BACTERIOLOGICAL ANALYSIS OF LOCALLY PROCESSED JUICES

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B. Sc. (HONS) IN MICROBIOLOGY

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CERTIFICATION

I hereby certify that this project was carried out by **Onyinye Theresa ANOCHIE (Miss)** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision

_____ _____

Prof. B. A. Omogbai **Date**
(Project Supervisor)

APPROVAL

This project work is accepted in partial fulfillment for the award of Bachelor of Science, B.Sc. (Hons.) in the Department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. S.E. Omonigho **Date**

(Head of Department)

DEDICATION

This research work is dedicated to God Almighty for his infinite mercy, abundant life and sound health He had endowed me with and also to my family for their care and understanding.

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Firstly, I am grateful to God Almighty for his love, protection, favours and also for his divine inspiration throughout my course of study.

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ABSTRACT

This research was undertaken in order to investigate the bacteria associated with locally processed juices and screen for their sensitivity to antibiotics and chitosan. Tigernut milk, zobo and ginger lemon juice samples were purchased from three (3) different shopping malls in Benin City and transported to the laboratory for bacteriological analysis using pour plate isolation method. Susceptibility of bacteria to antibiotics was carried out using disc diffusion method. Antimicrobial activity of chitosan against isolated organisms was carried out using agar well diffusion method. Total bacterial count on nutrient agar ranged from 13.5×10^3cfu/ml for zobo drink (ZN) to 114×10^3cfu/ml for tigernut milk (TNMFJ). On eosin methylene blue agar, total bacterial count ranged from 5×10^3cfu/ml for zobo drink (ZN) to 52×10^3cfu/ml for tigernut milk (TNMN). Organisms isolated include *Lactobacillus fermentii*, *Staphylococcus* spp and *Salmonella* spp. While *Lactobacillus fermentii* was the least prevalent (60%), *Salmonella* spp was the most prevalent (100%). The pH ranged from 3.56 in zobo drink (ZN) to 4.78 in tigernut milk (TNMFJ). Tigernut milk (TNMF) had the highest value of total dissolved solids (2.27mg/ml) while ginger lemon juice (GLN) had the least (0.10mg/ml). The titratable acidity of juice samples ranged from 0.02mg/L in zobo juice (ZN) and ginger lemon juice (GLN) to 0.48mg/L in tigernut milk (TNMFJ). The bacterial isolates showed various levels of sensitivity and resistance to a variety of ten (10) antibiotics. Antimicrobial effects of chitosan against *Staphylococcus* spp and *Salmonella* spp showed that *Salmonella* spp was more susceptible to chitosan at a concentration of 25mg/ml. The results indicate that locally processed juices were contaminated with bacterial isolates and should be subjected to good manufacturing practice (GMP). The inhibitory effect of chisotan show it as a potential preservative in food.

CHAPTER ONE

1.0 INTRODUCTION

Research efforts have been concentrated on the development of beverages from local raw materials which has led to the consumption of both locally produced alcoholic and non-alcoholic beverages in the country.

Tiger nut (*Cyperus esculentus*) belongs to the Division-Magnoliophyta, Class-Liliopsida, Order-cyperales and Family-Cyperaceae and is a cosmopolitan, perennial crop of the same genus as the papyrus plant. Tiger nut has been cultivated since early times (chiefly in South Europe and West Africa) for its small tuberous rhizomes which are eaten raw or roasted, used as hog feed or pressed for its juice to make a beverage (Said *et al*., 2017).

Tiger nuts are valued for their highly nutritious starch content, dietary fibre and carbohydrate and are rich in sucrose (17.4-20.0%), fat (25.5%) and protein (8.0%) (Belewu and Abodunrin, 2008). Tiger nut is also rich in mineral elements such as sodium, calcium, potassium, magnesium, zinc and traces of copper (Belewu and Abodunrin, 2008). Tiger nut had been reported to be a health food, since its consumption can help prevent heart disease and thrombosis and is said to activate blood circulation (Chukuma *et al.*, 2010). It assist in reducing the risk of colon cancer (Adejuyitan *et al.,* 2009). The nutritional contents of tiger nut stimulates its utilization in the preparation of tiger nut beverage so as to provide protein-rich drink at affordable price in place of animal protein which is scarce and expensive.

Zobo is a local, cheaper soft drink made from Hibiscus sabdariffa calyx which is a herbaceous medicinal plant grown in the tropics (Adesokan *et al.,* 2013). The demand for zobo drinks is due to its low prices, nutritional and medicinal properties (Oboh and Elusiyan, 2004; Osueke and Ehirimand, 2004). It is served indoors or at special occasion to people of various tribes and

tradition, in Nigeria.

The concentrated zobo is a dehydrated water extract of the dried petals of the Rosselle plant (*H. sabdariffa*). It is red in colour and is a rich source of vitamins and minerals such as calcium and iron. It is prepared by first boiling the leaves of the Rosselle (that is the dried leaves), spiced up with ginger and cloves, sweetened with sugar to produce the sorrel red drink. This is then dehydrated to concentration (67%). This reddish liquid extract can be reconstituted as a drink. Zobo drink is relished for its colour and flavor and is increasingly becoming a substitute for the more expensively produced soft drinks and juices (Oliver, 1980). Studies have shown that the colour of the drink influence consumer preference for the drink and the variety used for the production of zobo drink (Olayemi *et al.,* 2011) The calyces of *H. sabdariffa* have been found to be rich in vitamins, natural carbohydrates, proteins, vitamin C and other antioxidants (Onuorah *et al.,* 2014) and also minerals (Onuorah *et al.,* 2014; Okafor *et al.,* 2018). According to the work of Egbere *et al.* (2007), the proximate composition of zobo drink is 88.88% water, 0.046% protein, 0.16% fat, 0.16% crude fibre, 0.21% ash and 10.64% carbohydrates.

Zobo drink if well prepared and packaged as a concentrate can compete favorably with most of the imported non-alcoholic beverages available in the country, considering the increasing acceptance, socio-economic potentials, vitamin C and other minerals content (Bamishaiye *et al.,* 2011). The bacterial species commonly found in zobo include; *Pseudomonas* species, *Escherichia coli, Staphylococcus* species, *Streptococcus* species and *Bacillus* species (Amusa *et al.,* 2011)

Ginger (*Zingiber officinale*) is a herbaceous aromatic perennial plant which possesses medicinal properties due to its bioactive compounds (Sanwal *et al.,* 2010), anti-oxidants (Singh and Gupta, 2013), anti-inflammatory activities (Badreldin *et al.,* 2008). Effective anti-oxidants in ginger such

as gingerols, zingerone and vitamin C contents may have capacity to blood thinning and reduce cholesterol levels that may make it useful for treating heart disease. The pungency of ginger is due primarily to the gingerols and shoagaols and the zingiberol, zingiberene, pheallndrene and linalool constituents which account for the aroma of ginger (Malhotra and Singh, 2006).

Lemon (*Citrus limon*) a yellow, prolate fruit with five to ten seeds, botanically a berry is known throughout the world. Lemon was introduced into Spain and Africa between the years 1000 and 1200CE. It is further distributed through Europe by the crusaders who found it growing in Palestine. In 1494 the fruit was being cultivated in the Acores and shipped largely to England. As a cultivated tree, lemon is now grown to a limited extent in most tropical and sub-tropical countries.

Chitosan is obtained by deacetylation of chitin. It consists of D-glucosamine linked to N-acetyl D-glucosamine by b-1, 4-glycosidic bond. The distribution of these subunits depends on the method of preparation of chitosan. In chitosan, degree of deacetylation ranges from 40 % to 98 % and the molecular weight ranges between $5x10^4$ Da and $2x10^6$ Da.

Intense research and development work is being carried out on chitosan as it is considered to be a material of great futuristic potential with immense possibilities for structural modifications to impart desired properties and functions. The presence of reactive amino groups at C2 atom and the hydroxyl group at atom C3 and C6 on chitosan is useful in a wide application in various industries. The positive attributes of excellent bio-compatibility and admirable bio-degradability with ecological safety and low toxicity with versatile biological activities such as antimicrobial activity and low immunogenicity have provided ample opportunities for further development.

Chitosan can be extracted from insects, yeast, mushroom, cell wall of fungi, and marine shellfish such as crab, lobster, krill, cuttlefish, shrimp, and squid pens.

The increase in consumption of shellfish and the expansion of aquaculture have led to a tremendous increase in the quantity of shrimp and prawn being processed and hence in the amount of waste available for chitin/chitosan production. Using mycelium waste from fermentation processes as a source of chitin and chitosan still remains a vast and as yet untapped potential source.

1.1 Aims and Objectives

To isolate and identify bacteria present in locally produced juice beverages such as tiger nut milk, zobo and ginger lemon drink sold in Benin metropolis, Edo state.

To determine the susceptibility of the isolates to chitosan and antibiotics.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tigernut (*Cyperus esculentus)*

Cyperaceae or sedges are a family of monocotyledonous angiosperms found worldwide both in tropical and temperate regions. They make up the seventh largest angiosperm and third largest monocotyledonous family. Only 10% are used by humans and mostly in the tropics such as in Thailand and Southern India where they are cultivated for matting and for basketry (Simpson *et al.,* 2011). To the untrained eye, they appear similar to grasses (Gramineae family). *Cyperus* L., a large genus belonging to the Cyperaceae family comprises of more than 500 species and *Cyperus esculentus* is one of these. It is a perennial plant that develops rhizome producing tubers. The plants are on average 24-90 cm tall, depending on the origin. Stems of the plant are triangular, about 2 mm wide, with leaves reaching up to 30 cm long and 8 mm wide (Ezeh, 2015).

Cultivation of tigernut requires mild climate and sandy soil. Reproduction of tigernut is by seeds and pollination is by wind (Bamishaiye and Bamishaiye, 2011). Tigernut grows widely as a grass in wet places. Tigernut is planted in April and harvested in November. The planting period is grouped into major (April-July) and minor (September-November). During harvesting of tigernut tubers from the field, foreign materials such as stones, animal droppings and other extraneous materials mix with the tubers. Since the tubers of tigernut can be eaten raw, removal of foreign materials and thorough washing of the tubers with potable water is required before raw tigernut tubers can be chewed (Ayeh-Kumi *et al.,* 2014). Although the cultivation and utilization of tigernut dates back to many centuries ago in ancient Egypt, the plant had successfully been introduced to other parts of the world through human activity spanning many centuries. Tigernut

requires irrigation almost every week until the tubers are due for harvesting (Bamishaiye, 2011; Abano and Amoah, 2011). It has been established through several researches that mineral contents and acceptability of tigernut-milk drink is affected by the location and planting period of tigernut (Asante *et al.,* 2014).

Tigernut is well distributed in Chile, Brazil and USA. It naturally grows in Ghana, Nigeria and Sierra Leone (Sanchez-Zapata *et al.,* 2012). Cultivation of tigernut takes place in other West African countries such as Cameroon, Senegal, Guinea and Cote d'ivore. In Nigeria, tigernut is grown mainly in the northern region and the tuber is available in the market all year round (Bamishaiye and Bamishaiye, 2011). Tigernut can also grow in the middle belt of Nigeria (Nwaoguikpe, 2000). It grows luxuriantly in wet marshes and areas close to streams (Bamishaiye and Bamishaiye, 2011). The geographical spread of four varieties of yellow nutsedge was described by Schipper *et al.* (1995). According to their study, the variety *Cyperus esculentus* is abundant in Africa and Southern Europe; variety heermannii is rarely seen growing in many countries except in SouthWest region of USA, variety *C. macrostachyus* grow abundantly in Central America whereas *C. leptostachyus* is present in both the Old and the New World.

In Egypt, it is used as a source of food, medicine and perfumes (De-Vries, 1991). Tigernut is commonly known as earth almond, chufa and chew-fa and zulu nuts. It is known in Nigeria as Aya in Hausa, Ofio in Yoruba and Akiausa in Igbo where three varieties (black, brown and yellow) are cultivated. Among these, only two varieties, yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size, attractive colour and fleshier body. The yellow variety also yields more milk upon extraction, contains lower fat and more protein and possesses less anti-nutritional factors

especially polyphenols (Okafor *et al.,* 2003). Tigernut can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called Horchata De Chufas or tigernut milk.

2.2 Tigernut Milk

Tigernut milk is an edible light brown coloured liquid extract obtained from tigernut tubers. In Spain and Ghana, tigernut milk is known as ←chufa de horchata→ and ←Atadwe→ respectively (Nwobosi *et al.,* 2014). Tigernut milk is a sweet, non-dairy, nutritious, energetic and diuretic drink. It contains reasonable quantity of minerals predominantly phosphorus and potassium. Tigernut milk has vitamins C and E (Gambo and Dau, 2014). Tigernut milk has reasonable quantity of carbohydrate, fats and proteins required to meet daily human nutrition needs.

