and general acceptability by a panel of nine judges of regular amala consumers using the Hedonic scale product. The sensory scores were analyzed statistically.

3.15 Data Analysis

Data generated were expressed as mean value ± standard error of mean (SEM). Among groups, comparisons of mean were performed by the analysis of variance (One-way ANOVA) test for statistical significance differences at $P< 0.05$. Mean values was separated by Duncan Multiple Range Test (DMRT). All data was evaluated using the Statistical Package for Social Science (SPSS version 25).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Identity of yeast isolated from palmwine

The identities of yeasts isolated from palmwine are shown in Table 4.1. The microscopic, morphological properties and biochemical characteristics of yeast isolated from palmwine using sugar fermentation test revealed a total of nine isolates of which three belongs to the genera *Candida* while the remaining strains belong to the genera *Sacharomyces, Torulopsis and Pichia.* The identities of the yeast isolates were cross-matched with those of standard taxa. All the test isolates were able to ferment glucose while only *Torulopsis* sp was able to ferment lactose. Also *Saccharomyces cerevisiae*

fermented sucrose, maltose, galactose, but could not ferment xylose. *Candida ethanolica* did not ferment maltose, sucrose, galactose and xylose while *Pichia* sp was able to ferment maltose, sucrose and xylose but was negative to galactose fermentation. Appendix I and \II shows the colonial morphology of some of the isolates on SDA and the result of sugar fermentation test for the isolates.

Table 4.1: Identity of Yeast Isolated from Palmwine

Cultural characteristics	Microscopic characteristics	Glucose	maltose	sucrose	lactose	galactose	xylose	Organisms suspected
Dull creamy and butyrous colony	Large spherical/ovoid budding cell	+	+	+	-	+	-	*Saccharomyces cerevisiae*
White to creamy colony with rough surface.	Spherical shaped non-buded cell	+	+	+	-	+	-	*Saccharomyces* sp
Smooth, wrinkled surface, white to creamy colony which is slightly convex in shape	Round and oval in shape with the absence of true mycelium and pseudomycellium.	+	-	-	-	-	-	*Candida* spp
White to creamy colony with smooth surfaces and margins,	Ovoid to sausage-shaped vegetative cell which appear singly, in pairs or chains.	+	+	+	-	-	-/+	*Candida ethanolica*
White to creamy colony, smooth and convex in shape.	Round/oval in shape. Absence of mycelium and pseudomycelium.	+	+	+	-	-	+	*Pichia* spp
Smooth creamy colony.	Ovoid cell shape, multipolar-budding but no true or pseudomycelium formation.	+	-	+	+	-	+	*Torulopsis* sp

+: positive, -: negative

4.1.2 Yeasts Selected for Cassava Flour Enrichment Process

The result of the screening test for the selected yeast strains isolated from palm wine to grow in cassava medium is shown in Figure 4.1. Of the nine yeasts isolated, four isolates designated and identified as *Candida ethanolica* (PW2b), *Candida ethanolica* (PW3b), *Candida ethanolica* (PW4) and *Saccharomyces cerevisiae* (PW6) exhibited high ability to grow and utilize cassava flour (lafun) as a carbon source. While other isolates *Saccharomyces* sp, *Torulopsis* sp and *Pichia* spp would need more than 72 hours to grow maximally in the cassava medium. These four strains were selected for protein enrichment experiments.

