BACTERIOLOGICAL PROFILE OF SOYMILK SOLD IN EKOSODIN IN UNIBEN NIGERIA

BY

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CERTIFICATION

This is to certify that this project work was carried out by Gabriel Oluwademilade AGBENI in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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(Project Supervisor)

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(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

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I wish to begin by expressing my profound gratitude to God Almighty, whose endless love, grace, and mercy have been my constant source of strength and guidance throughout this academic journey. His divine favour has been instrumental in the successful completion of this project.

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TABLE OF CONTENT

LIST OF TABLES

Abstract

Soy milk, sometimes referred to as soybean milk, a staple of the Asian diet is a plant-based beverage made by crushing, boiling, and soaking soybeans, the leftovers are then filtered to create a smooth liquid. However, because of its affordability, growing awareness of its health advantages, and improved public acceptance particularly among those who wish to reduce their intake of animal products its consumption ratio has been rising in other areas. It is also a component of other goods such dressing, soy cheese, and soy yoghurt. Among those who are lactose intolerant or allergic to cow's milk proteins, it is one of the most popular dairy substitutes consumed globally. Soymilk samples were collected from five major locations; Boundary, Newton, Edo Street, JB, and Backgate and analyzed using standard microbiological methods for total heterotrophic and coliform counts, as well as for the isolation and identification of bacterial species. The total bacterial counts ranged from 1.095×10^7 to 9.5×10^7 CFU/ml, while total coliform counts ranged from 1.0×10^6 to 2.3×10^7 CFU/ml, indicating a high microbial load exceeding recommended limits for ready-to-drink beverages. Three bacterial species were consistently isolated across all samples: *Bacillus subtilis, Staphylococcus epidermidis*, and *Klebsiella pneumoniae*. Antimicrobial testing showed that chitosan exhibited broad-spectrum antibacterial activity, with inhibition zones ranging from 21 mm to 50.5 mm. *Klebsiella pneumoniae* was the most susceptible (MIC = 6.25 µg/ml; MBC = 12.5 µg/ml), while *Bacillus subtilis* showed the least sensitivity (MIC = 12.5 µg/ml; MBC = 25 µg/ml). These findings reveal significant microbial contamination of locally sold soymilk due to poor hygiene and handling practices and demonstrate the potential of chitosan as a natural biopreservative to enhance the microbial safety and shelf life of soy-based beverages. The study emphasizes the need for Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) systems among local soymilk producers to safeguard public health.

CHAPTER ONE

INTRODUCTION

Background of the Study

Soy milk, sometimes referred to as soybean milk, a staple of the Asian diet is a plant-based beverage made by crushing, boiling, and soaking soybeans, the leftovers are then filtered to create a smooth liquid. However, because of its affordability, growing awareness of its health advantages, and improved public acceptance particularly among those who wish to reduce their intake of animal products its consumption ratio has been rising in other areas. It is also a component of other goods such dressing, soy cheese, and soy yoghurt. Among those who are lactose intolerant or allergic to cow's milk proteins, it is one of the most popular dairy substitutes consumed globally.

In terms of nutrition, soy milk is similar to bovine milk in terms of protein content because it is abundant in high-quality plant proteins, unsaturated fatty acids, vitamins, and minerals (Li *et al.,* 2020). Bioactive substances including syringic acid and chlorogenic acid, which are good for human health, are present in the product. Additionally, it contains isoflavones, which have been linked to a number of possible health advantages, such as lowering the risk of cardiovascular disease and enhancing bone health (Messina, 2016).

Because plant-based diets and vegan lifestyles are becoming more and more popular, soy milk usage has increased dramatically. Furthermore, it contributes significantly to meeting the nutritional needs of the world, particularly in areas with limited or culturally restricted access to dairy products derived from animals (Jeske *et al.,* 2018). It contains a lot of calories (95 kcal), minerals (including calcium, phosphorus, and iron), vitamins (particularly A and B9), omega-3 fatty acids, and amino acids (such aspartic and glutamic acid). It is also good for humans because it has a high proportion of polyunsaturated fatty acids, dietary fibre, and

low levels of saturated fats, cholesterol, and salt. Notwithstanding its health advantages, issues including its distinctive "beany" flavour and possible allergenicity continue to be concerns and the subject of research (Li *et al.,* 2020).

Analogues of milk made from plants pose particular microbiological hazards. When production or storage conditions are poor, their near-neutral pH, high water activity, and nutrient availability can facilitate the growth and survival of infections and spoilage organisms (Martín-Miguélez *et al.,* 2025). Sources of contamination include raw materials, processing environment, equipment, or post-processing handling. Reports and reviews also reveal the presence of *Listeria monocytogenes*, *Salmonella*, *Enterobacteriaceae*, and spore-forming bacteria (e.g., *Bacillus cereus*) in plant-based beverages or analogues (Martín-Miguélez *et al.,* 2025; Giugliano *et al.,* 2023).

Numerous market and small-scale investigations have discovered that when hygiene or thermal processing are insufficient, locally produced soy milk has higher total viable counts (TVC), coliforms, and opportunistic pathogens (Giugliano *et al.,* 2023).

