BACTERIOLOGICAL QUALITY ASSESSMENT OF TIGERNUT TUBERS SOLD BY HAWKERS IN AUCHI TOWN, EDO STATE, NIGERIA

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BEING A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF APPLIED SCIENCE AND TECHNOLOGY AUCHI POLYTECHNIC, AUCHI, EDO STATE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN SCIENCE LABORATORY TECHNOLOGY, MICROBIOLOGY OPTION

DECEMBER, 2022

CERTIFICATION

We the undersigned, certify that this project work carried out by OKHAGBUZO JUDITH LUCKY with Matriculation Number AST/238200316, OKHAISHIE EMIKE ELIZABETH with Matriculation Number AST/2382030915 and OKOEGUALE JOY EFUA with Matriculation Number AST/2382070481 of the Department of Biological Science Laboratory, Auchi Polytechnic, Auchi.

We also certify that this work is adequate in scope and quality in partial fulfilment of the requirements for the award of Higher National Diploma (HND) in Microbiology Option.

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DEDICATION

This project work is dedicated to the Lord God Almighty, who has been our strength in carrying out this project work.

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Our most sincere gratitude goes to God Almighty who has brought us this far in our academic career. We give him all the thanks for the wisdom and knowledge in putting these scripts together and also for the successful completion of this project work.

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ABSTRACT

Bacterial contamination of ready to eat food sold by street hawkers have become a major health challenge as it is associated with pathogenic microorganisms resulting from poor hygienic practices. This study investigated the bacteriological quality standard of tigernut tubers sold in Auchi metropolis, Edo State, Nigeria. The tigernut tubers were obtained from three different locations; A, B and C. A total of three samples, one from each locations and a prepared control were analysed. Isolation was carried out using Nutrient agar, MacConkey agar and Salmonella-Shigella agar. The bacterial isolates obtained were suggestive of Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Salmonella species and Pseudomonas species based on their cultural characteristics and biochemical test. The total number of viable cell counts were 4.5 x 10^{-1} cfu/ml, 5.6 x 10^{-1} cfu/ml and 3.9 x 10^{-1} cfu/ml for sample A, B and C respectively. This study shows a high level of contamination of the tigernut tubers beyond the acceptable limit by the World Health Organisation (WHO) standard. The route of contamination is traceable to the lack of personal and environmental hygiene by the tigernut vendors. It is therefore, recommended that sellers of tigernut tubers should be educated on the importance of washing tigernut thoroughly with clean water before sale and also, the consumers of tigernut tubers should endeavour to make it routine to wash tigernut with clean water before consumption.

CHAPTER ONE

1.0 INTRODUCTION

Tigernut (*Cyperus esculentus*) is a crop of the sedge family widespread across the world. It is an underutilized crop which belongs to the division-Magnoliophyta, Classiliopcida, order-Cyperales and family-Cyperaceae. It is found in most of the Eastern Hemisphere, including Southern Europe, Africa and Madagascar, as well as the Middle East and the Indian subcontinent and of the same genus as the papyrus plant. It is called earth almond, Zulu nut. It is known in Nigeria as Aya in Hausa, Ofio in Yoruba and Akiausa in Igbo. It has three varieties (black, brown and yellow). The yellow variety is preferred because it yields more milk upon extraction, contains lower fat, less anti-nutritional factors especially polyphenols and more protein (Francis *et al.,* 2020).

Tigernuts (*Cyperus esculentus* L.) is a perennial plant which has scaly rhizomes at its base which give rise to hard spherical tubers. Rush nut, yellow nut-grass, Zulu nut, chufa, water grass, Earth almond and edible rush are common names given to tigernut which is actually a tuber and not a nut. The usefulness of tigernut tubers obtained from tigernut plant which include preparation of tigernut was comprehensively reported by Francis *et al.* (2020). Tigernut is rich in energy, fat, starch, glucose, fibre and protein. However, most soft drinks lack fibre, protein, minerals, vitamins and other essential nutrients. Instead, it contains refined cane sugars or corn syrups which is linked to obesity in children which could lead to type 2 diabetes, osteoporosis and very weak bones. Excess consumption of soft drinks over a long period could cause cancer, gallstones and teeth enamel erosion because of toxic substances such as benzene and phosphoric acid contained in most soft drinks. Interestingly, tigernut could be beneficial in managing and controlling type 2 diabetes since it contains natural sugar. It has been opined nutrition-related disorders could be prevented by consuming beverages.

Tigernut are natural tigernut milk, pasteurized tigernut milk, sterilized tigernut milk, ultra-high temperature tigernut milk and concentrated and condensed tigernut milk. Any of these products is recommended for those who experience milk allergies such as galactosemia and lactose intolerance (Nura *et al.,* 2016). A non-alcoholic drink obtained from tigernut tubers widely eaten raw by children, older persons and sportsmen in Nigeria, parts of West Africa and East Africa is known as tigernut beverage. In Northern Nigeria, it is popularly known as 'kunu-aya'. This product is white in colour, refreshing when it is chilled and consumed both in wet and dry season. Some researchers refer this product as a milk substitute (phyto milk) or vegetable milk because it is obtained from plants such as tigernut, soybean, bambara nut and baobab which have a high protein content (Maduka and Ire, 2018).