Interestingly, tigernut milk contains higher quantity of iron and magnesium than cow milk. However, the protein content of cow milk is higher than that of tigernut milk. The protein content of tigernut milk is 6.05% (Bamishaiye and Bamishaiye, 2011). The low protein content of tigernut milk can be boosted by blending it with milk from other sources. Bambaranut-tigernut-coconut milk beverage blends had been developed and its proximate composition and consumer acceptability determined by (Adedokun *et al.,* 2014).

Due to increasing cost of cow milk in developing countries, attention is shifting to non-dairy milk such as tigernut milk (Awonirin and Udeozor, 2014). More consumer depth knowledge about non-dairy milk is increasing due to factors such as dietary limitations, allergens, religious beliefs and moral principles of people of different tribes and races. In fact, tigernut milk is not only known for its nutritional benefits because recently it is also being considered as a medicinal drink (Gambo and Dau, 2014).

Tigernut milk help control heart attacks and thrombosis; cause improvement in blood circulation and contribute in reducing risk of developing colon cancer (Ukwuru *et al.,* 2011). (Asante *et al.,*

2014) were able to compare expected milk solids (EMS) from tigernut-milk from brown and black variety tigernut tubers for industrial applications.

It is interesting to note that tigernut milk does not contain lactose and casein. As a result of this, tigernut milk is recommended to individuals who do not have the ability to tolerate gluten or lactose present in cow milk. Tigernut milk without refined sugar added to it is a suitable drink for people suffering from diabetes. Tigernut milk contains arginine which liberates the hormone that produces insulin. This makes tigernut milk suitable for people suffering from diabetes. In recent times, more individuals are becoming increasingly concerned about excessive consumption of foods and beverages that have high fat and cholesterol content. Therefore, products low in fat and cholesterol is highly recommended to the general public. As for those individuals that are lactose intolerant, non-dairy products such as tigernut milk is strongly recommended. Tigernut milk is preferred by diabetics because it does not contain sodium, lactose sugar, casein protein, gluten and cholesterol. Apart from being a drink for everyone, tigernut-milk is a special drink suitable to meet the nutritional requirements of certain category of people such as diabetics, lactose intolerance individuals, sports men and women (Okudu and Ogubuike, 2016; Udeozor, 2012; Bristone *et al.,* 2009).

Tigernut-milk drink can be subjected to fermentation using yoghurt starter to produce tigernut-milk yoghurt. Varieties of non-dairy products are derived from fermentation of tigernut milk. They are naturally prepared tigernut-milk drink, pasteurized tigernut-milk drink subjected to pasteurization, tigernut-milk drink subjected to sterilization, tigernut-milk subjected to ultra-high temperature sterilization and condensed tigernut-milk drink (Wakil *et al.,* 2014; Bristone *et al.,* 2015; Ukwuru and Ogbodo, 2011). Concentrated and condensed tigernut milk is subjected to pasteurization, sterilization or ultrahigh temperature sterilization in order to reduce or completely

eliminate microbial contamination of the product so that the shelf life of the product is prolonged (Krichene *et al.,* 2016; Wakil *et al.,* 2014).

2.2.1 Preparation of Tigernut Milk

For the Nigerian tiger nut milk to be successfully prepared, the following ingredients are needed; tigernut, filtered water, salt, flavorings such as vanilla powder, maple syrup, cinnamon, dates are optional.

Tools needed: Grinder, Milk extractor, Stainless bowls, Sieves.

2.2.2 Steps taken in making Tigernut Milk

Stones, bad tigernuts and unwanted materials were removed from the batch of tigernuts to be extracted, the sorted tigernuts were washed and soaked in clean water, dried tigernuts are soaked for 24hrs while fresh tigernuts (un-dried tigernuts) can be soaked for 4-6hrs this is to help soften the tigernuts for easy grinding and also easy removal of any adhered dirt to the tigernuts. The soaked tigernuts is sieved from the water, washed in clean water and blended using a blender, the ground tigernuts is filtered with a muslin cloth, to give the milk and the residue, the milk is filtered to ensure there is no residue in the milk. Extracted tigernut milk is dispensed in air-tight bottles or jar and stored in the refrigerator to prevent spoilage by activities of spoilage microorganisms.

Tigernut milk could be sold at supermarket, Schools, Offices, Churches e.t.c.

2.2.3 Microbial Contamination of Tigernut Milk

Microbial contamination of tiger nut milk usually occurs as a result of unhygienic preparation, use of contaminated raw materials and crude utensils (Umar *et al.,* 2014; Osuntogun and Abiola,

2014). Microbial contamination of tigernut milk could be traced to microbial contamination of tigernut tubers used for production. A study carried out by (Arranz *et al.,* 2006) identified aflatoxin B1 from samples of horchata sold in Southern Europe. This is an indication that the products were contaminated with fungi that produce mycotoxins. The bacterial load of tigernut juice sold in some localities within Katsina metropolis in Nigeria is quite high (Osuntogun and Abiola, 2014).

In Spain where tigernut beverages have been commercialized, a comparative study between microbiological quality of commercial and home-made tigernut beverage was determined. Findings from that study revealed that total viable count of all commercially prepared tigernut beverages were below the detection limit. However, *Enterobacteriaceae* (3.41-5.47 log cfu/ml), *Escherichia coli* (2.69 log cfu/ml), *Bacillus* species (1.79-2.47 log cfu/ml), yeasts (2.69-4.47 log cfu/ml) and moulds (3.63-4.47 log cfu/ml) were present in home-made tigernut beverages. These values did not exceed legislated levels (Sebastian *et al.,* 2012). In a related study, Onovo and Ogaraku (Onovo and Ogaraku, 2007) identified microorganisms associated with exposed and unexposed tigernut-milk. Results from their study revealed that frequency of occurrence of *Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, Fusarium solani, Saccharomyces L cerevisiae, S. fubiligera and Candida pseudotropicalis* isolated and identified from tigernut-milk samples 13.04, 21.74, 13.04 and 4.35 %, respectively. On average, they reported that bacterial load in exposed and unexposed tigernut-milk samples are 1.2×10^{3} cfu/ml and 0.2×10^{-3} cfu/ml, respectively. The presence of these microorganisms in tigernut-milk is considered a threat to public health because they are capable of producing toxic metabolites which can cause ill health in humans.

2.2.4 Health Benefits from Tigernut Consumption

Tigernut milk has been found to be good for arteriosclerosis since it contains Arginine which is a precursor of nitric oxide that helps the vein to expand. Tigernut milk without sugar can be taken by a diabetic because it contains carbohydrates which has a base of sucrose and starch (without glucose) and also for its content of Arginine which liberates the hormone that produces the insulin. Tigernut milk is also an ideal drink for people who are not able to take gluten and also for those who are unable to take cow's milk and derivatives. It could also be recommended for those who have heavy digestions, flatulence and diarrhea because it provides us a lot of digestive enzymes like catalase, lipase and amylase. Considering the nutritive and health benefits of the underutilized tigernuts, there is the need for increased utilization and awareness of its health benefits. Moreover it is suggested that products from tigernuts should be encouraged so as to solve the problem of protein-calorie malnutrition in Africa more so that high price of imported milk and milk products (for instance) coupled with poor milk production in Nigeria in particular and Africa in general seem to have made consumers more ready to accept milk produced from plant sources.

2.3 Zobo (*Hibiscus sabdariffa)*

Zobo drink, a non-alcoholic local beverage is produced from the dried petals of *Hibiscus sabdariffa* (Linn Roselle) by boiling and filtration (Ameh *et al.,* 2009). *Hibiscus sabdariffa* (Roselle) is a vegetable plant of West Africa origin. It has however been noted that the crop is native to India but was introduced to other part of the world such as central Africa, West Indies, Australia, Africa and many tropical countries as it is best grown in tropical and subtropical regions (Omemu *et al.,* 2006). It has the most widespread acceptance in the Roselle producing areas of the Nigerian savanna region where it is grown as a vegetable crop (Omemu *et al.,* 2006).

The Roselle is an annual herbaceous, upright plant growing up to two metres belonging to the family Malvaceae. Its habitat is variable and the leaves also vary in shape and size. The flowers are usually yellowish, sometimes occurring with dark red pigmentation at the center (Rice, 1990).

Zobo drink is one of the locally made beverages in Nigeria and Africa. It is prepared through an indigenous technology. People prefer zobo drink to the carbonated drinks because it is rich in natural carbohydrate, protein, antioxidants, vitamin C, calcium, magnesium and zinc. It is non-alcoholic, medicinal and has low glycemic index (Wong, 2002; Oboh *et al.,* 2011). Zobo is becoming acceptable in social gathering because it is economically affordable and attractive to many people more than soda (Olayemi *et al.,* 2011). Increase in religious and health campaigns against alcoholic beverages in Nigeria and the consequent decrease in the consumption of alcoholic beverages in certain areas has afforded zobo drink great potential as a local alternative to imported red wines in particular and alcoholic beverages in general (Egbere *et al.,* 2007).

Recently, zobo drink has become a main source of income in many homes both in rural communities and more in the urban areas where cottage business has increased due to support from the government through the poverty alleviation schemes, thereby alleviating poverty among the people (Essien *et al.,* 2011). Based on the numerous merits of zobo drink, many researchers had worked on examining its nutritional value, sensory quality and medicinal properties. Zobo was found to be very rich in vitamin C, other antioxidants and minerals (such Potassium, Sodium and Phosphorus) but low protein (Olayemi *et al.,* 2011; Egbere *et al.,* 2007; Fasoyiro *et al.,* 2005). This accounts for its limitation to solve protein energy malnutrition. Many researchers have reported on the preparation and preservation of zobo drinks with different food items, such as lime. (Nwachukwu *et al.,* 2007) revealed that total coliforms and total viable counts generally decreased in values following treatment of zobo drink samples with lime juice. Large scale

production of zobo drink is limited by its tendency for rapid deterioration. It has a shelf-life of approximately twenty-four hours following production, if not refrigerated. The drink is prone to microbial contamination which can lead to food spoilage (Omemu *et al.,* 2006) . A lot of factors such as, packaging mode before retailing, and the poor hygienic practices as well as lack of portable water, proper storage and waste disposal facilities at preparation and service points have resulted in poor unsanitary conditions and thus served as potential contaminants and increased risk to public health in commercial zobo drinks sold (Omemu and Aderoju, 2008).

2.3.1 Preparation of Zobo Drink

In the preparation of zobo drink, the following ingredients are needed; zobo(*Hibiscus sorrel*) leaves, piece(s) of ginger, glove(s) of garlic, enough water, pineapple cut, slices of citrus fruits; orange, lemon, lime (to garnish)

2.3.2 Steps taken in making Zobo Drink

Zobo leaves are washed into a pot, enough water is added and the contents of the pot covered. The zobo leaves are allowed to boil at medium to high heat for 5 minutes, ginger and garlic and more water is added the mix is allowed to boil for at least 30 minutes. This is the time it will take for the zobo leaves to be completely soft, the heat is turned off while the boiled leaves is set aside to cool completely. When cool, the zobo leaves are sieved and poured into a muslin cloth to remove tiny particles and leave only a smooth juice. Blended and sieved pineapple juice is then added, the mix can be sieved through a chiffon cloth again to ensure there is are particles left, garnishment and flavorings are added at this time and stirred. Ready to drink zobo juice is dispensed in air-tight bottles or jar and stored in the refrigerator to prevent spoilage by activities of spoilage microorganisms.

Zobo juice could be sold at supermarket, Schools, Offices, Churches e.t.c

2.3.3 Microbial Contamination of Zobo Drink

Typically, microorganisms invade food products from several perspectives ranging from exposure, handling and storage. (Ayandele, 2015) reported that spices usually added being an agricultural commodity may contain high level of microbial load. A large number of lactic acid bacteria, coliform, mould and yeast in zobo drinks have been reported. These microorganisms have implication for food spoilage and possible infection as they utilize the carbohydrate content of zobo drink for fermentation process to produce undesirable compounds, the fact that zobo is never subjected to any form of post-production treatment that can eliminate or at least reduce the bacteria load in the drink; it could be a potential source of health hazard. Also the activities involved in the cooling and subsequent dispensing of the drink into containers also represent a potential source of health hazard (Bulakarima *et al.,* 2017).

The microbial diversity found in zobo drink is mainly bacteria and fungi/yeast. The microbes that have been widely isolated from zobo drink sold in public places in Nigeria belong to the genera *Staphylococci, Escherichia, Proteus, Pseudomonas, Salmonella, Shigella, Enterobacter, Klebsiella, Serratia, Bacillus, Streptococci, Lactobacilli, Clostridium, Corynebacterium, Aeromonas, Micrococcus* (bacteria), *Aspergillus, Saccharomyces, Candida, Rhizopus, Fusarium, Mucor, Geotrichum* (fungi). To a large extent *Staphylococcus, Escherichia, Proteus, Pseudomonas, Klebsiella, Bacillus, Streptococcus, Aspergillus, Saccharomyces, Candida* are the predominant microbial isolates found in zobo drink consumed in Nigeria. Most of these microbes have invaded the products from the dried *H. sabdariffa* bought in the market (Omemu *et al.,* 2006).