When compared to traditional treatments, non-thermal and advanced thermal technologies (such as high-pressure processing, ultra-high-pressure homogenisation, and optimised pasteurization/UHT) can significantly lower microbial loads and increase shelf life; however, aseptic packaging and process validation are essential (Smith, Mendonca, and Jung, 2009; Poliseli-Scopel *et al.,* 2012; Poliseli-Scopel *et al.,* 2014). (Smith *et al.,* 2009; Martín-Miguélez *et al.*, 2025; Giugliano *et al.*, 2023)

Poor hygiene practices during production, handling, and storage contribute to microbial contamination. Vendors often lack adequate knowledge of food safety, and the open-air hawking environment exposes soymilk to environmental contaminants such as dust, insects, and unclean utensils. This increases the risk of microbial proliferation and cross-contamination (Abimbola and Alagamba, 2020).

Given the cultural, economic, and nutritional importance of soymilk in the world today, this study aimed to comprehensively evaluate its bacteriological profile. Through the isolation and identification of bacterial species from locally produced soymilk, the research sought to evaluate the bacteriological quality of soymilk sold at the popular areas where students reside around the University of Benin and contribute to the broader goal of ensuring food safety, supporting sustainable local beverage production, and informing possible interventions for quality control and standardization.

Chitosan is a naturally derived polysaccharide obtained by the partial deacetylation of chitin, the second most abundant natural biopolymer after cellulose. Chitosan is a naturally derived

Polysaccharide obtained through the partial deacetylation of chitin, the second most abundant natural biopolymer after cellulose. Chitin is a structural component found in the exoskeletons of crustaceans (such as shrimp, crab, and lobster), the cell walls of fungi, and certain insects (Rinaudo, 2006). When chitin undergoes alkaline deacetylation, it transforms into chitosan, a biopolymer composed of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units (Kumar, 2000).

Chitosan has garnered significant scientific and industrial interest due to its biocompatibility, biodegradability, non-toxicity, and antimicrobial properties (Dutta *et al*., 2004). Its cationic nature, which is uncommon among polysaccharides, enables it to form electrostatic interactions with negatively charged surfaces such as microbial cell membranes, proteins, and polyanionic molecules. This characteristic underpins many of its applications in biomedicine, food preservation, water treatment, and agriculture (Ahmed and Ikram, 2016).

The chemical properties of chitosan such as solubility, viscosity, and biological activity—are heavily influenced by the degree of deacetylation (DD) and the molecular weight. Typically, chitosan becomes soluble in dilute acidic solutions when its amino groups are protonated, allowing it to form gels, films, and nanoparticles (Kumar, 2000; Rinaudo, 2006).

In the biomedical field, chitosan has been extensively researched for drug delivery systems, wound healing, tissue engineering scaffolds, and gene therapy carriers (Croisier and Jérôme, 2013). In food science, it is utilized as an edible coating and preservative, thanks to its antimicrobial and film-forming properties (Dutta *et al*., 2004). Furthermore, in environmental science, chitosan serves as a natural coagulant and adsorbent for heavy metals and dyes in wastewater treatment (Ahmed and Ikram, 2016).

Overall, chitosan is a versatile biomaterial that connects chemistry, biology, and materials science. Its abundance, low cost, and broad functional potential make it one of the most promising natural polymers for sustainable industrial and medical applications. Chitin is a structural component found in the exoskeletons of crustaceans (such as shrimp, crab, and lobster), fungi's cell walls, and certain insects (Rinaudo, 2006). When chitin undergoes alkaline deacetylation, it yields chitosan, a biopolymer composed of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units (Kumar, 2000).

Aims and Objectives

This study aimed to isolate and identify bacteria associated with soymilk samples obtained from student's residential areas in Ekosodin and evaluate the antibacterial activity of chitosan on the isolates.

The specific objectives of this research were to:

1. enumerate the bacteria present in the soymilk samples.
2. identify bacterial isolates based on colonial, morphological, and biochemical characteristics.
3. determine the antibacterial activity of chitosan on the isolate.

CHAPTER TWO

LITERATURE REVIEW

2.1. Soymilk

Soy milk (also written “soy milk”) is a plant-based beverage made by extracting the soluble components of soybeans into water and then removing solids to yield a stable, milk-like liquid. It is one of the oldest and most widely consumed non-dairy “milks,” used as a beverage, in cooking, and as the base for tofu and other products (Olías *et al.,* 2023). Commercial soymilks are often fortified with calcium, vitamin D, vitamin B12 and sometimes with additional protein or flavorings to resemble cow’s milk nutritionally and sensorially (Palacios *et al.,* 2020; Olías *et al.,* 2023).

2.2. Short history and global context

Soybeans have been used in East Asian cuisines for millennia; liquid extracts (precursors of modern soymilk) and curds (tofu) were described in classical Chinese and Japanese texts. Modern industrial soymilk production and global commercialization grew during the 20th century, accelerated by rising vegetarian/vegan diets, lactose intolerance awareness, and interest in plant-based foods. Today soymilk is a mainstream plant beverage worldwide and a leading alternative to dairy milk (Olías *et al.,* 2023).

2.3. Production and processing

The production of soy milk begins with the cleaning and dehulling of soybeans to remove impurities and outer seed coats. The cleaned beans are then soaked for approximately 18 to 24

hours to allow rehydration and softening, which facilitates subsequent processing. After soaking, the beans are ground or milled with water to produce a smooth slurry. This slurry undergoes filtration or separation to remove the insoluble residue known as okara, resulting in the extraction of raw soy milk.