Tigernut (*Cyperus esculentus L.*) is a tuber that grows freely and is consumed widely in Nigeria and in various parts of West and East Africa. It has long been recognized as one of the best nutritional crops used to augment diets with its rich iron and calcium contents for body growth and development, since a substantial intake has reduced reported cases of various health related conditions such as cardiovascular disease, diabetes, cancer and obesity, and also was found ideal for children, older persons and sportsmen. Tiger nuts with its inherent nutritional and therapeutic advantage could serve as alternative to cassava in baking industry (Austin, 2020).

Root crops (tubers) are important sources of nourishment and a vital ingredient in healthy and balanced diets, but they harbor varied loads of microbial contaminations while passing from farm to table. These various microbial contaminations can be contacted before, during or after harvest (Ike *et al.,* 2017). Fruits and vegetables may become contaminated by infected field-workers, food preparers, consumers, cross contamination, use of contaminated irrigation water, use of inadequate composted manure or contact with contaminated soil. They serve as good source of food borne illness when contaminated with pathogenic microorganisms

during harvesting, handling, transporting and display in street markets. Food is generally a fertile ecosystem, in which microorganisms compete for nutrients (Ike *et al.* 2017). Various microorganisms find their way into foods; they are either introduced from the soil in which they were grown, or during harvest, packaging, storage and handling (Udeozor and Awonorin, 2014).

Tigernut could provide a basis for rural industries in Africa. It is an important food crop for certain tribes in Africa, often collected and eaten raw, baked as a vegetable, roasted or dried and ground to flour. The ground flour is mixed with sorghum to make porridge, ice-cream, sherbet or milky drink (Maxwell *et al.,* 2019). It is mostly consumed raw as snack without knowledge of the food and nutritional quality. It has also been found to possess good therapeutic quality. Moore (2012), stated that "the expansion of tiger nut milky drinks will significantly help the research linking tiger nut milk to healthier cholesterol levels and other non-dairy manufacturers (Ukpabi and Ukenye, 2015). This could also gain a boost from an increased consumer interest in health foods".

It has been revealed that lack of effective antimicrobial treatments at any step from planting to consumption suggested that pathogens introduced at any point may be present on the final food product. Some diverse microbial species associated with tiger nuts include *Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, Fusarium solani, Saccharomyces cerevisiae, Saccharomyces fubiligera* and *Candida pseudotropicalis* (Adejuyitan, 2011). The presence of pathogenic *Escherichia coli*, *Streptococcus faecalis* and *Staphylococcus aureus* usually constitute a direct proof of faecal contamination of irrigation water (Eke-Ejiofor and Awaji, 2018).

Tigernuts are sold on the streets usually in uncovered wheelbarrows that are exposed to atmospheric elements (Adejuyitan, 2011). They are widely patronized by the populace based on the numerous health benefits, especially among males due to its perceived aphrodisiac

property. It is also a favourite snack among children due to its sweet nutty flavour. The sellers often use bare hands and rusted cups to dispense the nuts into polythene wrappers to consumers, which most times are eaten immediately. There is need to place emphasis on food safety, such that foods from a source like the street market should be protected from contamination and spoilage during subsequent handling, packaging, and storage while on transit. Therefore, this study was targeted at evaluating the bacteriological quality assessment of tigernut sold by hawkers in Auchi Polytechnic.

1.2 Statements of Research Problem

Root crops (tubers) are important sources of nourishment and a vital ingredient in healthy and balanced diets, but they harbor varied loads of bacteria contaminations while passing from farm to table. Fruits and vegetables may become contaminated by infected field-workers, food preparers, consumers, cross contamination, use of contaminated irrigation water, use of inadequate composted manure or contact with contaminated soil. They serve as good source of food borne illness when contaminated with pathogenic microorganisms during harvesting, handling, transporting and display in street markets. Various microorganisms find their way into foods; they are either introduced from the soil in which they were grown, or during harvest, packaging, storage and handling.

1.3 Justification of the Study

Attributable to the increase cases of food infection and intoxication to human and inadequate sanitary condition observed in processing of many locally made foods and drinks. In Nigeria, hawking of ready-to-eat food in streets and markets even along the road for travellers is very common. These vendors enjoy huge patronage from different societal classes. Unfortunately, none of these hawkers or vendors is licensed or monitored by relevant agencies saddled with the responsibility of ensuring the safety of our foods and drinks. Thus, owing to the manner and conditions these vendors operate, there is possibility that some of the ready-to-eat nutritional drinks and food may be contaminated by foodborne pathogens. Some gastro-

intestinal illness characterized by diarrhea, abdominal cramps, and vomiting which may be assumed of been unknown aetiology may arise from drinks contaminated with microorganisms. Many picnic suppers and banquets have come to a disastrous end which home prepared foods and drinks serves not only as food and drinks for guest, but also as the vehicle for transmitting staphylococcus food poisoning.

1.3 Scope of the Study

The scope of the study covers the Bacteriological Quality Assessment of Tigernut Sold by Hawkers in Auchi Polytechnic. The scope will also cover the bacterial assessment of various bacterial associated with tiger nut juice in the study area.

1.4 Aim and Objectives of Study

The aim of this research was to determine the bacteriological quality assessment of tigernut sold by hawkers in Auchi Metropolis, Edo State, Nigeria. The specific objectives were;

1. To isolate bacteria associated with Tigernut.
2. To identify isolate using cultural characteristics and Gram's reaction
3. To characterize isolates using biochemical test
4. To make adequate recommendation on the research findings.

