EVALUATION OF THE NUTRITIONAL AND BACTERIOLOGICAL QUALITIES OF SELECTED UNDERUTILIZED LOCAL DRINKS IN NORTHERN NIGERIA

A Project Submitted in Partial Fulfillment of the Requirements

for the Award of the

Degree of Bachelor of Science in Biochemistry

BY

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DECLARATION

I hereby declare that this work is the product of research efforts undertaken under the supervision of Mr. Miracle Uwa Livinus and has not been presented and will not be presented elsewhere for the award of a degree or certificate. All the sources have been duly acknowledged.

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Khadija Bashir Hayatu Date

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CERTIFICATION

This is to certify that this study was carried out by Khadija Bashir Hayatu (1090) in the Department of Biochemistry, School of Science and Information Technology, Skyline University Nigeria, under my supervision.

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APPROVAL

The panel of examiners recommends the candidate Khadija Bashir Hayatu (1090) for the award of the Degree of Bachelor of Science in Biochemistry subject to effecting all the corrections pointed out during the oral examination.

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External Examiner Date

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Internal Examiner Date

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Project Supervisor Date

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Head of Department Date

DEDICATION

This report is dedicated to Almighty Allah for his guidance and protection during and even after this work. He has been the source of my strength throughout this program. I also dedicate this report to my amazing parents who their endless love, prayers and encouragements.

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ABSTRACT

This study was aimed to evaluate the Nutritional and Bacteriological Qualities of Selected Underutilized Local Drinks in Northern Nigeria. Sorrel and tiger nut juice samples were produced and analyze for physical, sensory, nutritional and microbial analysis using standard methods. The result of the physical analysis showed that the pH of the tiger-nut juice samples was within the range of 6.07 and 6.41. The samples also contained vitamins and minerals such as Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn at varying concentrations Total bacterial counts in the samples ranged between $0.25x10^5$ to $6.28x10^5$ cfu/ml while the total coliform count (TCC) varied from $0.98x10^4$ to $1.42x10^4$ cfu/ml. Six bacterial isolates, *E. coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Staphylococcus* and *Bacillus* species were identified in the study samples through the biochemical and morphological tests. The high bacterial load of the samples is an indication that samples were contaminated and this can pose a health hazard to the consumers. Hence, effort should be geared towards improving the sanitation and hygienic practices during production to minimize contamination and spoilage.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Nigeria is a country with an incredibly diverse range of clans, dialects, and food sources (Iwuoha and Eke, 1996; Blench, 2012). Nigeria's range of food supplies may not be quite as diverse as the evidence that practices, acceptances, and respects were among the essential factors influencing how food was prepared, inclined to be served, required to be healthy, and classified as food (Ayeomoni, 2011; Cox and Anderson, 2013). Numerous specialists accounted for various assemblages and assortments of natural food sources in Nigeria (Iwuoha and Eke, 1996; Onimawa, 2010). Contrasts in food preparation techniques and tendencies are common within a single clan (Ayeomoni, 2011). For instance, inside a bunch of the food called Kunu (non-cocktail), there are different subsets of Kunu. Gaff *et al*. (2002) and Solange *et al*. (2014) announced classifications of grain refreshments. For instance, there are many subsets of the dish known as Kunu (non-cocktail) inside a group of Kunu. Classifications of grain refreshments were released by Gaff *et al*. in 2002 by Solange *et al*. (2014). For example, both *Kunun gyada* and *Kunun zaki* have a variety of assortments. One type of Kunu's planning process may differ fundamentally from one location to the next; the name and final result may also vary depending on the circumstances. The use of numerous raw components may also lead to the production of another assortment. The super-requested Malvaceae family includes *Hibiscus sabdarffa*, and it is accepted that it originated in East Africa (Ilondu and Iloh, 2007). Other Hibiscus varieties are grown for the strands they generate, while *Hibiscus sabdariffa* plants are developed and used as a vegetable and tea. It goes by a number of names, including

Roselle and Sorrel in English, and is referred to informally as zobo in northern Nigeria (Adebayo-Taye and Samuel, 2000).

Native food sources have a crucial role in people's ability to survive in Nigeria. According to Onimawo (2010), networks in Nigeria have formed their own preferences and eating habits over time and would generally stick to what is well-known. Traditional cuisine options are available and many in Nigeria According to Ayeomoni (2011), the word "food" has become a part of social convention. It assumes a crucial role in the lives of living souls. It is a source of happiness, comfort, and security as well as a representation of kindness, a gauge of social standing, and rigid standards. The food we choose, how it is prepared, served, and even how we eat it all have an impact on our own social heritage or way of life. This drink has both health and medicinal benefits, according to studies by Fadahunsi *et al*. (2013) and Solange *et al*. (2014), and it is frequently used in many traditional festivities and services. As a result, these drinks and other aged non-cocktails (like Kunun tsiro) might be used as an alternative in mediations to reduce the risky consumption of alcohol and other psychoactive substances (drugs, opiums, heroin, cocaine, pot and high cocktails). While these food sources serve those purposes, other local food types can also be used to address diet-related problems, such as battling hunger and starvation, unhealthiness, and non-contagious illnesses.

Nigerian local food variety actually have amazing nourishing and rejuvenating potentials that one can't just ignore. Involving indigenous food items from Nigeria in dietary intercessions has a number of benefits. Although the value of strange food may not be obvious right away, using strange eating habits over time may have an impact on one's inclinations while also depriving them of nutritious meals (Macdonald *et al*., 2011; Cox and Anderson, 2013). The feeling of anxiety toward the uncharted world is a small problem for those who consume native foods.

Hereditarily altered food, which is commonly seen as being foreign (peculiar) goods around the world, may have problems such as a change in nutritional value, a source of potential toxins, and the presence of hypersensitive compounds. Other urgent challenges include those that are strict, social, and moral (Uzogara, 2000; Olaniyan *et al*., 2007). Many senior Nigerians now acknowledge that the use of some of the most cutting-edge diets may not be the only factor contributing to the high mortality rate among young Nigerians. As opposed to those shipped from outside, nutrition mediations using local food kinds are swift and affordable. These food sources are very inexpensive and simple to prepare. Additionally, it is difficult to learn anything about the history of those imported food types, including the sources of the surprise innovation used to create them as well as their unprocessed substance sources. The administrative organizations routinely expose such food supplies for modification, debasement, misbranding, and other issues. Another advantage of eating locally grown food is that many food crops grown in Nigeria use minimal synthetic fertilizers and occasionally none at all, only faeces, to be spread on ranches. The purpose of this investigation is to examine the nutritional and bacteriological properties of a few underutilized neighboring beverages in northern Nigeria based on this concept.

1.2 Statement of Problem

One of Nigeria's underutilized food crops for a long time has been *Cyperus esculentus* and *Hibiscus sabdariffa.* The former is typically consumed unprocessed as bite and without distinction as a key food crop that has exceptional potential in making due, preventing and curing hunger (macronutrient and micronutrient deficiencies), or problems with food insufficiency. However, the last approach is well established for pulse administration. Nutritionists have demonstrated that the main health problems can be resolved by utilizing the

nutritional and economic potential of local food resources. Tawny and tiger nut juice are two examples of underutilized food beverages with enormous potential for domestic and commercial application. Despite its accessibility, there is little information on the nutritional and bacteriological qualities of these commonplace foods and drinks. An advantage that the client can recognize and value is provided by an effective product (NUTRA, 2005).

1.3 Justification

The necessity of fabricating the generation and usage of locally accessible food resources has recently been mentioned in several public and international fora. Among the underutilized foods and beverages that are readily available in Nigeria are kunun aya and zobo. By deceiving people about their potential financial and health benefits, these foods and beverages could be used to deal with significant stimulating issues. The results of this study will provide information on the utilization of kunun aya and zobo. This will go a long way toward extending their use, which will ultimately result in extending their production at both the family and public levels in order to guarantee food security. Additionally, it is expected that by studying its game plan, *Cyperus esculentus* and *Hibiscus sabdariffa* can be used to prevent and treat a number of non-communicable diseases like cancer, diabetes, hemorrhoids, cardiovascular diseases, the primary cause of circulatory strain, lower cholesterol levels, promote liver health, etc.

1.4 Aim of the Study

The goal of this study is to determine the nutritional and bacterial qualities of a few locally produced drinks that are underutilized in northern Nigeria.

1.5 Objective of the Study

i. Making kunun aya (*Cyperus esculentus*) and zobo (*Hibiscus sabdariffa*) drinks

ii. To analyze the zobo and kunun aya beverages' sensory, physical, and nutritional makeup.

iii. To isolate and identify bacterial contaminant in zobo and kunun aya beverages.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Indigenous and Underutilized Food Crops

Plants of every color and kind have been used as food for both people and animals from the beginning of time. Numerous crops have historically served as sources of food, medicine, and nourishment. Today, both non-human animals and humans regularly consume close to ten thousand (10,000) indigenous food species worldwide. Again, over the past five centuries, there has been a rise in human interaction and worldwide trade, and as a result, around 20% of these crop species are now employed over the world and form the basis of the majority of agricultural activities (FAO, 2015). However, many of the plant species that are grown for food around the world are either underutilized, neglected, or both. According to studies, eating these crops' micronutrients improves health and lowers the likelihood of illness in both women and young children (under the age of five). Indigenous and underutilized crops have advantages for human health, in addition to enhancing local populations' access to food (Agulanna, 2020).

Review of the available literature revealed that majority of the crops are rich in nutrients since they have therapeutic values, despite commercial research showing that indigenous and underutilized crops have helped boost household income by reducing poverty (Abukutsa-Onyago, 2014). Additionally, the crops aid in addressing local residents' health requirements and fighting malnutrition. There has been a dependence on a few widely grown crops or plant species for the past 200 years or so. While this has helped to support a constantly expanding human population, it has also resulted in the omission of many other species that might have

improved global food security and the world's agricultural systems. At the moment, only three crops—maize, wheat, and rice—provide a significant share of the calories and protein requirements of humanity. Since this situation puts humanity in grave risk, everyone must take quick action, especially in order to support crop variety in global agriculture. Global food security is limited by a number of issues, including the high reliance on a small number of critical crops (Amujoyegbe *et al*., 2015).