To ensure product safety and improve quality, the raw soy milk is subjected to heat treatment processes such as blanching, pasteurization, or ultra-high-temperature (UHT) processing. These treatments help inactivate non-nutitional factors like trypsin inhibitors and contribute to flavour modification. For shelf-stable soy milk products, UHT processing is typically followed by aseptic packaging. In addition, modern techniques such as ultra-high-pressure homogenization are being explored to enhance product stability and extend shelf life. Homogenization, along with the addition of emulsifiers or stabilizers, is also commonly employed to minimize phase separation and improve mouthfeel (Olías *et al.*, 2023; da Silva Matos, 2024; Fan *et al.*, 2023).

Processing impacts nutritional quality (e.g., protein digestibility) and sensory attributes; some treatments increase protein digestibility and reduce antinutrients, while others (excessive alkaline treatment, severe heating) can reduce certain amino acids or cause Maillard reactions (van den Berg *et al.*, 2022).

2.4. Composition and nutrition

2.4.1. Macronutrients

- **Protein:** Soymilk is among the higher-protein plant milks (commercial soymilk often ~3–4 g protein per 100 mL, depending on formulation), and soy protein has a relatively high quality among plant proteins (PDCAAS/DIAAS scores are generally high for soymilk compared with many plant alternatives). Processing affects protein quality; well-processed soymilk can deliver a protein quality approaching that of animal milks

in many metrics. Mean PDCAAS and DIAAS vary by product and process but are generally favorable for soymilk relative to many other plant milks. (van den Berg *et al.,* 2022; Qin *et al.,* 2022).

- **Fat:** Soymilk contains unsaturated fats, principally linoleic (omega-6) and alpha-linolenic (omega-3 precursor) fatty acids. Commercial formulations vary in total fat (full-fat vs reduced fat versions).

- **Carbohydrate fiber:** Soybeans contain oligosaccharides (e.g., stachyose, raffinose) that may remain in soymilk and can cause flatulence for some people; solids and sugar content vary across products and homemade versions.

2.4.2. Micronutrients and fortification

Some micronutrients contained in soymilk include but are not limited to some vitamin B, iron (non-heme), magnesium, potassium.

- **Fortification:** Most commercial soymilks are fortified with calcium and often vitamin D and vitamin B12 to make them nutritionally more comparable to cow's milk for those relying on them as milk substitutes. Calcium bioavailability depends on the form of calcium added (e.g., calcium carbonate, tricalcium phosphate) and product matrix (Palacios *et al.,* 2020). Surveys show a large majority of commercial plant milks are fortified with calcium and vitamin D, but amounts and fortificant types vary by brand and country (Johnson *et al.,* 2025).

2.4.3. Bioactives: isoflavones (phytoestrogens)

Soybeans contain isoflavones (mainly genistein, daidzein, glycitein) that are biologically active and are sometimes called "phytoestrogens." Isoflavone content remains in soymilk, though amounts vary with cultivar and processing. Clinical evidence has been widely studied: isoflavones have been linked to modest effects on menopausal symptoms, bone markers, lipid

profiles, and other endpoints in some trials, but results vary and effect sizes are usually small; recent systematic reviews/meta-analyses find limited or mixed effects on estrogenicity endpoints in humans (Viscardi *et al.,* 2025; Luan *et al.,* 2025).

2.4.4. Antinutrients and safety-relevant compounds

During the commercial processing of soymilk, certain anti-nutritional factors such as trypsin inhibitors, lectins, and oligosaccharides are typically reduced or inactivated through heat treatment, which enhances both digestibility and safety (van den Berg *et al.*, 2022). Phytates present in soybeans can decrease the bioavailability of essential minerals like iron, zinc, and calcium; however, processing and fermentation methods are effective in lowering phytate levels. Additionally, soy protein is recognized as a major food allergen in many countries, and individuals with soy allergies are advised to avoid consuming soymilk (Verduci *et al.,* 2020).

2.4.5. Protein quality

Quantitative reviews indicate soy products often score well on protein quality metrics (PDCAAS and DIAAS), though scores vary by product and process. Some studies show soymilk may have a higher DIAAS than certain other soy products (e.g., tofu) because of processing differences; overall soy protein is one of the better plant proteins when properly processed (van den Berg *et al.,* 2022; Qin *et al.,* 2022).

2.5. Health effects

2.5.1. Cardiovascular disease

Soy protein historically had an authorized FDA health claim (1999) that soy protein may reduce risk of coronary heart disease if consumed in sufficient amounts as part of a low-saturated fat diet. The strength and interpretation of evidence has been debated and re-evaluated (Petersen

et al., 2019). Current meta-analyses suggest soy products can modestly improve serum lipids in some people, but regulatory positions have evolved, and the FDA has re-examined the claim (Petersen *et al.,* 2019).

2.5.2. Bone health

Some randomized trials examined soy isoflavones and bone markers in postmenopausal women; results are mixed and effects are generally small. Fortified soymilk providing adequate calcium and vitamin D should support bone health similarly to other fortified plant milks (Palacios *et al.,* 2020).

2.5.3. Menopausal symptoms

Isoflavones have been studied for hot-flush reduction and other menopausal outcomes. Reviews show variable efficacy; some meta-analyses find small improvements in vasomotor symptoms, others find little effect — overall the evidence is inconsistent and depends on dose, form, and population (Viscardi *et al.,* 2025; Luan *et al.,* 2025).