2.3.4 Health Benefits from Zobo Consumption

Ginger (*Zingiber officinale*), one of the ingredients used in zobo production, contain effective anti-oxidants in ginger such as gingerols, zingerone and vitamin C contents, which may have capacity to blood thinning and reduce cholesterol levels that may make it useful for treating heart disease (Fahlberg, 1969).

The calyces of *H. sabdariffa* have been found to be rich in vitamins, natural carbohydrates, proteins, vitamin C and other antioxidants (Onuorah *et al.,* 2014) and also minerals (Onuorah *et al.,* 2014; Okafor *et al.,* 2018).

2.4 Ginger Lemon

Ginger (*Zingiber officinale*) is a herbaceous, aromatic, perennial plant with the characteristics of growing horizontal or fibrous rhizomes. They are cultivated in many countries, though this plant prefer moist, tropical conditions. Ginger thrives in well drained soils like sandy loam, clay loam, red loam or lateritic loam. A friable loam with a pH of 6.0 to 6.5 rich in humus is optimal for production of zingiber. The crop prefers a temperature range of 19➔28◦C and a humidity of 70–90% (Jayashree *et al.,* 2015). Before cultivation, the land needs to be ploughed four to five times. Ginger is propagated by portions of rhizomes known as seed rhizomes. Preserved seed rhizomes are carefully cut into small pieces of 2.5-5.0cm length weighing 20-25g; each of the pieces should have one or two buds. China, Indonesia, Nigeria, Philippines, Thailand and India are the main ginger producers. Ginger reaches full maturity in 210-240 days after planting.

Harvesting of ginger for vegetable purposes starts after 180 days based on the demand. As ginger is used in many different forms and high amounts are consumed in the countries where it is grown or is used to provide seeds for the next crop, it is difficult to get accurate figures of trade of

zingiber. To enhance the productivity of crops, in some countries, at the time of planting, people use decomposed cattle manure or compost at 25-30 t/ha, either by broadcasting over the beds prior to planting or applying them in the pits at the time of planting. Rhizome seed generation is difficult and expensive. Therefore, people are looking for other techniques to cultivate zingiber cost effectively. A transplanting technique in ginger by using single bud sprouts (about 5g) is recommended to produce high quality planting material with reduced cost. The technique involves raising transplants from single sprout seed rhizomes in the pro-tray and planting in the field after 30-40 days. This technique is greatly useful for reducing the quantity of rhizome seed (Jayashree *et al.,* 2015). To improve the quality, in some countries, crop rotation and intercropping techniques are also used. Ginger is usually intercropped in coconut, areca nut, coffee and orange plantations in Kerala and Karnataka (Jayashree *et al.,* 2015). Changes in soil fertility for improved growth of ginger under different quantities of pigeon pea hedgerow alley management produced a significant increase in ginger yield (Nwaogu, 2014). Another important technique is propagation by using internodal cuttings (Davidson, 2006). Aeroponic cultivation of ginger has also been introduced (Hayden *et al.,* 2004) as well as micropropagation and cytogenetic assessment (Archana *et al.,* 2013).

Lemon (*Citrus limon*) a yellow, prolate fruit with five to ten seeds, botanically a berry is known throughout the world. Lemon was introduced into Spain and Africa between the years 1000 and 1200CE. It is further distributed through Europe by the crusaders who found it growing in Palestine. In 1494 the fruit was being cultivated in the Acores and shipped largely to England. As a cultivated tree, lemon is now grown to a limited extent in most tropical and subtropical countries. Lemon trees for commercial planting are propagated by grafting or budding the desired variety on seedlings of other citrus species such as the sweet orange, grape fruit, mandara orange,

sour orange or tangelo. The relatively cool climatic zones of coastal Italy and California are especially favorable for lemon cultivation. The trees are commonly grown in orchards where they are spaced 5-8metres (16-26feet) apart. Lemon trees usually bloom throughout the year and the fruit is picked six to ten times a year. Full sized fruits for commercial purposes is about 50mm (2 inches) in diameter. The fruit is usually picked while it is green and after curing may be kept 3 months or more in storage.

Among the important by products of lemon are Citric acid, citrate of lemon, lemon oil, lemon juice and pectin. Lemon oil is used in the production of perfumes, soaps and as flavoring extract, this is an important industry in Silicy. Citric acid is used in the beverage manufacturing, pectin has long been an important material in the making of fruit jellies and it is also being used in medicine in the treatment of intestinal disorders as an anti-hemorrhagic.

Lemon juice (lemon fruit extract) alongside ginger is used in the making of functional beverage with antioxidant properties, consumed by health conscious people.

2.4.1 Preparation of Ginger Lemon Juice

For the Nigerian Ginger lemon juice to be successfully prepared, the following ingredients are needed: Ginger, lemon fruit, water

2.4.2 Steps taken in making Ginger Lemon Juice

Ginger tuber is grated and added to boiling water and allowed to boil for 20 minutes, the heat is turned off and boiled ginger set aside to cool completely. When cool, the boiled ginger is sieved and poured through a chiffon cloth to remove tiny particles and leave only a smooth juice. Lemon fruit is squeezed for its juice, the lemon juice is added to the cooled ginger drink, the mix can be

sieved through a chiffon cloth again to ensure there is no particles left. Garnishment or artificial flavorings of are added to the mix and stirred. Ready to drink ginger lemon juice is dispensed in air-tight bottles or jar and stored in the refrigerator to prevent spoilage by activities of spoilage microorganisms.

Ginger lemon juice could be sold at supermarket, Schools, Offices, Churches e.t.c.

2.4.3 Health Benefits of Ginger Lemon Drink

Ginger lemon drink is a ready to be served beverage, Ginger lemon drink offers the following health benefits;

Improved immunity

Lemon and ginger are normally good sources of vitamin c, antioxidants, fibre, vitamin B-6, magnesium and potassium, this components have immunity boosting properties and can guard against some bacteria.

Anti-hypercholesteraemic effect

Ginger extracts interfers with cholesterol biosynthesisleading to decreasing cholesterol levels. Ginger extracts have antilipidemic effects, by reducing thermogenesisand high lipids levels. It also helps to increase serum HDL-cholesterol (Al-Awwadi, 2010; 2013).

Regulation of blood glucose and lipid levels

Ginger is very effective in lowering blood glucose level when same has been taken in dried form. It also decreases cholesterol and triglyceride level. Long term usage helps to increase high-density lipoprotein cholesterol concentrations (Afzal *et al.*, 2011; Li *et al*., 2012).

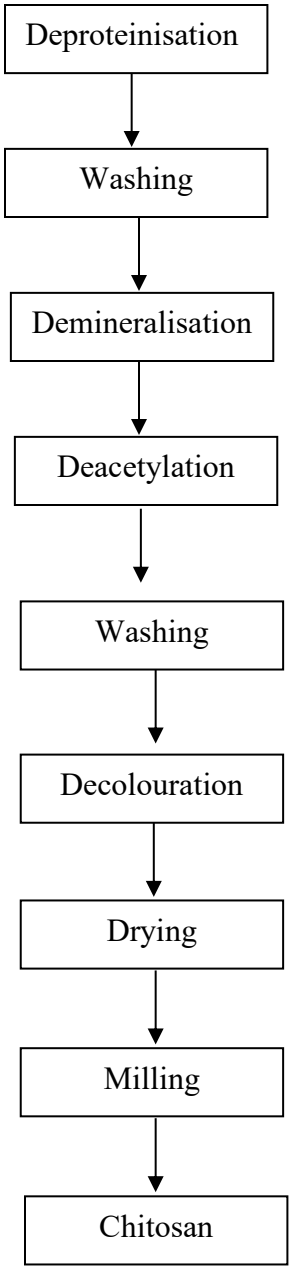
Menstrual cramps (dysmennorhea)

The powerful anti-inflammatory action on prostaglandin synthesis help in menstrual cramps (Mahmoud *et al*., 2012; Kubra *et al.*, 2013).

2.5 Chitosan

Majority of chitosan available globally is produced by the USA, Japan, Norway, Thailand, India, Australia, and Poland. The production of chitosan involves various steps such as preparation of the chitin from the biological material followed by the deacetylation that would result in chitosan. Thus, typical production of chitosan from crustacean shell generally consists of four basic steps: demineralization, deproteinization, decoloration, and deacetylation. Demineralization and deproteinization steps are interchangeable in terms of order. The exoskeleton of crustacean is a major starting material used for commercial production of chitosan.

Typical flow chart for manufacture of chitosan is given below



Demineralization

The mineral content in the exoskeleton of crustacean is not the same for all species of crustaceans. Demineralization is generally carried out using acids such as hydrochloric acid, nitric acid, acetic acid, or formic acid (up to 10 %) at room temperature with agitation to dissolve calcium carbonate as calcium chloride. However, hydrochloric acid is the preferred acid and is used at a concentration of 0.2-2M for 1-48h at temperatures varying from 0°C to 100°C. Demineralization for 1-3 h using dilute (1-8 %) hydrochloric acid at room temperature produces appreciable amounts of calcium chloride. A solid-to-solvent ratio of 1:15 (w/v) is usually used. The ash content of the demineralized shell is an indicator of the effectiveness of the demineralization process.

Deproteinization

Chitin occurs naturally in association with protein. The protein is bound by covalent bonds to chitin through aspartyl or histidyl residues, or both, thus forming stable complexes (Attwood and Zola, 1967). Deproteinization of chitin is usually carried out by alkaline treatment. The shells are treated with sodium or potassium hydroxide at 65-100°C at a minimum shell-to-alkali ratio of 1:4 for periods ranging from 1 to 12 h. Under these conditions, the protein becomes detached from the solid component of the shrimp waste. Relatively high ratios of solid-to-alkali solution of 1/10 or 1/20 with proper agitation are used to increase the deproteinization efficiency. To prevent oxidation of the products, the process is usually carried out in a nitrogen atmosphere and in the presence of sodium borohydride ($NaBH_4$). After completion of deproteinization step, the protein hydrolysate is removed easily by separation of the solids from the protein slurry by filtration. Prolonged alkaline treatment under severe conditions causes depolymerization and deacetylation.

Decoloration

Chitin obtained after the demineralization and deproteinization of shell waste is a colored product. For commercial acceptability, the chitin needs to be decolorized or bleached to yield cream white chitin powder (No *et al.,* 1989). The pigment in the crustacean shells forms complexes with chitin. (Fox, 1973) found one 4-keto-ß-carotene and three 4,40-diketo-ß-carotene derivatives firmly bound to the exoskeletal chitin of red kelp crab. The level of association of chitin and pigments varies from species to species among crustacean. The residues are decolorized using solvents and/or oxidants (Acosta *et al.,* 1993). During the process of decoloration, the chemical used should not affect the physicochemical or functional properties of chitin and chitosan. (No *et al.,* 1989) were able to prepare a near-white-colored crawfish chitin by extraction with acetone, which was dried for 2h at ambient temperature, followed by bleaching with 0.315 % (v/v) sodium hypochloride solution (containing 5.25 % available chlorine) for 5 min with a solid-to-solvent ratio of 1:10 (w/v), based on dry shell.

Deacetylation

Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. There are several critical factors that affect the extent of deacetylation including temperature and time of deacetylation, alkali concentration, prior treatments applied to chitin isolation, atmosphere (air or nitrogen), ratio of chitin to alkali solution, density of chitin, and the particle size. Considering all these as necessary conditions, the ideal process condition of deacetylation should yield a chitosan that is not degraded and is soluble in dilute acetic acid in minimal time (Muzzarelli *et al.,* 1980). The N-acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus, alkaline methods must be employed for N-deacetylation (Muzzarelli, 1977). Severe alkaline hydrolysis treatments are required due to the resistance of groups imposed by the

trans arrangement of the C2-C3 substituents in the sugar ring. It is generally achieved by treatment with concentrated sodium or potassium hydroxide solution (40-60 %) usually at 80-140^{o}C for 30 min or longer using a solid-to-solvent ratio of 1:10 (w/v) to remove some or all of the acetyl groups from the polymer (No and Meyers, 1989). Sodium hydroxide is the preferred alkali. After deacetylation, the chitosan is washed to completely remove alkali and is dried to give flakes. The material should be low in protein and ash. Production of chitosan by chemical processes has several disadvantages such as environmental pollution, inconsistent molecular weights, and degree of acetylation.

2.5.1 Novel Methods for Preparation of Chitosan

The conventional harsh conditions used for extraction could adversely affect the quality of the chitin. Novel methods are being developed to replace conventional demineralization and deproteinization to extract chitin from crustacean waste. The use of enzymes in the deproteinization step has been extensively studied. Shrimp waste deproteinized using *Aspergillus niger*, washed, dried, and then demineralized using acetic or lactic acid produced by fermentation from low cost biomass such as cheese whey, has been reported (Rinaudo, 2006). A number of microorganisms such as *Bacillus subtilis, Lactobacillus helveticus, Pseudomonas aeruginosa, Lactobacillus paracasei, Lecanicillium fungicola, and Penicillium chrysogenum* have been utilized for demineralization (Choorit *et al.,* 2008; Oh *et al.,* 2008). These microorganisms are responsible for the precipitation of organic salts such as calcium lactate, which is easily removed from media by wash out.