Due to its potential influence and contribution to food security, overdependence on a few key crops remains a significant concern in AfricaFor instance, the advent of the Green Revolution in Nigeria led to the development of an agricultural model that emphasized the cultivation of a small number of staple or cash crops. According to the FAO analysis, increasing a country's food base rather than decreasing it will lead to growth or higher food productivity (FAO, 2015). For instance, despite an increase in cereal production, the gains have been inconsistent globally and within individual nations and regions. Again, the increase in cereal output in sub-Saharan African nations over the past thirty years or so has not kept pace with population growth. As a result, the continent continues to import a lot of crops (Abukutsa-Onyago, 2014). Indigenous and underutilized crops are used as a counterbalance to other staples, whether they be foods or supplements, in many rural African communities. Additionally, they serve as backup plans for food shortages or crises (FAO, 2015). These food or supplement categories significantly increase peoples' incomes, particularly females. Underutilized crops also assist farmers in coping with unfavorable meteorological conditions and economic difficulties in marginal economies, where poverty and food insecurity are pervasive. Indigenous or rural groups typically have access to food varieties that are frequently unknown to or outside the expertise of professionals or city dwellers. The food and health needs of rural people depend

on these native, underutilized, or neglected crops, which are outside the knowledge base of the agricultural and food sectors. They contribute significantly to social and agricultural development in rural areas. To ensure food security and agricultural expansion, crop diversification is essential. Continued disregard for them will have disastrous effects on both food security and global agribusiness (Agulanna, 2020).

2.2 Importance of Indigenous and Underutilized Crops

From the aforementioned, it is obvious that neglected, native, or undervalued crops can play a significant role in promoting food security not only in Africa but also internationally if they are properly utilized. For instance, although there are around 10,000 edible plant species listed in the literature, only five major crops are thought to be responsible for providing 60% of the global human energy intake. The significant rise in global population over the past 20 years has also increased the need for food globally (Abukutsa-Onyago, 2014).

The lack of variety in diet has recently had a detrimental effect on people's health and wellness. Native and underutilized foods can help reverse this bad trend by providing the essential nutrients for human wellbeing and good health, which will lower the prevalence of sickness and malnutrition among local populations worldwide (Amujoyegbe *et al*., 2015). Thus, incorporating native animals into the food chain is a crucial step toward improving general human health and welfare. Therefore, indigenous and underutilized crops should play a significant role in the global food supply. These native and underutilized plants can be used to increase human wellbeing as well as food security. Wild indigenous plants not only contribute to the local food supply but also preserve cultural uniqueness (Agulanna, 2020).

2.3 Tiger Nut

Cyperus esculentus, also known as tigernut sedge, chufa sedge, yellow nut sedge, and earth almond, is a species of sedge that is native to warm, mild, to subtropical climates in the Northern Hemisphere. Tigernut is a versatile crop that grows well in a range of climatic and soil circumstances. The subtropics, warm climate zones, and jungles may very possibly be where it is found. It is abundant in Western Africa, but it is a severe weed of cotton, grains, potatoes, and sisal in Eastern Africa. It is populated, among other places, in South America, Europe, and Asia. Each tuber weighs between 2 and 26 g and produces 50 and 250 tubers each plant (FAO, 1988).

Tigernut belongs to the *Cyperales* order, *Liliopsida* class, *Magnoliph*yta division, and *Cyperaceae* family. *Cyperus esculentus* belongs to the order Commelinales and to the family *Cyperaceae*. When compared to other New World nut sedge species, *Cyperus esculentus* can be distinguished by its determined direct earthy colored spikelets and intensely covering scales. The three-sided stem of the yellow nut sedge has a mild green-yellow hue. Rhizomes that end in tubers are the primary means of proliferation, despite the fact that they can generate viable seed (Deatra, 1999; HBR, 2005).

2.3.1 Nutritional and Health Importance of Tiger Nuts

The tigernut is a rich source of iron, potassium, and phosphorus. Additionally, there is magnesium, calcium, zinc, copper, sodium, and manganese (TTSL, 2005). Phosphorus is inadequately absorbed from the stomach because it is constantly linked to a chemical called phytate in plants. The majority of the minerals in the bones and teeth are calcium and phosphorus (P). It aids in the association of ATP, which is necessary for "authorizing" glucose,

unsaturated fats, and several other substances, as well as the enhancement of mental performance. The impact of phosphate on the human body is crucial. It supports the sharpness and alkalinity rule by acting as a filler (Moore, 2004).

Potassium maintains awareness of the electrolyte and chemical concordance between tissue cells and the circulation (K). K is the essential neuronal component in the intracellular way to acting. Along with important physiological functions like heartbeat, nerve conduction, and intense pressure, it is locked in by several enzyme activities. Foods frequently contain iron (Fe) in a tangled pattern. L-ascorbic corrosive aids in iron maintenance. L-ascorbic acid is a reducing agent that completely converts Fe to a structure that is even easier to hold. Similar to how an acidic medium helps with Fe intake. Fe also helps in the anticipation of illness Every human tissue contains zinc, which has a variety of functions in the body. It is bound to a variety of energy-producing exercises, such as those that utilize proteins, lipids, and carbohydrates. Additionally, it functions in cell division, blood transfer of carbon dioxide and oxygen, and opposition. Given its enormous range of physiological functions, zinc's required secondary effects include a delay in wound healing, reduced hankering, a hindered safe structure, and regrettable improvement (Wardlaw and Kessel, 2002; Moore, 2004).

Magnesium is similarly linked to other protein systems, including those that remember ATP, the body's main source of energy. Magnesium plays a comparable role in energy production, protein interaction, and solid removal (Moore, 2004). In studies, type 2 diabetes and cardiovascular disease have both been linked to low magnesium levels (Al Delaimy *et al*., 2004). (Lopez-Ridaura *et al*., 2004).

Numerous tigernuts and their subordinates are present in sugars, mono-, di-, and polysaccharides (TTSL, 205; Moore, 2004). There are very large amounts of protein, oleic

destructive (a monounsaturated unsaturated fat with a higher security from produced breakdown), and fat available (TTSL, 2005). Tigernuts are rich in vitamins and minerals, especially phosphorus and potassium, and have a lipid component similar to that of olive oil (FAO, 1988; Moore, 2004). Tigernut oil is regarded as a food oil similar to, but superior to, olive oil in terms of quality because of its smooth, exquisite flavor. Because of its higher level of Vitamin E (alpha-tocopherol) and higher concentration of polyunsaturated unsaturated fat and gammatocopherol than other oils, Moore (2004) oil has a more stable oxidative strength (TTSL, 2005). The health advantages of tiger nuts, which include a high monounsaturated unsaturated fat content, high vitamin E levels, and prebiotic qualities, may be required (NUTRA, 2005; Moore, 2004). Vitamin E is essential for the support of cell layers since it protects the body from free radical attack and acts as a disease prevention specialist. Additionally, it might have a role in preventing cellular growth, hence enhancing skin beauty. Skin aggravation and other "wrecks" of the skin can be treated with vitamin E. It is particularly important in oxidatively targeted body parts including the lungs and red platelets. Although studies in this area is currently ambiguous, vitamin E's anti-oxidant characteristics could lessen the likelihood of adverse development and cardiovascular disease (Wardlaw and Kessel, 2002; Moore, 2004). When taken in supra-dietary levels, vitamin E has been shown to aid conditions connected to oxidative stress, such as cardiovascular disease, conditions that pose a hazard to development, Alzheimer's disease, and Parkinson's disease (Brigelius-Flohe, 2002).

levels in, as far as possible the bet of blood bunches, cause vein extending, and prevent Tigernut oil is beneficial because it increases "wonderful" cholesterol (HDL cholesterol) while decreasing "terrible" cholesterol (LDL cholesterol) (HDL-cholesterol). It can also lessen arteriosclerosis brought on by oily substances. Tigernuts may have a significant impact on the

balance and feeding therapy of cardiac diseases because to their high concentration of monounsaturated unsaturated fats (Oleic damaging) (Moore, 2004; TTSL, 2005). Tigernut oil has a significant impact on stomach-related releases (gastric, pancreatic, and bile) because it contains a lot of oleic acid, which is the most amazing promoter of the cholecistokinine relationship (TTSL, 2005). Tigernuts may aid in preventing respiratory setbacks, circulatory issues, and blood clotting Dissolvable glucose, which is abundant in tigernuts and aids in preventing undesirable development. Actually, a few experts discovered that they slow the rate of colon infection. Tigernuts have a strong cell support activity since they have a lot of water-soluble flavonoid glycoside in them (a phytochemical). Utilizing disease prevention specialists could safeguard the safe systems of malnourished individuals. Cell-supporting blowouts may be able to prevent the development of AIDS from HIV tainting (ONRG, 2005).

Because of their rich fiber content and delicious flavor, tigernuts are actually ideal for cutting back on processed foods. Tigernut assimilation is considerably impacted by its high fiber content (TTSL, 2005). This is a direct outcome of fiber's ability to speed up stomach-related fluids, promote procrastination, and speed up movement in the processing parcels, which prevents obstruction.

Because oligosaccharides feed probiotic bacteria and promote the health of the digestive system, tigersnut may have prebiotic qualities (NUTRA, 2005). Moore (2004) claims that although oligosaccharide levels in tigernut have not been quantified, they have been identified in the creamy beverage "horchata." As potential prebiotics, oligosaccharides, which are short-chain carbohydrates, have demonstrated the most responsibility.