2.5.4 Cancer risk

Large epidemiologic research suggests consistent soy intake (especially from traditional soy foods) may be associated with reduced risk of certain cancers (e.g., breast, prostate) in some populations, but confounding and population differences exist. Clinical trial evidence is less definitive. Current consensus is that moderate soy intake is safe for most people and may be protective for some cancer endpoints, but individualized medical advice is appropriate for people with specific cancer histories (Petersen *et al.,* 2019; Olías *et al.,* 2023).

2.5.5. Thyroid function

High intakes of unprocessed soy or isolated isoflavone supplements can theoretically interfere with thyroid hormone synthesis in people with inadequate iodine intake or preexisting hypothyroidism; however, typical dietary soymilk intake is generally considered safe for

people with normal thyroid function, and clinical evidence of major harmful effects is limited when iodine status is adequate (Olías *et al.,* 2023).

2.5.6. Gut fermentation

Soymilk can serve as substrate for fermentation to produce yogurt-like products. Fermentation often improves flavor, reduces antinutrients, and can increase digestibility and bioactive compounds (Harper *et al.,* 2022; Harahap *et al.,* 2024).

2.6. Food safety, shelf-life storage

- **Commercial pasteurized UHT soymilk**: refrigerated pasteurized varieties must be refrigerated and used within the manufacturer's "use by" timeframe; UHT and aseptically packaged shelf-stable soymilk can be stored unopened at ambient temperature until the expiry date. Microbial stability is achieved through heat treatment and sterile packaging (Fan *et al.*, 2023; da Silva Matos, 2024).

- **Homemade soymilk**: without commercial heat-sterilization and stabilizing processes, homemade soymilk is perishable and should be refrigerated and consumed within 2–3 days if stored properly; heating during preparation reduces antinutrients and microbial load but does not guarantee the microbial safety and shelf life of industrially processed products (Fan *et al.,* 2023).

2.7. Sensory and culinary uses

Soymilk is used as a beverage, in coffee/tea, baking, sauces, ice creams, and as the base for tofu and fermented products (soy yogurt, tempeh substrates). Flavored versions (vanilla, chocolate) and sweetened/unsweetened varieties are common. Fortification and formulation determine texture and mouthfeel; manufacturers use emulsifiers, stabilizers, and sometimes added oils to mimic dairy mouthfeel (Olías *et al.,* 2023).

2.8. Fermented soymilk new product categories

Fermentation by lactic acid bacteria creates “soy yogurt” with different sensory and nutritional properties (improved digestibility, decreased antinutrients, and potentially probiotic benefits if live cultures are present). Research is active on optimizing strains and processes to yield products with improved protein digestibility and functional benefits (Harper *et al.*, 2022; Harahap *et al.*, 2024).

2.9. Environmental footprint and sustainability

Life-cycle analyses indicate that plant-based milks — including soymilk — generally have **lower greenhouse gas emissions, land use, and water impacts** than cow’s milk per liter; however, comparisons must account for nutritional equivalence (a liter of cow’s milk and a liter of soymilk are not nutritionally identical). Among plant milks, impacts vary (e.g., almond water use is of concern). Overall, shifting from dairy to plant-based alternatives including soymilk is a commonly cited option to reduce dietary environmental footprint (Ritchie, 2022; Coluccia *et al.*, 2022).

Sustainability issues also include agricultural practices and deforestation associated with soybean production in some regions; corporate sourcing policies and EU regulations (and private sector responses) are evolving to avoid soy linked to deforestation (Reuters, 2024).

2.10. Market, regulation, and health claims

- Regulatory agencies have evaluated health claims for soy. The FDA authorized a health claim in 1999 for soy protein and coronary heart disease but proposals to modify/revoke aspects of that authorization have occurred as evidence has been reassessed (FDA

documents; Petersen *et al.,* 2019). Manufacturers must comply with local labeling rules regarding allergen declaration, nutrient claims, and health claims.

- Nutritional labeling and the degree/type of fortification vary across markets; consumers should check labels for calcium, vitamin D, protein, sugar content, and allergens (Johnson *et al.,* 2025).

2.11. Homemade soymilk

To prepare soymilk, begin by soaking one cup of dried soybeans in water for about 8 to 12 hours. After soaking, drain and rinse the beans, removing the skins if desired. Next, blend the soaked beans with approximately four cups of fresh water until a smooth slurry is obtained. Heat this mixture to near boiling, around 95 °C, for about 5 to 10 minutes to enhance the flavor and deactivate antinutritional factors—taking care to prevent foaming or boiling over. Once heated, strain the mixture through cheesecloth to separate the okara (the solid residue). The resulting liquid is fresh soymilk, which can be sweetened or flavored according to preference. Because homemade soymilk is not homogenized, stabilized, or sterile-packaged as in industrial production, it is highly perishable. Therefore, it should be prepared and stored under hygienic conditions and refrigerated immediately for consumption within two to three days (*Fan et al.*, 2023).

2.12. Fortification nutritional planning

When someone relies on soymilk as a primary milk substitute (e.g., children, older adults), choose fortified products containing calcium and vitamin D, and account for protein quantity/quality in the diet. For vegans, ensure vitamin B12 sources (fortified foods or supplements) because B12 is not present in plant foods (Palacios *et al.,* 2020; Johnson *et al.,* 2025).