1.5 Definition of Terms

Some terms expressions or technical work used in the process of the research write up is clearly defined and their meaning was well explained. Some of these terms include;

Microorganism or Microbe: It is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells.

Assessment: The action of assessing someone or something.

Quality: It is an attribute or a property characteristic of an object in philosophy.

Tigernuts, also known as chufa, yellow nutsedge or earth almonds, are not actually nuts, but rather edible tubers. They are the size of a chickpea but wrinkly with a chewy texture and sweet nutty flavor similar to coconut.

CHAPTER TWO

2.0 LITERATURE REVIEW

Tigernut (*Cyperus esculentus*) relatively lesser underutilized crop, many of which are potentially valuable as human and animal food, has been identified to maintain a balance between population growth and agricultural productivity particularly in the tropical and sub-tropical areas of the world (Adejuyitan, 2011). In Spain, the "horchata" industry is of considerable economic importance. The annual value of tiger nut production is close to 3.3 million euro. In recent years, the popularity of "horchata" has been extended to other countries, such as the United Kingdom, France, Portugal, Argentina, and United States of America.

According to Ike et al. (2017), the plant is a tuber that grows freely and is consumed widely in Nigeria, other parts of West Africa, East Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula. Furthermore, tiger nut milk has been classified as a medicinal drink due to its highly energetic and diuretic, rich in mineral, predominantly phosphorus, potassium and also vitamins C and E contents. It is known in Nigeria as "Aya" in Hausa, "Ofio" in Yoruba and "Akiausa" in Igbo where these varieties (black, brown and yellow) are cultivated.

Fig : Diagram of Tigernut
Source: Wikipedia.com

In Ghana, commercial cultivation of tiger nuts is found in areas such as Aduamoa and Esereso in the Kwahu areas of the Eastern Region. In the Central Region, it is cultivated at Bawjiase and surrounding villages in the Awutu Senya East District, Ampenyi and its surrounding villages in the Komenda Edina Eguafo District, Twifo Praso and surrounding villages in the Twifo Praso Lower Hemang District and New Ebu and surrounding villages in the Abura Aseibu Kwamankese District. Also, the crop is cultivated in commercial quantities in Adansi Danyameso in the Adansi South District in the Ashanti Region, Tanoso and surrounding villages in the Sunyani District of the Brong Ahafo Region and Tampiong in the Savelugu Nantom District in the Northern Region (Taiwo, 2017). According to an earlier study conducted by Tetteh and Ofori (1998) in Kwahu Aduamoa on the cultivation of the tubers, shows that women constituted about 70% of the farmers whilst the men were about 30%.

2.1 Brief History of Tigernuts

Tigernuts are not actually nuts but tubers found on the root of a sedge plant. It was first discovered 4000 years ago and comes in several varieties. The tubers were originally cultivated by ancient Egypt's populations at the Nile valley. Their cultivation was subsequently extended throughout other areas with temperate climate and fertile soil. Reports have shown that tigernuts came to Spain from Africa. Tigernuts are edible tubers with a sweet nutty flavour. Other common names for these tubers are “earth almond” and “yellow nut sedge”. They are quite hard and are generally soaked in water before consumption (Egbebi and Seidu, 2011).

In Egypt and the Mediterranean nut sedges were used as sources of food, medicine and perfumes. Tigernut tubers were routinely roasted and consumed by nursing mothers. The dried ground tubers were used in coffee and chocolate drinks. Oil extracted from the tubers was an ingredient in soap making as well as a lubricant for fine machinery. The leafy plant parts of the nut sedge were fed to livestock. Egyptians made very efficient use of the nut sedge. They used them in cultivation as early as 2400 BC. One such example of tigernuts is depicted in a wall painting of an Egyptain tomb in 15th century BC (Deatra, 1999). In the painting, workers are

shown to be weighing the nuts while a scribe records their work. In another part of the same tomb, instructions were written for eating the tubers as sweets after grinding and adding honey. Tigernut tubers have been found in the tombs and are considered to be locally domesticated in Egypt. This gives the impression that the tubers were greatly valued by the Egyptian people as a food source.

2.2 Botany of Tigernuts

Cyperus esculentus (tigernut sedge/chufa sedge/yellow nut sedge/earth almond) is a species of sedge, native to warm temperate to subtropical regions of the Northern Hemisphere.

Tigernut is a highly adaptable crop and grows well under a wide range of climatic and soil conditions. It is found throughout the tropics, subtropics and warm temperature regions. It is cultivated in Western Africa, but is a serious weed of cotton, cereals, potatoes and sisal in Eastern Africa. It is also grown in South America, Europe and Asia. The tuber grows 50- 250 tubers per plant and weigh 2-26 g per tuber (Falola *et al.,* 2011).

Tigernut is an annual or perennial plant, growing to 90 cm tall, with solitary stems growing from a tuber. The stems are triangular in section, and bear slender leaves 3-10 mm wide. The flowers of the plant are distinctive, with a cluster of flat oval seeds surrounded by four hanging leaves positioned 90 degrees from each other. The plant foliage is very tough and fibrous, and is often mistaken for a grass (Chukwu *et al.,* 2013). Tigernut plant produces edible yellow to yellow brown spike-lets flowers, mostly only 1 cm to 1.5 cm long. The root system is by yellowish rhizome, ending in single tubers of 5-20mm in length, with a thin brown outer skin which darkens with maturity. In its non-flowering state it resembles *Cyperus rotundus* which is dark brown, slightly fragrant, unpleasant tasting tubers produced in a chain and blunt tipped leaves with no shoulders. The leaves of tigernut (*Cyperus esculentus leptostachyus*) are long, narrow, shiny, light green, arranged in 3 rows around the triangular stem often with characteristic pointed tip separated from the rest of the leaf by a distinct shoulder.