Oligosaccharides may help with the absorption of the minerals calcium and magnesium, according to evolving analyses. These effects were observed daily estimates ranging from 5 to

10 g. (Delzenne, 2003). Tigernuts' amino-destructive profile is controlled by arginine. Despite the fact that it is everything but a big amino destructive, arginine is a prohibitively important amino destructive. It is essential for both the developing organism and the infant. In adult disorder stages, it might anticipate a section, especially when tissue is being isolated, such in sepsis or damage (Wu *et al*., 2000). The study of arginine is still a fascinating field of food research, but it should be noted that some of the effects might call for prescription dosages that are astronomically higher than what our typical diet provides (Moore, 2004).

Due to the fact that arginine is a precursor to nitric oxide, it is believed to have enormous amounts of clinical benefits (NO). Endothelial cells in the circulatory system produce NO, a vasodilator that plays a crucial role in the regulation of cardiovascular structure. High plasma cholesterol, diabetes, and hypertension prevent this "endothelium-decided loosening up." In animal studies, Pieper *et al*. (1996) shown that oral administration of L-arginine helped alleviate endothelium laxity in diabetic rodents. On the other hand, Guigliano *et al*. (1997) discovered that giving diabetic males an intravenous mixture of L-arginine (3-5g) helped slow their heartbeat. When administered intravenously, arginine at a dose of 21 grams per day prolonged endothelium-decided relaxation in people with high blood cholesterol. The amount of arginine administered intravenously is noticeably greater than the amount consumed during a typical eating regimen (Moore, 2004).

Tigernuts are free of cholesterol and gluten. They are almost sodium-free (TTSL, 2005). Tigernuts have a high fulfilled of oleic destructive and significantly influence cholesterol levels due to their high vitamin E content, as demonstrated by cogent investigation on "healthy and dietetic pieces of tigernuts" (Farré, 2003), "stomach related pieces of tigernuts" (Bixquert, 2003), and "effects of tigernuts on heart infections and related perspectives" (Valls, 2003).

Tigernuts are excellently supporting, sensible for diabetics, and absolutely ideal for teenagers, the more experienced, and contenders. Since quite some time, it has been ensured that tigernuts have beneficial properties to combat respiratory ailments as well as a few gastrointestinal illnesses (CVNews, 2006). Tigernuts may reduce the risk of colon disease and are ideal for diabetics and persons who are overweight, according to Zimmermann (1987).

According to widespread reasoning in Valencia, Spain (CVNews, 2006), horchata is currently viewed as a decent entrails fix. Horchata is a tigernut-based, sweet-tasting vegetable milk that can be used instead of cow's milk or as a sustaining beverage. Given the following qualities, horchata is a superior alternative to vegetable milk:

i. People who are sensitive to cow-like milk and its byproducts or who are gluten intolerant (celiacs) should drink the appropriate milk.
ii. It supports the reduction of LDL ("terrible") cholesterol and the addition of HDL ("amazing") cholesterol due to its high content of oleic acid and Vitamin E, which impacts lipids.
iii. Arteriosclerosis is avoided by the high concentration of oleic destructive and amino destructive arginine.
iv. Diabetics can benefit from it.
v. Since it contains stomach-related proteins, it is recommended for those who experience stomach problems, gas, or internal dissociation (lipase, catalase and amylase).
vi. There are plenty of phosphorus, potassium, calcium, magnesium, and iron.
vii. It has enormous amounts of vitamins C and E. (TTSL, 2005 and Moore, 2004).

2.3.2 Economic Importance of Tiger Nut

In a few areas of Africa, Europe, and Asia, tigernut is promoted for its good tubers. The plants are a troublesome weed that Egyptians have used as food and medicine. In damp soils or along sandy coastlines, tigernuts are grown for their delicious tubers (ONRG, 2005). Tigernut tubers can be used to make cellulose, vegetable oil, and flour in addition to being eaten raw, seared, or ground into flour (FAO, 1988). In the Mediterranean region of Spain, where tubers are used to make horchata, tigernut is a common collect. Tiger nut concentration, which has a watery, watery appearance and a great, distinctive vanilla and almond flavor, can be used to bars. Unfortunately, tigernut milk removal, also called "horchata," is not well known in Nigeria.

For export to Nigeria, tigernut is filled in Maradi state, Eastern Niger. Comparatively speaking, this generates greater income than other cash crops like cowpea and peanuts. In Northern Nigeria, Ghana, and Togo, tigernut is currently popular as a sweet meat dish or as a side dish served raw. Reliable sources indicate that these nations, as well as others like the Ivory Coast, send 2300 tons of tigernut tubers to Spain (ONRG, 2005). Tigernut seed may be used to break down control seed mixtures and restore wetland ecosystems.

In the United States, tigernut is typically packed to draw in and care for animals, particularly wild turkeys. Tigernuts are revered by turkeys, who will repeatedly return to a plot of them during the winter months or until other food sources are available. To stuff the animals and work on the type of meat, tigernuts have also been spread out in pig and crowd fields (Wikipedia, 2005; HIS, 2005; Anne and Grossman, 1998). Tigernuts have long been known to be a major source of food for cranes and birds. Ducks will pounce for them precisely when wetland fields are spilled over (ONRG, 2005).

According to Burden (2005), tigernuts weigh about 44 pounds per bushel, and a hectare can produce between 0.5 and 1.5 tons of oil. Tigernut may provide high-oleic vegetable oil that is harmful and high-carb tuber cakes for commercial usage. Some manufacturers claim that tuber oil can be utilized in a similar way to how olive oil is. The iodine concentration of tigernut oil places it in the category of a non-drying, dazzlingly unsaturated oil that may be utilized for cooking and as a defining ingredient in companies that produce cleaning agents, salad dressings, frozen yogurt, and other non-food items using vegetable oil (Umerie *et al*., 1997).

Tigernut oil has been determined to have the same fuel value as soybean oil, according to Barminas *et al*. (2001). Tigernut oil has an incredibly high energy density. The tigernut oil's physical and fuel characteristics have been studied, and the researchers found that its actual features are similar to those of other vegetable oils. They admit that this oil might also be utilized to make biodiesel. The waste development after oil extraction could be handled into syrups, flours, even trained creatures handles (Zhang *et al*., 1996; Barminas *et al*., 2001). Tigernut oil can be used in the material industry to waterproof fabric fibers (TTSL, 2005).

Egyptians transported paper, sails, fabric, mats, ropes, and shoes using a sedge plant known as Cyperus papyrus. In the Peruvian Amazon, native women frequently use a local kind of Cyperus as a standard method of contraception. This is a direct outcome of a structure that develops on the roots of Amazonian plants and shares oxytoxic (fruitless) properties with the critter that develops on rye, the ergot.

2.4 Sorrel (Zobo)

There are more than 300 kinds of hinted-at hibiscus utilized as decorative plants in the family Hibiscus, which has a place with the Malvaceae. It has lobed leaves that are occasionally used

for greens and can reach heights of up to 5-7 feet. The few leaves and stems are a kind of rose green. The large sepal, known as a calyx, that encloses the seed boll in the blossom is the actually edible component. With the exception of one grouping where it is estimated to range from 12 to 1 12 creeps, the calyx size fluctuates with each grouping (James, 1994). Although the origin of *H. sabdariffa* isn't fully understood, it is known to be close to Malaysia, where it normally evolved, and to have likely crossed into Africa at a young age. It has largely been circumscribed in the Tropics and Subtropics of the two hemispheres, and it has naturalized in a number of West Indian and Central American locales. It became popular in the West Indies originally and grew mostly as an ornamental plant. *H. sabdariffa* is mostly another species found in Malaysia. It arrived in Malaysia in the middle of the 1990s. The Department of Agriculture in Terengganu pioneered its commercial planting in 1993, and it has since extended to other states. The district is now only about 150 acres in size (Osman *et al*., 2011).

Numerous parts of *H. sabdariffa*, such as seeds, leaves, and dirt-derived products, are employed in diverse food sources. The large red calyces are the most notable of them. Various nations have utilized the large calyces of *H. sabdariffa* as food or a culinary additive for jams, syrups, desserts, puddings, cakes, and wines (Christian *et al*., 2006). The crucial component of its flowers that has an unpleasant taste and is typically employed in the creation of cold and hot beverages as well as a food colorant is the red eager calyx (Maganha *et al*., 2010).

2.4.1 Nutritional and Medicinal Importance of Sorrel (Zobo)

The global interest in healthy beverages has increased in response to the rising consciousness in wellbeing and wealth. Due to its purported medical benefits, zobo drink is widely used in Nigeria (Ezekiel, 2016; Chukwu and Akaninwor, 2017). Nevertheless, Zobo drinks are

distributed using pineapple strips, pineapple flavor, and orange taste (Gbadegesin and Gbadamosi, 2017). Organic pineapple products are a plentiful source of vitamins and phytochemicals that are beneficial to health (Ehler, 2011). Adelekan *et al*. (2014) evaluated how the flavor of Zobo beverages was enhanced by the addition of pineapple juice, Iyeye natural product (Spondiasmombin), and pepper organic product (Denettiatripetala) (Ananas comosus). Fasoyiro and others (2005) inspected the synthetic structure and tactile nature of Zobo enhanced with orange, apple and pineapple. Anyway they improved the Zobo drink with sugar. There is as yet restricted information on the impact that improvement of Zobo drink with pineapple and orange organic product juice might have on the supplement, phytochemical and tactile properties of Zobo drink. Thusly, this study will assess the supplement creation, phytochemical and tactile properties of Zobo drinks, subbed with pineapple and orange natural product juices.

