THE MICROBIOLOGICAL PROFILE OF PLANT-BASED MILK DRINKS LOCALLY MADE IN BENIN CITY, EDO STATE, NIGERIA

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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

FEBRUARY, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **Esohe EFE** in the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

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(Head of Department)

APPROVAL

This project work was carried out by Esohe EFE in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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Prof. (Mrs.) F.I. Akinnibosun. **Date**

(Head of Department)

DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies throughout my period of study.

ACKNOWLEDGEMENTS

I wish to give my profound gratitude to God Almighty for His faithfulness, goodness and grace throughout my life and academic journey.

I wish to acknowledge whole heartedly my project supervisor **Dr (Mrs) A.S. Ogbebor** for her patience and understanding towards me and the success of this project. May God Almighty richly bless you ma for your efforts.

My deepest appreciation goes to my incredible mother, **Mrs. Izehiuwa Izevbokun**, and my amazing brother, **Oghosa Efe.** Your unwavering support, both emotional and financial, carried me through every step of my educational journey. I couldn't have done it without you. I love you both more than words can express. You are my greatest blessings.

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To my role model, **Park Jimin.** Your words and music have been a guiding light, teaching me strength, compassion, and the courage to grow into a better version of myself. Thank you for inspiring me, not just as an artist, but as a person. Apobangpo

To all my dear friends especially Esohe and Juliet, thank you for being there since day one. Your love, support, and presence have meant the world to me. We finally made it, and I'm so grateful to have shared this journey with you.

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ABSTRACT

The popularity of the plant- based milk drinks is on the increase as substitutes to the traditional dairy products due to the growing consumer awareness of the health, sustainability and ethical concerns. Although plant-based milk drinks have nutritional advantages, there are major challenges in production, storage and distribution and has been associated with higher chances of food-borne disease. Some studies have indicated bacterial contaminants in plant-based milk drinks including soya milk, kunu and coconut milk. This research paper sought to examine the bacterial and fungal contamination profile of plant-based milk drinks during storage. The milk drinks are the plant-based ones which were bought in three Benin City markets and they consist of the samples of kunu, soya, tiger nut and coconut milk. The enumeration and isolation of bacteria and fungi were done using the pour plate method. Identification was done using the cultural, morphological and biochemical characteristics of the isolates. The heterotrophic bacterial counts had a mean of 1.90+-0.53x105 (soya milk) -82.40+-6.90 x 105 cfu/mL (coconut milk). The total *Salmonella*-Shigella count was between 1.10+-0.48 x 105 cfu/mL (tiger nut milk) and too numerous to count (coconut milk). The average fungal counts were between 2.40+-0.25 (soya milk) -44.00+-0.00 x 105 cfu/mL (tiger nut milk). The identified bacterial isolates are Bacillus cereus (16.00%), (25.00%), Pseudomonas sp. (8.33%), Klebsiella pneumoniae (25.00%), Bacillus subtilis (16.60%), *Staphylococcus* sp. (25.00%) and *Salmonella* sp. (16.60%). Saccharomyces sp. (16.60%), Mucor sp. (8.33%), *Penicillium* sp. (16.60%) and *Aspergillus flavus* (25.00%) were the most common genera of the fungi. The average pH of the samples were between 3.90+-0.00 (coconut milk) -5.90+-0.14 (kunu). Antibiotics susceptibility testing showed that different patterns of resistance were exhibited by different bacterial isolates with multi drug resistance observed in Gram-positive and Gram-negative bacteria. The numbers and types of microbial population represented poor standard of production as a grave health risk among the population. The findings can serve as a worthy basis in the execution of the social health intervention to enhance the safety of the plant-based milk drinks and other locally prepared beverages in Benin City and other areas.

CHAPTER ONE

1.1 Background of the Study

Due to the increasing consumer awareness about the importance of health, sustainability, and ethical concerns, the consumption of plant-based milk drinks has been on the rise as an alternative to traditional dairy products (Walther *et al*.,2024). These drinks such as soya milk, almond milk, rice milk, and coconut milk have been taken since ancient times as ordinary drinks and during religious practices (Pingali *et al*., 2013). Their consumption has grown in the past years all around the globe with the rise of the demand of vegan and vegetarian food, along with lactose-free food. This change is particularly clear in the developing markets such as Nigeria, where economic and environmental issues drive people towards more convenient, plant-based alternatives (Acquah *et al*., 2023).

Milk is a liquid that is rich in nutrients, which have been traditionally known to be beneficial because of their protein, vitamins, and minerals that aid in growth and health. It is mainly composed of water (87.4%), the rest of the composition is proteins, fats, carbohydrates, vitamins and minerals (Amy, 2008). Milk is a very important component of a balanced diet because it contains essential nutrients, including calcium, phosphorus, and vitamins A and D (Pereira, 2014). Despite cow milk being the standard of dairy products, plant-based milk may have similar or even better nutritional value, which is determined by the source (Walther *et al*., 2022).

The most common type of plant-based milk is coconut milk, which is made out of the grated meat of the coconut (*Cocos nucifera*). Coconut palm is called the tree of life because it is extensively used in tropical areas where it is used as a source of food, beverage, oil, medicine, fiber and other essentials (Nulu *et al*., 2006). Coconut milk contains medium-chain triglycerides (MCTs), which are also reported to possess antimicrobial and anti-inflammatory properties (St-Onge and Bosarge, 2008). The health benefits of coconut milk are that it is beneficial to the heart and it is a source of energy that is quick (Zhao *et al*., 2014).

Another popular plant-based milk is soya milk which has become popular because of its nutritional content. Soybeans (*Glycine max*) have been known to contain high quality protein content that is similar to cow milk (Belewu and Belewu, 2007). Soya milk is also rich in the essential amino acids and includes unsaturated fats which are healthy to the heart (Adegoke *et al*., 2002). Further, soy milk is heart healthy because of the presence of polyunsaturated fatty acids such as omega-3 and the absence of cholesterol (Food and Agriculture Organization, 1999; William and Akiko, 2000). It has been demonstrated that the consumption of soybean lowers the risks of cardiovascular diseases, and also assists in the control of blood pressure (Desroches *et al*., 2004). Also, there is evidence that soy products can reduce the occurrence of some forms of cancer and enhance the overall metabolic wellbeing (Hodge *et al*., 2007).

The nutritional benefits notwithstanding, plant-based milk drinks have major challenges in production, storage, and distribution. The plant based milk beverage like Soya milk, Kunu and Coconut milk have been reported to have bacterial contaminants. The microorganisms that have been identified in plant-based milk products include, *Staphylococcus*, *Lactobacillus, Clostridium, Streptococcus, Corynebacterium, Bacillus, Pseudomonas* species and *Escherichia coli* (bacteria), and *Fusarium oxysporum, Aspergillus flavus, Aspergillus niger* and *Penicillium oxalicum* (Agboke *et al*., 2011; Essien *et al*. 2011).

One of the most prevalent bacteria in soya milk is *Escherichia coli* which is commonly associated with fecal contamination. Its existence normally signifies poor hygiene in the processing process. Some pathogenic strains like *E. coli* O157:H7 have the ability to cause extremely serious gastrointestinal diseases, hence the need to observe sanitary practices in the production plants (Jay *et al*., 2005). Another major bacterial contaminant is *Listeria monocytogenes* that is a psychotrophic pathogen that can grow at refrigeration temperatures (Farber and Peterkin, 1991). The bacteria is particularly harmful to high-risk groups, such as pregnant women, infants, and immunocompromised people. Likewise, *Salmonella* species are often linked to contaminated raw materials or improperly maintained equipment, which is a significant threat of foodborne infections (Carrasco *et al*., 2012). *Bacillus cereus* spore-forming bacteria is one of the spolilage bacteria that can survive the unfavourable processing and storage conditions resulting in the production of toxins and food poisoning (Ceuppens *et al*., 2011).

Fungi, such as yeasts and molds, also play an important role in the microbiological issues of production of plant based milk drinks. *Candida tropicalis* and *Candida parapsilosis* have been found to cause spoilage leading to the development of off-flavors and reduced quality (Fleet, 2007). Particularly concerning are such types of molds as *Aspergillus* and *Penicillium*, which may generate mycotoxins that are harmful to health when ingested in the long term (Pitt and Hocking, 2022). Although the uncontrolled growth of *Saccharomyces cerevisiae* in soya milk is advantageous in controlled fermentations, it may cause spoilage when not controlled (Bhalla and Savitri, 2017).

The causes of microbial contamination of plant based drinks are complex. Poor heat treatment in the manufacturing process does not usually kill heat resistant microorganisms like Bacillus cereus (Giffel *et al*., 1996). Unhealthy hygiene, such as using contaminated equipment and water, bring the pathogens such as *E. coli* and *Salmonella*. Poor storage conditions, particularly high temperature, provide a condition favourable to the growth of spoilage organisms like

Listeria monocytogenes. Moreover, long shelf life products are at increased risks of fungal contamination when poor or incorrect use of preservatives.

Although the consumption of plant-based milk drinks is on the increase in Nigeria because of their nutritional benefits and the possibility of reducing protein-calorie malnutrition, microbiological hazards tend to undermine their production. Poor hygiene practices, lack of proper processing facilities and poor temperature control during storage may lead to contamination by pathogens and spoilage organisms (Akinsemolu and Onyeaka, 2024). This may cause foodborne diseases and spoilages and decreases the shelf life and safety of these beverages.

There is a gap in the research on the microbiological quality of plant-based milk drinks manufactured in Nigeria. It is important to understand the contaminants of microbes and their commonness in these products in order to enhance food safety and come up with preventive strategies. This is due to the absence of extensive microbiological research that would facilitate the provision of safe consumption standards and regulations of such products. Plant-based milk drinks offer a promising solution to protein-calorie malnutrition, particularly in regions where animal protein sources are scarce or costly (Ali, 2010). The use of legumes such as soybeans and coconuts to produce these beverages can improve nutritional intake and support local economies by creating opportunities for small-scale farmers and producers (Nwokolo, 1996; Sharma *et al*.,2024). However, without adequate microbiological safety measures, the health benefits of plant-based milk are undermined by the potential for microbial contamination.

1.2. AIM AND OBJECTIVES

The aim of this study was to evaluate the microbiological profile of locally produced plant-based milk drinks in Benin City, Edo State, Nigeria.

The specific objectives were to:

1. enumerate and isolate bacteria and fungi from plant based milk drinks
2. identify the bacteria and fungi present in the samples.
3. Determine the pH of plant-based milk drinks sold around Benin city.
4. Determine the antibiotics susceptibility pattern of the bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. PLANT-BASED MILK DRINKS

Plant-based milk drinks are non-dairy beverages prepared using different types of plants, including nuts, grains, seeds, or legumes, as substitutes to the traditional dairy milk (Reyes-Jurado *et al*., 2023). The drinks are made by dissolving the soluble parts of the plant materials and then emulsifying them to attain a milk-like texture. Plant-based milk beverages have risen to a significant role in food systems of the world because of their nutritional value, affordability, and the ability to solve dietary limitations and inclinations (Sharma *et al*., 2024). These plant-based milk substitutes include legumes, nuts, seeds and grains, which are viable alternatives to animal milk, particularly in lactose intolerant and milk allergic individuals, or those who are vegan. Plant-based milk production and consumption in Nigeria has been triggered by the rising health benefits of plant-based diets and the necessity to have low-cost protein sources (Sethi *et al*., 2016).