Tigernut is classified in the division: Magnoliophyta; class: Liliopsida; order: Cyperales; family: Cyperaceae. *Cyperus esculentus* is in the order Commelinales and the family Cyperaceae. *Cyperus esculentus* can be distinguished from other species of New World nut sedge by its persistent linear brown spikelets that have closely overlapping scales. The stem of yellow nut sedge is triangular and has a light green-yellow color. Rhizomes that terminate in tubers are the main means of reproduction, although it does produce viable seed.

2.3 Ecology of Tigernuts

Tigernut is common in seasonally wet grassland, irrigated crops, damp grassland, and along banks, but at the same time is considered fairly drought resistant. It does not tolerate shade. Best yield are obtained with moderately high temperature throughout the growing season, and well distributed rainfall. High temperature of 27 - 30 ºC, with low nitrogen levels favors tuber formation. Light sandy loamy soils of pH 5.5 - 6.5 are preferred, but can grow in any soil provided it is well drained. Alluvial soils containing relatively high quantities of Manganese (Mn), sulfur (S), calcium (Ca), Magnesium (Mg), and boron (Bo) are particularly suitable. It is tolerant of salty soils. Short photo periods of 8-12 hours favor tuber formation and long photo periods of more than 16hrs favor vegetative growth (Cappucino and Sherman, 2010).

Tigernut cultivation requires sandy soil and a mild climate. Tubers are soaked in water for 24 - 36 hours before being planted out, either by hand or using a drill. In United States of America, tubers which had been chilled were found to germinate better and to produce more sprouts per tuber. Tubers may be planted at 10-15cm intervals along rows 60-90cm apart, about 2.5-4cm deep. At closing spacing, 1 tuber per hole is used, with 2 per hole at wider spacing seed rates (Adejuyitan, 2011). Tigernuts are planted during March, April and May and must be irrigated every week until they are harvested in November and December. Harvest time may take 90-120 days only and at the end of dry season. Immediately after harvest, tigernuts are washed with water in order to remove any sand and small stones. Once the Tigernuts have been

cleaned, they are dried out in order to preserve them. This is a natural process that requires 1-3 months. Temperature and humidity levels are carefully monitored during this period. The Tigernuts are turned over every day to ensure uniform drying. Small and damaged tigernuts are removed before packaging and utilization.

2.4 Nutritional Composition of Tigernuts and its Products

Chandrasekara and Josheph (2016) showed that tigernut tubers are rich in starch (20-30% of DW) and fat (20-28% DW) with small quantities of protein which is about twice of that of cassava. Tigernuts have relatively higher fat content and gross energy, and in this regard compared better with nuts than that of cereals which also belong to the same other Cyperales. Research has been done on the oil extracted from the seeds of yellow nut sedge (*Cyperus esculentus* var. *esculentus*) as a non-conventional oilseed. This study was used to determine oil substitutes for more conventionally used oil types such as soybean, palm and olive oils. Non-conventional oils would be less expensive and therefore more available to poorer (developing) countries.

Tigernut oil is 80% unsaturated fatty acid, mainly oleic (64.2 – 68.8 %) and this shows that tigernut oil has a good potential as a substitute for imported olive oil (Ayeh, 2014). Fat in diets provide twice much energy as carbohydrate or protein, thus low fat diets are recommended to aid weight control. Different types of fat (fatty acids) have different effects on health and the risk of diseases states such as coronary heart disease (CHD). Saturated fatty acids (SFA) increase levels of blood cholesterol and should be avoided whenever possible.

Tigernut is a good source of phosphorous, potassium and iron. It also contains magnesium, calcium, zinc, copper, sodium and manganese (Ukpabi and Ukenye, 2015). Phosphorus found in plant is usually bound to a compound called phytate meaning that it is poorly absorbed from the gut into the body. Phosphorous (P)**,** together with calcium, constitutes the bulk of the mineral substance of the bones and teeth. It plays a part in the formation of ATP (an energy compound indispensable for "activating" glucose, fatty acids, etc) and in

improvement of intellectual performance. Phosphate is important in the body. It helps regulate acidity/alkalinity by acting as a buffer.

Potassium (K) is important in maintaining electrolyte and chemical balance between the tissue cells and the blood. K is the most important neural element in intracellular behaviour. It plays a part in numerous enzymatic reactions and in important physiological processes, such as cardiac rhythm, nervous conduction, and muscular contraction. Iron (Fe) in food is often in a complex form. Vitamin C aids in the absorption of iron. Vitamin C is a reducing agent and changes Iron (Fe) into a more easily absorbed form. An acid medium also helps Fe absorption. Consequently, Iron (Fe) helps prevent anaemia. Zinc has a wide variety of functions in the body and is found in all body tissues. It is involved in many enzyme reactions including those involved in energy generation from carbohydrate, fat and protein. It also has a role in cell division, the transport of carbon dioxide and oxygen in the blood and also in immunity. Since it has a wide range of role in the body, symptoms of zinc deficiency are also wide-ranging and include a delay in wound healing, poor appetite, a suppressed immune system and poor growth (Taiwo, 2017).