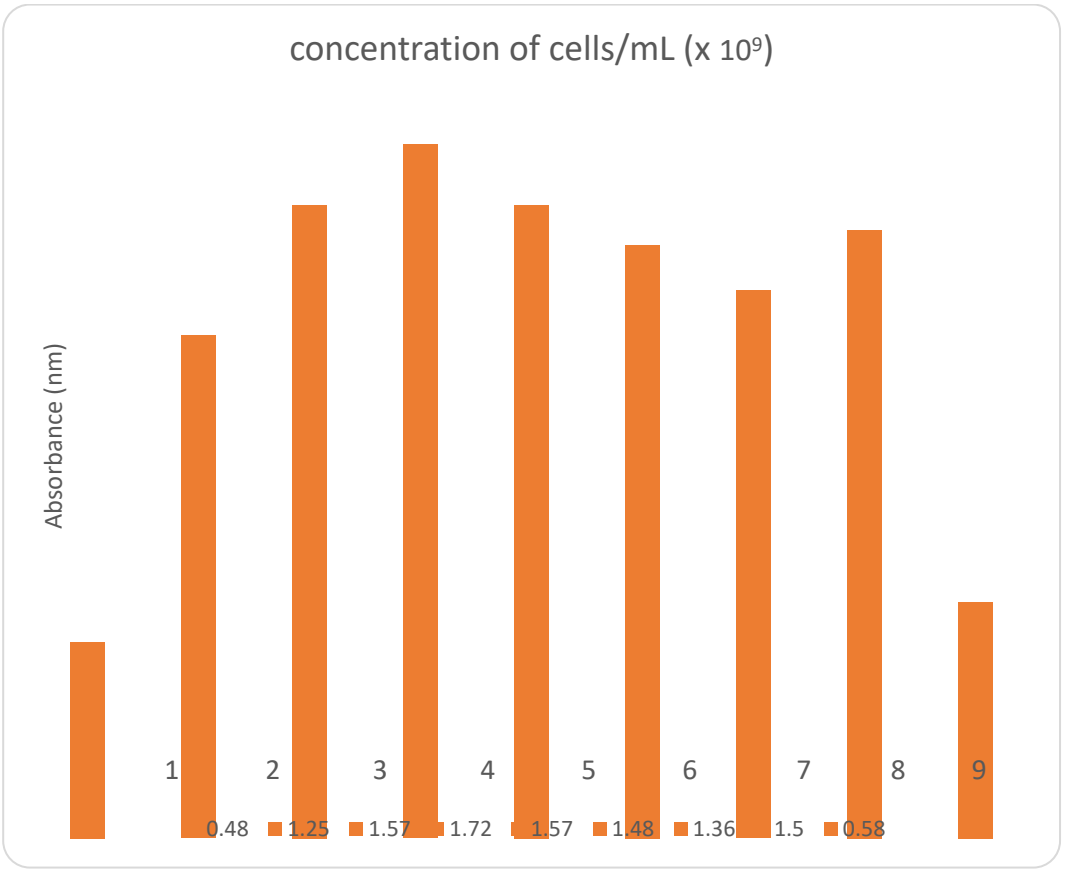

Figure 4.1: **Screening test for yeast isolate**

4.1.3 Molecular Identity of Selected Yeast Isolates

The result of molecular characterization using 16rRNA of the four (4) selected yeast isolates are shown in Table 4.3. The isolates were categorized and identified using the highest percentage similarity with the organism of the nearest homology, based on database information accessible on the National Centre for Biotechnology Information (NCBI) site and the Basic Local Alignment Search Tool (BLAST). Three of the isolates are from the *Candidiaceae* family and the genus *Candida*. *Candida ethanolica* was found in isolate Pw2b, *Candida ethanolica* in isolate Pw3b, and *Candida ethanolica* in isolate Pw4, whereas *Saccharomyces cerevisiae* was identified in isolate Pw6. The summary of molecular characteristics of the tests organisms are presented in Plate II

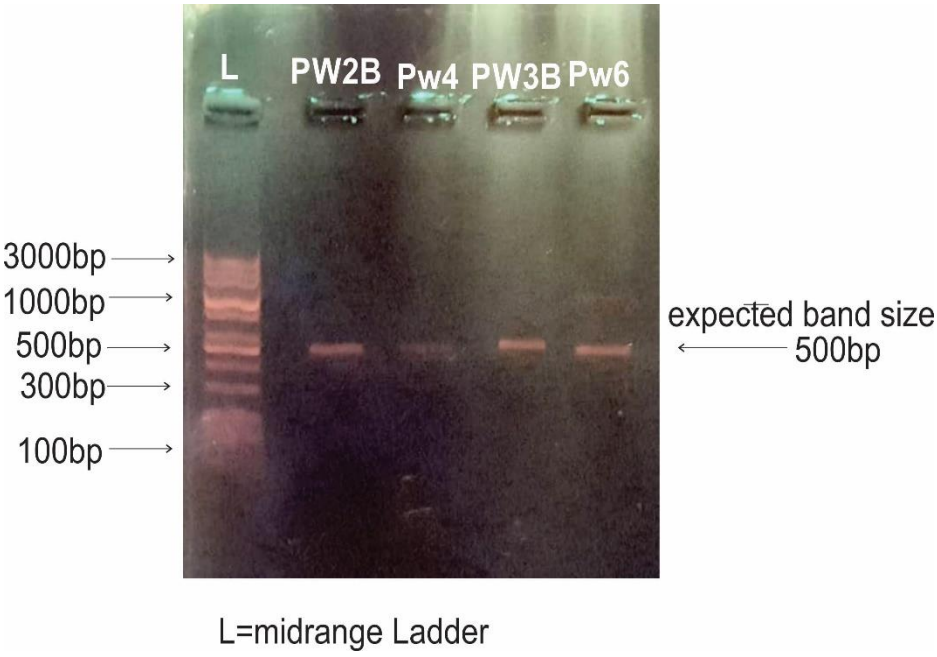

Plate II: Gel electrophlorograph showing the positive amplification of sabr6 (fungi) isolate using the ITS universal primers. A band size of about 500bp indicated a positive amplification.