The popularity of plant-based milk beverages is unprecedented in the world due to the health-aware customers and the issues of environmental sustainability. It is reported that plant-based milk takes up about 15 percent of the total milk sales in the global market, with soy, almond, coconut, and rice milk taking over the market (Ramsing *et al*., 2023). These beverages are not only healthy but also meet the current food trends that focus on natural, less processed, and eco-friendly food products.

Soybeans, coconut, and tiger nuts (Cyperus esculentus) are the main local raw materials in the production and consumption of plant-based milk in Nigeria. These resources are easy to access, affordable and they are culturally incorporated in the traditional diets. An example is soya milk, which has been a dietary staple among the Nigerians because of its affordability and protein composition, which makes it an essential dietary ingredient in the treatment of protein-energy malnutrition (PEM) in low-income groups (Ndudi *et al*., 2024). Another popular plant-based milk product is coconut milk, which is valued due to its creamy texture, high flavor, and nutrient content, such as essential fatty acids, vitamins, and minerals (Pandiselvam *et al*., 2024).

The most important factors in the production of plant-based milk are microbiological safety and nutritional value. Although these beverages have a lot of health advantages, they can be spoiled by improper processing, storage and handling which results in microbial contamination and spoilage. Some of the most common microbial contaminants of plant-based milk are *Escherichia coli*, *Salmonella* spp., Listeria monocytogenes, and yeast, which can be very dangerous to human health when ingested in contaminated forms (Mishra *et al*., 2024). It is imperative that microbiological quality is maintained by following strict hygienic standards, appropriate methods of pasteurization, and appropriate storage conditions.

The plant-based milk drinks have also a great potential in overcoming the nutritional issues that many Nigerians have. Since malnutrition and food insecurity are still high in Nigeria, the milk substitutes offer convenient sources of protein, vitamins, and minerals, especially in areas where the animal milk is costly or inaccessible (Nwokolo, 1996). In addition, the production of plant-based milk is consistent with the objectives of sustainable agriculture since it has a smaller environmental footprint than dairy milk because it consumes less water, less land, and less greenhouse gases (Poore and Nemecek, 2018).

Plant-based milk drinks are undergoing a phase of rising popularity, but they have some issues with consumer acceptance, regulatory control, and quality management. Consumer tastes, texture and perceived nutritional value are some of the factors that determine consumer preferences and market penetration. Also, there are no uniform laws regulating the production of plant-based milk in Nigeria, which poses challenges in quality and safety control (Sharma *et al*., 2024). These issues need to be resolved by conducting sensitization programs, investing in research, and developing regulatory frameworks to maximize the potential of plant-based milk drinks in Nigeria.

The plant-based milk beverages play a crucial role in filling the nutritional gap in Nigeria and providing a sustainable, low-cost, and health-promoting substitute to animal-based milk. They are important not only in nutrition but also in the areas of public health, environmental sustainability, and socio-economic development, and therefore, an essential part of changing the food system in Nigeria (Alcorta *et al*., 2021).

2.2 HISTORICAL BACKGROUND OF PLANT-BASED MILK DRINKS

Plant-based milk drinks have a long history, as thousands of years ago, the practice was practiced by different cultures utilizing the available plant resources to make milk-like drinks. These are drinks based on grains, nuts, seeds, and legumes, and their main purpose was to be a sustainable and healthy substitute of animal milk. Plant-based milk has over the years shifted the traditional homemade preparation to industrially manufactured beverages that address the current dietary and environmental issues. The historical development of the plant-based milk drinks gives an insight into their cultural, nutritional, and societal values.

Plant-based milk has its roots in the ancient societies that depended on the natural resources they had. An example of these is soya milk, which is recorded in the Chinese history as early as the Han dynasty (circa 1365 AD). The health benefits and affordability made it be initially consumed as traditional Chinese medicine. Soya milk has been part of Chinese food over the centuries, and since then, it has become popular in other regions of Asia and even other countries (Shurtleff and Aoyagi, 2014). On the same note, the tiger nut milk or horchata de chufa is said to have been invented in ancient Egypt (Maduka and Ire, 2018). The drink is popular in Spain, where it was used to cool and also to provide natural sweetness during the Islamic reign of the Iberian Peninsula. Another early example is coconut milk that was used extensively in Southeast Asia, the Pacific Islands, and India centuries ago. It was a staple food, as well as a crucial component of culinary practices, providing not only great flavors but also practical health applications (Nath *et al*., 2021).

Besides their nutritional content, plant-based milk drinks also had great nutritional, cultural and religious value (Hidalgo-Fuentes *et al*., 2024) . As one example, in Hindu and Buddhist cultures, milk made out of almonds and sesame seeds, and other vegetable products, was commonly used instead of milk of animals because of vegetarianism. Likewise, tiger nut milk became part of the Islamic culture especially during Ramadan, as it gives energy and hydration to the fasting people.

The commercial production of plant-based milk drinks started in the early 20 th century and was a transition between the traditional and commercial production. Soya milk was the first to be introduced with Li Yu-ying setting up the first soya milk factories in Paris and Shanghai. These activities brought soya milk into the Western markets and established the basis of the plant-based milk market worldwide (Shurtleff and Aoyagi, 2014). Almond and rice milk also became popular by the end of the 20 th century, particularly in Europe and North America. Their popularity was predetermined by the increasing incidence of lactose intolerance and the increasing popularity of cholesterol-free diets (Sethi *et al*., 2016).

The modern evolution of plant-based milk drinks relied on the technological advances. Advances in emulsification, pasteurization, and aseptic packaging enhanced the texture, taste and shelf life of these beverages making it possible to produce and distribute them in large quantities. Their popularity was also boosted by the increasing environmental sustainability consciousness. Plant-based alternatives are much less harmful to the environment than dairy milk, which consumes less water and land and generates fewer greenhouse gas emissions (Carlsson Kanyama *et al*., 2021).

The plant-based milk is an increasing demand in the world over the last several decades due to the shifting preferences and dietary habits of consumers. The number of health-conscious people that tend to find alternatives to products that are perceived to be healthier and with less saturated fats and cholesterol is on the rise. Also, the emergence of veganism and flexitarian diets has made plant-based milk one of the main characteristics of the contemporary diet (Sharma *et al*., 2024). The beverage sector has reacted by expanding the choices, providing oat milk, cashew milk, hemp milk, and others to cover a wide range of viewers.

2.3 TYPES OF PLANT-BASED MILK DRINKS

The plant-based milk has been diversified, and this has resulted in a broad selection that addresses the preferences of the diet, culture, and health. Plant-based milk has compositional, nutritional and sensory characteristics that are unique to each type, and they can be used as substitutes to animal-derived milk. The following is a discussion of the key varieties of plant-based milk drinks, how they are produced, their nutritional value, and their drawbacks.

2.3.1. Coconut Milk

Coconut milk is a liquid that is versatile and creamy and is formed by the grated meat of fully grown coconuts (*Cocos nucifera*). It is a popular ingredient of the cuisines of tropical and subtropical areas, such as Asia, the Caribbean, and some parts of Africa. Coconut milk is made through grating the white coconut meat, mixing it with water and filtering the mixture to retrieve the juice. Coconut milk comes in two major forms, including powdered coconut milk and liquid coconut milk, depending on the consistency that one wants (Zafisah *et al*., 2018).

2.3.1.1. Composition of Coconut Milk

Coconut milk is the grated pulp of mature coconuts that is known to have a high composition of medium-chain triglycerides (MCTs), the most common fatty acid of which is lauric acid. Lauric acid has been well-reported to have antimicrobial and antiviral effects and also to be able to give a quick energy boost (Pehowich *et al*., 2000). The MCTs of coconut milk are special because of the fact that they are quickly absorbed and metabolized and do not have to be stored as fat in the body hence providing an instant source of energy.

In addition to lipid, coconut milk is a source of small yet beneficial quantities of protein and dietary fiber that help to make one feel full and maintain a healthy digestive system. It is also a source of important micronutrients, including potassium, magnesium, and iron (Weerakoon *et al*., 2024). Potassium is important in the regulation of blood pressure and cardiovascular health, whereas magnesium is important in muscle activity and nerve conduction. Iron, in its turn, plays a crucial role in the transportation of oxygen in the body, which helps in the overall metabolism (Bhutto *et al*., 2005; McLean and Wang, 2021).

Coconut milk is unique compared to other plant-based milk alternatives in that it contains low carbohydrates. This feature renders it especially appropriate to people who adhere to low-carbohydrate or ketogenic diets. Its specialty in terms of nutrient profile offers not only energy but also a range of physiological processes, which is why it has become an invaluable and versatile food ingredient in the new diet (Pandiselvam *et al*., 2024).

2.3.1.2. Nutritional Benefits of Coconut Milk

The MCTs of coconut milk, especially lauric acid have been associated with better cholesterol levels. Studies indicate that moderate consumption of coconut milk has the potential to raise the levels of high-density lipoprotein (HDL) (good cholesterol) and decrease the levels of low-density lipoprotein (LDL) (bad cholesterol), and this may lower the risk of heart disease (Nevin and Rajamohan, 2004). Nevertheless, it is necessary to make intake balanced because of its high saturated fat levels that can be dangerous to people with a tendency to cardiovascular problems. Lauric acid has shown strong antimicrobial effects, with the ability to prevent the growth of many pathogens, such as bacteria, viruses, and fungi (Dayrit, 2015). The following properties render coconut milk a helpful dietary ingredient in keeping the gut healthy and boosting the immune system in the body.

Coconut milk is a very good dairy-free alternative to those with lactose intolerance or cow milk protein allergies. It has a creamy texture and a mildly sweet taste, which makes it a universal in cooking, baking, and beverages (Sekar *et al*., 2020).

2.3.1.3. Health Benefits of Coconut Milk

The MCTs of coconut milk are not metabolized like the long-chain fatty acids; they are quickly turned into energy. This aspect renders coconut milk a good dietary supplement to athletes and people who are in need of prolonged energy levels (Pehowich *et al*., 2000). It is also proposed by some studies that MCTs can be beneficial in increasing fat oxidation, which helps in weight management when a balanced diet is taken.

The coconut milk plays an important role in the world cuisines. It is used in the curries, soups and desserts in Southeast Asia. It is consumed in the stews and celebratory drinks, such as coconut eggnog in the Caribbean. In addition to the use in culinary, coconut milk is widely used in plant-based diets, vegan dishes, and functional health products (Patil and Benjakul, 2018).

Coconut milk is a healthy and flexible alternative to milk that is vegetarian. It is also high in medium-chain triglycerides (MCTs), which have been known to have the energy-enhancing and metabolism-enhancing effects (Nevin and Rajamohan, 2004). It also consists of lauric acid, which is a fatty acid that has strong antimicrobial and antiviral properties (Dayrit, 2015). These qualities have helped coconut milk to be a worthy inclusion in different eating habits, especially in enhancing gut and immune health (DebMandal and Mandal, 2011).