Magnesium is also involved in many enzyme systems and in particular those involving the currency of energy in the body Adenosine triphosphate (ATP). Magnesium is also required for the synthesis of proteins, the production of energy and muscle contraction. Research studies have suggested that a low intake of magnesium may increase the risk of coronary heart disease (Musa and Hamza, 2013) and type 2 diabetes.

2.5 Nutritional and Health Importance of Tigernuts

Tigernuts and its products are rich in carbohydrates, mono-, di-, and polysaccharides (Udeozor and Awonorin, 2014). They contain relatively high levels of protein, oleic acid (monounsaturated fatty acid which has a bigger resistance to chemical decomposition) and fat. Tigernuts have excellent nutritional quality with a fat composition similar to olive oil and rich mineral content, especially phosphorus and potassium. Tigernut oil has a mild, pleasant flavour

and is considered as food oil similar but superior in quality to olive oil. The polyunsaturated fatty acid content (linoleic acid & linolenic acid) is enough to cover daily minimum needs of about 10g (Taiwo, 2017) oil has high content of Vitamin E (alpha-tocopherol), and thus higher oxidative stability than other oils**,** due to its content of polyunsaturated fatty acids and gammatocopherol.

Tigernuts may need to rely significantly on its health benefits, promoting a rich monounsaturated fatty acid content, high vitamin E levels and prebiotic qualities (Okereke, 2015). Vitamin E, an antioxidant which protects the body from free radical attack, is vital for the maintenance of cell membranes. It may also play an important role in delaying cells from aging thereby improving the elasticity of skin. Vitamin E is good for treatment of acne and other skin "alterations". It is particularly important in areas of the body exposed to oxidative stress such as the lungs and the red blood cells. Vitamin E may reduce the risk of cancer and CHD due to its role as anti-oxidant, however research in this area is currently inconclusive (Umaru, 2014). In supra-nutritional doses, Vitamin E has been claimed to benefit diseases associated with oxidative stress including cardiovascular disease and cancer.

Tigernut oil has therapeutic properties as it reduces "bad" cholesterol (LDL cholesterol) and increases the "good" one (HDL-cholesterol). It can also reduce levels of triglycerides in blood, reduce risk of formation of bloody clots, produce an opening in veins and prevent arteriosclerosis. Tigernuts may play an important role in the prevention and nutritional therapy for cardiac pathologies, due to its high content of monounsaturated fatty acids (Oleic acid) to improve metabolism and health (Musa and Hamza, 2013). Tigernut oil exhibits positive effects on digestive secretions (gastric, pancreatic and bile), due to high content of oleic acid, the most powerful stimulator of production of Cholecistokinine (Okereke, 2015). Tigernuts may prevent heart attacks, thrombosis and activate blood circulation. The high contents of soluble glucose in tigernuts prevent cancer. Recently, some investigators discovered that they reduce the risk

of suffering colon cancer. Tigernuts have relative antioxidant capacity, because they contain considerable amount of water-soluble flavonoid glycoside (a phytochemical). Consumption of antioxidant could protect the immune system of malnourished populations.

2.6 Economic Importance of Tigernut

In some parts of Africa, Europe and Asia, tigernut is grown for its edible tubers. Tigernuts may be regarded as an obnoxious weed that has been used historically as food and medicine by the Egyptians and Native Americans. Even today the Egyptians cultivate tigernuts in moist soils or sandy shores for their edible tubers (Onuoha *et al.*, 2017). Tigernut tubers may be consumed raw, roasted, or ground into flour as well as being used to produce vegetable oil, and cellulose. Tigernut is a representative crop of the Spanish Mediterranean region, where tubers are used to make *horchata*. The milky-looking aqueous extract of tiger nuts has a pleasant and characteristic flavor of vanilla and almonds and could be sold in Pubs. Unfortunately, popularity of tigernut milk extract or "horchata" has not extended to Nigeria.

In Maradi State, Eastern Niger, tigernut is cultivated for export to Nigeria. Revenues from this exceed those from the typical cash crops such as cowpea and groundnut. Nowadays, tigernut is cultivated in Northern Nigeria, Ghana and Togo where it is made into a sweet-meat or used uncooked as a side dish. These countries, and some others including the Ivory Coast, export 2300 tons of tigernut tubers every year to Spain (Badau *et al.*, 2018). Tigernut could be used in seed mixes for wetland restoration, mitigation and erosion control.

Ogodo et al. (2018), reported that tigernuts weigh about 44 pounds per bushel with oil yields from 0.5 to 1.5 tons/hectare. Tigernut is potentially a commercial source of high-oleic acid vegetable oil and high-carbohydrate tuber cakes. Some authors believe that the tuber oil could be exploited in the same way as olive oil. The iodine level of tigernut oil comes under a non-drying oil which is substantially unsaturated, which could be utilized for cooking and may find application as a raw material in industries for manufacturing soap, vegetable oil-based ice cream, salad cream and other non-food application.