Deproteinization is also carried out with the aid of proteolytic activities of some microorganisms. The calcium, magnesium, and potassium acetates obtained as by-products are suggested as possible de-iceing agents, while the calcium and potassium lactates could find applications as

food preservatives. Enzymatic deacetylation by using fungal chitin deacetylase also has commercial potential.

2.5.2 Characterization of Prepared Chitosan and its Properties

Molecular Weight

One of the most fundamental parameters characterizing a macromolecule is its molecular weight. Knowledge of the molecular weight of polysaccharides is of fundamental importance for the understanding of their applications and their role in living systems. The molecular weight of chitosan depends largely on the conditions of deacetylation and can be determined by methods such as chromatography (Bough *et al.,* 1978), light scattering (Muzzarelli, 1977), and viscometry (Maghami and Roberts, 1988). Viscometry is the simplest and most popular method to determine molecular weight of chitosan. The method however has the disadvantage of not being absolute because it relies on the correlation between the values of intrinsic viscosity with those of molecular weight. Chitosan is available commercially with molecular weight ranging from 10,000 to 1,000,000 Da.

Viscosity

Viscosity of chitosan increases with increase in its molecular weight and concentration. Increasing the degree of deacetylation also increases the viscosity (Skaugrud, 1991). This can be explained by the fact that high and low deacetylated chitosan have different conformations in aqueous solution. Chitosan has an extended conformation with a more flexible chain when it is highly deacetylated because of the charge repulsion in the molecule. However, the chitosan molecule has a rod-like shape or coiled shape at low degree of deacetylation due to the low charge density in polymer chain.

The viscosity of chitosan solution is also affected by factors such as concentration and temperature. As the chitosan concentration increases and the temperature decreases, the viscosity increases. Chitosan viscosity decreases with an increased time of demineralization due to depolymerization (Moorjani *et al.,* 1975). Similarly, (No *et al.,* 1999) demonstrated that chitosan viscosity is considerably affected by physical (grinding, heating, autoclaving, ultrasonication) and chemical (ozone) treatments. Viscosity of chitosan solution stored at 4 C is found to be relatively stable.

Solubility

Solubility characteristics of chitosan are based on its degree of deacetylation. High degree of deacetylation shows higher solubility, and low degree of deacetylation shows poor solubility (Heux *et al.,* 2000). It has swelling characteristics due to much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains. Chitosan solubility depends on the amount of protonated amino groups in the polymeric chain and, therefore, on the proportion of acetylated and non-acetylated D-glucosamine units. Its cationic nature is unique relative to other neutral or negatively charged polysaccharides. Chitosan is a strong base possessing primary amino group with a pKa value of 6.3. The pH of solution substantially alters the charged state and properties of chitosan (Yi *et al.,* 2005). At low pH, the amines get protonated and become positively charged and that makes chitosan a water-soluble cationic polyelectrolyte.

On the other hand, as the pH increases above 6, chitosan amines become deprotonated, and the polymer loses its charge and becomes insoluble. At higher pH, precipitation or gelation tends to occur, and the chitosan solution forms poly-ion complex with anionic hydrocolloid resulting in gel formation (Kurita, 1998). The soluble insoluble transition occurs at its pKa value around pH between 6 and 6.5. Chitosan can easily form quaternary nitrogen salts at low pH values. So,

organic acids such as acetic, formic, and lactic acids can dissolve chitosan. The most commonly used solvent for chitosan is 1% acetic acid at about pH 4.0 (Rinaudo *et al.,* 1999). Chitosan is also soluble in 1% hydrochloric acid and dilute nitric acid but insoluble in sulfuric and phosphoric acids. Thus, solubility of chitosan is related to the degree of deacetylation, the ionic concentration, pH, the nature of the acid used for protonation, and the distribution of acetyl groups along the chain, as well as the conditions of isolation and drying of the polysaccharide. The high molecular weight of chitosan, which results in poor solubility at neutral pH and its high solution viscosity, limits its use in the food, cosmetics, agriculture, and health industry (Xia *et al.,* 2011).

Degree of Deacetylation

Degree of deacetylation (DD) has often been cited as an important parameter that determines many physiochemical and biological properties of chitosans such as crystallinity, hydrophilicity, degradation, and cell response. Degree of deacetylation of chitosan is generally controlled by processing of the native polymer with alkali and with increasing time and temperature to obtain the highest degree of deacetylation (>90) materials. During the deacetylation reaction, the acetyl group of the chitin reacts with NaOH and produces an amine group. This is a reversible reaction, and when NaOH concentration is increased, the reaction is biased toward the forward direction by producing more chitosan. As a result, deacetylation will increase. In the deacetylation process, acetyl groups are removed from the polymers randomly, resulting in a final polymer that has a random distribution of acetyl glucosamine and glucosamine units. The biopolymer is characterized as either chitin or chitosan according to the deacetylation which is determined by the proportion of D-glucosamine and N-acetyl D-glucosamine. Various methods have been reported for the determination of the degree of deacetylation of chitosan such as

(1) Spectroscopy (infrared, ultraviolet, or 1H, 13C, 15N nuclear magnetic resonance)

(2) Conventional methods (various types of titration, conductometry, potentiometry, ninhydrin assay, adsorption of free amino groups of chitosan by picric acid)

(3) Destructive methods (elemental analysis or acid or enzymatic hydrolysis of chitin or chitosan) followed by colorimetric methods or high-performance liquid chromatography, pyrolysis gas chromatography, and thermal analysis using differential scanning calorimetry. Of these, 1H NMR has been found to be simple, rapid, and more precise than many of the other methods (Rinaudo, 2006).

Crystallinity

One of the major physical characteristics that determine the functional properties of chitosan is the crystallinity (Trang *et al.,* 2006). Crystallinity has been found to have an effect on metal sorption. (Piron *et al.,* 1997) found that the crystallinity of chitosan controlled the sorption rate and total uptake of uranyl, concluding that sorption was only possible in the amorphous domains and not in the crystalline domains. The crystallinity of the polymer can also control the accessibility of the amine groups (Guibal, 2004). The crystallinity of chitosan is determined by X-ray diffraction (XRD) in which the pattern produced by the diffraction of X-rays through the closely spaced lattice of atoms in a crystal is recorded and then analyzed to reveal the nature of the lattice.

Complex Formation with metals

Chitosan exhibits superior metal ion sequestering ability than chitin. It has reactive amino group and hydroxyl group and chelates many transition metal ions. Chelation is related to the amino content as well as to the distribution of the amino group. The nature of the cation is very important in the mechanism of interaction (Rhazi *et al.,* 2002). Various processes such as

adsorption, ion exchange, and chelation have been considered as the mechanisms responsible for complex formation between metal ions and chitosan. The type of interaction prevailing depends on the metal, its chemistry, and pH. Under heterogenous conditions, at pH less than 6, chitosan acts as a poly (monodentate) ligand, while at a higher pH, it behaves as a poly (bidentate) ligand forming chelates. However, in solution, the formation of complexes in which two amino groups belonging to the same chain or different chains coordinated to the same metal ion can also take place.

2.5.3 Modifications of Chitosan

Chitosan can be modified to improve its physicochemical properties to suit various applications. Modification of chitosan is possible due to the presence of several functional groups in the polymer. It has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions The main goals of modifying chitosan are to provide derivatives that are soluble at neutral and basic pH values; to control hydrophobic, cationic, and anionic properties; as well as to attach various functional groups and ligands (Mourya and Inamdar, 2008). Strong intramolecular and intermolecular hydrogen bonds exist in chitosan to form random orientations. The dissociation and reorganization of these hydrogen bonds by chemical modification facilitate the production of novel molecular conformations in the forms of solutions, hydrogels, fibers, films, and sponges (Tokura *et al.,* 1996).

Acylation

A variety of acylation reactions are possible with chitosan. Acylation with long chain aliphatic carboxylic acid chlorides such as hexanoyl, dodecanoyl, and tetradecanoyl chlorides give derivatives with a high degree of acylation. N-acylation of chitosan with fatty acid (C6→C16) chlorides increased its hydrophobic character. Such acylated products are soluble in chloroform

(Fujii *et al.,* 1980). Chitosan with a higher degree of deacetylation is more susceptible for acylation owing to a decrease in hydrogen bonding. N-acyl chitosan has the ability for longer retention in body and resistance to digestible enzymes like lysozyme and chitinase and is more biocompatible than native chitosan (Hirano and Yagi, 1980).

Graft Copolymerization

Graft copolymerization reaction introduces side chains and makes various molecular designs possible, thus affording novel types of tailored hybrid materials composed of chitosan and synthetic bpolymers. The properties of the graft copolymers can be controlled by molecular structure, length, and number of side chains attached. Grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecule, the graft, onto the chitosan backbone. The swelling behavior of chitosan at different pH has been improved by graft polymerization of vinylic monomers such as acrylic acid, acrylamide, and acrylonitrile onto chitosan (Borzacchiello *et al.,* 2001; Mahdavinia *et al.,* 2004). Super absorbents (absorb aqueous solutions up to hundreds of times their own dry weight) have been prepared by grafting these resins with chitosan (Nge *et al.,* 2004) and have possible applications in infant diapers, feminine hygiene products, agriculture, and other specialized areas (Dutkiewicz, 2002). Different types of chitosan graft copolymers have been prepared for use as flocculants, paper-binder strengtheners, and slow-release drug carrier. Polyethylene glycol (PEG) has been grafted onto chitosan to prepare water-soluble chitosan derivatives that have been used as carrier of anticancer drugs. Phosphorylated chitosan synthesized by grafting mono (2-methacryloyl oxyethyl) acid phosphate onto chitosan improved antimicrobial activities (Jung *et al.,* 1999).

Carboxymethyl Chitosans

It is an amphoteric polymer, is a derivative of chitosan, and is prepared under controlled reaction conditions. It can be synthesized by reductive alkylation wherein the amino group of chitosan is reacted with the carbonyl group of aldehyde glyoxylic acid and then hydrogenated by reaction with $NaBH_4$ or $NaCNBH_3$ to give carboxymethyl chitosans. It can also be prepared by direct alkylation using monohalocarboxylic acids such as monochloroacetic acid in alkaline medium. Carboxymethyl chitosans have enhanced biological and physicochemical properties compared to chitosan and hence have promising biomedical applications (Mohan *et al.,* 2012).

N-methylene Phosphonic Chitosans

These are anionic derivatives with amphoteric character and are synthesized under various conditions and proved to have good complexing efficiency for cations such as Ca^{2+} and those of transition metals (Cu (II), Cd (II), Zn (II), etc.) (Heras *et al.,* 2001). The complexation provides corrosion protection for metal surfaces. These derivatives are also modified and grafted with alkyl chains to obtain amphiphilic properties that have potential applications in cosmetics.

Carbohydrate-Branched Chitosan

Carbohydrates can be grafted on the chitosan backbone at the C2 position by reductive alkylation: disaccharides such as cellobiose and lactose (having a reducing end group) are introduced, in the presence of a reductant, on chitosan in the open chain form. These derivatives are water soluble. Carbohydrates can also be introduced without ring opening on the C6 position. These derivatives are important as they are recognized by the corresponding specific lectins and thus could be used for drug targeting (Morimoto *et al.,* 2001).

Alkylated Chitosans

Alkylated chitosans are very important as amphiphilic polymers based on polysaccharides. They exhibit surface activity and increase considerably the viscosity of aqueous solution due to hydrophobic interchain interactions. Alkyl chitosans are compatible with neutral and cationic surfactants

2.5.4 Applications of Chitosan

Chitosan and its derivatives have varied applications in agriculture, food processing, biotechnology, chemistry, cosmetics, dentistry, medicine, textiles, veterinary medicine, and environmental sciences. The polyelectrolyte nature and the presence of reactive functional groups are responsible for the gel-forming ability, high adsorption capacity, biodegradability, and antimicrobial properties of chitosan which in turn are essential for its commercial applications.

2.5.4.1 Antimicrobial Activity

Chitosan displays a broad-spectrum antimicrobial activity against bacteria, molds, and yeasts. It is effective against both Gram-positive and Gram-negative foodborne microorganisms, including *Aeromonas hydrophila, Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Clostridium perfringens, Brochothrix* spp*, Enterobacter sakazakii, Lactobacillus, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia liquefaciens, Staphylococcus aureus, and Escherichia coli* O157H7; the yeasts *Candida, Saccharomyces, and Rhodotorula; and the* molds *Aspergillus, Penicillium, and Rhizopus.* The chitosan and its derivatives are effective against plant pathogenic bacteria such as *A. tumefaciens, C. fascians, E. amylovora, E. carotovora, P. solanacearum,* and *S. lutea and* fungi *A. alternata, B. fabae, F. oxysporum, P. digitatum, P. debaryanum, and R. solani* (Vishnukumar *et al.*, 2005; Venugopal, 2011).