Table 4.4: Molecular Identification of Selected Potential Yeast Isolates.

Scientific Name	Sample ID	Max Score	E Value	Identity %	Accession Number
Candida ethanolica	Pw2b	654	0.0	100.00%	NR077165.1
Candida ethanolica	Pw3b	648	0.0	100.00%	NR077165.1
Candida ethanolica	Pw4	654	0.0	100.00%	NR077165.1
Sacharomyces cerevisiae	Pw6	636	1e-179	96.26%	LC413771.1

4.1.4 Biomass of Selected Yeast Isolates used for Enrichment process

The identified selected yeast isolates were utilized singly to ferment cassava for cassava flour (lafun) production. Inoculum size range of approximately 3.5×10^2 Cfu/mL – 4.5×10^2 Cfu/mL as earlier described was used for each organism and 5 mL was used for the enrichment process. Table 4.3 shows the result of the final inoculum size obtained from the enrichment of cassava flour (lafun)

Table 4.4: Inoculum Size of Selected Yeast Isolates

Sample Name	Inoculum size(cfu/mL)	Isolate
Pw2b	3.5×10^2	Candida ethanolica (NR077165.1)
Pw3b	4.5×10^2	*Candida ethanolica* (NR077165.1)
Pw4	4.5×10^2	*Candida ethanolica* (NR077165.1)
Pw6	3.5×10^2	*Sacharomyces cerevissiae* (LC413771.1)

4.1.5 Proximate Nutritional Composition of Enriched and Unenriched Cassava Flour

The results of proximate analysis obtained from enriched and unenriched cassava flour are shown in Table 4.5. There were significant differences at ($P<0.05$) in the proximate compositions obtained for the enriched, unenriched and control samples obtained from Bosso market. Unenriched cassava flour had the highest moisture content when compared to all the enriched samples while the enriched sample (Pw3b) had the least moisture content. Enriched cassava flour (Pw2b) had the highest protein content while the control from Bosso market had the least protein content. Unenriched cassava flour had least crude fibre and ash contents when compared with enriched sample (Pw4) and control with the highest fibre and ash content. The commercial control obtained from Bosso market had the highest carbohydrate content while the enriched sample (Pw3b) had the least carbohydrate content. Unenriched cassava flour had the highest fat content when compared to the other group of samples analyzed while enriched sample (Pw6) had the least.

Table 4.5: Proximate components of Cassava Flour

Sample ID	% Moisture	%Crude protein	%Crude fibre	% Ash	% Fat	%CHO
PW2b	9.25±0.060^{b}	2.54±0.110^{d}	0.14±0.005^{b}	0.28±0.025^{a}	0.84±0.020^{ab}	88.00±01.00^{d}
PW3b	8.20±0.035^{a}	2.49±0.025^{c}	0.15±0025 ^{b}	0.48±0.045^{ab}	0.56±0.001^{ab}	88.36±0.85^{f}
PW4b	12.15±0.015^{c}	2.45±0.060^{c}	0.16±0.025^{b}	0.35±0.025^{ab}	0.92±0.020^{c}	83.94±0.020^{d}
PW6	12.04±0.020^{c}	2.12±0.020 ^{b}	0.15±0.020^{a}	0.52±0.020^{b}	0.56±0.015^{a}	84.60±0.185^{d}
UEF	15.07±0.070^{d}	2.13±0.025^{b}	0.07±0.015^{a}	0.14±0.030^{a}	1.03±0.025^{b}	81.65±0.190^{e}
CON	8.74±0.190^{a}	1.74±0.215^{a}	0.11±0.020^{ab}	0.53±0.030^{b}	0.64±0.040^{a}	88.41±0.005^{f}

Values are in ± mean S.E. (*S.E = Standard error of Mean*)

Values Within rows bearing the same superscript are not significantly different at the 5% level (P<0.05).