Many different types of particular medicines contain *H. sabdariffa.* It is guaranteed as a Thai standard treatment for kidney and bladder stones (Hirunpanich *et al*., 2006). Additionally, *H. sabdariffa* is reported to have diuretic properties that make it an effective public medication for the treatment of illness and provoking conditions (Dafallah and Al-Mustafa, 1996). (Chewonarin *et al*., 1999). In studies focusing on both humans and animals, the beneficial effects of *H. sabdariffa* removal use to reduce circulatory strain have been demonstrated (Faraji *et al*., 1999 and Onyenekwe *et al*., 1999). More recently, preliminary hypertension has been linked to *H. sabdariffa*'s antihypertensive action (Odigie *et al*., 2003). Additionally, studies on individuals in a similar manner shown how using *H. sabdariffa* had a calming impact. (Beltrán-Debón *et al*., 2010; Herrera *et al*., 2004 Additionally listed uses for H. sabdariffa extract include antibacterial, antifungal, diuretic, uricosuric, and gentle laxative

(Farnworth and Bunyapraphatsara, 1992). Additionally, the fragments of *H. sabdariffa* elimination exhibit immunomodulating, antileukemic, and disease-threatening properties (Muller and Franz., 1992 and Tseng *et al*., 2000). *Bacillus anthracis* and *Staphylococcus albus* have been demonstrated to be affected by *H. sabdariffa* seed oil (Gangrade *et al*., 1979). Additionally, it has been demonstrated that an ethanol concentration of the plant's dried leaves can suppress the growth of certain parasites, including *Aspergillus fumigatus*, *Rhizopus nigricans*, and *Trichophyton mentagrophytes* in vitro (El-Shayeb and Mabrook, 1984).

2.4.2 Antihypertensive

Rodents' hearts were protected from hypertension and hypertension-related damage by a watery concentrate of petals. Additionally, it has been seen that blend significantly lowers systolic and diastolic blood pressure in both shockingly hypertensive and normotensive animals. The systolic circulatory strain and the diastolic pressure were both reduced by 11.2 and 10.7 percent, respectively, by tea of calyces. Patients with difficult to control hypertension were examined for the reasonableness and efficacy of a standardized eliminate, which revealed a decrease in systolic and diastolic circulatory strain of over 10%.

The watery concentrations of the calyx revealed a part-subordinate reduction in the rats' mean vein strain. In hypertensive animals with isolated aortic rings, the concentrate has a vasodilator effect. These effects appear to be mediated through the restriction of calcium uptake into vascular smooth muscle cells and the endothelium-determined nitric oxide-cGMP-relaxant pathway. Regular tea consumption reduces circulatory strain in pre and mildly hypertensive adults and may demonstrate a suitable portion of the dietary adjustments advised for individuals at risk for developing hypertension. Strong circulatory strain has been found in a

standardized study to prevent development in hypertensive people. When distinguished and treated with Lisinopril, a novel twofold outwardly debilitated, reference-controlled primer demonstrated a fundamental reduction in circulatory strain in the hibiscus bundle.

2.4.3 Anticancer

Particularly in HL-60 cells, anthocyanins can induce apoptosis in disease-causing cells. Anthocyanins' effects on LDL oxidation in cell free structure and against apoptotic thresholds in RAW264.7 cells were used to assess how anti-oxidative they were. According to the study, this plant's anthocyanins can act as a chemopreventive specialist by regulating LDL oxidation and oxLDL-mediated macrophage death. The potential of protocatechuic destructive as a disease chemopreventive expert against disease advancement was demonstrated by its inhibitory effect on the development of mice skin.

2.4.4 Antihyperlipidemic

It was demonstrated that the plant extract had inhibitory effects on low-thickness lipoprotein oxidation and antihyperlipidemia in fructose and cholesterol-treated mice. It was discovered that the concentration of LDL and the ratio of LDL to HDL cholesterol had decreased. When used, dried calyx ethanol removal lowers rodents' lipid profiles. Additionally listed are the hypocholesterolemic and disease prevention specialized effects of liquid movements in hypercholesterolemic animals. The audit revealed a protective effect of roselle on LDL oxidation. Disease counteraction specialist effects of the liquid concentrates of dried calyx utilizing rat low thickness lipoprotein were studied. The plant's hypocholesterolemic effect and biochemical components showed that its association significantly reduces the production of serum GOT, GPT, basic and destructive phosphatase as well as total blood protein. After 9

weeks of association, these traits almost went back to their hidden levels. In rodents with heart-guarded effects, liquid concentrates of the red and green plant's petals reduce hard and rapid plasma cravings.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Samples

Spices, dry sorrel leaves (*Hibiscus sabdariffa*), and fresh tiger nuts (*Cyperus esculentus*) were purchased from the Bata market in Kano State, Nigeria. Ginger (*Zingiber officinale*) and cloves (*Evgenia coryphée*) are among the spices used. Additionally, dates, entire pineapples, cucumbers, dates, coconut, and sugar were bought. The study was conducted during May and June of 2022 at Department of Biochemistry, Skyline University Nigeria, Kano-Nigeria.

3.2 Sample Preparations and Analyses

3.2.1 Preparation of Tiger nut Juice

The study used a total of 2 kg of dried tiger nuts. To eliminate unwanted objects, bad nuts, and seeds that can influence the drink's flavor and storage quality, the tiger nut was sorted. The tiger nut was properly wetted to remove any soil that might have adhered, and it was then immersed in 3 L of distilled water at 30 °C for two hours. After that, it was blended at the highest speed for five minutes to create an almost smooth slurry using a kitchen blender in a 3:1 ratio (i.e., 3 L of water per kg of the nut). A muslin cloth was used to filter the homogeneous slurry, and it was squeezed until almost no extract was collected. A whole coconut flesh (grounded), ½ cup of date fruit (grounded), 1 teaspoon of ground ginger and 2 teaspoons of vanilla essence were added.

3.2.2 Preparation of Sorrel Drink

For the study, dried hibiscus leaves weighed around 1 kg. In order to remove foreign substances that can damage the drink's flavor and ability to store, the hibiscus leaves were sifted. The 2.5 liters of water was already boiling when the leaves were added. Cucumber, pineapple, and ginger were grated before being added and boiled for 30 minutes. Using a muslin cloth, the Hibiscus leaves were removed in order to gather the extract. After adding sugar, it was allowed to cool. The beverage is frequently kept cold.

3.3 Physical Parameter (pH) of the Study Samples

In a laboratory setting, the pH of the samples was measured using a pH meter (Hanna instrument type H1 98129) that had been calibrated using two buffer solutions, pH 4 and pH 7. The pH of each sample was then determined and noted.

3.4 Sensory Evaluation

The samples of sorrel and tigernut juice underwent sensory evaluation. 15 semi-trained panelists who were students and workers at Skyline University Nigeria who were familiar with sorrel juice and tigernut juice were given the samples separately. The panelists sampled the tigernut and sorrel juices and rated each sample on a 9-point Hedonic scale (Iwe, 2010), with 1 being the worst and 9 being the best. The sorrel and tigernut juice's appearance, color, texture, taste, flavor, and general acceptance are all evaluated.

3.5 Mineral and Vitamin Content Analyses of Study Samples

3.5.1 Mineral Content

Each dry review test was individually warmed to a sensitive temperature over a Bunsen burner fire until a significant amount of the natural matter was destroyed. This was also heated thoroughly for a few hours in a suppress warmer until white-dark debris was removed. The trash substance was cooled. The ashed substance was mixed with around 20 ml of purified water and 10 ml of the weakened hydrochloric corrosive. This mixture was bubbled, divided into a volumetric cup measuring 250 ml, thoroughly rinsed with hot water, cooled, and made up to volume. Utilizing relevant colourimetric, spectrophotometric, or titrmetric techniques, the minerals content of each specimen was examined (AOAC, 2005; Pye Unicam, 1970; Pearson, 1976). Iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn), copper (Cu), phosphorus (P), calcium (Ca), sodium (Na), potassium (K), and manganese (Mn) were all tested separately (P).

3.5.1.1 Sodium (Na)

The sodium content was determined using a colorimetric method. A standard weakened sodium arrangement will be made by diluting 10 ml of stock sodium solution for 500 ml of water (10 mg/l Na) and setting it away. A sodium stock solution was made by dissolving 1.271 g of sodium chloride in water and weakening to 1 liter (500 mg/g l Na). As a result of the readings, an adjustment diagram was prepared. A mixture of 5 ml of test and 5 ml of uranyl acetic acid derivative was added, mixed, and allowed to stand for 5 minutes. The sample was centrifuged, and the supernatant was collected. It was then mixed with 0.4 ml of potassium fericyanide and 1 percent acidic corrosive. With purified water and the typical weakened sodium, the colorimeter was calibrated to scale 0. Absorbance was perused and the sodium

content was determined utilizing the accompanying equation:

$$Na = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.1.2 Potassium (K)

Both a typical weaken potassium arrangement and a potassium stock arrangement were prepared. The reading information was used to generate an alignment chart. A mixture of 2 ml of sodium cobaltaltonitrate and around 2 ml of test was given to represent 45 minutes. The mixture received around 2 ml of water before being centrifuged for 15 minutes. The supernatant was obtained and mixed with 2 ml of ethanol with a 70 percent alcohol content. After the mixture had been centrifuged for five minutes, the supernatant was allowed to bubble in water for ten minutes. The concentration is diluted with 2 ml of purified water, 1 ml of potassium fericyanide, and around 1 ml of 1 percent choline hydrochloride. Still in the air using a colourimeter at 620 nm. The example arrangement was then perused and sodium content was determined as follows:

$$K = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.1.3 Calcium (Ca)

After precipitation as calcium oxalate, the titrimetric approach was not completely resolved (Pearson, 1976). One milliliter (1 ml) of ammonium oxalate solution was added to five mililitres (5 ml) of tests. Using an ammonium hydroxide solution, the pH was adjusted to 8 and then changed back to 5 using a weak acidic corrosive. The mixtures were centrifuged and tapped after being given 4 hours to settle. It was reheated and around 2 cc of mild sulfuric

corrosive was added. Then, using 0.02 N potassium permanganate (1 ml = 0.0004 g Ca), titration was performed.