The exact mechanism of antibacterial activity of chitosan is not fully understood and several factors contribute toward this. Three models have been proposed, to explain the antimicrobial action of chitosan. The most satisfactory model suggests that the antimicrobial effect of chitosan is due to its polycationic nature. In an acid environment, the NH_2 groups in the C_2 position of chitosan protonates to yield NH_3^+, which binds to negatively charged carboxylate ($\rightarrow COO\leftarrow$) groups located on the surface of the bacterial and fungal cell surfaces, causing disruption of the barrier properties of the outer membranes of the microorganisms followed by leakage of cell components (Tsai and Su, 1999). This hypothesis is supported by electron microscopy studies that show binding of chitosan to outer membrane of bacteria (Raafat *et al*. 2008). The pH of the microenvironment in which chitosan functions determines the relative concentrations (ratios) of unprotonated and protonated amino groups. At a pH ~ pKa, 50 % of amino group are protonated. At pH 5.5, the positively charged amino group contributes 90%, and at pH 4.5, 99%. The antimicrobial effectiveness of chitosan appears to be highest below pH 6.0, where the protonated form predominates and where chitosan is most soluble.

Second proposed mechanism is based on ability of chitosan to bind with microbial DNA, leading to inhibition of the mRNA and protein synthesis (Sebti *et al.,* 2005). In this hypothesis, chitosan molecules are assumed to be able to pass through the bacterial cell wall, composed of multilayers of cross-linked murein, and reach the plasma membrane. This theory is supported by confocal laser scanning microscopy where the presence of chitosan oligomers (a chain with few number of monomer units) inside *E. coli* exposed to chitosan under different conditions has been demonstrated (Lui *et al.,* 2001).

The third mechanism is based on ability of chitosan to chelate metals. It is well known that chitosan has excellent metal-binding capacities where the amine groups in the chitosan molecules

are responsible for the uptake of metal cations by chelation; this results in reduced microbial growth and toxin synthesis (Goy *et al.,* 2009). This mechanism is likely to be more efficient at high pH values where positive ions are bounded to chitosan, since the amine groups are unprotonated and the electron pair on the amine nitrogen is available for donation to metal ions.

The ability of chitosan to form gas-impermeable coating interferes with fungal growth. It inhibits different developmental stages such as mycelial growth, sporulation, spore viability and germination, and the production of fungal virulence factors (El- Ghaouth *et al.,* 1992). The derivatives of chitosan, such as N-trimethyl, sulfonated chitosan, and chitose oligomers, have been reported to demonstrate antibacterial activities against *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis, Klebsiella pneumoniae,* and *Proteus vulgaris* to different extents (Venugopal, 2011).

2.5.4.2 Factors affecting antimicrobial activity

The antimicrobial activity of chitosan depends on its molecular weight, degree of deacylation, pH of solution, and, of course, the target organism.

Molecular weight: The antimicrobial activity of chitosan increases as the molecular weight increases. However, it is difficult to find a clear correlation between molecular weight and antimicrobial activity of chitosan when comparisons are between different studies. This is mainly attributed to the fact that many investigators have used an uncertain term for low molecular weight (LMW) and high molecular weight (HMW) chitosan without indicating exactly its MW. There are reports that conclude positive, negative, and neutral effects of MW on antimicrobial activity of chitosan (Badawy and Rabea, 2011).

Degree of deacetylation: The antimicrobial activity of chitosan is directly proportional to the degree of deacetylation of chitosan. The increase in degree of deacetylation means the increased

number of amino groups on chitosan. As a result, chitosan has an increased number of protonated amino groups in an acidic condition and dissolves in water completely, which leads to an increased chance of interaction between chitosan and negatively charged cell walls of microorganisms (Sekiguchi *et al.,* 1994).

The pH: The antimicrobial activity of chitosan is strongly affected by the pH. At lower pH, there is an increase in the number of protonated amino groups on chitosan in addition to the ↑hurdle effect↓ of inflicting acid stress on the target organisms (Badawy and Rabea, 2011).

Temperature: The incubation temperature also has an effect on the antimicrobial activity of chitosan. Higher temperature (37^0C) has been shown to enhance its antimicrobial activity compared to refrigeration temperatures (Kong *et al*., 2010).

Cations: Antimicrobial action of chitosan is inhibited by divalent cations in the order of $Ba^{2+} > Ca^{2+} > Mg^{2+}$. It is proposed that the cations form complexes with chitosan and consequently the reduced available amino groups of chitosan lead to the reduced bactericidal effect (Badawy and Rabea, 2011).

Chitosan possess a number of characteristics that make it a suitable antimicrobial polymer for various industrial applications. These include the following: easy and abundant availability, long-term storage stability at the temperature of its intended application, it does not decompose to and/or emit toxic products, it is not toxic or irritating to handlers and it is biocidal to a broad-spectrum of pathogenic microorganisms.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Samples

Tiger nut milk, zobo and ginger lemon locally produced ready-to-serve juice samples were purchased three (3) from different shopping malls in Benin City and immediately transported to the microbiology laboratory. These malls were chosen because they are highly patronized by consumers. The samples collected were for bacteriological analysis.

3.2 Preparation of Culture Media

All media were prepared according to manufacturer's instruction. The media used were Nutrient agar and Eosin Methylene Blue agar.

3.2.1 Nutrient Agar

This is used to culture non- fastidious organisms and for bacterial heterotrophic plate count. This medium was prepared from commercially available dehydrated powder, available from most suppliers of culture media. In the preparation, 28g of nutrient powder was dissolved in 1litre of distilled water in a conical flask covered with cotton wool wrapped with aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^{o}C for 15 minutes. The medium was cooled to 45-50^{o}C and then dispensed aseptically into sterile petri dishes.

3.2.2 Eosin Methylene Blue Agar

This media is a slightly selective and differential plating medium for the isolation of gram-negative enteric bacteria. In the preparation, 37.4g of eosine methylene blue agar powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool wrapped in aluminum foil paper. This is stirred and autoclaved at 121^{o}C for 15 minutes and then cooled to

45-50^{o}C. A portion of the medium (20ml) was poured into a sterile Petri dishes and allowed to solidify.

3.2.3 Mueller-Hinton agar

This is a microbiological growth medium that is commonly used for antibiotics susceptibility testing specifically disk diffusion tests. In the preparation, 38g was dissolved in 1litre of distilled water in a conical flask covered with cotton wool wrapped in aluminum foil paper. This is stirred thoroughly and sterilized by autoclaving at 121^{o}C for 15 minutes and then cooled to 45-50^{o}C. A portion of the medium (20ml) was poured into a sterile Petri dishes and allowed to solidify.

3.3 Determination of Total Bacterial Load

One (1 ml) milliliter of each sample was added to 9 ml of sterile normal saline test tubes, and from which 10-fold serial dilution was carried out to achieve 10^3 dilution. After carrying out the 10-fold serial dilution, 0.1ml of the appropriate diluent was spread on sterile Nutrient agar and Eosin Methylene Blue agar. The cultured plates were incubated at 37^{o}C for 24 hours .After incubation, the colonies in each plate was counted and expressed as colony forming unit per millimeter (cfu/ml). Hence the number of bacterial present in 1ml of the sample was calculated with the formula:

$$\text{Cfu/ml} = \frac{\text{number of colonies}}{\text{Volume plated x dilution factor (0.1ml)}}$$

3.4 Isolation and Identification of Bacteria isolated from the Samples

Streaking method was employed to isolate discrete colonies of bacteria from the mixed culture. Eosin Methylene Blue, was employed for the isolation of bacteria for the purposes of identification. Eosin Methylene Blue was used for the selective isolation of enteric coliforms. All plates were incubated at 37 ℃ for 24 hours. Identification of bacteria isolates was based on standard cultural, morphological and biochemical methods.

3.5 Cultural Characteristics

Each colony morphology e.g., size, shape, margin, elevation, consistency, color, transparency was determined.

36 Morphological Test

3.6.1 Gram staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.

3.6.2 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A

few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.6.3 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

3.6.4 Urease test

The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37^{o}C for 24-48 hours. Urease positive cultures produced a red-pink colour due to changes in the colour of the indicator

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

3.6.5 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37^{o}C for 24-48 hours. The development of deep blue colour after incubation indicate a positive result.

3.6.6 Hydrogen Sulphide (H_2S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe^{2+}) or lead (Pb^{2+}) ion as H_2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produces reacts with sulphure metal salt (ferrous sulphate) forming a visible insoluble black sulphide precipitate.

3.6.7 Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas or acid only, the test is used as an aid in their differentiation. The growth medium used was peptone water and the peptone water was prepared in a conical flask and the indicators; phenol red was added. The mixture was dispensed into test tubes containing Durham tubes. The tubes with their content were sterilized by autoclaving at 121^0C for 15 minutes. 1% solution of the sugar was prepared and sterilized separately at 115^0c for 10 minutes. This was then ascetically dispensed in 5ml volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37 ^{0}C. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from light green to yellow colour while gas production was indicated by the presence of gas in the Durham's tubes.

3.7 Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index was calculated using the formula below

MAR index = (a / b)

where a represents the number of antibiotics to which the isolates were resistant and 'b' represents the total number of antibiotics to which the isolate was exposed (Apun *et al.*, 2008). MAR index values > 0.2 indicate existence of isolate from high – risk contaminated source while MAR index values ≤ 0.2 show bacteria from source with less contamination.

3.8 Physicochemical analysis

3.8.1 pH Determination

The pH of the flour samples were determined at the point of sampling using pH meter. The value of each sample were taken after submerging the pH probe in the flour samples and holding for a couple of minutes to achieve a stabilized reading.(Singh *et al.,*2012).

3.8.2 Determination of Titratable Acidity

Fifty (50ml) milliliters of sample was pipetted into a clean 250ml conical flask and 2 drops of phenolphthalein was added. The solution was then titrated against the prepared 0.1MNaOH to obtain pink end point.

3.9 Antibiotic susceptibility test

Test organisms were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. Ten (10) different commercial antibiotic discs were used. The antibiotic discs were carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates were inverted and incubated for 37°C for 24 h. The diameter of the zone of inhibition were measured in millimeters (mm) using a meter rule. The experiments were carried out in triplicates to minimize probability of error.

Plate 1: Preparing Juice samples for physicochemical analysis

Plate 2: Prepared Juice samples set for physicochemical analysis

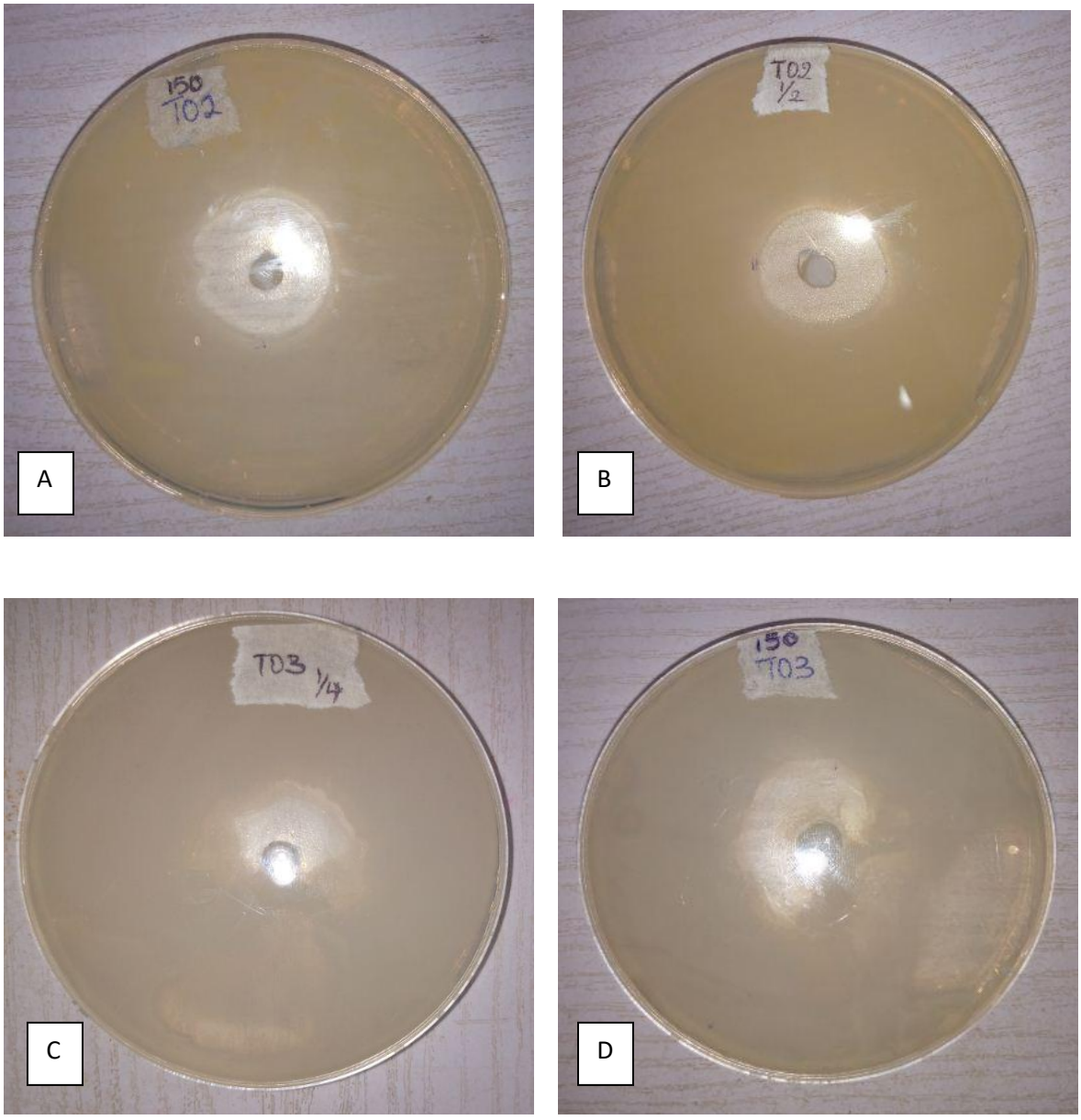


Plate 3: Chitosan activity on bacterial isolates (a) & (b)*Staphylococcus* spp (c) & (d) *Salmonella* spp