PW2b: *Candida ethanolica*, PW3b: *Candida ethanolica* (No32), PW4: *Candida ethanolica*, Pw6: *Sacharomyces cerevissiae,* UEF: unenriched cassava flour, CON: control

4.1.6 Microbial Load of Cassava Flour (lafun)

The results revealed that the total viable bacterial counts for the enriched, unenriched and commercial control obtained from Bosso market ranged from 5.17±0.040^{a} - 11.11±0.010^{d} while the fungal counts ranged from 3.14±0.010^{a} - 9.37±0.155^{d}. There were no coliform counts obtained for both enriched, unenriched and the commercial control of cassava flour. Enriched cassava flour (pw6) had the highest fungal count while Commercial control obtained from Bosso market had the highest bacterial count when compared with the rest of the samples. Enriched cassava sample (pw4) and (pw2b) had the least bacterial and fungal counts. Table 4.7 shows the microbial load of enriched, unenriched and commercial control of cassava flour (lafun).

Table 4.6: Microbial Load of Enriched and Unenriched Cassava Flour

S/N	Sample ID	Bacterial load ($\times 10^4$cfu/g)	Fungal load (×103cfu/g)
1	**Cf pw2b**	5.17 ± 0.040^{a}	3.14 ± 0.010^{a}
2	**Cf pw3b**	7.07 ± 0.050^{b}	8.39 ± 0.125^{c}
3	**Cf pw4**	4.18 ± 0.070^{a}	7.12 ± 0.005^{b}
4	**Cf pw6**	9.02 ± 0.020^{c}	9.37 ± 0.155^{d}
5	**CfUNE**	9.18 ± 0.035^{c}	9.23 ± 0.020^{c}
6	**CfCC(Bosso)**	11.11 ± 0.010^{d}	9.33 ± 0.095^{d}

Values are in ± mean S.E. (*S.E = Standard error of Mean*)

Values Within rows bearing the same superscript are not significantly different at 5% level ($P<0.05$).

Cf: Cassava flour, PW2b: *Candida ethanolica*, PW3b: *Candida ethanolica* (No32), PW4: *Candida ethanolica*, Pw6: *Sacharomyces cerevisiae*, UNE: unenriched, CC: commercial control.

4.1.7 Identity of Bacteria Isolated from Cassava Flour (Lafun)

The identities of bacteria isolated from cassava flour using cultural characteristics of the isolates obtained on both nutrient and MacConkey agar, are shown in Table 4.8. The morphological and biochemical characteristics of the bacterial isolates was also identified The organisms isolated include *Bacillus cereus, Micrococcus* sp, *Staphylococcus aureus, Bacillus mycoides* and *Streptococccus* sp. *Bacillus cereus, Staphylococcus aureus, Bacillus mycoides* and *Streptococcus* sp were oxidase negative while *Micrococcus* sp was oxidase

positive. All the test isolates were catalase positive except *Streptococcus* sp which was catalase negative. *Bacillus cereus* and *B. mycoides were able to* hydrolyse starch while *S. aureus, Micrococcus* sp *and Streptococcus* sp were negative to starch hydrolysis. All the test isolates were Gram-positive cocci and rod shaped organisms. *Bacillus cereus, B. mycoides* and *Micrococcus* sp were coagulase negative while *S. aureus* and *Streptococcus* sp were coagulase positive. *B. cereus* and *Micrococcus* sp were methyl red negative while *S. aureus* and *B. mycoides* were methyl red positive. *Bacillus cereus, S. aureus* and *B. mycoides* were positive to both Voges-Proskauer and citrate utilization test while *Streptococcus* sp and *Micrococcus* sp were negative to the test. *Bacillus cereus* and *S. aureus* were positive to urease test while *Streptococcus* sp and *Micrococcus* sp were urease positive.

Table 4.7: Identity of Bacteria Isolated from Cassava Flour (Lafun)

GR: Gram's reaction, SH: Shape, COG: Coagulase test, OX: Oxidase test, CT: Catalase test, MR: Methyl red test, MOT:

Growth on Nutrient Agar	MacConkey Agar	GR	SH	COG	SH	OX	CT	MO	MR	VP	CI	UR	SUG			FER	HEM		Suspected organisms
													L	G	S	α	β		
Large, irregular spreading Colony	ND	+	Rod	-	+	-	+	+	-	+	+	+	+	+	+	-	+		*Bacillus cereus*
Small, circular colony without pigmentation.	ND	+	Cocci	+	+	-	-	-	+	-	-	-	-	+	+	-	+		*Streptococcus* sp
Circular small colonies with golden yellow pigment	ND	+	Cocci	+	-	-	+	-	+	+	+	+	+	+	+	-	-		*Staphylococcus aureus*
Circular small colonies with golden yellow pigment	ND	+	Cocci	-	-	+	+	-	-	+	-	+	+	+	-	-	-		*Micrococcus* sp
Milk white, rhizoid colony with fine thread	ND	+	Rod	-	-	+	+	-	+	+	-/+	-/+	+	+	+	+	-		*Bacillus mycoides*

Motility test, UR: Urease test, HEM: Haemolysis test, SH: Starch hydrolysis, sugar fermentation test, S: Sucrose sugar fermentation test, G: Glucose sugar fermentation test, VP: VogesProskauer test, CI: Citrate utilization test, +: Positive, -: Negative ,ND: not detected.