Okorie et al. (2014) reported that the calculated fuel value of tigernut oil is comparable to that of soybean oil. Tigernut oil has high energy density. Researchers have measured the physical and fuel properties of oil extracted from the tigernut, and concluded that the physical properties are similar to those of other vegetable oils. They have suggested that this oil may also be used as bio diesel fuel. The waste residue after oil extraction could be further modified producing syrups, flours, or livestock feeds (Maduka and Ire, 2019).

Another specie of sedge plant called *Cyperus papyrus* has been used by Egyptians to make paper, sails, cloth, mats, ropes, or plaited into sandals. In the Peruvian Amazon, reportedly there is a native species of *Cyperus* is used widely by tribal women as a natural contraceptive. This property has been attributed to a certain mold that grows on the root of the Amazonian species that has oxytoxic (abortive) properties similar to Ergot, a fungus that grows on rye.

2.7 Anti-Nutrient Factor of Tigernuts

Nutritional quality of a food may be dictated mainly by its chemical composition and the presence of anti-nutritional factors, such as phytic acid, tannin and trypsin inhibitor. Phytic acid, a principal storage ubiquitously distributed in plants was reported to be about 724 mg per 100 g by Umar et al. (2016). However, reports have been made by researchers that fermentation, hydrothermal treatment and some other processing methods are able to nullify or reduce this antinutrient effect (Ukpabi and Ukenye, 2015). Therefore the level of antinutrient in raw tigernuts could be reduced by processing.

There are no reported cases of tigernut toxicity. However, Ochratoxin A (OTA) has been found as a contaminant in tigernut. Ochratoxin A (OTA) is a mycotoxin produced by different species of *Aspergillus* and *Penicillium*. It is found as natural contaminants in many foodstuffs including cereals, dried fruits, cocoa, wine, poultry eggs and milk. OTA is immunosuppressive, teratogenic, genotoxic and mutagenic (Taiwo, 2017). The problem with mycotoxin contamination in herbal plants is that they are consumed directly, unlike other

products such as maize and groundnuts, which may undergo some processing before eating. Besides, Okereke (2015) reported the presence of aflatoxins in tigernut at toxicologically unsafe levels. Akukwe and Ogbodo (2015) detected aflatoxins in 35% of tigernut with concentrations ranging from 10-120g/kg collected from different parts of Nigeria, and the incidence of *Aspergillus flavus* and aflatoxin contamination was found to be correlated.

2.8 Tigernut

Tigernuts, also known as chufa, yellow nutsedge, or earth almonds are not actually nuts, but rather edible tubers. They're the size of a chickpea but wrinkly with a chewy texture and sweet nutty flavor similar to coconut (Alibi *et al.,* 2016). It has a short shelf life which could be attributed to poor hygienic practices during preparation, packaging, storage and distribution which predisposes the product to microbial contamination. Studies have shown that non-alcoholic drinks prepared at home using traditional methods or commercially by industries are predisposed to microbial contamination. According to a study carried out by (Chukwu *et al.,* 2013), no sample of commercially prepared tigernut beverage contained viable microorganisms but *Escherichia coli, Bacillus* spp., *Shigella* sp., yeasts and moulds were isolated from homemade tigernut beverage. Hiko and Muktar (2020) reported the presence of *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis* and *Salmonella* sp. from tigernut beverage (kunu aya) sold in the Auchi.

Hundreds of residents of Auchi metropolis, consume local beverages especially tigernut drinks daily primarily to quench thirst as well as providing stimulatory effects rather than its food value (Ibrahim *et al.,* 2016). The unregulated production of this drink has led to uncertainty in strict adherence to food safety protocols during the process chain which could result to food borne illnesses and diseases.

Fig 1: Diagram of Tigernut
Source: Wikipedia.com

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Auchi Polytechnic, Auchi, Etsako West LGA, Edo State, Nigeria. Auchi is located in the northern part of Edo State within the coordinates of latitude 07^0 04^1N and longitude 06^0 16^1E. It is situated in the south-south geographical zone of Nigeria with a population of over 500,000 people according to the 2015 population census. It is approximately one hundred and thirty kilometer (130km) away from Benin City, the capital of Edo State. Auchi is the headquarters of Etsako West Local Government and has witnessed territorial development owing to rural-urban migration. It is bounded to the north by Jattu, to the south by Aviele, to the east by Iyakpi and to the west by Owan Local Government Area. It is also the seat of the Federal Polytechnic, Auchi.