Nevertheless, it is crucial to moderate and carefully choose minimally processed varieties to reap all benefits of it without undermining health. Excessive intake of commercially processed coconut milk, which might have additives like emulsifiers and stabilizers, may have a detrimental effect on health (Raghavendra *et al*., 2006). Further studies on its long-term health effects and new methods of its processing will only cement its position in the contemporary nutrition, and its benefits will be in line with the health-conscious dietary trends.

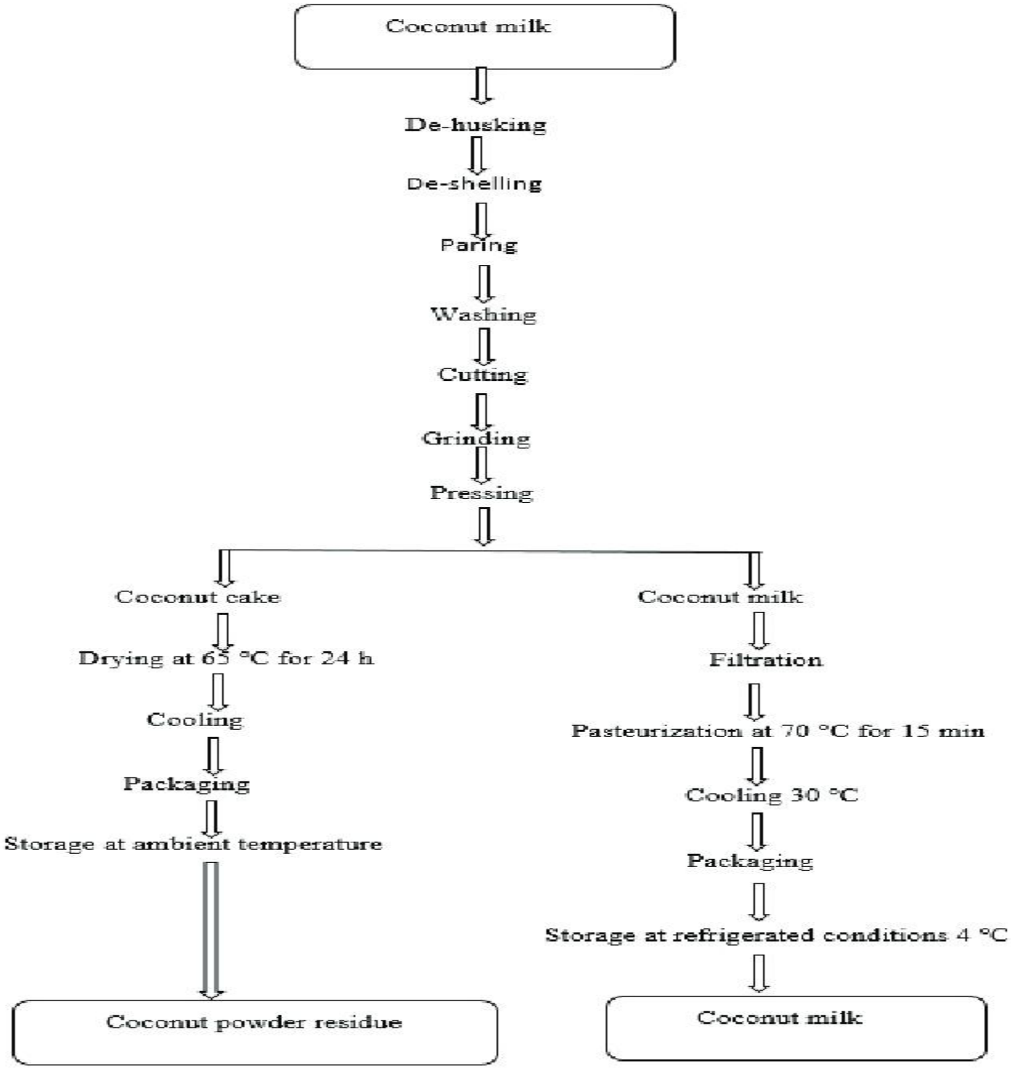

Figure 2.1. Flow chart of coconut milk production (Yakum *et al*., 2024).

2.3.2. Soya Milk

Soya milk, a product of soaked and ground soybeans (Glycine max), has become one of the most popular and nutritionally beneficial milk substitutes of animal milk in the whole world. Having a long-term history of Asian cuisine, soya milk has entered the homes and food items of all countries worldwide, being valued by its balanced structure and possible health advantages. Nevertheless, there are some challenges associated with its production which must be addressed in order to ensure quality and safety.

2.3.2.1. Composition of Soy Milk

It is observed that soya milk is nutritionally rich where its protein level compares to that of cow milk. The existence of high-quality plant protein is one of the most important characteristics as it has all the essential amino acids required by human health. This renders soya milk a great source of proteins to people who practice vegetarian or vegan diets. Moreover, polyunsaturated fats, such as omega-3 fatty acids, can be found in soy milk, and such fats are associated with heart health and the prevention of inflammation (Rizzo *et al*., 2018).

The other feature of soya milk that is of significance is that it contains a high content of isoflavones- plant compounds that are antioxidant and also have estrogen-like properties. Genistein and daidzein are isoflavones that may help in the possible health-promoting properties of soy milk by modifying cell mechanisms and enhancing protective properties against chronic diseases (Messina, 2016). These compounds have been researched on in the case of minimizing the risk of some hormone-related cancers such as breast and prostate cancer. In order to increase its nutritional value, commercially packaged soya milk is usually enriched with key nutrients like calcium, vitamin D and B vitamins. This fortification is used to imitate the nutritional value of cow milk and assists the nutritional requirements of people that do not take dairy products. Additional vitamin B12 and riboflavin which are vital in energy production and red blood cell formation may also be added to fortified soya milk (Belewu and Belewu, 2007).

2.3.2.2. Health Benefits of Soy Milk

Soya milk has been researched a lot concerning its ability to prevent the risk of chronic diseases especially heart disease. Studies have indicated that a daily intake of the soy products such as soy milk has the potential to reduce the level of low-density lipoprotein (LDL) cholesterol and enhance the overall health of the arteries. A research study conducted by Anderson *et al*. (1995) emphasized that soy protein is an important factor in lowering the total cholesterol and LDL cholesterol levels which are beneficial in enhancing cardiovascular health.

The soya milk isoflavones are very important in balancing hormones. These are phytoestrogens because of their estrogenic nature, which could help relieve the symptoms of menopause, including hot flushes and night sweats (Messina, 2016). Further, research is currently underway on how soy isoflavones may protect against hormone-dependent cancers, including breast and prostate cancer. Some of the epidemiological studies have found that the intake of soy-based foods reduces the incidence of these cancers, making dietary soy potentially protective (Messina, 2016).

Strengthened soy milk may be a good source of calcium, which is necessary to have strong and healthy bones. This renders it a very viable choice to those who do not take dairy products because of dietary limitations, lactose intolerance or due to personal choices. Bone density and bone protection against osteoporosis is achieved by consuming enough calcium, coupled with vitamin D, especially in older populations and those susceptible to bone-related disorders (Belewu and Belewu, 2007).

The composition of soy milk is such that it is a good addition to a balanced diet that can be used to control weight. The protein content aids in the feeling of fullness which can assist in the control of appetite as well as in weight loss or weight maintenance when eaten as a part of a calorie controlled diet. Although it has numerous benefits, there are numerous challenges that affect the production of soya milk, which affect the quality of this milk and the acceptance by the consumers. One of the weaknesses of soya milk is that it has a natural beany taste, and some consumers do not like it. This unpleasant taste is explained by the existence of some volatile compounds and amino acids which occur in soybeans. To prevent this, manufacturers tend to employ enzymatic treatments to counter undesired flavors or add flavoring agents like vanilla or sweeteners to enhance palatability (Hussain *et al*., 2016).

Soy is among the typical allergens, which is why soya milk cannot be used by people with soy allergies (Savage *et al*., 2010; Sicherer and Sampson, 2014). This food allergenicity limits the availability and acceptability of soy milk by some groups (Taylor and Hefle, 2001). Manufacturers need to label their products clearly to educate consumers on the possible allergens and provide them with alternatives in case they cannot take soy (Boyce *et al*., 2010).

Phytic acid and trypsin inhibitors are some of the antinutritional factors that can be found in soybeans and may cause interference with the absorption and digestion of nutrients. An example is the phytic acid that attaches to minerals such as calcium, iron and zinc making them less bioavailable. Protein digestion can be blocked by trypsin inhibitors which disrupt the activity of the enzymes in the digestive tract (Messina, 2016). The antinutritional factors can be considerably minimized by modern processing methods, including soaking, fermentation, and cooking, and the overall nutritional value of soy milk can be better (Rizzo *et al*., 2018). Soy production also has an environmental effect. Mass production of soybean, especially in other parts of the world such as South America, has been attributed to deforestation and environmental degradation. Although the environmental footprint of soy milk is smaller than that of dairy milk, sustainable farming methods are required to reduce the ecological harm and make soy a raw material sustainable in the long term (Coluccia *et al*., 2022).

Soya milk is a healthy and safe substitute of milk, which is characterized by good-quality protein, essential fatty acids, and useful isoflavones (Riaz, 2006;Messina, 2016). Its health effects include those that support cardiovascular health to those that promote hormonal balance and bone health (Liu *et al*., 2005; Setchell and Clerici, 2010). Nevertheless, off-flavors, allergenicity, and antinutritional factors are some of the challenges that need to be processed and made known to the consumers (Kwok *et al*., 2002; Redondo-Cuenca *et al*., 2008). The future will require sustainable practices and production methods to improve the quality, safety, and availability of soy milk (Sethi *et al*., 2016;Ribeiro *et al*., 2021).

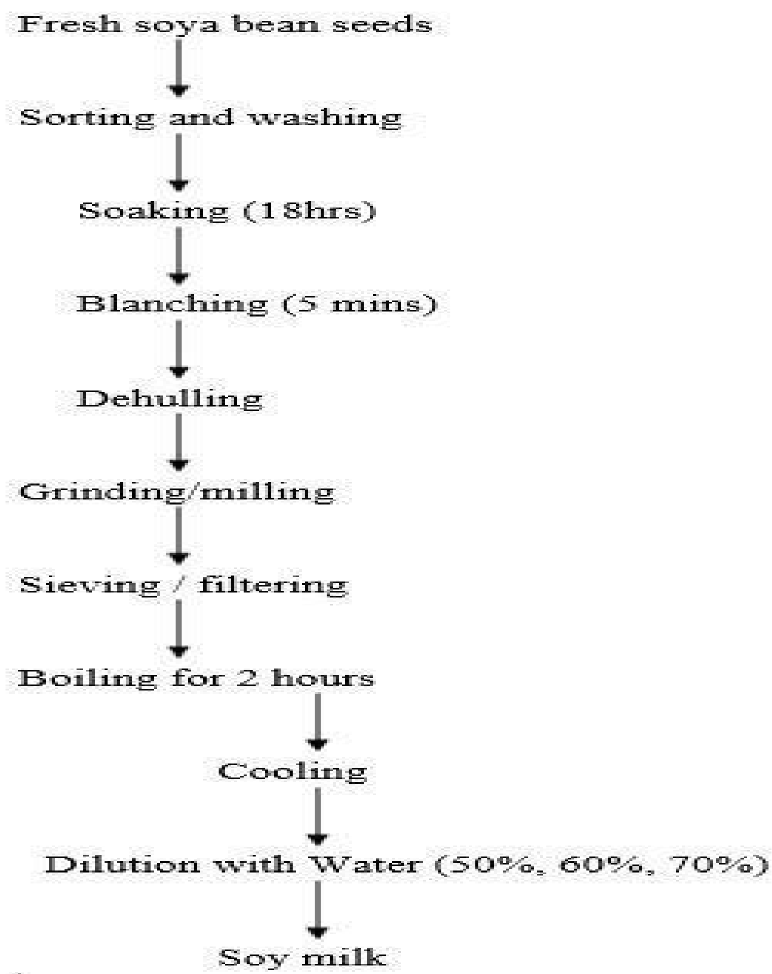

Figure 2.2. Flow chart of soyamilk production (Kale *et al*., 2012).