4.1.8 Identity of Fungi Isolated from Cassava Flour (Lafun)

One genera of mould and two genera of yeasts namely, *Aspergillus*, *Saccharomyces and candida* were isolated from the cassava flour on the basis of their colonial morphology and microscopic characteristics. The identities of the isolates were cross-matched with those of standard taxa. Table 4.8 shows the morphological characteristics of fungi isolated from the cassava flour (lafun).

Table 4.9: Identity of Fungi Isolated from Cassava Flour (Lafun)

Cultural characteristics	Microscopic characteristics	Organisms suspected
Smooth, wrinkled surface, white to creamy colony which is slightly convex in shape	Round and oval in shape with the absence of true mycelium and pseudomycellium.	*Candida* spp
Dull creamy and butyrous colony	Large spherical/ovoid budding cell	*Saccharomyces cerevissiae*
White to creamy colony with rough surface.	Spherical shaped non-buded cell	*Saccharomyces* sp
Black colony on SDA	Septate hyphae, dark brown to black and rough walled	*Aspergillus niger*

4.1.10 Frequency of Occurrence of Microbial Isolates from Cassava Flour (Lafun)

Bacillus cereus and other species of *Bacillus* were the most frequently isolated organisms (33%, 25%) from all the cassava flour (lafun) samples while *Staphylococcus aureus, Streptococcus* sp and *Micrococcus* sp were the least. *Candida* sp was the most frequently occurred fungi isolate on all the samples while *Aspergillus niger* was the least.

Table 4.10a Frequency of Occurrence of bacteria Isolated from Cassava Flour

Isolates	Number of occurence	% of occurrence
Bacillus cereus	5	35
Bacillus mycoides	4	25
Staphylococcus aureus	3	16
***Streptococcus* sp**	2	12
***Micrococcus* sp**	2	12

Table 4.10b Frequency of Occurrence of fungi Isolated from Cassava Flour

Isolates	Number of occurrence	% of occurrence
Aspergillus niger	3	30
Saccharomyces cerevissiae	3	30
***Candida* sp**	4	40

4.1.11 Sensory attributes of Prepared Amala

The results of sensory attributes of the prepared amala (enriched, unenriched and control) using Hedonic scale are presented in Table 4.11. Statistical analysis of the data showed that there were significant differences ($P<0.05$) among the enriched, unenriched and commercially prepared amala. However, the texture and taste of the enriched amala was preferred to that of the commercially prepared amala after 4 days of fermentation thus, good acceptability of the sensory parameters (colour, taste, aroma, texture). This finding indicates that there was higher general acceptability and the taste of the Enriched amala than other amala (unenriched and control) analyzed.

Table 4.11: Sensory Attributes of the Prepared Amala

Values are in ± mean S.E. (*S.E = Standard error of Mean*)

S/N	Sensory Attribute	Commercial control	Unenriched flour	Enriched flour
1	**Colour**	6.44 ± 0.185^{c}	8.60 ± 0.065^{d}	8.71 ± 0.015^{cd}
2	**Taste**	5.34 ± 0.080^{b}	6.45 ± 0.015^{b}	8.29 ± 0.125^{b}
3	**Texture**	6.54 ± 0.025^{c}	8.00 ± 0.110^{c}	7.90 ± 0.010^{a}
4	**Aroma**	4.47 ± 0.055^{a}	5.57 ± 0.085^{a}	9.00 ± 0.015^{d}
5	**General acceptability**	7.36 ± 0.100^{d}	7.77 ± 0.060^{c}	8.44 ± 0.045^{bc}

Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5% level ($P<0.05$)

4.2 Discussion

4.2.1 Identity of yeasts isolated from palm wine

In the present study, a variety of yeasts were isolated from palmwine. The result obtained in this study revealed that palmwine contains yeasts particularly *Candida* sp and *Saccharomyces cerevisiae*. This could be as a result of high sugar content of palm wine which encourages the proliferation of the yeasts in the drink. Umeh *et al*. (2017) reported that *S. cerevisiae* constituted 89-92 % of total microbial isolates present in palm wine. Other species of yeasts such as *Pichia* and *Torulopsis* sp were also isolated in this study. This was in agreement with the findings of Onwumah *et al.* (2019) who isolated similar species of yeast such as *Candida* sp, *Pichia* sp and *Saccharomyces cerevisiae* from palm wine. In this study, a total of four yeast isolates were selected for the enrichment process. This was as a result of the screening test obtained on the isolates potential to utilize cassava flour as a carbon source. This revealed that *Candida ethanolica* (NR077165.1), *Candida ethanolica* (NR077165.1), *Candida ethanolica* (NR077165.1) and *Sacchromyces cerevissiae* (LC413771.1) exhibited maximum growth in cassava medium within 72 hours. This means that these organisms have competent degradative enzyme system for the utilization of the carbon source. The result was in agreement with the findings of Bala *et al.* (2012) who enriched gari with different strains of *S. cerevisiae.*