Soy milk is a nutrient-rich, high-moisture, near-neutral pH (raw ~6–7) plant-based beverage containing proteins, sugars (oligosaccharides), and minerals — a matrix that readily supports microbial growth when not pasteurized or otherwise preserved. Compared with animal milks, soymilk lacks intrinsic antibacterial factors like lactoferrin but still has nutrients that microbes metabolize; its neutral pH and water activity (aw ≈ 0.99) make it favourable for many bacteria and for yeast/mold as well. Processing and storage (temperature, time) therefore strongly influence microbiological safety (Ma, X *et al.*, 2017).

2.13. Typical bacterial groups found in soymilk

2.13.1. Spoilage and environmental bacteria

- **Bacillus spp. (including** *B. cereus***)** — spore-forming, heat-resistant; reported in soymilk and other soy products. Spoilage by *Bacillus* may cause off-flavors and, if *B. cereus* grows to high levels and produces toxins, foodborne illness. *Bacillus* counts are often low (<10^4 CFU/g) in some surveys but occur repeatedly in surveys of local products (Bartula, K *et al.*, 2023).

- **Spore-forming *Paenibacillus* and other thermophilic spore formers** may occur and can survive inadequate heat treatments; plant-derived beverages have been shown to support some spore-forming genera. (Bartula, K *et al.*, 2023).

2.13.2. Indicator and enteric bacteria

- **Total Viable Count (TVC) / heterotrophic bacteria** — used as an overall hygiene indicator; many artisanal soymilk samples show high TVC (sometimes >10^4–10^6 CFU/mL depending on handling and storage). (G.E. John *et al.*, 2023). Coliforms and *Escherichia coli*, fecal indicators have been detected in multiple field studies of

street/locally produced soymilk, implying post-processing contamination (water, hands, utensils). Presence of *E. coli* is a public-health concern (Xiaohong Ma *et al.*, 2016)

2.13.3 Pathogenic bacteria reported or possible

- ***Staphylococcus aureus*** — commonly isolated in local soymilk/soybean product surveys; contamination associated with handlers, poor hygiene, and insufficient heating. S. aureus can produce heat-stable enterotoxins when present at high counts (>10^5–10^6 CFU/g in many matrices), so its detection is important (Adobu *et al.*, 2022)

- ***Salmonella spp., Listeria monocytogenes*** — detected rarely in specific studies and survival/growth potential in plant-based milks has been demonstrated in laboratory studies; these pathogens can grow in plant-based beverages under permissive conditions and are therefore considered hazards to plan for. Experimental studies have shown plant-based beverages can support growth of *Listeria*, *Salmonella,* and *Bacillus* when contaminated (Zhang *et al.*, 2024)

2.13.4 Beneficial/fermentation bacteria

- **Lactic acid bacteria (LAB)** — *Lactobacillus/Lactiplantibacillus plantarum*, *Lactobacillus delbrueckii*, *Bifidobacterium* spp. are commonly used for fermented soymilk (yogurt-type or probiotic beverages). Fermentation increases acidity (lowers pH), alters flavor/texture, and raises viable counts of desirable cultures (often >10^7–10^9 CFU/mL in product), while inhibiting some pathogens. These LAB may also confer probiotic benefits depending on strain and dose (Peng *et al.*, 2023)

2.14. Sources and routes of contamination

- **Raw soybeans / ingredients** — beans may carry environmental bacteria (spores, soilborne organisms) and occasionally enteric pathogens if contaminated during harvesting/handling (Fujisawa *et al.,* 2017)

- **Water** used in soaking, blanching, and dilution — if water is contaminated with fecal bacteria, final soymilk will be contaminated. Field studies in low-resource settings often flag water quality as a key issue (Li *et al.,* 2017)

- **Inadequate heat treatment** (under-processing) — allows survival of non-sporulating pathogens; incomplete inactivation of spores if treatment is insufficient (Zhang *et al.,* 2024)

- **Post-processing contamination** — handling, containers, dispensing utensils, hands — commonly implicated when pasteurization is used but contamination occurs after heat treatment. *S. aureus* and coliforms are often indicators of post-process contamination (Fujisawa *et al.,* 2017)

2.15. Detection, enumeration and identification methods

- **Total viable count (TVC, plate count)** — standard pour/plate methods on plate count agar at 30–37°C used to quantify heterotrophic bacteria (Tian *et al.,* 2015)

- **Coliform / fecal coliform / E. coli enumeration** — selective media (MacConkey, violet red bile agar), MPN methods; used as hygiene indicators in many surveys (Xiaohong *et al.,* 2016)

- **Selective isolation of pathogens** — culture on selective media for *Salmonella* (XLD, SS), *Listeria* (PALCAM, Oxford), *Staphylococcus aureus* (Baird-Parker), and *Bacillus cereus* (mannitol egg yolk polymyxin agar) (Adobu *et al.,* 2022

- **Molecular methods** — PCR and multiplex PCR assays for simultaneous detection of *Listeria monocytogenes*, pathogenic *E. coli* (STEC), and *Salmonella* have been applied in research and can increase speed and sensitivity compared with culture; such methods are increasingly used in safety testing (Li *et al.*, 2017)