2.3.3. Kunu

Kunu is a non-alcoholic, non-carbonated, traditional beverage, which is commonly manufactured and consumed in Nigeria especially in the north (Abubakar *et al*., 2013; Agbo *et al*., 2013). According to Aseibai *et al*. (2015), kunu is an umbrella term that covers different non-alcoholic drinks based on cereals. However, contrary to zobo, which is prepared using only one plant, kunu may be prepared using a variety of grains, including sorghum (Sorghum bicolor), millet (Pensisetum typhoides), maize (Zea mays), rice (Oryza sativa), wheat (Triticum aestivum), and acha (Digitaria exilis). The type of kunu that is made depends on the raw materials that are used, and as such there are a few variations such as Kunun zaki, Kunun gyada, Kunun akamu, Kunun tsamiya, Kunun baule, Kunun jiko, Amshau, and Kunun gayamba, the most common one being Kunun zaki (Aseibai *et al*., 2015).

Agbo *et al*. (2013) noted that kunu is also a good source of carbohydrates, vitamins and minerals, but it is usually low in protein and some vital amino acids. It is stated that kunu prepared of sorghum has starch, protein, fats, fiber, and a variety of amino acids (Antai *et al*., 2017). The content of starch in millet and guinea corn is more than 82 percent (Adeware and Fapohunda, 2012). Although the traditional methods are mostly used in the production of kunu, an industrial production process with branded products also exists. It is prepared by wet milling grains with spices, sieving, partial gelatinization, adding sugar, bottling and subsequent sale. The beverage is prepared after fermentation that takes a duration of 6 to 24 hours.

2.3.3.1. Nutritional Values Of Kunu Drink

It has been reported that the nutritional composition of kunu is 2.31 -3.63% (protein), 3.55 - 3.63% (fat), 1.16 - 1.21% (ash), 82.92 -83.55% (carbohydrate) (Abiodun *et al*., 2017).

Glutamic acid (4.49-11.66g/100g) has the highest concentration in kunu followed by the lowest concentration of cysteine (0.34-1.45g/100g) (Adelekan *et al*., 2019). The minimal concentration of amino acid other than tryptophan was indicated to be when rice was taken as a substrate to make kunu beverage (0.44-1.40 g/100g). In addition, the amino acids cysteine, valine, isoleucine and methionine are also found in trace levels compared to the FAO (Food and Agriculture Organization) and WHO (World Health Organization) reference protein amounts (Adelekan *et al*., 2019).

2.3.3.2. Health Benefits Of Kunu Consumption

Kunu beverage assists in reducing blood sugar and managing blood glucose levels among individuals with diabetes. This is done by activating the pancreas to secrete a reasonable level of insulin. Consumption of a cup of kunu in the morning and evening is beneficial as it enhances ovulation in women and improves their likelihood of conceiving. In addition, the kunu beverage dilates blood vessels to the extent of reducing blood pressure. Kunu is hypertensive and it works in reducing excess blood cholesterol. Excessive cholesterol in the blood may lead to stroke and coronary heart diseases. Kunu is heart healthy and brain healthy (Abiodun *et al*., 2017).

Kunu is a good and safe pregnancy. It contains a lot of vitamins and folic acid. Folic acid is beneficial to pregnant women as it helps in the prevention of spinal bifida and congenital defects in newborns. Kunu enhances the production of oxytocin hormone, a hormone that triggers the release of milk by the breasts. Kunu helps nursing and breastfeeding mothers to breastfeed easily. Kunu prevents indigestion and enhances appropriate food digestion. Kunu drink is a high fibre diet that facilitates bowel movements and constipation treatment (Adelekan *et al*., 2019).

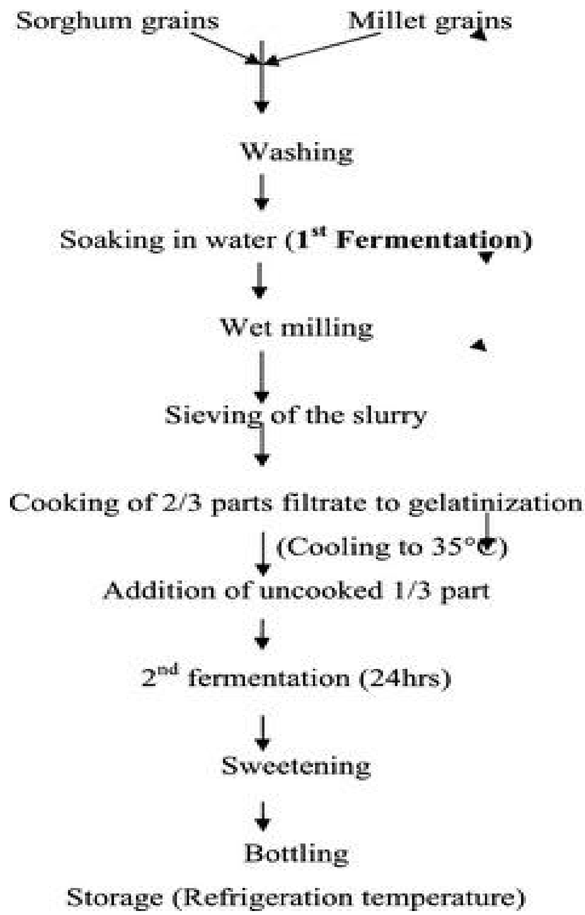

Figure 2.3. Flow chart of kunu production (Oluwajoba *et al*., 2013).

2.4. OTHER PLANT-BASED MILK DRINKS

Kunun is a non-alcoholic drink, which is common in Nigeria and other West African regions (Chukwuma *et al*., 2020). It is usually made of grains like millet, sorghum or maize, and spices like ginger, cloves and in some cases sweet potatoes are also added to the preparation to make it more palatable and nutritious. Kunu is a good nutritious beverage that contains carbohydrates, vitamins and minerals, and offers a speedy source of energy to its consumers (Olatoye *et al*., 2023). It is made by soaking, grinding, fermenting, and sieving the cereal grains to give a creamy and slightly fermented beverage. Research has shown that probiotics are present in kunu because of the fermentation process, which is capable of maintaining gut health. Also, it is commonly used to rehydrate, and is believed to be an excellent source of antioxidants, which has led to its possible effect in alleviating oxidative stress (Egwim *et al*., 2013).

Zobo or hibiscus tea is a colorful red beverage prepared out of dried calyces of Hibiscus sabdariffa. It is a well-known drink in Nigeria, which is hailed as a refreshing drink and health-giving drink. Zobo is very nutritious, especially in vitamin C and includes phenolic compounds that have antioxidant and anti-inflammatory effects (Ologundudu *et al*., 2009). Zobo is prepared by boiling of the dried calyces that discharges the red color and bioactive components into the water. Ginger, cloves and pineapple are ingredients that can be used to add flavor and additional health benefits. It has been discovered that zobo can be used to treat blood pressure, enhance digestion, and maintain good heart health (Edo *et al*., 2023).

The tigernut milk is made out of the tubers of Cyperus esculentus, also referred to as tigernut properties (Maduka and Ire, 2018). It is a lactose-free, naturally sweet and creamy drink, which is a perfect substitute of lactose intolerant people. Tigernut milk is full of fiber and healthy fats, iron, magnesium, vitamins E and C, which make it an antioxidant and anti-inflammatory (Maduka and Ire, 2018). Tigernut milk is made by soaking the tubers and combining them with water and then straining to make a smooth liquid. Tigernut milk is said to have potential in improving digestion, control blood sugar levels and improving the skin health because it contains high levels of vitamin E (Ndubuisi, 2009).

2.6. MICROBIAL CONTAMINATION OF SOME PLANT BASED MILK DRINKS

2.6.1. Microbial Contamination of Locally Prepared Kunun Drink

According to a study conducted by Essien *et al*. (2011), it was found that Lactobacillus plantarum, Bacillus cereus, Streptococcus faecium, *Escherichia coli*, Micrococcus acidophilus, *Staphylococcus* aureus and fungi like Aspergillus niger, Fusarium oxysporum and *Penicillium* oxalicum were some of the microorganisms that were associated with locally prepared kunun drink. Saccharomyces cerevisiae and Candida mycodema were also detected in the samples of kunun. It is not surprising that these microorganisms are present since most of them are able to grow in areas that have a lot of fermentable sugars, leading to the production of acid during the fermentation process (Amusa and Odunbaku, 2009).

Enterobacter, Shigella, *Escherichia coli*, Klebsilla, Micrococcus, Proteus, Leuconostoc, Bacillus, Citrobacter and *Staphylococcus* are some of the microbial isolates present in Kunu zaki sold (Kelechi *et al*., 2020). According to Edward *et al*. (2019), microbial diversity in freshly processed and hawked drinks of kunu was Lactobacillus plantarum, Bacillus subtilis, B. cereus, Streptococcus faecium, Streptococcus lactis, *Staphylococcus* aureus, Micrococcus acidophilus, *Escherichia coli*, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida mycoderma, Aspergillus Microbial isolates were reported in Adebayo-Tayo *et al*. (2016) in Kunun drink sold in Calabar, Nigeria as *Staphylococcus*, Streptococcus, Bacillus, Pseudomonas species and *Escherichia coli* (bacteria), Fusarium, Aspergillus, *Penicillium*, and yeast Fungi species (Edward and Ezekiel, (2019)). *Escherichia coli*, *Staphylococcus* aureus, Streptococcus pyogenes, Rhizopus nigricans and *Penicillium* species were reported in Kunu aya sold in Kaduna State University, Kaduna State (Musa and Hamza, 2013).

Aspergillus niger, *Penicillium* oxalicum and Fusarium oxysporum are fungi that are present in food and cereals with high carbohydrate content such as sorghum (Essien, 2011). These fungi are characterized by their strong ability to survive on spores and their occurrence in kunun may be attributed to contamination of air especially with hawked kunun which is usually not well covered (Adegoke and Skura, 1994). Saccharomyces cerevisiae and Candida mycodema are important in flavor formation are not surprising in both hawked and freshly prepared kunun (Odunfa and Adeyeye, 2005).