CHAPTER FOUR

RESULTS

The total bacterial count of the juices is shown in Table 1. Total bacterial load on nutrient agar ranged from 13.5×10^3cfu/ml in zobo drink (ZN) to 114×10^3cfu/ml in tigernut milk (TNMFJ). On eosin methylene blue agar, total bacterial load ranged from 5×10^3cfu/ml in zobo drink (ZN) to 52×10^3 cfu/ml in tigernut milk (TNMN). Table 2 summarizes the cultural morphological and biochemical characterization of bacterial isolates. Organisms isolated include *Lactobacillus fermentii*, *Staphylococcus* spp and *Salmonella* spp. In table 3 is presented the percentage distribution of the bacterial isolates, *Salmonella* spp was present in all juice samples (100% prevalence), *Lactobacillus fermentii* was present only in tigernut juice samples; TNMN, TNMF, TMNFJ (60% prevalence), *Staphylococcus* spp was present in all juice samples with the exception of tigernut milk (TNMN) (80% prevalence). Table 4 shows the physico-chemical values of all the juice samples. The pH ranged from 3.56 in zobo drink (ZN) to 4.78 in tigernut milk (TNMFJ). The highest total dissolved solids (TDS) value of 2.27mg/ml was obtained in tigernut milk (TNMF), ginger lemon drink (GLN) had the least mean value of 0.10mg/ml. The titratable acidity of the juice samples ranged from 0.02mg/L in zobo drink (ZN) and ginger lemon drink (GLN) to 0.48mg/L in tigernut milk (TNMFJ). *Lactobacillus fermentii* isolates were sensitive to Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxacillin, Rocphin, Cirpofloxacin, Streptomycin and Erythromycin are intermediately sensitive to Septrin. *Staphylococcus* spp on the other hand were sensitive to Gentamycin, Ampiclox, Zinnacef, Rocphin, Cirpofloxacin, Streptomycin, Septrin, Erythromycin and completely resistant to Perfloxacin and Amoxacillin. *Salmonella* spp were sensitive to Perfloxacin, Gentamycin, Cirpofloxacin, resistant to Ampiclox, Zinnacef, Amoxacillin, Rocphin and intermediately resistant to Streptomycin, Septrin and

Erythromycin (Table 5). The susceptibility of bacterial isolates to chitosan is presented in Table 6. It presents the inhibitory effects of chitosan at different concentration on *Staphylococcus* spp and *Salmonella* spp. The minimum inhibitory concentration of chitosan on *Salmonella* was at 25mg/ml.

Table 1: Total bacterial count from juice samples

Juice samples	Bacterial count ($x10^3$cfu/ml)	
	Nutrient Agar	**Eosin Methylene Blue Agar**
ZN	13.5	5.0
GLN	14.5	5.0
TNMN	110.0	52.0
TNMF	94.0	51.5
TNMFJ	114.0	42.5

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolates

Parameter	1	2	3
Cultural			
Shape	Round	Round	Round
Colour	Cream on NA	White on NA	
Size	Medium	Small	Small
Elevation	Raised	Flat	Flat
Transparency	Transparent	Transparent	Opaque
Morphological			
Gram stain	Positive	Positive	Negative
Cell type	Rod	Cocci	Rod
Cell arrangement	Cluster	Cluster	Chains
Biochemical			
Urease	-	-	-
Indole	-	-	-
Citrate	-	+	-
Catalase	-	-	-
Coagulase	-	-	-
Oxidase	-	-	-
H_2S	-	-	+
Oxidative FermentationTest			
Glucose	A	A	A
Sucrose	-	A	A
Lactose	-	AG	-
Isolates	*Lactobacillus fermentii*	*Staphylococcus* spp	*Salmonella* spp

A: Acid, AG: Acid and Gas, - (no reaction**)**

Table 3: Distribution of bacterial isolates among the different juice samples

Juice samples	Eosin Methylene Blue Agar	Nutrient Agar	
	Salmonella spp	*Lactobacillusfermentii*	*Staphylococcus* spp
ZN	+	–	+
GLN	+	–	+
TNMN	+	+	–
TNMF	+	+	+
TMNFJ	+	+	+
% Distribution	**100**	**60**	**80**

Key: + = Present, – = Absent, ZN = Zobo drink, GLN = Ginger Lemon drink, TNMN = Tigernut milk (Sample N), TNMF = Tigernut milk (Sample F), TNMFJ = Tigernut milk (Sample J)

Table 4: Physico-chemical values of the different juice samples

Parameter	pH	Total Dissolved Solids (mg/ml)	Titratable Acidity (mg/L)
ZN	3.56	1.28	0.02
GLN	4.56	0.1	0.02
TNMN	4.74	0.13	0.11
TNMF	4.18	2.27	0.47
TNMFJ	4.78	1.68	0.48

Key: ZN = Zobo drink, GLN = Ginger Lemon drink, TNMN = Tigernut milk (Sample N), TNMF = Tigernut milk (Sample F), TNMFJ = Tigernut milk (Sample J)

Table 5: Antibiotic susceptibility test of the bacterial isolates

	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MAR Index
Lactobacillus fermentii	S	S	S	S	S	S	S	S	I	S	0.0
Staphylococcus spp	R	S	S	S	R	S	S	S	S	S	0.1
Salmonella spp	S	S	R	R	R	R	S	I	I	1	0.4

Key:

CN = Gentamycin	30µg	S =Streptomycin	30 µg
AU = Augmentin	10 µg	OFX = Tarivid	10 µg
AM = Amoxacillin	30 µg	PEF = Pefloxacin	30 µg
CPX = Ciprofloxacin	30 µg	Resistance (R) = 0-10mm	
SP = Sparfloxacin	10 µg	Intermediate (I) = 11-16mm	
CH = Chloranphenicol	30 µg	Sensitive (S) = 17mm and above.	
SXT = Septrin	30 µg	MAR = Multiple antibiotic resistance index MAR index values > 0.2 (high →risk contamination source) MAR index values ≤ 0.2 (low risk contamination source).	

Table 6: Zone of inhibition (mm) of chitosan against *Staphylococcus* spp and *Salmonella* spp

		Zone of inhibition (mm)	
	Concentration (mg/ml)	*Staphylococcus* spp	*Salmonella* spp
15g/150ml	100	21	20
20ml/20ml	75	19	22
20ml/20ml	50	14	18
20ml/20ml	25	12	17
20ml/20ml	12.5	11	0.0

CHAPTER FIVE

5.0 DISCUSSION

High bacterial counts were obtained from all samples of juice used in this work. Total bacterial load ranged from 13.5×10^3cfu/ml zobo (ZN) to 114×10^3cfu/ml tigernut milk (TNMFJ) on nutrient agar and 5×10^3cfu/ml zobo (ZN) to 52×10^3cfu/ml tigernut milk (TNMN) on eosin methylene blue agar. It is observed in this work that locally processed juices were highly contaminated with bacteria. Bacterial contamination of locally processed juices may be due to spices usually added (Ayandele, 2015). Cooling and subsequent dispensing into drink containers (Balakarima *et al*., 2017) may also have contributed to the contamination

Following cultural, morphological and biochemical characterization, the bacteria isolated included *Salmonella* spp, *Lactobacillus fermentii* and *Staphylococcus* spp. This result is not in consonance with the report of Ogodo *et al*. (2018) who however isolated not only *Salmonella* spp from tigernut milk but also potentially pathogenic bacteria such as *Shigella* spp, *Micrococcus* spp and *Citrobacter* spp. The presence of this bacteria reflects poor hygienic and sanitary levels in juice processing and management. Consumption of contaminated juices can induce food borne intoxication and disease such as fever and diarrhea caused by *Salmonella*. This is a serious public health concern as it may lead to death of consumers.

Lactobacillus fermentii, however are hetero-fermenters and probiotic bacteria found in the mouth, gastrointestinal tract and vagina of humans. This probiotic strain of bacteria offers a number of potential benefits, including reducing cholesterol and boosting immunity.

Isolated bacterial species were variedly distributed among the different juice samples. *Salmonella* spp was present in all juice samples (100% prevalence), *Lactobacillus fermentii* was present only in tigernut juice samples; TNMN, TNMF, TNMFJ (60% prevalence), *Staphylococcus* spp was present in all juice samples with the exception of TNMN (80% prevalence). All the samples were found to be acidic (3.56 - 4.78), this suggests a corresponding prolonged shelf life since low pH creates an unfavorable environment for microorganisms to thrive (Sodini *et al.*, 2002; Lutchmedial *et al.*, 2004). This level of acidity of zobo and tigernut milk has been reported (Foline *et al*., 2011; Awonorin and Udeozor, 2014). The inhibitory action of chitosan was more prominent in *Salmonella* spp than *Staphylococcus* spp at 25mg/ml. Chitosan inhibit and kills the bacteria *Salmonella,* hence can be used as a food preservative. Chitosan is an effective antimicrobial that helps to remove any extraneous bacteria, thereby ensuring safety of the juices. Chitosan helps to prevent foodborne intoxication due to faecal contamination by inhibiting or killind *Salmonella* which is a causative bacteria.

5.1 CONCLUSION

This work has shown that locally processed juices are highly contaminated with bacterial isolates. It has also shown the degree of sensitivity or resistance of bacterial isolated with various antibiotics. The level of bacterial contamination observed in all the samples analyzed is a major health concern, therefore there is need to maintain adequate hygienic conditions during processing and preparation of these ready-to-drink beverages. The ability of chitosan to inhibit the bacterial isolates shows their potential as food preservative.

REFERENCES

Abano, E. E. and Amoah, K. K. (2011). Effect of moisture content on the physical properties of tigernut (*Cyperus esculentus*). *Asian Journal of Agricultural Resolutions,* **5**(1): 56-66.

Acosta, N., Jimenez, C., Borau, V. and Heras, A. (1993). Extraction and characterization of chitin from crustaceans. *Biomass Bioenergetics* **5**: 145-153.

Adedokun, I. I., Okorie, S. U. and Barizaa, B. (2014). Evaluation of proximate, fibre qualities and consumer acceptability of bambaranut, tigernut-coconut milk beverage blends. *International Journal of Nutrition, Food Science* **3**(5):430-437.

Adejuyitan, J.A., Otunola, E.T., Akande, E.A., Bolarinwa, I.F. and Oladokun, F.M. (2009). Some physicochemical properties of flour obtained from fermentation of tigernut (*Cyperus esculentus*) sourced from a market in Ogbomoso, Nigeria. *African Journal of Food Science* **3**: 51-55.

Adesokan, I.A., Abiola, O.P., Adigun, M.O. and Anifowose, O.A. (2013). Analysis of quality attributes of *Hibiscus sabdariffa* (zobo) drinks blended with aqueous extract of ginger and garlic. *Africa Journal of Food Science* **7**(7): 174-177.

Afzal, M., Al-hadidi, D. Menon, M. Pesek, J. and Dhami, M.S. (2011). Ginger: An ethnomedical, chemical and pharmacological review. *Drug Interaction* **18**:159-190.

Al-Awwadi, N.A.J. (2010). Effects of *Achillea santolina* extracts and fractions on human platelet aggregation *in vitro* and on rat arteriovenous shunt thrombosis in vivo, *Third Quarter Medical Journal* **15**: 12-16.

Al-Awwadi, N.A.J. (2013). Anti-diabetics effect of *Achillea santolina* aqueous leaves extract, **4**(7):151-156.