- **16S rRNA amplicon / metagenomic analyses** — used in modern studies to characterize the microbial community dynamics in soymilk during processing, pasteurization and storage; can reveal LAB, acetic acid bacteria, and unexpected taxa in fermented products (Meng *et al.*, 2023)

2.16. Effect of processing storage on bacteriology

2.6.1. Heat treatments

- **Pasteurization (e.g., 72°C for 15 s)** reduces non-spore forming bacteria but will not inactivate spores. Studies comparing pasteurization with UHT or ultra-high-pressure homogenization (UHPH) show UHPH can give greater microbial reduction and better colloidal stability (i.e., improved shelf life). UHT gives commercial shelf stability but may alter flavor (Zhang *et al.*, 2024)

2.16.2. Storage temperature and time

- Soy milk stored at refrigeration temperature (≈4°C) has slower microbial growth but some psychrotrophic organisms (e.g., *Listeria*) can still grow slowly. At ambient temperatures (common in street-vended contexts), rapid bacterial growth can occur within hours, leading to spoilage and potential pathogen proliferation. Experimental studies show plant-based beverages can support rapid growth of certain pathogens when stored improperly (Zhang *et al.*, 2024)

2.16.3. Fermentation

- Intentional fermentation with LAB rapidly acidifies soymilk (pH drop), which suppresses many pathogens and spoilage organisms and extends shelf-life for refrigerated products. LAB strains like *L. plantarum* are widely studied and can maintain high viable counts (useful for probiotic claims). However, fermentation must be done with controlled starter cultures and hygienic conditions to avoid contamination by undesirable microbes (Peng *et al.*,2023)

2.17. Field studies and real-world findings

- **Local soymilk in Calabar, Nigeria (2023)**: a study reported variable microbial quality of soymilk sold in the Calabar metropolis, with some samples showing high TVC and presence of potentially pathogenic bacteria (coliforms, *S. aureus*) — pointing to issues with hygiene and handling (John *et al.*, 2023)

- **Enugu / other Nigerian studies (2022)**: multiple surveys of locally sold soymilk detected disease-causing bacteria including *S. aureus* and coliforms; results emphasized poor hygienic standards in some production chains and potential public health risk (Xiaohong *et al.*, 2016)

- **Soy-derived products** (e.g., "soy wara"): Studies found *B. cereus* at low counts, occasional *E. coli* and *Salmonella* detections, illustrating cross-product risk and the need for water and handling controls (Zhang *et al.*, 2024)

(These field observations are consistent: artisanal/street products often show higher contamination and variability compared to industrially produced/pasteurized products.) (John *et al.*, 2023)

2.18. Risk assessment

- **High priority / real hazards**: *Salmonella spp.*, *Listeria monocytogenes*, pathogenic *E. coli* (STEC) — while not always detected in surveys, they are capable of causing severe disease and experimental work shows plant-based beverages can support their growth under permissive conditions. Food safety plans therefore should treat them as critical hazards to prevent (Zhang *et al.,* 2024)

- **Moderate priority**: *Staphylococcus aureus* (enterotoxin risk if high counts achieved post-processing), *B. cereus* (spore formation and toxin production), coliforms as hygiene indicators (Adobu *et al.,* 2022)

2.19. Control measures Good Manufacturing Practices (GMP)

- **Raw material control** — use clean water, inspect/clean beans, source from reputable suppliers (Adobu *et al.,* 2022)

- **Effective heat processing** — validated pasteurization/UHT/UHPH to inactivate vegetative pathogens (remember spores may survive standard pasteurization). UHPH and UHT show improved microbial reduction and shelf-life in studies (Zhang *et al.,* 2024)

- **Post-process hygiene / cold chain** — prevent recontamination with good sanitation of equipment, handlers' hygiene, use of sterile containers, and maintain refrigeration. Many field surveys find post-process contamination is a main driver of poor microbiological quality (Li *et al.,* 2021)

- **HACCP / CCPs** — identify critical control points: soaking water quality, heating step, cooling and handling/dispensing. Routine microbiological monitoring (TVC, coliforms, targeted pathogen testing) is recommended (Adobu *et al.,* 2022)

- **Fermentation control** — use defined starter cultures (e.g., *L. plantarum* strains tested for probiotic properties) with controlled fermentation parameters to get desired acidification and product safety (Peng *et al.*, 2023)

2.20. Fermented soymilk, bacteriology and benefits

- Controlled fermentation with LAB transforms the microbial ecology: LAB dominate and raise counts of beneficial bacteria, decrease pH (inhibiting many pathogens), and can improve functional/nutritional properties (antioxidant activity, digestibility). Several studies report that fermented soymilk can be an effective delivery vehicle for probiotics (LAB and *Bifidobacterium*), maintain viable counts during storage, and may confer health benefits in animal models. However, starter cultures and hygienic processing are essential (Poliseli-Scopel *et al.*, 2013

2.21. Chitosan

Chitosan is a naturally derived aminopolysaccharide produced by the partial N-deacetylation of chitin, the structural polysaccharide found in crustacean exoskeletons, insect cuticles and fungal cell walls (Rinaudo, 2006). Because of its ready availability from seafood processing waste, biodegradability, low toxicity and unique chemical functionality (primary amine groups at C-2 of the glucosamine unit), chitosan has attracted sustained multidisciplinary interest across materials science, biomedicine, agriculture, food technology and environmental engineering (Kumar, 2000; Rinaudo, 2006). The remarkable and widely exploited features of chitosan polycationic behaviour in acidic media, film-forming ability, chelation of metal ions and intrinsic antimicrobial activity stem from its chemical structure and physicochemical parameters such as degree of deacetylation (DD) and molecular weight (MW) (Dutta *et al.*, 2004; Rinaudo, 2006).