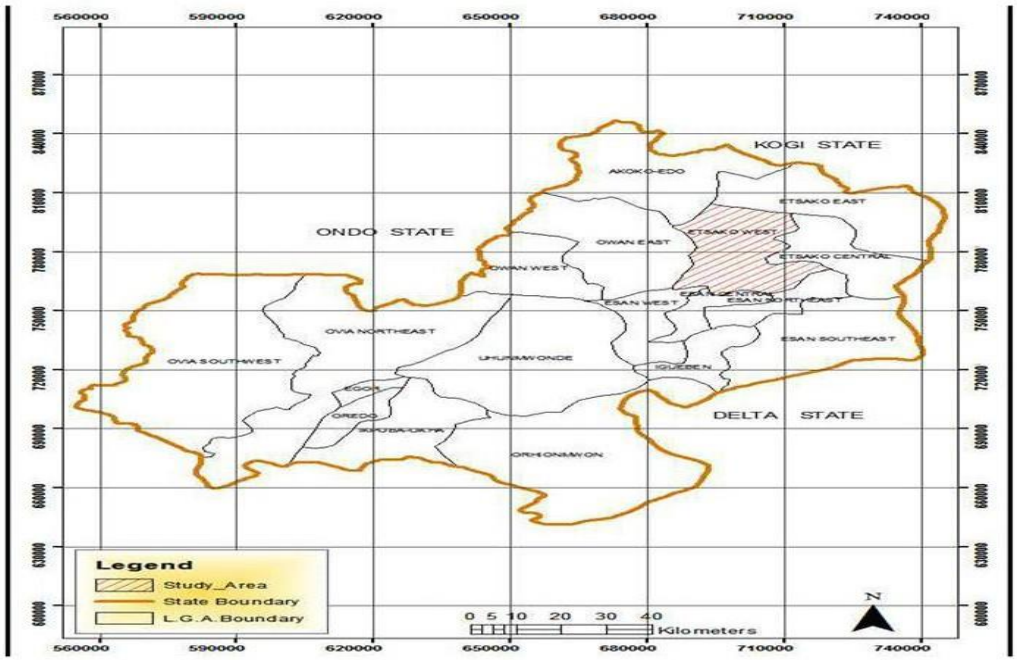

Figure 3.1: Map of Edo state showing Etsako West LGA (Source: Produced from Arcmap10.1.2013).

3.2 Sample Collection

Three samples of tigernut were purchased from three popular markets in Auchi (market sample coded A-C). The samples were aseptically transferred and immediately transported to Auchi Polytechnic Microbiology Laboratory for analysis as described by Ayandele (2015). Sample A (Uchi market), B (Campus One) while C (Campus Two).

3.3 List of Apparatus and Experimental Reagents

3.3.1 Materials:

Colony Counter, Weighing Machine, Electronic Weighing Balance, Nutrient Agar, Syringe, MacConkey Agar, Salmonella Shigella Agar, Conical Flask, Distilled Water, Beaker, Petri-dish, Hand-Glove, Wire Loop, Test-Tube, Incubator, Microscope and Autoclave.

3.3.2 Reagents:

Crystal violet, Iodine, Safranin and Acetone.

3.4 Sterilization of Material

All materials that were used in the course of this project such as glasswares were properly washed with detergent and water to remove dirty and contaminations and dried properly. The wash glasswares was sterilized in a portable laboratory autoclave at a temperature of 121^0C for 15 minutes as described by Bukar et al. (2015). All media used was also sterilized in the autoclave at a temperature of 121^oC for 15minutes according to Nwachukwu and Osuocha (2014).

3.5 Preparations of Culture Media

3.5.1 Nutrient Agar (NA)

Nutrient Agar was prepared by dissolving 14g of nutrient agar powder in 500 ml of distilled water in a clean flask. The mouth of the flask was plugged with non-absorbent cotton wool wrapped with aluminium foil paper that was extended up to the neck of the flask as described by Sylvester et al. (2015). The flask was placed on a bunsen flame and allows to boil and mix completely. It was sterilized in an autoclave

at 121^{0}C for 15 minutes and allowed to cool to 45^{0}C and aseptically dispensed into Petri-dishes. Nutrient agar was used for the total bacterial aerobic plate count (Roseline *et al*., 2006).

3.5.2 MacConkey Agar (MA)

This agar was prepared by dissolving bile salt, Then 23.5g of the powder was dissolved in 500ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121°C for 15 minutes and allowed to cool to a temperature of 45-50°C before pouring into plates. This was used to determine coliforms as described by Cheesebrough (2004). This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia et al. (2012).

3.5.3 Salmonella Shigella Agar (SS Agar)

This agar was prepared by dissolving 18.075g of SS Agar powder in 500ml of distilled water in a clean flask. It was autoclave at 121°C for 15 minutes and allowed to cool to a temperature of 45-50°C before pouring into plates. This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia et al. (2012).

3.6 Sample Extraction

1ml of tigernut were measure into 9ml of sterile 0.1% peptone water as diluents to make a 1:6 dilution, further six fold serial dilution were made and examined by means of the pour plate method. The plates were marked for easy identification and 1ml of the dilution used for the inoculation and incubation (Yeboah-manuu *et al.,* 2010).

3.7 Bacteriological Analysis of Samples

Total bacterial count was determined by pour plate techniques using standard methods. Nutrient agar medium was used for the enumeration of bacteria in the samples. The total bacteria count was obtained by incubation aerobically at 37^{0}C for 24hours. MacConkey and Salmonella Shigella Agar was used and there was no positive result was associated with acid

and gas production on incubation at 37^0C for 48hours. Morphological features and biochemical reactions patterns were used for the identification of bacterial isolates (Fawole and Oso, 2001).

3.7.1 Viable Cell Count

The representative petri-dish incubated was visualized under a colony counting machine and was used to count the total bacterial count (labtech, India) and result was expressed as colony forming unit per milliliter (cfu/ml) at the end of the count as reported by Olayemi et al. (2011).

3.7.2 Identification of Bacterial Isolates

Identification of the bacteria isolate was performed using classical methods based on their morphological and biochemical characteristics with reference to systematic manual of bacteriology described by Cheesbrough (2004).

3.7.3 Gram staining Technique

Gram staining reaction has the wide application that is capable of distinguishing virtually all bacteria into one of two large group gram positive or gram negative as described by Dr. Hans Christian Gram (1884). Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was added in drops. Lugols iodine was added for 45 seconds decolorized with acetone and washed with water. It was then sun dried examined at x l00 under oil immersion as described by Bello et al. (2014). The color of the experiment appeared as purple and its result was Gram-positive.