4. 2.2 Yeast selected for cassava enrichment

A total of four yeasts species was utilized for the enrichment process. This was based on their ability to grow in cassava medium owing to the fact that they were able to degrade starch, a complex carbohydrate to produce α-amylase or an

inducible/constitutive amylase to produce simple sugars which can then be readily metabolized (Ekundayo and Okoroafor, 2012; Guira *et al*., 2016). This was in agreement with the findings of Bala *et al.* (2012) who selected different strains of *Saccharomyces. cerevisiae* for the enrichment of gari .

4.2.3 Proximate Nutritional Analysis of Cassava Flour Samples

The moisture content of the samples varied significantly ($P<0.05$) with the highest value (15.07±0.070) in unenriched cassava flour. This is in agreement with the moisture content (15.68±0.26) obtained by Fawole *et al*. (2021) for spontaneous fermentation of cassava for lafun production. The moisture content of the enriched cassava flour ranged from 8.20±0.035 - 12.15±0.015 while that of the commercial control had a moisture content of 8.74±0.190 This results was also similar when compared with the moisture content of cassava flour (9.2 - 12.3 % and 11 - 16.5 %) reported by Charles *et al*. (2005) and Shittu *et al*., (2007) respectively. This indicated that fermentation process brought about both increased and reduced moisture content in the samples. Decrease in moisture content is an indicator for stable shelf life of the end product and this could be due to the uptake of water by the fermenting substrate which resulted in their soft and porous texture after fermentation (Onyinlola and Onilude, 2018). Similar reduction, as observed in this study, had earlier been linked to activities of fermenting microorganisms, since water is essential for growth and cell metabolism as well as loss of moisture along with the leaching of nutrients (Tiruha *et al*., 2014). However,Oyinlola *et al*. (2018) reported moisture reduction as a function of many factors such as temperature, time, humidity, etc. Contrary results were reported (Ogueke *et al*., 2010; Adegbehingbe, 2014)

during the fermentation of African locust bean and melon seed where moisture content was confirmed to have increased and it was related to hydrolytic activity of the fermenting organisms releasing moisture as part of their

metabolic product. In the present study, the crude protein contents of the samples varied significantly ($P<0.05$) with the highest value (2.54±0.110) in enriched cassava flour fermented using *C. ethanolica* while commercial control had the least value (1.74±0.215). Enrichment of the cassava flour with the isolates significantly increased the protein content of the final products. This may be as a result of yeasts proteins secretion (Oseni and Akindahunsi, 2011) and increase in growth and proliferation of yeasts in the form of single cell proteins (Boonnop *et al*., 2009). Researchers had noted that solid state fermentation with yeast increased crude protein (CP) content of food materials with better values than was found in this work. Day and Morawicki (2018) reported a crude protein increase from 9% - 27% in fermented grain sorghum using *S. cerevisiae* and *Lactobacillus amylovorus*- an amylolytic LAB. Similarly, earlier studies on solid-state fermentation of cassava pulp and cassava peels using *Aspergillus niger* and *Saccharomyces cerevisiae* increased the protein content from 7.91 – 9.04 % and 14.14 – 16.74 % respectively (Iyayi and Losel, 2001). Using mixed cultures of bacteria and fungi, Ahaotu *et al*. (2011) reported increase in protein content (13.7 mg/g/dry matter - 15.4 mg/g/dry matter) for fermented gari. Kolapo and Sanni (2009) documented a protein content of 1.0 – 1.5 % in naturally fermented gari. This was low when compared with the result obtained in this study. However, fawole *et al*. (2021) reported protein content of 1.13±0.01 - 1.63±0.00 for *lafun* samples fermented using *Lactobacillus plantarum* and *Saccharomyces boulardii*. This was also lower when compared with the protein content of 2.13±0.025 - 2.54±0.110 obtained for both enriched and unenriched cassava flour. The low protein values obtained in the present study when compared to other findings could be as a result of dewatering after submerged