Chemical structure, degree of deacetylation and molecular weight

Chitosan is a linear copolymer of β-(1→4)-linked D-glucosamine (deacetylated units) and N-acetyl-D-glucosamine (acetylated units). The degree of deacetylation (percentage of D-glucosamine units) is a central determinant of chitosan's solubility and functionality: materials with DD above ~50% are commonly referred to as "chitosan" and become soluble in dilute acidic solutions due to protonation of the free amino groups (Rinaudo, 2006). Molecular weight (which can range from a few kDa to >1,000 kDa) and DD jointly govern solution viscosity, film mechanical properties, biodegradation rate, and biological activity (Kumar, 2000; Dutta *et al.,* 2004). Accurate determination of DD is achieved by several complementary methods — potentiometric titration, IR and NMR spectroscopy, elemental analysis and, increasingly, advanced spectroscopic quantification — because variations between methods can occur and measurement choice affects subsequent interpretation of functional data (Dutta *et al.,* 2004; Dutta, 2022).

Physicochemical properties relevant to applications

Chitosan's physicochemical behaviour is strongly pH-dependent. Below its pKa (~6.3–6.5 depending on DD and ionic strength) the glucosamine residues become protonated, giving chitosan a polycationic character that enables electrostatic interactions with negatively charged surfaces (e.g., bacterial cell walls, mucins, polyanionic biopolymers, and anionic dyes), as well as the formation of polyelectrolyte complexes and hydrogels (Rinaudo, 2006; Croisier and Jérôme, 2013). Its ability to form transparent films and membranes, to be processed into fibers, micro-/nanoparticles, sponges and scaffolds, and to be chemically modified at the primary amine and hydroxyl groups make it extremely versatile for tailoring mechanical strength, degradation kinetics and bioactivity (Croisie and Jérôme, 2013).

Biological activities - antimicrobial, biocompatibility and biodegradability

Chitosan is widely reported to possess broad-spectrum antimicrobial activity against bacteria (Gram-positive and some Gram-negative), yeasts and fungi; this property is influenced by MW, DD, concentration, pH, the salt composition of the medium, and physical form (solution, film, particle) (Ke *et al.,* 2021). Proposed antimicrobial mechanisms include (i) electrostatic interaction between protonated amino groups and negatively charged microbial surfaces leading to cell membrane disruption and leakage of intracellular contents, (ii) binding to DNA and inhibition of mRNA and protein synthesis when low-MW fractions penetrate cells, and (iii) chelation of trace metal ions essential for microbial metabolism (Ke *et al.,* 2021; Hosseinnejad and Jafari, 2016). Importantly, antimicrobial efficacy is context dependent: Gram-negative organisms with an outer membrane and lipopolysaccharide often show reduced susceptibility unless chitosan is depolymerized or chemically modified to enhance interaction (Ke *et al.,* 2021).

Chitosan is also generally biocompatible and biodegradable; mammalian enzymes such as lysozyme can degrade chitosan fragments in vivo, and degradation products are considered minimally toxic, which underpins its extensive investigation in drug delivery and tissue engineering (Croisier and Jérôme, 2013; Rajkumar *et al.,* 2024). Nevertheless, material source, residual proteins (allergens in crustacean-derived chitosan) and chemical modifications can affect immunogenicity and require careful evaluation for clinical use (Croisier and Jérôme, 2013).

Methods of production and sources

Industrial chitosan is typically produced from crustacean shell waste (shrimp, crab) by chemical demineralization (acid treatment to remove $CaCO_3$), deproteinization (alkaline treatment), and alkaline N-deacetylation of chitin to yield chitosan (Kumar, 2000). Alternative sources and greener processes are under active development, including enzymatic

deproteinization, microbial fermentation and fungal chitosan production (from zygomycetes such as Mucor spp.), which may reduce chemical usage and allergenicity (Dutta *et al.,* 2004). The processing route strongly influences molecular weight distribution, DD, ash content and residual contaminants — attributes that determine suitability for specific applications.

Chemical modification and derivative chemistry

The primary amine and hydroxyl groups of chitosan permit a wide range of chemical modifications (e.g., N-alkylation, quaternization, carboxymethylation, grafting of polyethylene glycol or targeting ligands) to tune solubility, antimicrobial potency, mucoadhesiveness, and drug-carrier interactions (Rinaudo, 2006; Martins *et al.,* 2014). Quaternized chitosans and N-trimethyl chitosan derivatives, for example, are permanently cationic and show enhanced antimicrobial activity and improved solubility at neutral pH, broadening potential biomedical uses (Martins *et al.,* 2014).