3.7.4 Biochemical Test

3.7.4.1 Catalase Test

Catalase test was carried out using a drop of hydrogen peroxide 2 ml of 3% hydrogen peroxide (H_2O_2) was placed in a clean test tube. A sterile wire loop was used to pick a colony of the test organism and mixed with 2 ml of 3% hydrogen peroxide (H_2O_2) in the test tube and observed for the production of gas bubbles which indicates a positive reaction. This test was used to identify *Staphylococcus aureus* and *Bacillus subtilis* (James, 2001).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

The results of the bacteriological analysis of the Tigernut sold in Auchi is tabulated below. Table 1 represent the cultural, morphological and biochemical characteristics of the bacterial isolates, table 2 represent the total number of viable colony count and table 3 represent the percentage occurrence of isolates in each sample.

4.1 Results

Table 1: Cultural, morphological and biochemical characteristics of the bacterial isolates

Cultural Characteristics	**Gram Reaction**	**Catalase Test**	**Probable Oragnism**
Smooth oily lactose farmenter	+	+	*Escherichia Coli*
Smooth cream colony	+	+	*Staphylococcus spp*
Large grey white mucold colonies	+	+	*Salmonella spp*
Grey white irregular colonies	+	+	*Pseudomonas spp*
Smooth cream irregular colonies	+	+	*Bacillus subtilis*

Note: + = Positive, - = Negative

Table 2: Total number of viable colony count of the sample isolates

Sample	Dilution Factor	Number of Colonies on Agar Plates	Total Numbers of Colony Forming Unit (Cfu/ml)
A	10^{-1}	45	4.5×10^{-1}
	10^{-3}	24	2.4×10^{-3}
B	10^{-1}	56	5.6×10^{-1}
	10^{-3}	31	3.1×10^{-3}
C	10^{-1}	39	3.9×10^{-1}
	10^{-3}	14	1.4×10^{-3}
CTR	10^{-1}	6	0.6×10^{-1}
	10^{-3}	3	0.3×10^{-3}

Key: A= Uchi Market, B= Campus One, C= Campus Two, CTR = Control

Table 3: Percentage occurrence of isolates in each sample

Sample	*E. coli*	*Staphylococcus spp*	*Salmonella spp*	*Pseudomonas spp*	*Bacillus subtilis*
A	+	+	-	-	+
B	-	+	+	-	-
C	-	+	-	+	+
Total %	33.3	100	33.3	33.3	66.6

KEY: - = Negative and + = Positive

4.2 Discussion

Pathogenic microorganism remains the major contaminant found in Tigernut. In this study, the bacterial isolates obtained were suggestive of *Escherichia coli*, *Staphylococcus aureus, Pseudomonas spp* and *Bacillus spp* and the total average number of viable cell count ranged from 4.5 x 10^{-1} CFU/ml to 2.4 x 10^{-3} CFU/ml using the different dilution factor. Each of these results represents the value which corresponds with the result obtained by Egbere et al. (2007). All samples indicated significant level of contamination and the presence of bacteria may be due to both pre and post contamination. Although all the samples were contaminated with varying levels of bacterial counts that can be classified as unsatisfactory as reported by Zumbe et al. (2014).

The percentage occurrence of bacterial isolates in table 3 shows that *Staphylococcus spp* has the highest occurrence of isolates with 100%, followed by *Bacillus subtilis* with 66.6%. while *E. coli* has the least occurrence of 33.3%, *Salmonella spp* with 33.3% and *Pseudomonas spp* with 33.3%. The occurrence of these microorganisms is largely due to their presence in nature (Bristone *et al.*, 2018). Their association with foods such as tigernut may be as a result of poor hygiene or poor sanitary condition as reported by Raima (2013).

According to Omemu *et al.* (2006), *Staphylococcus spp* is a common contaminant of foods and other similar preparations if good hygienic practices are not employed. *Staphylococci spp* as normal flora of human inhabit the nostrils, hands, skin, mouth and dresses etc. They might easily gain access to tigernut without good sanitary practices. Those who sell Tigernut locally do not consider the use of good water as a means of reducing contamination therefore water may also serve as source of contamination introducing *staphylococcus* and other bacterial into the Tigernut, especially the water used for washing and rinsing of tigernut. *Salmonella spp*. might be present as a result of the handling processes from contaminated hands and water.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The result of this study shows a high level of contamination of tigernut tubers sold by hawkers in Auchi town. The route of contamination was traceable to the source of water used lack of environmental and personal hygiene. It also revealed the presence of some bacteria among the different samples of tiger nuts drawn from different vending locations, which if left unchecked could pose serious health risks that might lead to food borne outbreaks.

5.2 Recommendation

It is advisable that an adequate campaign should be organized for the major stakeholders (vendors/hawkers and consumers) in order to educate them on the inherent risks associated with the handling of tigernut. Therefore, it is recommended that;

1. Hawkers of tigernut should endeavour to wash them properly before selling to the consumers.
2. Consumers of tigernut should be able to practice good sanitary hygiene.
3. There is need to place emphasis on the safety of tigernut, such that the tigernut from a source like the street hawkers and market sellers should be protected from contamination and spoilage during subsequent handling, packaging, and storage while on transit.

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