3.5.1.4 Iron (Fe)

5 ml of the tiger nut mineral ash solution was mixed with 3 ml of acetate buffer, 2 ml of 2.5 percent hydrochinon, and 2 ml of - dipyridyl. A few drops of ammonia were used to correct the mixture's pH, and the preparation was measured at 250 nm using a photometric colorimeter (Pye Unicam, 1970). The calculations for iron were as follows:

$$Fe = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.1.5 Magnesium (Mg)

To 5 ml of the treated ash solution from the tiger nut sample, one mililitre (1 ml) of magnesium buffer and 2.5 ml of eriochrome blue black tea were added. For ten minutes, this was permitted to stand. A colorimeter was used to measure absorbance at 520 nm (pye Unicam, 1970).

Following is the calculation for magnesium:

$$Mg = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.1.6 Zinc (Zn)

Zinc was determined using the dithizone technique. To 5 ml of mineral ash sample solution, 2.5 ml of 0.2 M acetate buffer and 0.5 ml of 0.1 N sodium thiosulphate were added. After adjusting the pH to between 4 and 5.5, 5 cc of dithizone solution was added. Four minutes were spent shaking the mixture, after which it was left to stand and separate. The remaining

material was read at 535 nm after the supernatant was decanted away (AOAC, 2005). Following is the calculation for zinc:

$$Zn = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.1.7 Copper (Cu)

The mineral ash sample solution received exactly 1 ml of the versenate citrate mixture, 2 drops of the phenolpthaline indicator, and a few drops of strong ammonia (till pink). Additionally, 5 ml of carbon tetrachloride and 1 mililitre of 0.1 percent diethyl diethylene carbamite were added. The mixture was then stirred for 5 minutes and allowed to separate. At 440 nm, the absorbance was measured (AOAC, 2005). Following is a calculation for copper:

$$Cu = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.1.8 Phosphorus (P)

The amount of phosphorus was determined using the VanadoMolybdate technique. To a 5 ml solution of mineral ash sample, four (4) drops of ammonia, 2.5 ml vanadylmolybdate, and 2.5 ml distilled water were added. A colorimeter was used to measure absorbance at 470 nm (Pearson, 1976; AOAC, 2005). The calculation for phosphorus was as follows:

$$P = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.2 Determination of Vitamin Content in Study Samples

Spectrophotometric techniques were used to determine the amounts of vitamins A and E. (AOAC, 2005). The 2, 4 dinitrophenyl hydrazine method of Roe and Kuethe, described by Ball (1994), was used to measure vitamin C (ascorbic acid).

3.5.2.1 Determination of Vitamin A (Retinol)

A test tube containing one gram (1g) of the sample was weighed and macerated for ten minutes with 20 milliliters of n-hexane. The top hexane extract was then split into two 3 ml portions and placed into a dry test tube before being evaporated to dryness. Then, 2ml of 50% trichloroacetic acid (TCA) in chloroform and 0.2ml of acetic anhydride chloroform reagent were both added. At 620 nm, the absorbance was measured after 15 and 30 seconds.

In order to calculate, the following formula was used:

$$Vitamin\ A = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.2.2 Determination of Ascorbic Acid (Vitamin C)

Freshly macerated study materials weighing one gram (g) each were independently liquidized with 50 ml of distilled water and filtered. With 0.5 ml of chloroform and 1 ml of 10% trichloroacetic acid, the sample filtrate was homogenized. After centrifuging, the mixture was left to settle. The clear supernatant liquid was removed, combined with 0.4 ml freshly made color reagent (5 ml 2, 4 dinitrophenyl hydrazine, 0.1 ml 5 percent cupric sulphate, and 0.1 ml 10 percent thiourea), and then incubated for an hour at 56 °C in a water bath. For three

minutes, this was chilled in an ice bath. Each tube received a slow addition of 85 percent sulphuric acid that was extremely cold, mixed, and allowed to sit at room temperature for 30 minutes.

$$Vitamin\ C = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.5.2.3 Determination of Vitamin E

Prior to analysis, 50 ml of distilled water was used to independently liquidize and filter one gram (1 g) of the dry study materials. To extract the oil fraction, the filtrate was diluted with petroleum ether by around 10 ml. After boiling the supernatant for an hour in a water bath while adding 5 ml of 1.5 M alcoholic potash, the supernatant was removed and allowed to evaporate. After adding 5 ml of petroleum ether and 5 ml of distilled water, the mixture underwent a 10-minute centrifugation. Once more, the supernatant was removed and left to evaporate. The following ingredients were added: 3 ml of ethanol, 1 ml of 0.2 percent ferric chloride, and 1 ml of 0.5 percent alcohol. It was measured at 520 nm (AOAC, 2005). Calculation was made using the following formula:

$$Vitamin\ E = \frac{Absorbance of sample \times Concentration of standard solution \times Dilution factor}{Absorbance of standard solution \times Sample volume}$$

3.6 Microbial Screening

3.6.1 Serial Dilution

Transferring 1 ml of each sample into a test tube with 9.0 ml of sterile, distilled water, shaking the tube, and labeling it 1:10 was the initial step in the serial dilution procedure. Then, 1.0 ml (one mile) was transferred from this tube into a another tube marked 1:100, which had 9.0 ml of sterile distilled water. Using sterile syringes diluted up to one to 104, the procedure was repeated with this as well.

2.6.2 Preparation of Culture Media

28.0 grams of supplement agar powder were dissolved in 1 liter of distilled water to create nutrient-rich agar. The heating of the blend entirely broke apart the medium. The medium was cleaned by autoclaving it at a strain of 15 lb for 15 minutes at 121 °C and then letting it cool to 45 °C. Agar that was high in supplements was put into petri dishes and chilled at 8°C until it set. MacConkey agar was prepared according to the manufacturer's (Titan Biotech Ltd.) instructions. 49.5 grams of dried media were suspended in 1 liter of distilled water. The heating of the blend entirely destroyed the medium. The medium was autoclaved for 15 minutes at a tension of 15 lb at 121 °C, then permitted to cool to 45 °C to disinfect it. The MacConkey agar was set into petri dishes and kept up with in the cooler at 8 °C until it set.

3.6.3 Determination of Total Count of Bacteria

The bacterial enumeration was carried out using nutrients agar (NA) and MacConkey agar (MA), both of oxoid grade, in accordance with Cheesbrough's pour plate method (2005). About 1 cc of the serially diluted samples were used to inoculate each triplicate plate. The

culture plates were then incubated for the following 24 hours at 37 °C. After 24 hours of incubation, the colonies on each plate were counted using a GallenKamp colony counter.

3.6.4 Isolation of Bacteria

Purified cultures, such as Nutrient and MacConkey Agars, were produced by sub-culturing distinctive colonies formed from the serial dilution on different selective media.

3.6.5.1 Gram-staining

On clean, grease-free slides, thin smears of the isolates' fresh, pure cultures were prepared every 24 hours, labeled correctly, dried in the air, and fixed by running it three times over the Bunsen burner flame. Crystal Violet solution was applied to the smear and allowed to operate for 60 seconds before being removed with purified water. The smear was then decolorized with 70 percent Alcohol for 15 seconds before being flooded once again with Lugol's iodine, which works as a mordant for 30 seconds, and washed off with distilled water. Safranin was applied to a counter stain, allowed to operate for 60 seconds, and then immediately removed with distilled water. In order to observe the smear, a drop of immersion oil was applied after the smear had dried. The slides were then put on a microscope and observed through a 10X magnification lens. Pink or red color signified Gram negative organisms, while the color purple indicated Gram positive organisms (Fawole and Oso, 2004).

3.6.5.2 Biochemical Tests

Motility

Gently touch a colony of a young (18 to 24 hour) culture that is forming on agar media with a needle. Just a third to half inch deep, stab the tube once in the centre. Make sure to keep the

needle in the same line it entered the medium while removing it. Incubate at 35°–37°C for up to 7 days, checking daily. Search for a sizable growth zone that extends beyond the inoculation line. Negative results are confirmed by growth that is restricted to the stab-line, has sharply defined boundaries, and leaves the surrounding medium plainly transparent. Positive results are indicated by diffuse, hazy growths that extend throughout the medium and make it slightly opaque.

Catalase

With the use of sterile inoculating, a small amount of 24 hour-old culture was placed into a clean slide. Next, a drop of a 3 percent hydrogen peroxide solution was added to the slide. Those bacteria that produce catalase produced white frothy bubbles of oxygen gas, but those that do not make catalase did not produce any bubbles (Cheesbrough, 2006).

Citrate

In the medium used for this experiment, sodium citrate serves as both the only source of carbon and energy. Simmon's citrate medium was the employed medium. The medium was produced as directed by the manufacturer. In 100 ml of distilled water, 2.4 g of citrate agar was dissolved. Each tube received 10 ml of citrate medium, was covered, sterilized, and allowed to cool in a tilted posture. The organisms were streaked once across the surface of the tubes to inoculate them, and they were then incubated for 24 hours at 37 °C. Citrate utilization is positive when the color changes from green to blue, and it is negative when the color remains green (Cheesbrough, 2006).

Indole

When the amino acid tryptophan is degraded by bacteria that have tryptophanase, the resulting molecule is called indole and it contains nitrogen. This test is used to assess an organism's capacity to break down the amino acid tryptophan to produce the chemical indole. Each test isolate was individually inoculated into sterile test tubes with 3ml of tryptone water to conduct the test. After 48 hours of incubation at 37°C, 0.5 ml of Kovac's reagent was applied. After one minute, the setup was shaken for inspection. The interphase was pink or crimson, indicating the synthesis of indole (Cheesbrough, 2006).