2.6.2. Microbial Contamination of Locally Prepared Zobo Drink

According to a study by Ezeigbo *et al*. (2016), the total aerobic bacteria count of the retailed zobo drinks was between 0.3 x 106 cfu/ml and 4.4x 106 cfu/ml with the total coliform count being 0.1 x 105cfu/ml to 6.5 x 105 cfu/ml. Zobo drinks are associated with bacteria like Echerichia coli, *Staphylococcus* spp, Lactobacillus spp, Bacillus spp and Pseudomonas spp (Ezeigbo *et al*., 2016). Microbial contamination in zobo drinks was also found to be caused by fungi like Aspergillus niger, *Aspergillus flavus*, *Penicillium* sp. and Saccharomyces sp. (Ekanem *et al*., 2018).

Escherichia coli is an indication of fecal contamination, which is not surprising because of the use of unclean water sources and unsanitary environments in production (Okeke *et al*., 2000). The *Staphylococcus* species might be of cross-contamination through food, utensils, or human contact, but there is no specific data on the prevalence (Okeke *et al*., 2000). Bacillus species contaminated zobo may cause abdominal cramping and nausea. Also reported are Enterobacter and Klebsiella species that are usually of water sources that are contaminated with fecal matter (Nweze, 2010). Candida species such as yeasts have the capacity to make esters and phenolic acids that change the taste of zobo beverages. Saccharomyces cerevisiae is capable of fermenting starch to glucose to produce ethanol and carbon dioxide, which affects the organoleptic characteristics of the drink (Osuntogu *et al*., 2004).

Added spices like pineapple juice and lemongrass have been found to prevent the growth of microbes and contamination by the pathogenic bacteria like Pseudomonas aeruginosa (Oboh *et al*., 2004). Thus, it is strongly suggested that spices should be added to zobo drinks.

2.6.3. Microbial Contamination of Locally Prepared Soya Milk

Microorganisms are everywhere and soya milk is not an exception to microbial contamination. Bacillus cereus, Bacillus subtilis, *Staphylococcus* aureus, Clostridium species and Lactobacillus species and Corynebacterium species have been associated with soya milk locally prepared (Agboke *et al*., 2011). Bacillus cereus presence implies that there might be some contamination with the environment during preparation since this bacterium can cause food poisoning, which is manifested in diarrhea and emesis which can result in severe complications or death (Farinde *et al*., 2010).

The presence of *Staphylococcus* aureus can be interpreted as the lack of hygiene in the production process, and the *Escherichia coli* can be taken as the evidence of fecal contamination. The presence of the Lactobacillus species is not astonishing because they are involved in the fermentation processes. Streptococcus mitis may induce pharyngitis that resembles scarlet fever in case of large doses (Wong *et al*., 2002).

2.7. FACTORS ENHANCING MICROBIAL CONTAMINATION IN SOYMILK DRINKS

Various factors during production, handling, and storage of plant-based milk drinks can affect microbial contamination of these beverages. These factors should be understood so as to reduce the risks that are involved in taking contaminated beverages.

2.7.1. Quality of Raw Materials

Raw materials quality is a very important factor in microbial contamination. The naturally occurring microorganisms, including bacteria and fungi, may be found in fresh plant-based ingredients, including soybeans, cereals, or fruits (Gustaw *et al*., 2021). As an example, species of Aspergillus and *Penicillium* are widespread on plant-based products and may develop during processing (Bamidele *et al*., 2022). Raw materials that are not washed or kept properly also pose additional risks of contamination by microbes (Aadil *et al*., 2019).

2.7.2. Hygiene and Sanitation Practices

Poor hygiene and sanitation during the preparation and handling of plant-based milk beverages are also major causative factors of microbial contamination. Research has revealed that unsanitary conditions in the processing process, including inadequacy in washing of equipment and contact with contaminated surfaces may harbor pathogenic organisms (Mishra *et al*., 2024). *Staphylococcus* aureus and Bacillus cereus in the drinks are frequently associated with contaminated utensils or hands or processing facilities (Jahan *et al*., 2019; Oranusi *et al*., 2003).

2.7.3. Fermentation and Production Methods

One of the most common ways to improve the flavor and preserve plant-based milk is fermentation. Nevertheless, the uncontrolled fermentation may result in the proliferation of dangerous microorganisms. As an example, during controlled fermentation, Lactobacillus and Saccharomyces species are useful, but the uncontrolled appearance of *Escherichia coli* and Clostridium species may take place under unsanitary conditions (Ekanem *et al*., 2018). Microbial diversity in the end product is also influenced by the type of fermentation process, the time taken and the temperature (Agboke *et al*., 2011).

2.7.4. Temperature and Storage Conditions

Inadequate temperature regulation in storage and transportation may greatly enhance the chances of microbial proliferation. The plant-based milk drinks are very prone to spoilage and contamination when kept at higher temperatures than the recommended range. Heat may promote the growth of mesophilic and thermophilic bacteria, including Lactobacillus and Bacillus (Wong *et al*., 2002; Okeke *et al*., 2000). A quick development of microbes and spoilage can also occur when the storage is done in poorly insulated or non-refrigerated containers (Osuntogu *et al*., 2004).

2.7.5. Water Quality and Use

The quality of water applied in the manufacture of plant-based milk is a major factor that can improve microbial contamination. Research has observed that water obtained in untreated or shallow wells has the potential to transmit pathogens, including *E. coli* and Klebsiella, to the end product. Water should be treated and purified properly to avoid microbial contamination of water in the vegetable drinks (Mishra *et al*., 2024).

2.7.6. Cross-Contamination

Plant-based milk may be contaminated by cross-contamination in the process and packaging stage. This can occur when raw materials, finished products or packaging materials are exposed to contaminated surfaces or machinery or other products. Shared equipment and handling techniques can transmit contaminants like *Staphylococcus* and Pseudomonas species (Pakdel *et al*., 2023; Mishra *et al*., 2024).

2.7.7. Additives and Preservatives

The safety of plant-based milk drinks in terms of microbes can be affected by the use of additives and preservatives. Although preservatives are used to prevent the growth of microbes, when used in incorrect proportions or where effective antimicrobial agents are not used, they may cause contamination. Conversely, some of the natural preservatives, including lemongrass and pineapple extracts, have demonstrated antimicrobial activities that have the potential to increase the shelf life of these drinks (Pandey and Negi, 2018).

2.8. Health Implications of Microbial Contamination in Plant-Based Milk Drinks

Plant-based milk beverages are subject to microbial contamination, which is a significant health issue to the population of a specific area, especially in those locations where hygiene is not practiced and food safety protocols are not properly enforced. These traditional drinks which in most cases are made using raw materials and processes that are not strictly controlled in terms of quality, may serve as carriers of dangerous microorganisms and this has a very serious effect on the health of consumers. The adulterated milk drinks of plant origin can cause outbreaks of food diseases, and some of them are severe and may lead to hospitalization or even death. Children, pregnant women, the elderly, and immunocompromised people are particularly vulnerable to the health hazards of contaminated drinks (Mukuna, 2020).

The health effects of microbial contamination of these beverages include acute infections and chronic illnesses. Gastrointestinal infections due to acute exposure to bacterial pathogens, including *Escherichia coli*, *Salmonella*, and Bacillus cereus, are characterized by diarrhea, vomiting, and abdominal pain. When these symptoms are severe they may result in dehydration, electrolyte imbalance and malnutrition. These infections can develop life-threatening complications especially among children and the elderly in areas with low access to healthcare and other supportive treatments (Bryan, 1988; CDC, 2016).

Fungal contamination is also a very threatening risk especially because fungi like Aspergillus and *Penicillium* produce mycotoxins. The long-term exposure to mycotoxins, in particular, aflatoxins, is an important societal problem in developing countries such as Nigeria, where the traditional food production practices are common, and control measures are weak. Aflatoxins are very toxic substances, which have been implicated in liver cancer, stunted childhood growth, and weakening of the immune system, which exposes the affected people to infections and other diseases (Pitt and Hocking, 2009; IARC, 2012). The long-term health impacts of exposure to mycotoxins demonstrate the necessity of strict monitoring and better safety measures in the manufacturing and distribution of plant-based milk beverages.

In addition to the direct health impacts, the socioeconomic impacts of microbial contamination of plant-based milk drinks are more far-reaching. Regular eruption of foodborne diseases may exert pressure on the health care systems, resulting in higher treatment expenses and decreased labor force output. In the resource-strained areas, the financial cost of managing preventable diseases takes away money used in other important public health activities, thus continuing the poverty and poor health status cycles (FAO/WHO, 2009).

The fact that plant-based milk beverages are contaminated also compromises consumer trust in conventional foodstuffs. This may adversely affect the local economies that rely on the manufacture and sale of such drinks especially in the localities where such drinks are a major source of livelihood to small-scale producers (Siddiqui *et al*.,2024).

2.9. Strategies for Controlling Microbial Contamination in Plant-Based Milk Drinks

Plant-based milk beverages are becoming more popular as substitutes to dairy milk, mainly due to their health advantages and the ability to conform to the dietary limitations, such as lactose intolerance and veganism. Nonetheless, such drinks are rich in nutrients, and they can be easily affected by microbes (Mishra *et al*., 2024). To achieve microbial safety and quality, it is necessary to adopt effective control measures. Microbial contamination of plant-based milk drinks is mitigated by the following methods:

A common method is thermal processing, which implies the use of heat to decrease the number of microbes. Pasteurization, which is commonly done at 63degC to 85degC and at different time periods, is an effective method of inactivating spoilage and pathogenic microorganisms without affecting the nutritional and sensory properties of the beverage. In the case of shelfstable products, temperatures above 100degC are used in sterilization, which guarantees a long shelf life (Silva, 2023).

Another advanced technique is high-pressure processing (HPP) which exerts pressure between 100 and 600 Mpa on microorganisms to inactivate them without heat. The method maintains the nutritional quality that research has shown its effectiveness in combating spoilage microbes, molds, and yeasts, and has potential in plant-based drinks and flavor of the beverage as well as providing a wide-spectrum of microbial protection. (Amsasekar *et al*., 2022).

Natural acidification of plant-based milk drinks by using citric or lactic acid modulates the pH of the beverage to an environment that cannot support the growth of microorganisms. Other preservation techniques are usually used alongside acidification to improve its efficacy (Ay *et al*., 2012). Natural and synthetic preservatives are very crucial in controlling microbes. Preservatives, such as potassium sorbate and sodium benzoate, which are commonly used, prevent the growth of microbes. Natural alternatives, including essential oils obtained with neem and bergamot, were also found to have antimicrobial activity against several types of spoilage organisms (Abdi-Moghadam *et al*., 2023).Aseptic processing and packaging entails sterilization of the product and container individually followed by filling in a sterile environment. This reduces post processing contamination and guarantees microbial safety, especially when storing over a long period (Lewis and Deeth, 2008).