fermentation during Lafun production. In addition to protein enrichment, yeast fermented *Lafun* also showed a remarkably high ash (0.28±0.025 -0.52±0.020) and fibre contents (0.14±0.005 – 0.16±0.025) which was significantly different ($P<0.05$) when compared with the ash and fibre values obtained for unenriched sample (0.14±0.030 and 0.07±0.025). The ash level after yeast fermentation could be attributed to the level of incomplete utilization of the nutrients present in the raw material by the yeasts (Eromosele *et al*., 2017). On the contrary, Onyinlola and Onilude (2018) reported high ash and fibre content of (0.78% -1.03%) and (1.93% - 2.24%) in fermented Usi with starter cultures of *Lactobacilli* and Fawole *et al*. (2021) also reported high ash (2.26% - 15.20%) and fibre (3.14% - 15.80%) values for fermented lafun using *Lactobacillus plantarum* and *Saccharomyces boulardii*. Ash content is a factor of mineral availability in the fermented food. The decrease in ash values observed in this study could be ascribed to leaching of soluble mineral elements into the fermenting medium or as a result of enzymatic hydrolysis of food components into their absorbable forms while low fibre values obtained in this study could be as a result of the softening of the fibrous tissues during fermentation and also attributed to microbial bio-conversion of carbohydrates and lignocelluloses into protein. Furthermore, carbohydrate contents of the samples are also significantly different ($P<0.05$). The unenriched cassava flour had the least carbohydrate content of 81.65±0.190 when compared to the enriched samples which ranged from $83.94±0.020^{d}$ - 88.00±01.00 and the commercial control with the highest value of $88.41±0.005^{f}$. This could be attributed to fact that the unenriched cassava flour had high moisture content when compared to the rest of the cassava samples. This was similar to the findings of Onyinlola and Onilude, (2018) who reported carbohydrate

content (88.4 % - 89.6 %) for fermented *Usi*. The general increase in total carbohydrate of the enriched cassava flour and commercial control could be a factor of decrease in moisture content as suggested by Igbabul *et al*. (2014) during the fermentation of cocoyam flour or the proportionate increase in protein content (Oboh *et al*., 2002) since increase in crude protein could be attributed to the ability of the organisms to convert carbohydrate during fermentation to protein or breakdown of carbohydrates into simple sugars and organic acids. In addition, unenriched cassava flour had higher crude fat content (1.03±0.025) when compared with the other samples while the least fat value (0.56±0.001) was obtained for the sample enriched with *S. cerevisiae*. This was similar to the fat content (0.58±0.07 - 1.22±0.00) obtained by Fawole *et al*. (2021) for fermented *lafun* but slightly higher than the crude fat value of 0.25 % and 0.39 % reported by Onyinlola and Onilude, (2018) for fermented usi.

4.2.3 Microbiological Analysis of Cassava Flour (Lafun) Samples

In the present study, the microbiological safety of the enriched and unenriched cassava flour samples were also determined and compared with a commercial control obtained from Bosso market. The total bacterial count obtained from the cassava samples were 5×10^4cfu/g, 7×10^4 cfu/g, 4×10^4cfu/g, 8×10^4cfu/g, 9×10^4cfu/g and 1.1×10^5cfu/g for enriched cassava flours (*Candida ethanolica* (NR077165.1), *Candida ethanolica* (NR077165.1), *Candida ethanolica* (NR077165.1) and *Sacchromyces cerevissiae*(LC413771.1)), unenriched cassava flour and the commercial control. The total fungal count obtained from the cassava samples were 3×10^3 cfu/g, 7×10^3 cfu/g, 5×10^3 cfu/g, 2×10^3 cfu/g, 8×10^3cfu/g and 9×10^4 cfu/g for