Methyl-Red (MR)

This experiment depends on the compartments' ability to provide corrosive by maturing the starch in the growth medium. The test medium, glucose phosphate, was prepared in accordance with the producer. In clean, sterilized test tubes, 1g of glucose, 0.5 percent KH2PO4, 0.5 percent peptone, and 100ml of distilled water were added to make precisely 5ml of glucose phosphate stock. After being inoculated with the test organic entities, the cylinders underwent a 48-hour hatching process at 37 ^{o}C. When 5 drops of pointer (methyl red arrangement) were introduced to the 5 ml culture toward the end of hatching, a variety change was seen. Red variety arrangements produced favorable results, whilst yellow tone arrangements produced unfavorable results (Olutiola *et al*., 2000).

Coagulase

On a spotless glass slide, a drop of regular saline was placed to conduct the test. The complex was gently rocked after a little amount of the isolate was emulsified in a drop of normal saline

and a drop of plasma was added to the suspension. The isolate agglutinated upon observation in 10 (ten) seconds, suggesting a positive coagulase test result (Cheesbrough, 2006).

3.7 Statistical Analyses

Statistics were applied to all the data. The statistical software for social sciences (SPSS) version 20 was used to examine the differences between the vitamin and mineral contents of the sorrel and tiger nut samples. The values were presented as mean standard deviation (SD). P-values less than 0.05, or at a 95 percent confidence level, were used to determine whether the results were significant (P 0.05).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Physical Evaluation of the Tigernut and Sorrel Juice

All of the samples' pH readings, which ranged from 6.07 to 6.41 and indicated an acidic pH, are shown in Figure 4.1. Samples of tiger nut juice had pH levels that were greater than those of sorrel beverage. With a pH of 6.41, TN2 had the highest pH, followed by TN1 (6.35), SJ2 (6.32) and SJ1 (6.07).

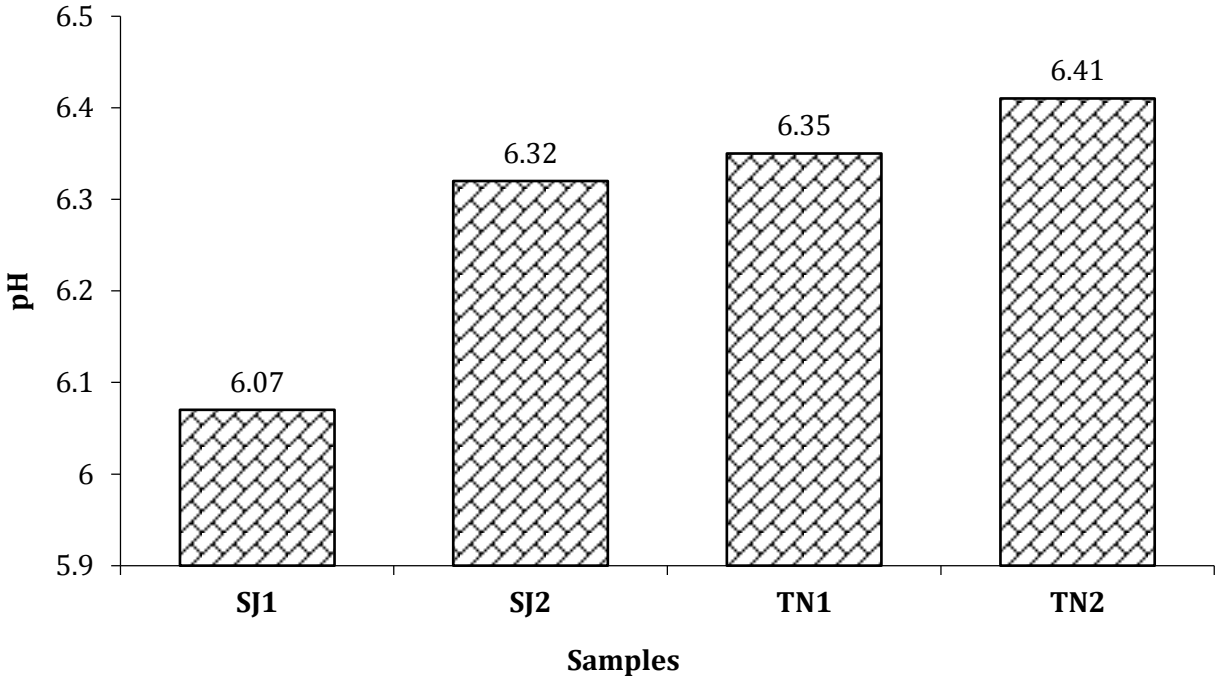

Figure 4.1: pH of Tigernut and Sorrel juice samples

4.1.2 Sensory Evaluation of Tigernut and Sorrel Juice

In Table 4.1, sample SJ1 had the highest mean score for colour, and is closely followed by sample SJ2, TN2 and TNI. From the result, SJ1 had the highest mean score (8.40±0.89) for aroma followed by SJ2 (8.00±0.71), TN1 (7.60±1.14) and TN2 (7.60±0.89). In terms of taste of the samples, TN1 had the highest mean score of 8.40±0.89 followed by SJ2 (8.20±1.30), TN2 (8.00±0.71) and SJ1 (7.80±0.84). The result showed that SJ1 had the highest mean score of 8.20±0.84 for sample appearance. All the samples had overall acceptability.

Table 4.1: Sensory Evaluation of Tigernut and Sorrel Juice

Samples	Colour	Aroma	Taste	Appearance	Overall Acceptability
TN1	7.40±0.55	7.60±1.14	8.40±0.89	8.00±0.71	3.00±0.00
TN2	8.00±1.00	7.60±0.89	8.00±0.71	7.80±0.84	3.00±0.00
SJ1	9.00±0.00	8.40±0.89	7.80±0.84	8.20±0.84	3.00±0.00
SJ2	8.00±1.22	8.00±0.71	8.20±1.30	7.60±1.67	3.00±0.00

Keys: TN – Tiger nut juice; SJ - Sorrel juice

4.1.3 Vitamin and Mineral Constituents of Study Samples

The sorrel and tiger nut juice's mineral composition is displayed in Table 4.2. The findings of the current study showed that different amounts of minerals, including calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), sodium (Na), and zinc (Zn), are found in both sorrel and tiger nut kinds. Further analysis revealed that tiger nuts had higher mineral concentrations than sorrel, with values for Ca, Cu, Fe, Mg, Mn, P, and K of 135.007.81, 0.9.000.36, 4.040.12, 68.075.00, 25.204.53, and 399.6710.60mg/100g, respectively. However, the Zn concentration of sorrel was higher than that of tiger nut (2.050.22mg/100g). Similar to Figure 4.1, Figure 4.2 displays the vitamin components of samples of tiger nuts and sorrel. The results showed that sorrel had higher vitamin A and vitamin E contents than tiger nuts, at 0.640.08 and 3.671.54 mg/100g and 0.640.08 and 7.475.74 mg/100g, respectively. Tiger nuts, however, had higher vitamin C contents than sorrel, at 9.62.18 mg/100g and 7.475.74 mg/100g, respectively.

Table 4.2: Mineral Constituents of Study Samples

Constituents (mg/100g)	**Sorrel**	**Tiger nut Juice**	***p-value***
Calcium (Ca)	129.78±2.07	135.00±7.81	0.326
Copper (Cu)	0.77±0.15	0.9.00±0.36	0.587
Iron (Fe)	3.93±0.15	4.04±0.12	0.398
Magnesium (Mg)	66.4±2.95	68.07±5.00	0.645
Manganese (Mn)	23.53±2.2	25.20±4.53	0.597
Phosphorus (P)	184.00±3.46	189.13±3.05	0.126
Potassium (K)	393.00±2.65	399.67±10.60	0.350
Sodium (Na)	73.67±2.52	73.67±2.52	-
Zinc (Zn)	2.05±0.22	1.98±0.17	0.696
Na/K	0.19±0.01	0.18±0.01	0.678
Ca/P	0.71±0.02	0.71±0.03	0.882

Values are mean±SD of triplicate estimation.