Hygiene in the production process is important in contamination prevention. Compliance with the principles of good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) will help to ensure purity of equipment, water quality, and personal hygiene, which will minimize the risk of microbes (Codex Alimentarius Commission, 2020). Irradiation is a less popular technique, but with a regulatory and consumer-based issue, and this is an effective way of destroying microbial populations by exposing the beverage to controlled doses of ionizing radiation. Biopreservation takes advantage of antimicrobial compounds that are naturally found such as bacteriocins and organic acids to prevent microbial growth. Examples of biopreservatives that have been applied in plant-based beverages are nisin and natamycin (Chakraborty and Dutta, 2022).

The management of microbial contamination in plant-based milk beverages can be achieved with the help of a combination of strategies in order to achieve the highest efficiency. The methods used are determined by the composition of the product, the shelf life required and the regulatory requirements. Continued research and innovation is necessary to improve the safety and quality of these ever more popular beverages.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The experiment was carried out in Benin City, Edo State, Nigeria, where the microbiological analysis of locally produced plant-based milk drinks was carried out. Benin City is a busy city in which there are a variety of agricultural and commercial activities. The samples used in this study were sampled in 4 major markets within the city including Oluku, Oba and Uselu Markets. The markets were chosen because of their large consumer traffic and the fact that they represent the locally produced plant-based milk drinks.

3.2 Collection of Samples

One dozen (12) samples of plant-based milk drinks were obtained. These contained three (3) samples of soya milk, tiger nut milk, kunu, and coconut milk in the said markets. The samples were taken to the laboratory in an ice-packed cooler to ensure that they are not damaged and that there is minimal change in the microbes as they are transported to the laboratory. The microbiological analysis was initiated within a period of two hours of sample collection.

3.3 Sterilization of Materials

The materials including glassware were washed in detail using detergent and rinsed using distilled water (conical flasks, measuring cylinders, McCartney bottles, Bijou bottles). Then they were sterilized in an oven at $16^{0}C$ at 1hr to remove any possible contaminants.

3.4 Preparation of Culture Media

The culture media that was used in this experiment were Nutrient Agar (NA), *Salmonella*-Shigella Agar(SSA), and Potato Dextrose Agar (PDA). These media were ready as per instructions of the manufacturers. The necessary volumes of powdered media were dissolved in distilled water, mixed and sterilized in an autoclave at 121^0C in 15 min. The ready media were left to cool to 45-50degC and then poured into sterile Petri dishes.

3.5 Isolation of Microorganisms

To each of the samples 10 mL was vortex mixed in 90 mL of sterile peptone water. Dilutions were done in series up to 104 dilution. Based on the 10-4 dilution, 1 mL was subculture on Nutrient Agar and *Salmonella*-Shigella Agar to enumerate bacteria and Potato Dextrose Agar to isolate fungi using the pour plate technique. Nutrient Agar (NA) and *Salmonella*-Shigella Agar(SSA) plates were incubated at 37degC in 24 hrs to establish the total number of bacteria and coliform number of bacteria respectively. Fungal isolation was done by incubating Plates with Potato Dextrose Agar (PDA) at 25degC over 3-5 days. After incubation, a digital colony counter was used to count the colonies. The findings were in colony-forming units per milliliter (CFU/mL).

3.6 Subculturing and Purification of Isolates

The agar plates were observed and distinct colonies were carefully selected on the basis of morphological features like size, color and texture. To get pure isolates, each colony type was subcultured on new Nutrient Agar plates by the streaking technique. The plates were incubated at 37degC during 24 hours. Nutrient Agar slants with pure cultures were preserved at 4degC ready to be identified.

3.7 Identification of Isolates

Detection of microbial isolates was done through Gram staining and biochemical test according to the methodology of Cheesbrough (2006). The biochemical tests that have been conducted are catalase, oxidase, indole, coagulase, Methyl red, Voges Proskauer, citrate, sugar fermentation and oxygen relationship test.

3.7.1. Gram Staining

Using a wire loop, a thin smear of the isolates was transferred onto various slides and left to dry and then they will be heat fixed and allowed to cool. After that, the various smears were stained with crystal violet stain (30-60 sec) and then sprayed off using clean water. The smears were then dipped into Lugol iodine (30-60sec) and washed quickly with clean water. The smears were decolourised instantly using alcohol and washed out instantly using clean water. Next the smears were stained with safaranine 30-60 sec and rinsed with clean water. The stained smears were then left to air dry. The stained smears were dried then a drop of oil immersion was added to the stained smears and observed under a microscope (x100 oil objective lens) to verify the microscopic characteristics of the organisms such as the Gram reaction, morphology (Cheesbrough, 2006). In the case of the fungal isolate, lactophenol cotton blue stain was dropped at the centre of a clean slide. A piece of the fungus was then picked with the help of a wireloop and some of the fungus were taken and put in the drop of the stain and teased and covered with a coverslip. No pushing or tapping of the coverslip was done to prevent the dislodging of the conidia of the conidiophores. The stained isolate was then observed using the microscope using x10 and x40 objective lens to study its morphological features (Cheesbrough, 2006).

3.8 Determination of pH values of the samples

The pH value of the samples was determined using a Hanna 98127 pH meter from which the readings were carried out.

3.8 BIOCHEMICAL TESTS

3.8.1. . Catalase Test

A drop of hydrogen perioxide (H_2O_2). was added to the discrete colonies of each of the isolates and a wooden stick was used to emulsify the bacterial colonies. A positive result was indicated by the presence of bubbles of gas (Cheesbrough, 2006).

3.8.2. Indole Test

A small sample of each of the isolates was inoculated in 5 ml of prepared peptone water that was sterilised in various test tubes using a wire loop. Then the test tubes with the organisms were incubated at 37 deg C during 48hr. Following incubation period, 3-4 drops of indole reagent referred to as Kovac reagent were added and gently shake. The positive result provided a red surface layer after 10min whereas the negative result provided no red surface layer after 10minutes as per Cheesbrough (2006).

3.8.3. Oxidase Test

One drop of oxidase reagent was prepared and 2-3 drops of the prepared reagent were put in a disposable petri dish. Discrete colonies of the isolates were picked with the help of a wooden stick and smeared on the filter paper separately. A positive reaction produced a purple-blue colouration after 10 sec but a negative reaction produced no colour after 10 sec as per Cheesbrough (2006).

3.8.4. Coagulase Test

A drop of distilled water was added on each end of a slide and a colony of the test organism was emulsified in each of the drops to create a thick suspension. A loopful of plasma was then poured into one of the suspensions and swirled. Cheesbrough (2006) found that there was positive result indicating the presence of clumping after 10 sec and the negative result indicating no clumping after 10 sec.

3.8.5. Citrate Utilization Test

The Simon citrate agar was prepared as per the specification of the manufacturer in a disposable Petri dish; it was inoculated with the test organism and incubated in the incubator at 37 o C, over a period of 3 days. The change of colour between the green and blue colour meant a positive result and a negative result was still green.

3.8.6. Sugar Fermentation Test

The medium of fermentation was prepared and sterilized having the indicator and durham tube with no air bubbles in them. Autoclaving of the sugar solution was performed at pressure of 10 ibs/ sq inch and 0.5 ml of the sugar was added to sterile peptone water. The test organism was inoculated in the fermentation tubes. All the sugar was kept under negative control. Colour change was observed after incubating the tubes at 370C over the period of 24-48 hours.

3.8.7. Starch Hydrolysis Test

Nutrient agar that had been impregnated with 0.3 percent of soluble starch was prepared, mixed and poured in sterile petri dishes to allow it to solidify. The isolates were then streaked on the solid medium in a discrete manner and incubated at 37 o C and 48 hrs after which they were flooded with 5-10 ml of Iodine. A positive result was denoted by blue-black coloration.

3.8.8. Voges Proskauer Test

The test was carried out to distinguish Bacillus sp. and enteric bacteria that ferment glucose and produce acetoin which undergoes oxidation reaction. 2 ml of sterile Methyl red-Voges Proskauer broth was inoculated with test organism and incubated at 37 0C, 24 hrs. One ml of 10 percent naphthol was added and mixed. Approximately 3 ml of KOH was added and swirled. It was then allowed to stand at room temperature (25 +- 20C) an hour. The positive result was represented by a pink to red colour (Omomowo *et al*., 2015).

3.8.9. Methyl Red Test

This test is employed to test the production of acid in the medium typically of coliform organisms that ferment dextrose quickly resulting in the drop in pH. Methyl red-Voges Proskauer broth was made. 10 ml of broth was put into test tubes and sterilized. It was then inoculated and incubated at 300C at 24 hrs. The culture was incubated and a drop of methyl red indicator was added after which a red colouration was observed indicating a positive reaction (Omomowo *et al*., 2015).

3.9 Antibiotics Susceptibility Test

The susceptibility and resistance of bacterial isolates were determined using the identified colony of bacteria that were subjected to standard antibacterial susceptibility testing (AST) to decode the resistance or susceptibility of bacteria to common antibiotics that are used to treat them in the locality. The Kirby Bauer disc diffusion test was used to perform the test. The antibiotics tested included **Amoxicillin (10 µg), Ciprofloxacin (10 µg), Gentamicin (10 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Erythromycin (10 µg), Ceftriaxone (30 µg), Trimethoprim-Sulfamethoxazole (30µg).** The disc diffusion method used in this study was done using the standard discs produced by Oxoid, UK. In this assay a fully grown bacterial culture (18-24 hrs old) was grown on Muller-Hinton Agar (MHA). The inoculum of

1.5 x 108 cells/ml McFarland standard was streaked on the MHA plates with a sterile loop and antibiotic discs were added with utmost care on the plates with the help of sterile forceps. The susceptibility values were taken following a 24-hr incubation period at 37 o C. Adhering to the standard or rules of AST that were developed in 2017 by CLSI (Clinical Laboratory Standards Institute). The area of inhibition surrounding each disc (measured with a meter rule in diameter) was measured and evaluated according to the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S). The equation applied in computing of MAR (Multiple Antibiotic Resistance) index is a/b where a is the number of antibiotics to which an isolate is resistant, and b is the number of antibiotics that have been tested.

3.10. IDENTIFICATION OF FUNGAL ISOLATES

3.10.1 Cultural Characteristics

Each colony morphology e.g., size, texture, color, reverse color was determined by physical examination.

3.10.2 Preparation of Pure Cultures

Pure cultures are prepared by adding a single colony into a petri dish containing 500 mL of sterile media (Garber 2009).

A pure culture was obtained by one colony which was subcultured onto the surface of a plate medium of potato dextrose agar. Following the attainment of a pure colony, the same colony was streaked on potato dextrose agar slant. These cultures were incubated at room temperature (25+-20C) over a period of 72hr.

3.10.3 Lactophenol cotton blue staining

Lactophenol cotton blue is a stain that is mostly used in preparing semi-permanent microscopic preparation of fungi. It stains the fungi cytoplasm and gives a light blue color, on which the wall of hyphae can be easily observed. It consists of four ingredients: phenol, which is used as fungicide; lactic acid, which is used as clearing agent; cotton blue, which is used as staining the cytoplasm of the fungus; and glycerine, which provides semi- permanent preparation. To begin with, a drop of lactophenol cotton blue stain was put on a clean slide. A small tuft of the fungus was then smeared on the drop on the slide by means of a sterile wire loop. A cover-glass was then overlaid on the slide and examined using a bright field microscope with a magnification of 400x.