enriched cassava flours (*Candida ethanolica* Pw2b, Pw3b,Pw4 and *Saccharomyces cerevisiae*) unenriched cassava flour and control respectively. The total bacterial count (10^4 cfu/g) studied as well as the fungal count (10^3 cfu/g) for all the cassava samples were within the acceptable limit (10^4 – 10^6 cfu/g) recommended by International Commission on Microbiological Specification for Foods (ICMSF, 2008). Hence the cassava flour may be recommended for the diet of people. This count was lower than the total microbial count (10^3-10^6 cfu/g) observed in *lafun* samples sold in Ogun market by Adebayo-oyetoro *et al*. (2013). It was similar to the findings of Alamu *et al.* (2021) who reported microbial count of 2×10^3 to 9×10^5 cfu/g for cassava flour sold in Zambia market and also .The low microbial count enumerated from the samples indicated that the cassava flours were slightly contaminated. Cassava flour (lafun) has the advantage of maintaining low count of the enumerated organisms because it is a dried food item. These microorganisms could have been introduced either in the pre or post processing stages. In the present study pathogenic as well as non pathogenic microorganisms were isolated in different percentage viz: *Bacillus cereus* (35%), *Bacillus mycoides* (25 %), *Staphylococcus aureus* (12 %), *Micrococcus sp* (12%), *Streptococcus sp* (12%), *Candida spp* (40 %), *Aspergillus niger* (30 %) and *Saccharomyces cerevisiae* (30 %). There were no coliform bacteria detected in all the samples analyzed. However, Bala *et al*. (2012) reported coliform count during the fermentation of gari with *Saccharomyces cerevissiae* which is in contrast with the findings of this study. The results of microbial analysis of cassava flour obtained in this study indicated that the laboratory samples are of good quality regarding food safety.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Four yeast strains isolated from palm wine were selected and used for the enrichment of cassava. They were molecularly characterized as *Candida ethanolica* (NR077165.1), *Candida ethanolica* (NR077165.1), *Candida ethanolica* (NR077165.1) and *Sacchromyces cerevissiae* (LC413771.1).

Cassava flour Pw2b enriched with *Candida ethanolica* (NR077165.1) shows a good potential for improvement of some of its nutritional components especially protein and fat content when compared to the remaining samples. The enriched cassava flours also showed higher sensory attributes (taste, texture and general acceptability) when compared to the unenriched and control samples.

The present study also showed that enriched and unenriched cassava flour had low microbial count when compared to the commercial control obtained from Bosso market. Furthermore, they were no coliform bacteria identified in all the samples analyzed.

This indicated that all the cassava flour samples were slightly contaminated. Yeast fermentation of cassava has excellent potential to produce safe, high quality cassava flour by enhancing the nutritional quality of the products obtained.

5.2 Recommendation

- There should be an enlightenment campaigns to the general public on the benefits of inoculation of cassava products with microorganisms such as yeast in order to boost consumption of protein, and prevent malnutrition associated with constant dependence on cassava based foods especially by the economically weaker populations. Since the inoculants (yeast) are chaep and easily accessible.
- Toxicological studies should also be carried out in order to check the safety of the final products on human health.
- Further works should concentrate on how to maximize protein enrichment of *Lafun* by complementing yeast fermentation with amyloglucosidase treatment, nitrogen (pepsin) supplementation that would enhance biosynthesis of amino acids by the fermenting organisms.
- Also the yeast isolates should be utilized in combined form so as to give a better yield in the protein content.
- Cassava flour '*lafun'* should be produced under aseptic condition so as to avoid contamination with microorganisms.
- Local processors and sellers of cassava flour (*lafun*) should be educated on the adverse effect of lack of proper hygiene and good sanitation.

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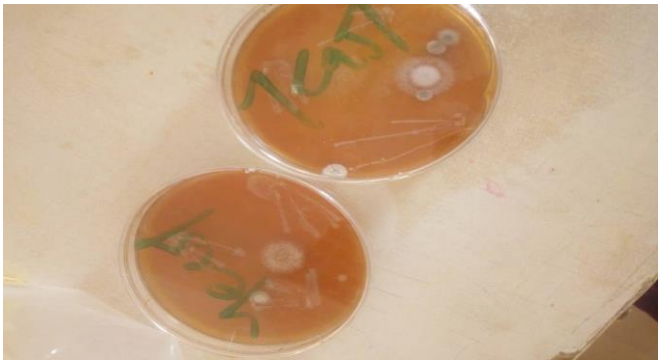
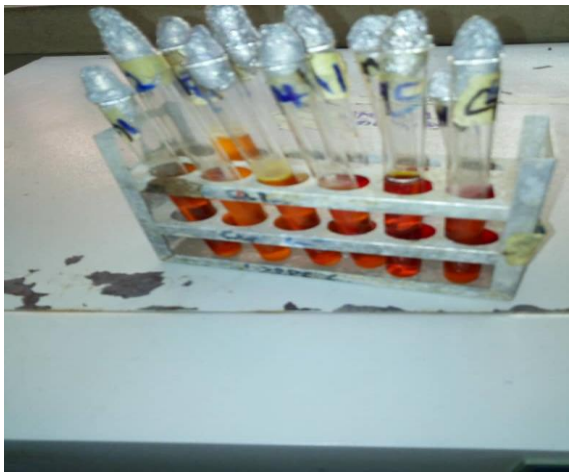
Appendices

Appendix I: colonial morphology of yeast isolates.



Appendix II: sugar fermentation test



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