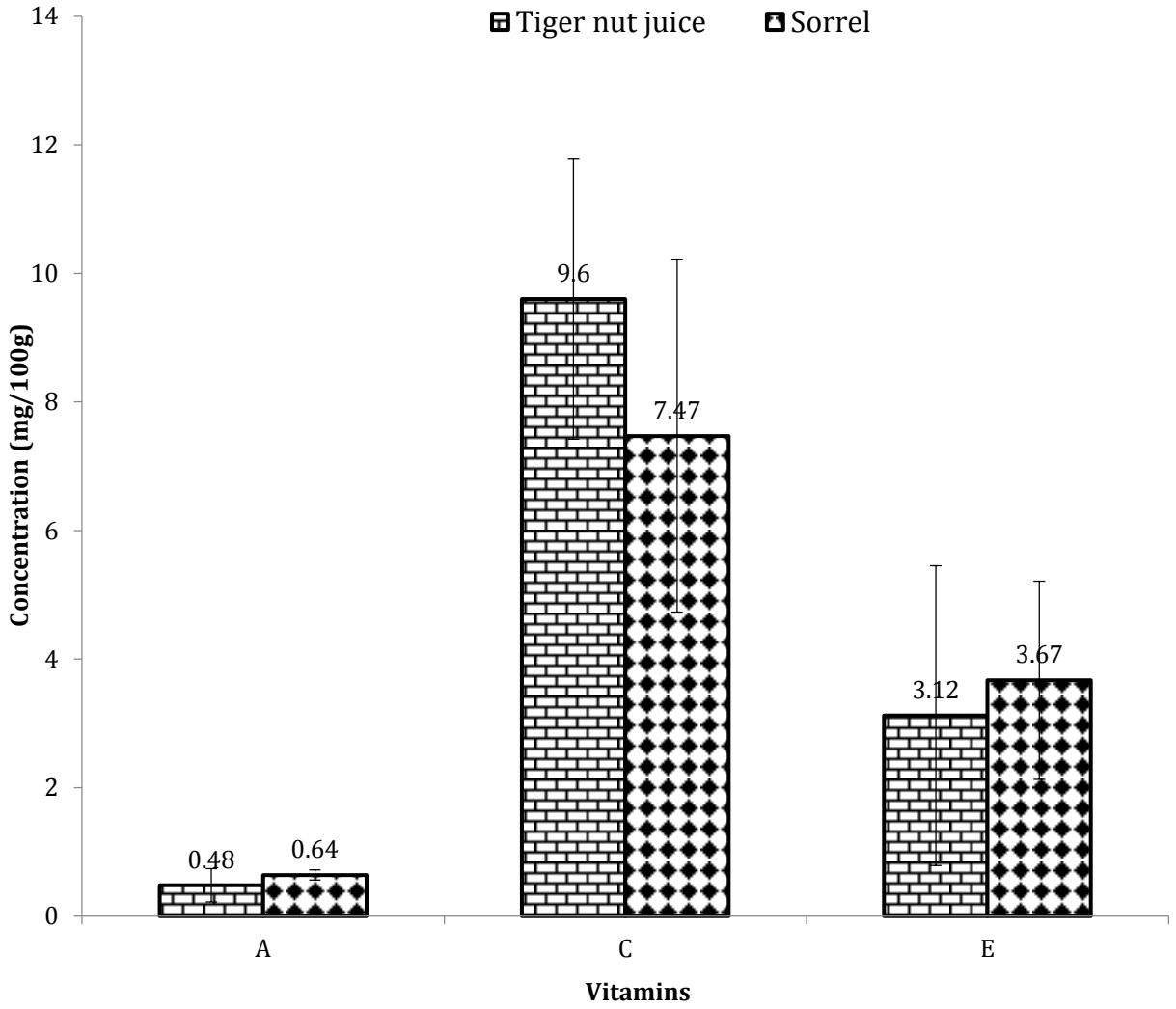

Figure 4.2: Vitamin Constituents of Study Samples.

Values are significant at $p < 0.05$.

4.1.4 Total Aerobic Bacterial Counts

Table 4.3 lists the sorrel and tiger-nut juice samples' total bacterial counts (TBC). The samples' total bacterial counts ranged from 0.25x105 to 6.28x105 cfu/ml. TCC ranged from 0.98x104 to 1.42x104 cfu/ml (total coliform count). TCC levels in samples of tiger nuts were higher than those in samples of sorrel drink.

Table 4.3: Total Aerobic Bacterial Counts in the Study Samples

Samples	**Total bacterial count ($\times 10^5$ cfu/ml)**	**Total coliform count ($\times 10^4$ cfu/ml)**
TN1	4.36	1.33
TN2	6.28	1.17
SJ1	0.41	0.98
SJ2	0.25	1.42

KEY: TN – tigernut juice; SJ – sorrel juice

The total coliform counts in the samples during the serial dilution are shown in Figure 4.3. In the first dilution (10^{-1}), the sorrel and tiger nut juice samples had the most colonies, followed by samples in the second dilution (10^{-2}), third dilution (10^{-3}) and fourth dilution (10^{-4}).

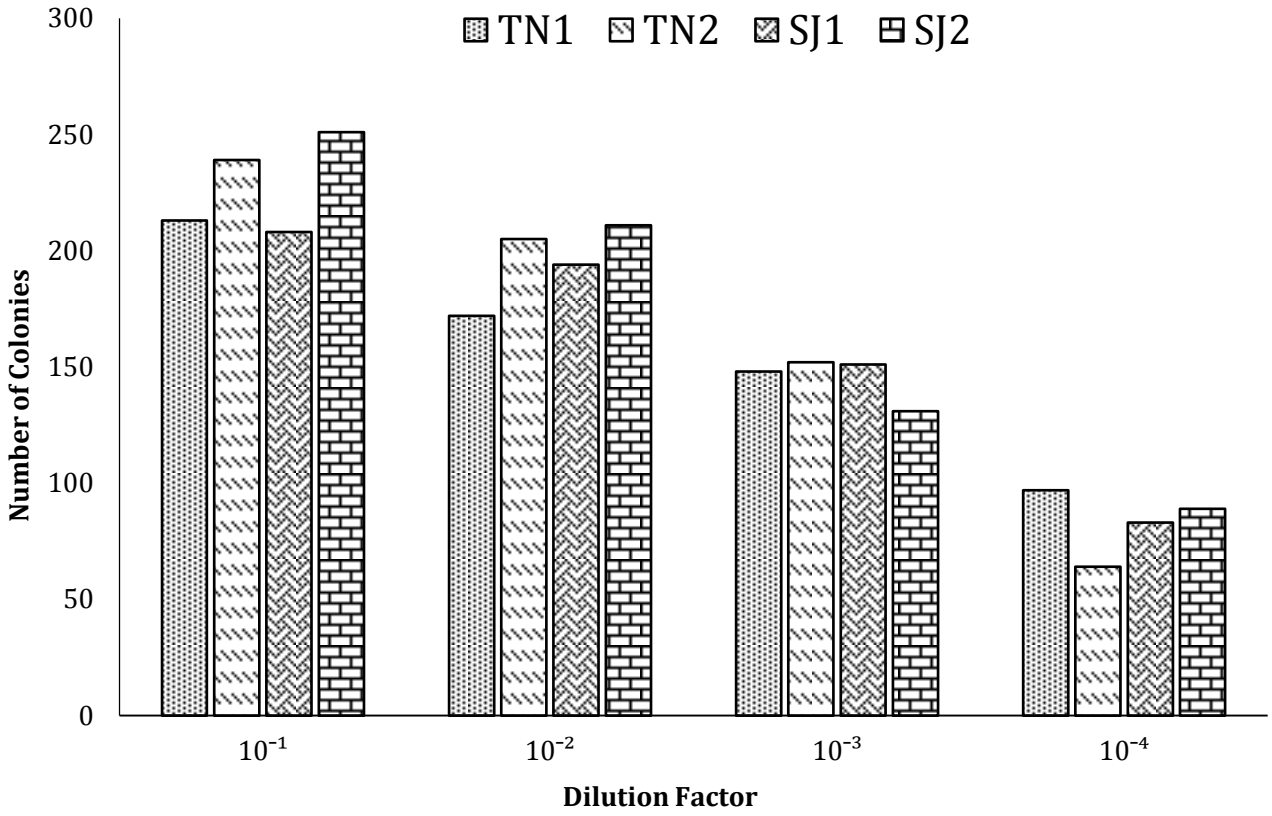

Figure 4.3: Number of colonies obtained from tiger nut and sorrel juice samples

4.1.5 Biochemical and Morphological Characteristics of Test Organisms

Four of the test organisms that were released from the roan and tiger nut juice tests had biochemical and morphological characteristics that indicated they were Gram-negative microorganisms, responded favorably to the catalase test, and were motile. *E. coli*, *Enterobacter*, *Klebsiella*, and *Proteus* species were the bacteria that were disengaged (Table 4.4). Additionally, by biochemical and morphological studies, two Gram positive microorganisms, *Staphylococcus* spp. as well as *Bacillus* spp., were found in the tawny and tiger nut juice.

Table 4.4: Biochemical and Morphological Characteristics of Bacteria Isolates from Study Samples

Samples	**Gram reaction**	**Motility**	**Methyl red**	**Indole**	**Citrate**	**Coagulase**	**Catalase**	**Probable Bacteria**
TN1, SJ1, SJ2	Negative short rod	+	+	+	-	-	+	*E. coli*
TN1	Negative short rod	+	-	+	+	+	+	*Enterobacter* spp.
SJ1, TN1	Positive short rod	+	+	-	+	+	+	*Staphylococcus* spp.
SJ2	Negative short rod	+	-	-	+	-	+	*Klebsiella* spp.
TN1	Negative short rod	+	-	-	-	+	+	*Proteus* spp.
TN1, TN2	Positive short rod	+	-	-	+	+	+	*Bacillus* spp.

Key: TN – tigernut; SJ – sorrel juice; + (positive); – (negative)

4.2 Discussion

4.2.1 Physical Analysis

Tiger-nut juice test C had the most notable pH value, 6.73, whereas test A had the lowest pH value, 6.11. The instances were neither acidic nor basic, according to the pH values, and these characteristics fall within the range of regular water, which is suitable for human consumption. The variations in pH of the tiger-nut juice tests may be attributed to the occurrence of microbiological activities and material reactions throughout age cycles. The examples were neither acidic nor basic, according to the pH readings, making them safe to consume. This causticity can be attributed to specific types of lactic corrosive microscopic organisms producing lactic corrosive (Akoma *et al*., 2006). According to Bolarinwa *et al*. (2009), commodities like zobo and squeezed oranges, as well as burukutu and pito, have comparable high acidic pH values (Kolawole *et al*., 2007). Even though these types of beverages have an acidic character, the causticity will typically increase as the maturation period lengthens and degradation sets in. The acidic notion of the examples may also be due to the possibility that the kunun aya had already started to decay before the time of purchase. This might have caused the production of certain metabolites and led to a pH fall in the object.

The ability of decay living forms to survive at extremely low temperatures is the biggest problem seen in the tiger-nut juice testing (Brooks and Asamudo, 2003). As evidenced by the high levels of bacteria found in the review, temperature ranges between 20.6 °C and 24.8 °C were observed in the current review, which are suitable for microbial development. Controlling ecological temperature may be helpful for reducing the lack of useful microbes in foods and beverages since temperature can affect bacterial endurance (Jaworska *et al*., 2011; Yang *et al*., 2018). In

general, high temperature severely reduces the rationality of microorganisms, but low temperature, akin to refrigeration, has been shown to be better for the endurance of particular probiotics (Champagne *et al*., 2011).