CHAPTER FOUR

4.0. RESULTS

The results for the total heterotrophic bacterial counts obtained from the locally made plant-based milk samples obtained from various market sources in Benin City are shown in Table 4.1 below. The plant-based milk drinks included Soya milk, Tiger nut milk, Kunu and Coconut milk and the market sources from which these samples were obtained are Oluku, Oba and Uselu markets. The mean heterotrophic bacterial counts ranged from 1.90±3.53 (Soya milk, Oluku Market) – 82.40±16.90×10^5 cfu/mL (Coconut milk, Oba Market).

The total *Salmonella*-*Shigella* counts in the plant-based milk samples are shown in Table 4.2. It was observed that the counts ranged from 1.10±8.48×10^5 cfu/mL (Tiger nut milk, Uselu Market) – TNC (Coconut milk, Oba Market).

The mean heterotrophic fungal counts of locally made plant-based milk samples are presented in Table 4.3. It was observed that the counts ranged from 2.40±4.24 (Soya milk, Oluku Market) – 44.00±0.00×10^5 cfu/mL (Tiger nut milk, Oba Market).

The cultural, morphological and biochemical characteristics of the bacteria isolated from plant-based milk samples in Benin City are shown in Table 4.4. The bacteria isolated from the samples include *Pseudomonas* sp., *Klebsiella pneumoniae*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus* sp., *Bacillus subtilis* and *Salmonella* sp.

Table 4.1: Mean heterotrophic bacterial counts (10^5 cfu/mL) of locally made plant-based milk sold in selected markets in Benin City

MARKETS	SOYAMILK	TIGER NUT MILK	KUNU	COCONUT MILK
	HETEROTROPHIC BACTERIAL COUNTS (CFU/mL)			
OLUKU	1.90±0.53	4.60±0.65	42.40±9.50	37.00±5.50
USELU	2.68±0.70	6.70±0.70	14.30±4.80	10.50±4.80
OBA	5.40±0.24	34.40±6.90	37.20±6.70	82.40±6.90

Table 4.2: Mean *Salmonella-Shigella* counts (10^5 CFU/mL) of locally made plant-based milk sold in selected markets in Benin City

MARKETS	SOYA MILK	TIGER NUT MILK	KUNU	COCONUT MILK
		***Salmonella-Shigella* Counts (CFU/mL)**		
OLUKU	4.10±0.78	3.90±0.70	1.82±0.24	13.80±0.83
USELU	7.70±0.78	1.10±0.48	1.19±0.94	8.30±0.70
OBA	35.00±0.00	64.00±5.70	TNC	TNC

Key: TNC= Too numerous to count

Table 4.3. Heterotrophic fungal counts (10^5CFU/mL) of locally made plant-based milk sold in selected markets in Benin City

MARKETS	SOYA MILK	TIGER NUT MILK	KUNU	COCONUT MILK
	Heterotrophic fungal counts (10^5CFU/mL)			
OLUKU	2.40±0.24	3.30±0.70	3.00±1.41	4.50±1.60
USELU	10.30±3.40	7.60±2.82	20.00±2.82	7.00±0.17
OBA	3.00±0.70	44.00±0.00	3.00±0.00	24.80±5.65

The distribution of bacterial isolates from locally made plant-based milk drinks in selected markets in Benin City is summarized in Table 4.5. *Klebsiella pneumoniae*, *Staphylococcus* sp. and *Escherichia coli* were detected in soya milk samples, while *Bacillus subtilis, Pseudomonas* sp., *Bacillus cereus,* and *Salmonella* sp. were absent. In tiger nut milk, *Escherichia coli*, *Bacillus cereus, Bacillus subtilis*, *Salmonella* sp. and *Staphylococcus* sp. were present, but *Klebsiella pneumoniae,* and *Pseudomonas* sp. were absent. Kunu milk samples showed the presence of *Bacillus cereus, Klebsiella pneumoniae, Pseudomonas* sp. and *Salmonella* sp., while *Bacillus subtilis, Escherichia coli* and *Staphylococcus* sp. were not detected. In coconut milk, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus* sp. were identified, while *Pseudomonas* sp., *Bacillus cereus* and *Salmonella* sp. were absent.

Table 4.6. shows the frequency of occurrence of the bacteria isolated. The bacteria with the highest frequency of occurrence were *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus* sp. which had the occurrence of 25.00% and the lowest was *Pseudomonas* sp. (8.33%).

The cultural and morphological characteristics of fungi isolated from plant-based milk samples in Benin City are presented in Table 4.7. The identified fungal species include *Mucor* sp., *Saccharomyces* sp., *Penicillium* sp. and *Aspergillus* sp.

Table 4.8. presents the distribution of fungal isolates from the locally made plant-based milk drinks in the selected markets. In soya milk, *Saccharomyces* sp., *Penicillium* sp. and *Aspergillus flavus.* were found, while *Mucor* sp. was absent. *Mucor* sp. were present in tiger nut milk, but *Saccharomyces* sp., *Penicillium* sp., or *Aspergillus flavus* were not detected. Kunu milk showed the presence of *Saccharomyces* sp., *Penicillium* sp. and *Aspergillus flavus* with no

Mucor sp. identified. Coconut milk samples contained only *Aspergillus flavus*, with no *Saccharomyces* sp., *Mucor* sp., or *Penicillium* sp. present.

Table 4.9 shows the frequency of occurrence for each of the fungus isolated. The organism with the highest frequency of occurrence was recorded to be *Aspergillus flavus* which had an occurrence of 25.00% and the lowest was *Mucor* sp. which had an occurrence of 8.33%.

Table 4.10a and 4.10b presents the antibiotic sensitivity patterns of Gram-positive and Gram-negative bacterial isolates in plant-based milk samples from Benin City. For Gram-positive bacterial isolates, *Staphylococcus* sp**.** exhibited resistance to Amoxicillin but was sensitive to all other antibiotics tested, resulting in a MAR Index of 0.10**,** indicating minimal resistance. *Bacillus cereus* was fully sensitive (S) to all antibiotics, with a MAR index of 0.00**,** suggesting no significant resistance. *Bacillus subtilis* showed intermediate sensitivity to Cephalexin and Ampiclox**,** and resistance to Ceftriaxone**,** leading to a MAR index of 0.20**,** indicating low resistance. For Gram-negative bacterial isolates, *Pseudomonas* sp. displayed resistance to Augmentin, Pefloxacin, Ceftazidime, and Trimethoprim-Sulfamethoxazole**,** resulting in a high MAR Index of 0.40**,** which signals considerable antimicrobial resistance. *Shigella* sp. exhibited resistance to Ceftazidime and Trimethoprim-Sulfamethoxazole**,** but was sensitive to other antibiotics, resulting in a MAR index of 0.20**.** *Klebsiella pneumoniae* showed resistance to Cephradine and Ceftazidime**,** with a MAR index of 0.30**,** while *Escherichia coli* demonstrated resistance to Augmentin**,** Ceftazidime**,** and Trimethoprim-Sulfamethoxazole**,** leading to a MAR index of 0.30

The mean pH values of plant-based milk samples ranged from 3.90±0.00 (coconut milk, Uselu Market) – 5.90±0.14 (Kunu, Oba Market) as shown in Table 4.11

Table 4.4: Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

Shape	**Irregular**	**Circular**	**Circular**	**Circular**	**Irregular**	**Irregular**	**Circular**
Size	Large	Medium	Medium	Medium	large	Medium	Small
Morphological							
Gram stain	+	-	-	-	+	+	-
cell type	Rod	Rod	Rod	Rod	Rod	Cocci	Rod
Arrangement	Disperse	Disperse	Pair/chains	Disperse	Disperse		Disperse
Color	Purple	Pink	Milky	Green	purple	Golden yellow	Pink
Biochemical							
KOH test	-	+	+	+	+	+	-
Indole	-	-	-	-	-	-	-
Citrate	+	-	-	+	+	+	+
Oxidase	+	+	+	+	-	-	-
Urease	+	+	+	-	+	+	-
Glucose	-	+	+	-	+	+	+
Maltose	+	+	+	-	+	+	-
Lactose	-	+	+	-	+	+	-
Methyl red	+	+	-	-	-	+	-
Voges-Proskauer	+	-	-	-	-	+	-
Catalase	+	+	+	+	+	+	+
Xylose	-	+	+	-	+	+	-
Mannitol	-	-	+	+	-	+	-
Gas formation	+	+	+	-	-	-	-
H2S formation	-	-	-	-	-	-	+
Identity	*Bacillus cereus*	*E. coli*	*K. pneumoniae*	*Pseudomonas* sp.	*Bacillus subtilis*	*Staphylococcus* sp.	*Salmonella* sp.

Table 4.7. Cultural And Morphological Characteristics of Fungal Isolates

	1	2	3	4
CULTURAL CHARACTERISTICS OF FUNGI ISOLATES	White fluffy colony with reverse side yellow	Cotton candy texture, white with pale yellowish brown reverse colour	Velvety, white to gray-orange, reverse is white to pale yellow	Greenish-yellow colony with reverse side yellow
NAME OF HYPHAE	Septate	Septate	Septate	Septate
COLOUR OF SPORE	Brown	White	Greyish	Brown
TYPE OF SPORE	Conidiospore	Conidiospore	Conidiospore	Conidiospore
POSSIBLE ISOLATE	*Saccharomyces* sp.	*Mucor* sp.	*Penicillium* sp .	*Aspergillus flavus*

Table 4.5: Distribution of bacterial isolates from locally made plant-based milk sold in selected market in Benin City

SAMPLES	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas* sp.	*Bacillus subtilis*	*Staphylococcus* sp.	*Salmonella* sp.
SOYA MILK	-	+	+	-	-	+	-
TIGER NUT MILK	+	+	-	-	+	+	+
KUNU	+	-	+	+	-	-	+
COCONUT MILK	-	+	+	-	+	+	-

Table 4.6: The frequency of occurrence for bacteria isolated from locally made plant-based milk samples

Isolates	No. of Occurrence	Percentage of Occurrence (%)
Bacillus cereus	2	16.60
Escherichia coli	3	25.00
Klebsiella pneumoniae	3	25.00
Pseudomonas sp.	1	8.33
Bacillus subtilis	2	16.60
Staphylococcus sp.	3	25.00
Salmonella sp.	2	16.60

Table 4.8: Distribution of fungal isolates from locally made plant-based milk sold in selected markets in Benin City

ISOLATES	***Saccharomyces* sp.**	***Mucor* sp.**	***Penicillium* sp.**	***Aspergillus flavus***
SOYA MILK	+	-	+	+
TIGER NUT	-	+	-	-
KUNU	+	-	+	+
COCONUT MILK	-	-	-	+

Key

+= Present

- = Absent

Table 4.9 : The frequency of occurrence for fungi isolated from locally made plant-based milk samples

Isolates	No. of Occurrence	Percentage of Occurrence (%)
Saccharomyces sp.	2	16.60
Mucor sp.	1	8.33
Penicillium sp.	2	16.60
Aspergillus flavus	3	25.00

Table 4.10a: Antibiotic sensitivity pattern of Gram-positive bacterial isolates in plant-based milk samples