Ameh, A.O., Isa, M.T., Ahmed, A.S. and Adamu, S.B. (2009). Studies on the use of Troma in improving the taste of the extract from *Hibiscus sabadariffa* Calyx. *Nigerian Journal of Pharmaceutical* **(8)**1: ISSN: 0189-823x

Amusa, N.A., Ashaye, O.A., Aiyegbayo, A.A., Oladapo, M.O., Oni, M.O and Afolabi, O.O. (2005). Microbiology and nutritional quality of hawked sorreldrinks (zoborodo) the Nigeria locally brewed soft drinks widely consumed and notable drinks in Nigeria. *Journal of Food, Agriculture and Environment* **3**(3): 47-50.

Apun, K., Chong, Y.L., Abdullahi, M.T. and Micky, V. (2008) Antimicrobial susceptibilities of *Escherichia coli* isolates from food animals and wildlife animals in Sarawak, East Malaysia. *Asian Journal of Animal Veterinary Advancements* **3**(6): 409-416.

Archana, D., Vigya, K. and Latha, R. (2013). Micropropagation and cytogenetic assessment of Zingiber species of Northeast India. *Biotechnology* **3**: 471-479.

Arranz, I., Stroka, J. and Neugebauer, M. (2006). Determination of aflatoxin B1 in tigernut based soft drink. *Food Additives Cont.* **1**: 1-15.

Asante, F.A., Oduro, I., Ellis, W.O. and Saalia, F.K. (2014). Effects of planting period and site on the chemical composition and milk acceptability of tigernut (*Cyperus esculentus L.*) tubers in Ghana. *American Journal of Food and Nutrition* **2**(3): 49-54.

Attwood, M.M. and Zola, H. (1967). The association between chitin and protein in some chitinous tissues. *Comprehensive Biochemistry Physiology* **20**: 993-998.

Awonorin, S.O. and Udeozor, L.O. (2014). Chemical properties of tiger nut-soy milk extract. *IOSR Journal of Environmental Science and Toxicology, Food Technology* **8**(3): 87-98.

Ayandele, A.A. (2015). Microbiological analysis of hawked kanun and Zobo drink within LAUTECH campus, Ogbomoso, Oyo state Nigeria. IOSR *Journal of Environmental Science and Toxicology and Food Technology* **9**(10): 52-56.

Ayeh-Kumi, P.F., Tetteh-Quarcoo, P.B., Duedu, K.O., Obeng, A.S., Addo-Osafo, K., Mortu, S. and Asmah, R.H. (2014). A survey of pathogens associated with *Cyperus esculentus* L. (tigernuts) tubers sold in a Ghanian city. *Biomedical Central, Resolution Notes* **7**(343): 1-9.

Badawy, M.E. and Rabea, E.I. (2011). A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. *International Journal of Carbohydrate Chemistry,* **13**: 11-23.

Badreldin, H.A., Blunden, G., Tanira, M.O. and Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): A review of recent research, *Food and Chemical Toxicology* **46**: 409-420.

Bamishaiye, E.I. and Bamishaiye, O.M. (2011). Tigernut as a plant, its derivatives and benefits. *African Journal of Food, Agricultural Nutritional Development* **11**(5): 5157-5170.

Bamishaiye, E.I., Olayemi, F.F. and Bamishaiye, O.M. (2011). Effects of Boiling Time on Mineral and Vitamin C Content of Three Varieties of *Hibiscus sabdriffa* drink in Nigeria. *World Journal of Agric Science* **7**(1): 62-67.

Belewu, M.A. and Abodunrin, O.A. (2008). Preparation of kunnu from unexploited rich food source: Tiger nut (*Cyperus esculentus*). *Pakistan Journal of Nutrition* **7**: 109-111.

Bough, W.A., Salter, W.L., Wu, A.C. and Perkins, B.E. (1978). Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. Chemical composition, viscosity, and molecular weight distribution of chitosan products. *Biotechnology Bioengineering* **20**: 1931-1940.

Bristone, C., Badau, M.H., Igwebuike, J.U. and Igwebe, A.O. (2015). Production of yoghurt from mixtures of cow milk, milk extract from soybean and tigernut. *World Journal of Dairy Food, Sciences.* **10**(2): 159-169.

Bulakarima, A.U., Bello, H.S., Isa, M.A. and Abbas, M.I. (2017). Isolation and identification of microorganisms from locally prepared zobo drink in Konduga town, Borno state, Nigeria. *Asian Journal of Research in Biological and Pharmaceutical Sciences* **5**(3): 117-122.

Chinma, C.E., Abu, J.O. and Abubakar, Y.A. (2010). Effect of tigernut (*Cyperus esculentus*) flour addition on the quality of wheat-based cake. *International Journal of Food Science Technology* **45**: 1746-1752.

Choorit, W., Patthanamanee, W. and Manurakchinakorn, S. (2008). Use of response surface method for the determination of demineralization efficiency in fermented shrimp shells. *Bioresource Technology* **14**: 6168-6173.

Chukwuma, E.R., Obioma, N. and Christopher, O.I. (2010). The phytochemical composition and some biochemical effects of Nigerian tigernut (*Cyperus esculentus L.*) tuber. *Pakistan Journal of Nutrition,* **9**: 709-715.

Davidson, A. (2006). The Oxford Companion to Food, 2nd ed.; Oxford University Press: Oxford, UK. 323p.

Dutkiewicz, J.K. (2002). Super-absorbent materials from shellfish waste-a review. *Journal of Biomedical Mater Resolution* **63**: 373-381

Egbere, O.J., Anuonye, J.C., Chollom, P.F. and Okpara, P.V. (2007). Effects of some preservation techniques on the quality and storage stability of Zobo drink (a Nigerian, nonalcoholic beverage from *Hibiscus sabdariffa*). *Journal of Food Technology* **5**: 225-228.

Ekeanyanwu, R.C. and Ononogbu, C.I. (2010). Nutritive value of Nigerian tigernut (*Cyperus esculentus L.*). *Agric. Journal* **5**: 297-302.

Essien, E., Monago, C. and Edor, E.A. (2011). Evaluation of the nutritional and microbiological quality of Kunun (a cereal based non-alcoholic beverage) in Rivers State, Nigeria. *The Internet Journal of Nutrition and Wellness* **10**: 1-10.

Ezeh, O. (2015). Edible oil from tigernut (*Cyperus esculentus* L.): Mechanical pressing and aqueous enzymatic extractions methods. *Journal of Food and Nutritional Sciences* **3**: 11-19.

Fahlberg, E. (1969). The Pungent principles of ginger and their importance in certain ginger products, *Journal of Food Technology* **21**: 570-571.

Fasoyiro, S.B., Babalola, S.O. and Owosibo, T. (2005) Chemical composition and sensory quality of fruit-flavored Roselle (*Hibiscus sabdariffa*) drinks. *World Journal of Agricultural Science* **1**: 161-164.

Foline, O., Adedayo, R., Muhummad, R. and Bamishaiye, E. (2011). The Nutritional Quality of Three Varieties of Zobo (*Hibiscus sabdariffa*) Subjected to the Same Preparation Condition. *American Journal of Food Technology* **6**: 705-708.

Fox, D.L. (1973). Chitin-bound keto-carotenoids in a crustacean carapace. *Comprehensive Biochemistry Physiology* **44**: 953-962.

Fujii, S., Kumagai, H. and Noda, M. (1980). Preparation of poly (acyl) chitosans. *Carbohydrate Resolution* **83**:389-393.

Gambo, A. and Dáu, A. (2014). Tigernut (*Cyperus esculentus*): Composition, products, uses and health benefits - A review. *Bayero Journal of Pure and Applied Sciences* **7(1):** 56-61.

Goy, R.C., De Britto, D. and Assis, O.B. (2009). A review of the antimicrobial activity of chitosan. *Polimeros* **19**: 241-247

Guibal, E. (2004). Interactions of metal ions with chitosan-based sorbents: a review. *Sep Purification Technology* **38**: 43-74.

Hayden, A.L., Brigham, L.A. and Giacomelli, G.A. (2004). Aeroponic Cultivation of Ginger (*Zingiber officinale*) Rhizomes. **In**: *Proceedings of the International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition*, Kissimmee, FL, USA.

Heras, A., Rodriguez, N.M., Ramos, V.M. and Agullo, E. (2001). N-methylene phosphonic chitosan: a novel soluble derivative. *Carbohydrate Polymer* **44**: 1-8.

Heux, L., Chauve, G. and Bonini, C. (2000). Non-flocculating and chiral-nematic self-ordering of cellulose microcrystals suspensions in non-polar solvents. *Langmuir* **16**: 8210-8212

Hirano, S. and Yagi, Y. (1980). The effects of n-substitution of chitosan and the physical form of the products on the rate of hydrolysis by chitinase from *Streptomyces griseus. Carbohydrate Resolution* **83**:103-108.

Jayashree, E., Kandiannan, K., Prasath, D., Rashid, P., Sasikumar, B., Senthil-Kumar, C. M., Srinivasan, V., Bhai, R. and Thankamani, C. K. (2015). Ginger. ICAR-Indian Institute of

Spices Research Kozhikode-673 012, Kerala; ICAR-Indian Institute of Spices Research: Kerala, India.

Jung, B.O., Kim, C.H., Choi, K.S., Lee, Y.M. and Kim, J.J. (1999). Preparation of amphiphilic chitosan and their antimicrobial activities. *Journal of Applied Polymer Science* **72**: 1713-1719.

Kong, M., Guang, X., Xing, C.K. and Park, H.J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. *International Journal of Food Microbiology* **144**: 51-63.

Krichène, D., Artieda, D.A., Zarrouk, M. and Astiasaŕan, I. (2016). Review on *Cyperus esculentus*: From food safety to pharmacotherapeutics. *International Journal of Pharmacy* **6**(2): 71-81.

Kubra, I.R. Murthy, P.S. and Rao, L.J. (2013). In vitro antifungal activity of dehydrozingerone and its fungitoxic properties. *Journal of Food Science* **78**(1):64-69.

Kurita, K. (1998). Chemistry and application of chitin and chitosan. *Polymer Degradable Stability* **59**: 117-120.

Lasekan, O. and Abdulkarim, S.M. (2012). Extraction of oil from tiger nut (*Cyperus esculentus L.*) with supercritical carbon dioxide (SC-CO2). *LWT-Food Science Technology* **47**: 287-292.

Li, F., Nitteranon, V., Tang, X., Liang, J., Zhang, G., Parkin, K.L. and Hu, Q. (2012). In vitro antioxidant and anti-inflammatory activities of 1-dehydro-[6]-gingerdione, 6-shogaol, 6-dehydroshogaol and hexahydrocurcumin. *Journal of Food Chemistry* **135**(2):332-337.

Liu, X.F., Guan, Y.L., Yang, D.Z., Li, Z. and Yao, K.D. (2001). Antibacterial action of chitosan and carboxy methylated chitosan. *Journal of Applied Polymer Science* **79**: 1324-1335.

Maghami, G.G. and Roberts, G.A. (1988). Evaluation of the viscometric constants for chitosan. *Macromolecular Chemistry* **189**: 195-200.

Mahmoud, M.F., Diaai, A.A. and Ahmed, F. (2012). Evaluation of the efficacy of ginger, Arabic gum, and Boswellia in acute and chronic renal failure. *Renal Failure* **34**:73-82.

Malhotra, S. and Singh, A.P. (2006). Medicinal properties of Ginger (*Zingiber officinale* Roscoe), *Natural Product Radiance* **2**(6): 296-301.

Mohan, C.O., Ravishankar, C.N., Lalitha, K.V. and Srinivasa-Gopal, T.K. (2012). Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (*Sardinella longiceps*) during chilled storage. *Food Hydrocolloid* **26**: 167-174.

Moorjani, M.N., Achutha, V. and Khasim, D.I. (1975). Parameters affecting the viscosity of chitosan from prawn waste. *Journal of Food Science Technology* **12**: 187-189.

Morimoto, M., Saimoto, H., Usui, H., Okamoto, Y., Minami, S. and Shigemasa, Y. (2001). Biological activities of carbohydrate-branched chitosan derivatives. *Biomacromolecules* **2**: 1133-1136.

Mourya, V.K. and Inamdar, N.N. (2008). Chitosan-modifications and applications: opportunities galore. *React Functional Polymer* **68**: 1013-1051.

Muhammad, N., Bamishaiye, E., Bamishaiye, O., Usman, L., Salawu, M.O., Nafiu M.O. and O. Oloyede, O. (2011). Physicochemical properties and fatty acid composition of *Cyperus esculentus* (tiger nut) tuber oil. *Biores Bull* **5**: 51-54.

Muzzarelli, R.A. (1977). Depolymerization of chitins and chitosans with hemicellulase, lysozyme, papain and lipase. **In**: Muzzarelli RAA GPM (ed) Chitin handbook. European Chitin Society, Grottamare. Pp. 153-165.

Muzzarelli, R.A., Tanfani, F. and Scarpini, G. (1980). Chelating, film-forming and coagulating ability of the chitosan-glucan complex from *Aspergillus niger. Biotechnology Bioengineering* **22**: 885-896.