4.2.2 Sensory Analysis

Significant variations were found in the Zobo and tiger nut juice samples' scent, color, flavor, consistency, and general acceptability. The results of this study's values show that the panelists accepted the two samples in every one of the assessed attributes. However, the panelists' top choice was the sorrel (zobo) drink. Therefore, this study demonstrates that the drink's contents result in a Zobo beverage with increased nutritional value that is generally seen as being acceptable.

4.2.3 Vitamin and Minerals

Tests on sorrel and tiger nuts revealed that they are abundant sources of nutrients and minerals that are essential for the growth and support of the organism. It is impossible to overstate the importance of nutrients in maintaining good health, and according to the current review, tawny and tiger nuts serve as excellent sources of essential nutrients like L-ascorbic acid, vitamin An, and vitamin E. The findings of Samson and Safiya (2013), who observed that tiger nuts were a rich source of nutrients A, C, and E in addition to nutrients B1, B2, and B9, were confirmed by the results of the continuing review. Vitamin E and L-ascorbic acid work together as cellular reinforcements to suppress free revolutionaries. L-ascorbic acid also aids in maintaining or reactivating vitamin E levels so that it can continue to function in tissues and immunological structures (L-ascorbic acid is essential for lymphatic flow within the resistive framework). The results presented make it abundantly evident that tiger nuts, which are frequently regarded as

waste items, are rich in iron (for blood development), calcium (for the development of solid bones and teeth), magnesium, and sodium, all of which can contribute to the prosperity of species. Samson and Safiya (2013) and Arafat *et al*. (2009) also found that tiger nuts contain only trace amounts of copper, manganese, zinc, and magnesium despite having high concentrations of sodium, calcium, and phosphorus. As a result, recipes for developing strong and healthy bones and teeth in newborn children may be useful. They said that it has a substantial amount of iron, which can support the growth of blood (Oladele and Aina, 2007). The results obtained after analyzing the data conclusively demonstrate that the mineral convergences in yellow tiger nuts are in the declining demand of K, P, Ca, Na, Mg, Mn, Fe, Zn, and Cu. Intake of calcium, phosphorus, magnesium, potassium, together with a moderate sodium intake, according to Davies (2012), is associated with protection against bone demineralization, blood vessel hypertension, insulin resistance, and generally cardiovascular risk.

4.2.4 Bacterial Load

After the research, it was discovered that the absolute bacterial count varied from 0.25×10^5 to 6.28×10^5 cfu/ml. This data has some similarities to that reported by Aboh and Oladosu (2014), which saw a rise in CFU concentration from 2.00 to 5.10 micrograms per milliliter. In a comparison study, Victor-Aduloju *et al*. (2020) found that tiger-nut milk sold in specific eateries in Awka, Anambra State, increased in microbe development from 6.73×10^5 CFU/ml to 8.20×10^5 CFU/ml. This result is comparable to a paper by Umar *et al*. (2014) that focused on the bacteriological makeup of "Kunun aya" (tiger nut juice), which is marketed on the premises of the Umaru Musa Yar'Adua University in Katsina Similar research was conducted by Makut *et al*. (2014), who found that the 'Fulani' ladies' handling techniques may have contributed to the high microbe counts of the privately handled cow milk products offered in Keffi Metropolis.

Although their examples were road distributed and may have been contaminated by the sellers, the numbers obtained in the current analysis were greater than those reported by Raimi (2013) and Ayandele (2015) who evaluated the bacterial nature of zobo drinks in Oyo and Osun states independently in Nigeria. Only zobo drinks with a bacterial load of less than 10^4 cfu/ml are deemed tolerable for human consumption by Qi *et al*. (2010).

The type and quantity of water supplied during handling could have also served as a source for presenting microbial toxins either in the water or on the implements used. This is in agreement with Amusa and Ashaye (2009) and Nwosu *et al*. (2014), who claimed that contaminated water, containers, and the unsanitary environment where the "Kunun zaki" were handled and sold were to blame for the presence of coliforms like E. coli in the "Kunun zaki." The fact that tiger-nut milk is not subjected to any kind of intense therapy or sanitization throughout the manufacturing process, which could diminish or kill microorganisms present in the beverage, may be responsible for the high overall reasonable include observed in the tiger-nut juice testing. Likewise, According to Musa and Hamza's report, this could be due to contamination from the processing technique, water used in the handling, as well as the overseers' personal hygiene (2013). It might also be as a result of how efficiently consumable water was used to arrange and wash the additional ingredients (ginger, date, and coconut), as well as the tiger nut itself. The presence of coliforms in the beverage is thought to be a symptom of microscopic organism contamination of human and animal origin that may have occurred during handling (Badua *et al*., 2018). Maintaining and storing practices could be blamed for various sources of contamination.

The existence of *Staphylococcus*, *E. coli*, *Enterobacter*, *Klebsiella*, *Proteus*, and *Bacillus* species is shown by the hypothetical isolation and identification of bacterial detaches. This demonstrates the microbial contamination of these neighboring drinks. The majority of the bacteria found in

this investigation were previously described in privately handled matured milk by Musa and Hamza (2013), Umar *et al*. (2014), Makut *et al*. (2013, 2014), Susan *et al*. (2014), and Falegan and Akele, (2014).The findings of this investigation were similar to those found by Ameh and Abubakar (2002), who isolated *S. aureus*, *Proteus* sp, *Streptococcus* sp, *Bacillus* sp, *E. coli*, and Yeast from freshly made zobo beverages. The discovery of *E. coli* in Zobo indicates the existence of waste, natural tainting, and other intestinal microbes. The detected species have grave general wellbeing recommendations. Generally speaking, the pH of kunun aya is too low to allow the growth of harmful microbes, however the presence of *Escherichia coli*, *Bacillus*, and *Staphylococcus* spp. could be a severe cause for concern. *S. aureus* is a characteristic etiological agent causing septic joint inflammation and a typical verdure of the skin, nose, mouth, palms, hairs, and body fluid film (Alice, 1976). *E. coli* is a significant member of the coliform family and is necessary for both human and animal digestive systems to function normally. Some strains of *E. coli* can result in urine lot contamination, infants with loose stools, and gastroenteritis (Abegaz, 2007). In their studies on kunun aya, Adesiyan *et al*. (1983) stated that the presence of organisms including *Bacillus cereus*, *S. aureus*, and *E. coli* could produce a beverage that is unfit for human consumption. Because most people involved in creation, bundling, and sales don't take precautions against potential risk, it is possible that contamination by these bacteria occurred during sifting and packaging. As a result, pollution may be very obvious. The existence of these organisms and their penetration into the food or beverage due to poor cleaning and disinfection is mostly to blame for the tainting of food items by specific types of microbes (Bibek, 2001).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study ascertained the nutritional and bacteriological qualities of selected underutilized local drinks in Northern Nigeria. Sorrel and Tiger nut juice samples were produced and analyze for physical, nutritional and bacterial load using standard methods. The result of the physical analysis showed that the pH of the samples was within the range of 6.07 and 6.41. The samples also contained vitamins and minerals such as Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn at varying concentrations .Total bacterial counts in the samples ranged between 0.25×10^5 and 6.28×10^5 cfu/ml while the total coliform count (TCC) varied from 0.98×10^4 to 6.28×10^4 cfu/ml. The high bacterial load of the samples is an indication that they were contaminated and this can pose a health hazard to consumers. Hence, there is need to employ standard hygienic measures during production for the commercial samples since the production process does not involve any thermal treatment.

5.2 Recommendations

The accompanying proposals have been made in view of the discoveries of his examination study:

1. The nutritive and bacteriological qualities of a few neglected neighborhood drinks in Northern Nigeria were the focus of this study. In order to determine what capacity implies for the nourishing and microbiological properties of the local drinks, the impact

of capacity should thus be focused on the examples to evaluate the duration of realistic usability of the item.

2. To ensure that the item is fit for use, preservation techniques should be used during sales and distribution. The information gathered from this investigation should serve as the basis for general guidance on the type of conservation strategy that will best preserve the nature of this object.

3. To protect students' health, officials and school personnel should mediate by establishing standards for the purchase of raw materials, production systems and practices, and individual health status. Makers and food merchants of kunun aya and zobo ought to be urged to use the specialized help of National Agency for Food, Drugs Administration and Control (NAFDAC) towards accomplishing quality principles. In this way, additional tests are advised to ensure the clients' overall health.

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Appendix I: Procedure for Media Preparation Used in Study

Gram's Iodine

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	300.0 ml
Ethyl alcohol	(95 %)
Ethyl alcohol	(100 %) 95.0 ml
Distilled water	5.0 ml

Kovac's Reagent for Detection of Indole

P-Dimethylaminobenzaldehyde	5.0 g
Amyl alcohol	75.0 ml
Hydrochloric acid (concentrated)	25.0 ml

Dissolved the p-Dimethylaminobenzaldehyde in the amyl alcohol and added the hydrochloric acid

Methyl Red Solution

Methyl red	0.1 g
Ethyl alcohol	300.0 g
Distilled water	200.0 ml

Dissolved the methyl red in the 95% ethyl alcohol and diluted to 500ml with distilled water

Nutrient Agar

Digest of animal tissue 5.00, beef extract 1.50**,** yeast extract 1.50, sodium chloride 5.00, agar 15.00 g/l) and final pH (at 25 °C) 7.4 ± 0.2 was prepared by suspending 28.0 g in 1000 ml of distilled water. Heated to boiling to dissolve the medium completely then sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.

MacConkey Agar

Peptic digest of animal tissue 1.50, casein enzymatic hydrolase 1.50, pancreatic digest of gelatin 17.00, lactose, 10.00, bile salts 1.50, sodium chloride 5.00, crystal violet 0.001, neutral red 0.03, agar 15.00 g/l) and the final pH 7.1 it was then prepared by suspending 51.5 g in1000 ml distilled water. Heat to boiling with gentle swirling to dissolve the agar completely then sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min, finally cooled to 50 °C and poured into sterile Petri-plates. The surface of the medium should be dry when inoculated.