ISOLATES	CEF	CN	AMX	S	AZM	RD	CPX	CTZ	LEV	E	**MAR Index**
Staphylococcus sp.	I	S	S	S	S	R	S	S	S	S	0.10
Bacillus cereus	S	S	S	S	S	S	S	S	S	S	0.00
Bacillus subtilis	S	I	I	R	I	S	S	R	S	S	0.20

KEY: Resistance (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) = 17mm and above, **RD** = Rifampicin, **LEV=**Levofloxacin, **S** = Streptomycin, **AZM** = Azithromycin, **AMX** = Amoxicillin, **CPX** = Ciprofloxacin, **E** = Erythromycin, **CTZ=**Ceftazidime, **CN** = Cephalexin, **CEF** = Ceftriaxone

Table 4.10b : Antibiotic susceptibility pattern of Gram-negative bacterial isolates in plant-based milk samples

ISOLATES	OFX	AUG	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF	**MAR Index**
Pseudomonas sp.	S	R	R	R	S	S	I	R	S	S	0.40
Salmonella sp.	S	S	S	R	S	S	S	R	S	S	0.20
Klebsiella pneumoniae	S	S	S	R	S	S	R	R	S	S	0.30
Escherichia coli	S	R	I	R	S	S	S	R	S	S	0.30

KEY: Resistance (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive (S) = 17mm and above, **OFX** = Ofloxacin, **AUG** = Amoxicillin-Clavulanate (Augmentin), **PEF** = Pefloxacin, **CTZ** = Ceftazidime, **CN** = Cephalexin, **CPX** = Ciprofloxacin, **CEP** = Cephradine, **TRX** = Trimethoprim-Sulfamethoxazole, **S** = Streptomycin, **CEF** = Ceftriaxone

Table 4.11: pH values of plant-based milk samples sold in selected markets in Benin City

SAMPLES	SOYA MILK	TIGERNUT MILK	KUNU	COCONUT MILK
OLUKU	4.30±0.14	4.50±0.00	4.40±0.28	4.10±0.28
USELU	5.40±0.21	4.60±0.14	3.50±0.07	3.90±0.00
OBA	4.10±0.14	3.80±0.07	5.90±0.14	4.20±0.07

CHAPTER FIVE

5.1 Discussion

Plant based drinks are becoming more popular across the world as a substitute to the conventional dairy beverages. This demand increase is, in great part, caused by the increase in the popularity of vegan, lactose-free, and dairy-free products (Sharma *et al*., 2024). Based on a vast range of plants, such as soybeans, nuts, seeds, and grains, these beverages are commonly appreciated due to their supposed health value, including reduced fat levels, lactose-free nature, and increased levels of vitamins and minerals (Popova *et al*., 2023). Plant-based beverages, including soya milk, tiger nut milk, kunu, and coconut milk, are widely produced and consumed in places such as Benin City, Nigeria, and are usually made in traditional ways. Although their consumption continues to rise, there has been the concern of the safety and microbial quality of these beverages (Giugliano *et al*., 2023). Since the majority of such beverages are locally manufactured in small-scale facilities, they can be contaminated at different production stages, such as raw material processing, processing, and storage (Mishra *et al*., 2024). This research, thus, set out to determine the quality of microbes in plant-based milk drinks that are widely used in Benin City.

The overall heterotrophic bacteria count of the plant-based milk samples was between 1.90+-0.53 (soya milk) -82.40+-6.90x 105cfu/mL (coconut milk). These results indicate that there is a high level of microbial contamination, which may be a result of poor hygiene in the course of production and storage. Other works of similar nature have also indicated high microbial loads in plant-based milk products. John *et al*. (2023) in Calabar Metropolis have determined the heterotrophic bacterial count as high as 5.30 x 105 cfu/mL in soymilk samples, which is an indication of low hygienic standards in production processes. Having a high level of bacteria, particularly in some samples of coconut milk and kunu, suggests possible failures in the hygiene and handling of the product, which may result in the development of microorganisms that cause food spoilage. The maximum number of bacteria in the case of coconut milk could be explained by the high level of moisture and the nutrient-rich composition of this product that provide the optimal conditions to be in favor of the growth of microbes (Adelekan *et al*., 2019). Also, the extraction process is labor-intensive and requires a lot of manual handling, use of non-sterile equipment and exposure to environmental contaminants, which complicates the risk of contamination. Coconut milk is also very perishable because lack of proper temperature during storage and transportation may enhance the growth of bacteria (Nevin and Rajamohan, 2024). The result indicates that the risk of foodborne illnesses may be higher due to improper hygiene practices during the production since some of the bacteria species related to high total heterotrophic bacterial counts are also known to be pathogenic (Musa and Hamza, 2013).

The *Salmonella* species in the plant-based milk drinks is also an important discovery, with these bacteria being the cause of foodborne diseases including salmonellosis and shigellosis (Bartula *et al*., 2023). There were 1.10+-0.48 x 105 *Salmonella*-Shigella in Tiger nut milk and too numerous to count in coconut milk. The most loaded samples in this study were kunu and coconut milk and this observation is comparable to the results of Mbachu *et al*. (2014) because the counts of microbial loads in the samples stored in kunu were found to be between 3.10x105 cfu/mL to TNC. The fact that these bacteria are present implies the possibility of fecal contamination, which could be caused by contaminated water or unsanitary handling methods (Anumudu and Anumudu, 2019). *Salmonella* infection may cause serious gastrointestinal problems, particularly in the most vulnerable groups of patients, including children, the elderly, and immunocompromised patients (Feasey, 2013). Their coexistence is a great danger to human health since the two bacteria are known pathogens that cause foodborne outbreaks.

Another issue that concerns microbial quality of plant-based milk drinks is fungal contamination, especially molds and yeasts. Fungal counts 2.40+-0.24 (soya milk) -44.0+-0.00x 105 cfu/mL (tiger nut milk). The fungi used in this research were Mucor sp., *Penicillium* sp., *Aspergillus flavus* and Saccharomyces sp., some of which are known to produce mycotoxins (aflatoxins and ochratoxins) that can be very harmful to human health (Pouris *et al*., 2024). The existence of these fungi indicates poor management of the environmental conditions such as temperature and humidity during production and storage (Etang *et al*., 2017). The increased Aspergillus and *Penicillium* species leads to concerns regarding the possible contamination of mycotoxins, and their long-term effects may be liver damage and cancer (Awuchi *et al*., 2022).

The microbial diversity of the studied plant-based beverages is presented in Tables 4.4 and 4.7. Bacterial (*Staphylococcus* sp., *Escherichia coli*, Bacillus cereus, *Salmonella* sp., Bacillus subtilis, Pseudomonas sp., Klebsiella pneumoniae) and fungal (Aspergillus sp., *Penicillium* sp., Mucor sp. and Saccharomyces sp.) microbial isolates were used. The large microbial diversity indicated that most of the microbes in the samples were also similar to those that are usually present in plant beverages and other food items with a comparable composition (Pehowich *et al*., 2000). The potential contamination sources are contaminated water to be used in processing, feedstock (whole cereals used to make kunu), and packaging materials such as empty plastic bottles that could be collected in different unsanitary places (Wong *et al*., 2002). The microbial diversity found in this study is in line with other studies on plant based drinks. Isolates reported by Ayo *et al*. (2004) included, Enterobacter, Shigella, *Escherichia coli*, Klebsiella, Micrococcus, Proteus, Leuconostoc, Bacillus, Citrobacter, and *Staphylococcus* in kunu-zaki sold in a poly-technic community in Nigeria. On the same note, Pseudomonas, Bacillus, Klebsiella, Candida albicans and Aspergillus were detected in soy milk in Calabar metropolis by Asuquo and Antai (2017).

The frequency of occurrence of the bacterial isolates used show that *Escherichia coli*, *Staphylococcus* sp. and Klebsiella pneumoniae were the most common bacteria that were isolated in the samples with frequencies of 25.00 each. Klebsiella pneumoniae contamination is normally caused by water and misuse of raw materials in processing. These bacteria are also present in ill-cleaned equipments and they are associated with foodborne diseases and food spoilage. Even though the majority of *E. coli* strains are not harmful, some of the strains like *E. coli* O15:H7 can lead to serious gastrointestinal diseases (Bielaszewska *et al*., 2014). Certain strains of *Staphylococcus* sp. may cause enterotoxin that may cause food poisoning. Human handlings introduce these bacteria species to plant-based milks and are located in localized contamination zones like improperly washed equipments and packaging (Deziderio *et al*., 2023).

Asppergillus flavus was the most common species found in all the samples in this study and had a frequency of 25. The prevalence of this fungus was high which showed that contamination could have been in the course of storage or raw materials. These fungi are also known to produce aflatoxins that are harmful (Adegoke and Sakura, 1994). Contamination of commercially available plant-based milk is uncommon because of its quality control. The risks are higher in small-scale or home-made production in case the raw materials are not screened properly (Pouris *et al*., 2024).

Ntukidem *et al*. (2020) found 9 bacterial isolates such as Citrobacter sp., Bacillus cereus, Micrococcus sp., Kiebsiella sp., Shigella sp., *Escherichia coli*, Pseudomonas sp., *Staphylococcus* aureus and Lactobacillus sp. and six fungal species such as Rhizopus oryzae, Aspergillus niger, *Penicillium* sp., Saccharomyces cerevisiae, Asper In a study conducted by Ebisintei and Salim (2024), Pseudomonas sp., Bacillus sp. and *Staphylococcus* species were detected in coconut milk.

Patterns of antibiotic resistance of microbial isolates also highlight the possible health hazards of contaminated plant-based milk beverages. The Pseudomonas sp. was resistant to various antibiotics, such as Augmentin and Pefloxacin and the multiple antibiotic resistance (MAR) index was 0.40. This implies that these isolates have a high antimicrobial resistance hence may restrict the treatment of infections caused by such bacteria. Bacillus cereus, on the other hand, was sensitive to all the antibiotics tested, which implies that the resistance pattern among the various microbial species varies. The resistance manifested in *Staphylococcus* and *Salmonella* species also increases the concerns of the possibility of multidrug-resistant infections (Doyle, 2015). These results highlight the necessity to pay close attention to the use of antibiotics in food production and underline the significance of the idea that antimicrobial agents should not be abused in the agricultural industry.

5.1. Conclusion

The microbial profile of plant-based milk drinks in Benin City showed that there was alarming contamination with the presence of a number of pathogenic microorganisms including *Escherichia coli*, *Salmonella* sp., *Staphylococcus* sp., and fungi including *Penicillium* sp. and *Aspergillus flavus*. The results indicate the necessity of a better approach to hygiene, a higher quality of production, and a tighter control of regulation to minimize the threat of food poisoning. Also, the existence of antibiotic-resistant bacteria demands a higher level of control of antibiotic use in agriculture and food production to avoid the transmission of resistant strains. These findings form an excellent basis on how to implement the public health strategies to enhance the safety of the plant-based milk beverages and other locally made drinks in Benin City and other places. The producers, regulators, and consumers should be sensitized about the dangers of consuming these drinks and actively involved in reducing contamination and ensuring the safety of the population.

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