Nge, T.T., Hori, N., Takemura, A. and Ono, H. (2004). Swelling behavior of chitosan/ poly (acrylic acid) complex. *Journal of Applied Polymer Science* **92**: 2930-2940.

No, H.K. and Meyers, S.P. (1989). Crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing streams. *Journal of Agriculture and Food Chemistry* **37**: 580-583.

No, H.K., Kim, S.D., Kim, D.S., Kim, S.J. and Meyers, S.P. (1999). Effect of physical and chemical treatments on chitosan viscosity. *Journal of Korean Society of Chitin Chitosan* **4**:177-183.

No, H.K., Meyers, S.P. and Lee, K.S. (1989). Isolation and characterization of chitin from crawfish shell waste. *Journal of Agriculture and Food Chemistry* **37**: 575-579.

Nwachukwu, E., Onovo, O.M and Ezeama, C.F. (2007). Effect of lime juice on the bacterial quality of zobo drinks locally produced in Nigeria. *Research Journal of Microbiology* **2**: 787-791.

Nwaogu, E.N. (2014). Soil fertility changes and their effects on ginger (*Zingiber officinale Roscoe*) yield response in an ultisol under different pigeon pea hedgerow alley management in South Eastern Nigeria. *African Journal of Agricultural Resolutions* **9**: 2158-2166.

Nwaoguikpe, R.N. (2010). The phytochemical, proximate and amino acid compositions of the extracts of two varieties of tigernut (*Cyperus esculentus*) and their effects on sickle cell hemoglobin polymerization. *Journal of Medicine, Medical Science* **1**(11): 543-549.

Nwobosi, P.N., Isu, N.R. and Agarry, O.O. (2013). Influence of pasteurization and use of natural tropical preservatives on the quality attributes of tigernut drink during storage. *International Journal of Food Nutrition, Science* **2**(1): 27-32.

Oboh, G and Elusiyan, C.A. (2004). Nutrient composition and antimicrobial properities of sorrel drinks (Zoborodo). *Journal of Medical and Food Science*, **7**: 340-342.

Oboh, H., Obahiagbon, F., Osagie, A. and Omotosho, A. (2011) Glycemic response of some local Nigerian drinks in healthy subjects. *Journal of Nutritional Sciences* **34**: 12-18.

Oh, K.T., Kim, Y.J., Van-Nguyen, N., Jung, W.J. and Park, R.D. (2008). Effect of crab shell size on bio-demineralization with lactic acid-producing bacterium, *Lactobacillus paracasei subsp. tolerans KCTC-3074. Biotechnology Bioprocess Engineering* **5**: 566-570.

Okafor, U.C., Edeh, J.I. and Umeh, S.O. (2018). Tablewine production from mixed fruits of soursop (*Annona muricata*) and pineapple (*Ananas comosus*) using yeast from palmwine. IOSR. *Journal of Environmental Science Toxicology and Food Technology* **12** (3): 52-56.

Okudu, H.O. and Ogubuike, L.A. (2016). Evaluation of chemical composition of candy developed from tigernut (*Cyperus esculentus*) milk. *African Journal of Food Science and Technology* **7**(1): 27-31.

Olayemi, F., Adebayo, R., Muhammad, R. and Bamishaiye, E. (2011). The nutritional quality of three varieties of Zobo (*Hibiscus sabdariffa*) subjected to the same preparartion condition. *American Journal of Food Technology* **6**: 705-708.

Oliver, B. (1980). Medicinal Plants in Nigeria. *The Nigerian College of Arts, Science and Technology*, Ibadan. 28p.

Omemu, A. M., Edema, M. O., Atayese, A. O. and Obadina, A. O. (2006). A survey of the microflora of *Hibiscus sabdariffa* (Roselle) and the resulting zobo juice. *African Journal of Biotechnology* **5**(3): 254-259.

Omemu, A.M and Aderoju, S.T. (2008). Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. *Food Control,* **19**:396➔402

Onovo, J.C. and Ogaraku, A. O. (2007). Studies on some microorganisms associated with exposed tigernut (*Cyperus esculentus L.*) milk. *Journal of Biological Sciences* **7**(8): 1548-1550.

Onuorah, S.C., Akudo, C.A., Okafor, N.A., Obika, I.E. and Okafor, U.C. (2014). Bacteriological quality of locally produced sorrel beverage (zobo) vended in Awka campus of Nnamdi Azikiwe University Awka, Nigeria. *Journal of Bioscience and Biotechnology* **4**: 14-17.

Osueke, J.C and Ehirimand, F.N. (2004). Chemical, nutritional and sensory analysis of Zobo drinks and selected soft drinks. *Journal of Agriculture, Food Science,* **2:** 21-24.

Osuntogun, B. and Abiola, O.O. (2004). Microbiological and evaluation of some non-alcoholic beverages. *Pakistan Journal of Nutrition* **3**:188-192.

Piron, E., Accominotti, M. and Domard, A. (1997). Interaction between chitosan and uranyl ions. Role of physical and physicochemical parameters on the kinetics of sorption. *Langmuir* **13**: 1653-1658.

Raafat, D., Bargen. K., Haas, A. and Sahl, H.G. (2008). Insights into the mode of action of chitosan as an antibacterial compound. *Applied Environmental Microbiology* **74**: 3764-3773.

Rhazi, M., Desbrie→res, J., Tolaimate, A., Rinaudo, M., Vottero, P. and Alagui, A. (2002). Influence of the nature of the metal ions on the complexation with chitosan- application to the treatment of liquid waste. *Europe Polymer Journal* **38**: 1523-1530.

Rice, R.P. (1990). Fruits and vegetables production is warm climates. 1st edition. *Macmillan press*. Pp. 40-65.

Rinaudo, M. (2006). Chitin and chitosan: properties and applications. *Programming Polymer Science* **31**: 603-632.

Rinaudo, M., Pavlov, G. and Desbrie→res, J. (1999). Influence of acetic acid concentration on the solubilization of chitosan. *Polymer* **40**: 7029-7032.

Sa'id, A.M, Abubakar, H. and Bello, B. (2017). Sensory and microbiological analysis of tiger nut (*Cyperus esculentus*) beverage. *Pakistan Journal of Nutrition* **6**:731-737.

Sánchez-Zapata, E., Fernández-Lopez, J. and Pérez-Alvarez, J.A. (2012). Tigernut (*Cyperus esculentus*) commercialization: Health aspects, composition, properties and food applications. *Compressor Reviews Food Science and Food Safety* **11**: 366-377.

Sanwal, S.K., Rai, N., Singh, J. and Buragohain, J. (2010). Antioxidant phytochemicals and gingerol content in diploid and tetraploid clones of ginger (*Zingiber officinale Roscoe), Scientia Horticulturae*, **124**: 280-285.

Schipper, P., Borg, S.J. and Bos, J.J. (1995). A revision of the infraspecific taxonomy of *Cyperus esculentus* (yellow nutsedge) with an experimentally evaluated character set. *Systematic Botany* **20**(4): 461-481.

Sebastiá, N., El-Shenawy, M., Mañes, J. and Soriano, J.M. (2012). Assessment of microbial quality of commercial and home-made tiger-nut beverages. *Letters in Applied Microbiology* **54**: 299-305.

Sebti, I., Martial-Gros, A., Carnet-Pantiez, A., Grelier, S. and Coma, V. (2005). Chitosan polymer as bioactive coating and film against *Aspergillus niger* contamination. *Journal of Food Science* **70**:100-104.

Sekiguchi, S., Miura, Y., Kaneko, H., Nishimura, S. I., Nishi, N. and Iwase, M. (1994). Molecular weight dependency of antimicrobial activity by chitosan oligomers. In: Nishinari, K. and Doi, E. (eds) *Food Hydrocolloids: Structures, Properties, and Functions*. Plenum. Pp. 71-76.

Simpson, D.A., Yesson, C.A., Couch, C.A. and Muasya, A.M. (2011). Climate change and Cyperaceae. **In**: Hodkinson, T., Jones, M., Waldren, S. and Parnell, J. (eds.) Climate change, ecology and systematics. 1st edn. Cambridge University Press.

Singh, S. and Gupta, A.K. (2013). Evaluation of phenolics content, flavonoids and antioxidant activity of *Curcuma amada* (mango ginger) and *Zingiber officinale* (ginger): research and reviews. *Journal of Chemistry* **2**(1): 32-35.

Singh, S., Kushwaha, B.P., Nag, S.K., Mishra, A.K., Singh, A. and Anele, U.Y. (2012). *In vitro* ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Animal Feed Science and Technology **178**(1): 2-11.

Skaugrud O. (1991). Chitosan: new biopolymer for cosmetics and drugs. *Drug and Cosmetics Industry* **148**: 24-29.

Tokura, S.S., Nishimura, N., Sakairi, N. and Nishi, N. (1996). Biological activities of biodegradable polysaccharide. *Macromolecule Symposium* **101**: 389-396.

Trang, S.T., Wah, W.T., Nguyen, T.Q., Chuen, H.N. and Wellem, F.S. (2006). Functional characteristics of shrimp chitosan and its membranes as affected by the degree of deacetylation. *Bioresource Technology* **97**: 659-663.

Tsai, G.J. and Su, W.H. (1999). Antibacterial activity of shrimp chitosan against *Escherichia coli. Journal of Food Protection* **62**: 239-243.

Udeozor, L.O. (2012). Tigernut-soy milk drink: Preparation, proximate composition and sensory qualities. *International Journal of Food and Nutrition, Sciences* **1**(4): 18-26.

Ukpabi, U.J. and Ukenye, E.A. (2015). An assessment of wholesomeness of imported tiger nut *Cyperus esculentus* used as snack food in Umuahia, Nigeria. *Malaya Journal of Biosciences* **2**(2): 132-138.

Ukwuru, M.U. and Ogbodo, A.C. (2011). Effect of processing treatment on the quality of tigernut milk. *Pakistan Journal of Nutrition* **10**(1): 95-100.

Ukwuru, M.U., Ibeneme, C.L. and Agbo, G.I. (2011). New product development from tigernut (*Cyperus esculentus*) and their sensory, proximate and microbiological evaluation. *Pakistan Journal of Nutrition* **10**(2): 101-105.

Umar, Z.D., Bashir, A. and Raubilu, S.A. (2014). Study on bacteriological quality of kununaya (Tigernut Juice) sold at Umaru Musa Yar→adua University (Umyu) Campus, Katsina. *International Journal of Environmental Sciences* **3**(2): 87-97.

Venugopal, V. (2011). *Marine Polysaccharides: Food Applications*. CRC Press/Taylor and Francis Group, Boca Raton

Vishukumar, A.B., Varadaraj, M.C., Gowda, L.R. and Tharanathan, R.N. (2005). Characterization of chitooligosaccharides prepared by chitosanolysis with the aid of papain and pronase, and their bacteriocidal action. *Biochemistry Journal* **391**: 167-175.

Wakil, S.M., Ayenuro, O.T. and Oyinlola, K.A. (2014). Microbiological and nutritional assessment of starter-developed fermented tigernut milk. *Food and Nutrition Science* **5**: 495-506.

Wong, P. (2002). Physicochemical characteristics of Roselle. *Nutritional Food Science* **32**: 68-73.

Xia, W., Liu, P., Zhang, J. and Chen, J. (2011). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocoll* **25**: 170-179.

Yang, T.C., Chou, C.C. and Li, C.F. (2002). Preparation, water solubility and rheological property of the N-alkylated mono or disaccharide chitosan derivatives. *Food Resolution International* **35**: 707-713.

Yi, H., Wu, L.Q., Bentley, W.E., Ghodssi, R., Rubloff, G.W., Culver, J.N. and Gregory, F.P. (2005). Biofabrication with chitosan. *Biomacromolecules* **6**: 2881-2894.

APPENDIX

Antibiotic susceptibility test of the bacterial isolates

Isolate	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	MAR Index
Lactobacillus fermentii	S(30SS	S(24)	S(20)	S(21)	S(21)	S(20)	S(26)	S(24)	I(15)	S(20)	0.0
Staphylococcus spp	R(0)	S(24)	S(18)	S(22)	R(0)	S(20)	S(30)	S(25)	S(20)	S(24)	0.1
Salmonella spp	S(24)	S(21)	R(0)	R(0)	R(0)	R(0)	S(30)	I(16)	I(16)	1(16)	0.4

Key:

CN = Gentamycin	30µg	S =Streptomycin	30 µg
AU = Augmentin	10 µg	OFX = Tarivid	10 µg
AM = Amoxacillin	30 µg	PEF = Pefloxacin	30 µg
CPX = Ciprofloxacin	30 µg	Resistance (R) = 0-10mm	
SP = Sparfloxacin	10 µg	Intermediate (I) = 11-16mm	
CH = Chloranphenicol	30 µg	Sensitive (S) = 17mm and above.	
SXT = Septrin	30 µg	MAR = Multiple antibiotic resistance index MAR index values > 0.2 (high – risk contamination source) MAR index values ≤ 0.2 (low risk contamination source).	