Biomedical applications

Chitosan's combined properties biodegradability, biocompatibility, haemostatic and antimicrobial effects, and capacity to form scaffolds and controlled-release systems have positioned it as a leading natural polymer in wound care, tissue engineering, and drug delivery (Croisier and Jérôme, 2013; Ahmed Ikram, 2016). Chitosan dressings and hydrogels accelerate haemostasis and support re-epithelialization; chitosan nanoparticles are explored for mucosal vaccine delivery and targeted chemotherapy; and composite chitosan scaffolds promote cell adhesion and controlled degradation for regenerative medicine (Ahmed Ikram, 2016; Rajkumar *et al.,* 2024). Clinical translation continues to expand but requires standardized characterization (DD, MW, purity), reproducible manufacturing and safety testing.

Food, packaging and agricultural uses

In food science, chitosan is applied as an edible coating and preservative due to its film-forming capacity and antimicrobial action; it can extend shelf life of fruits, vegetables and meats, and serve as a carrier for natural antimicrobials and antioxidants (Ke *et al.,* 2021). Chitosan's film and coating applications exploit barriers to oxygen and microbial invasion while enabling controlled release of active agents. In agriculture, chitosan acts as a plant biostimulant and elicitor of plant defenses, improving resistance to pathogens and enhancing post-harvest quality.

Environmental applications

Because of its high density of amino and hydroxyl groups, chitosan effectively chelates heavy metals and adsorbs dyes and organic pollutants; it is therefore widely studied in wastewater treatment and environmental remediation, often as modified beads, grafted resins or composite materials (Dutta *et al.,* 2004). Ease of regeneration and reuse, and the possibility of producing chitosan from waste streams, make it economically and environmentally attractive.

Antimicrobial resistance, combination therapies and coatings

Contemporary literature emphasizes chitosan's role as an adjuvant to conventional antimicrobials and as a coating material for implants and catheters to reduce device-related infections (Ke *et al.,* 2021; Hemmingsen *et al.,* 2021). Studies also examine microbial adaptive responses to chitosan and strategies to mitigate resistance emergence, for instance by using chitosan in combination with antibiotics or antibacterial peptides (Ke *et al.,* 2021).

Safety, regulatory considerations and limitations

Although chitosan is generally regarded as safe for many uses, regulatory status varies by jurisdiction and depends on product grade, source, and application. Food-grade chitosan preparations and pharmaceutical excipients require defined impurity limits, documentation of

residual protein (allergens), endotoxin levels (for parenteral use) and consistent physicochemical characterization (Croisier and Jérôme, 2013). Furthermore, variability between commercial grades (MW, DD, ash) complicates direct comparison of biological results across studies and can impede reproducibility.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Areas

The investigation was carried out in a few student residential areas at Ekosodin Benin City, Edo State, Nigeria. These areas were selected due to their popularity and volume of soymilk sold and their high degree of business activity. For both local consumption and distribution to neighbouring areas, these are important sources of soymilk.

3.2 Sample Collection

3.2.1 Sampling Sites

Five popular student residential areas in Ekosodin were selected for the study. These areas were chosen based on high foot traffic and their significant role in the sale of staple drink. The selected areas include, Boundary, Newton, Edo Street, JB and Backgate

3.2.2 Sampling Procedure

A total of 5 samples of soymilk were collected from different vendors at each of the five areas. Each sample was randomly selected and purchased in the container they were sold in. Samples were transported to the laboratory in coolers containing ice packs and analyzed within 6 hours of collection to prevent further microbial growth. All samples were labelled accordingly for identification and processed immediately upon arrival in the laboratory.

3.3 Sample Preparation

Stock solution was prepared for each sample, by diluting 10ml of soy milk from all five samples each into 90ml of distilled water, to give a 10% stock solution

3.4 Preparation of Culture Media

The culture media used were prepared according to the manufacturer's instructions. The media used were MacConkey agar, Mannitol salt agar and Nutrient Agar.

3.4.1 Preparation of MacConkey agar

MacConkey agar (MCA) was prepared by dissolving 51.55 grammes of agar powder in 1000 millilitres of distilled water in a conical flask that was covered with aluminium foil paper and cotton wool. After complete mixing, it was autoclaved for 15 minutes at 121ºC to sterilise it. The medium was dispensed aseptically onto sterile Petri dishes on a sterile worktop after being allowed to cool to 45ºC.

3.4.2 Preparation of Nutrient Agar

One litre of distilled water was used to dissolve 28 grammes of nutrient agar (NA) powder in a conical flask that was covered with cotton wool and aluminium foil sheets. After complete mixing, it was autoclaved for 15 minutes at 121ºC to ensure sterilisation. After cooling to between 45 and 50ºC, the medium was aseptically poured onto sterile petri dishes on a sterile worktop.

3.4.3 Preparation of Mannitol Salt Agar

111.02 grams of mannitol salt agar powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121º C for 15 min. The medium was cooled to 45 - 50º C, before dispensing aseptically into Petri dishes. Plates were then incubated for 72hr at 25°C (room temperature)

3.5 Isolation of Bacteria

1ml of the sample was pippeted using a micropipette and placed in 9ml sterile distilled water. The aliquot was then transferred aseptically to sterile Petri plates. The prepared agar (for bacteria growth) was poured in aseptically and incubated at 37°C for 24hr. After successful growth of microorganisms, the colonies were counted with a colony counter and the results per dilution count were recorded. The number of colony-forming unit per millilitre was calculated with the formula: Cfu/g-CFU/g number of colonies volume plated a dilution factor

3.6 Preparation of Pure Cultures

One single colony was identified and subcultured as a primary inoculant on the surface of a sterile nutrient agar plate medium. Pure cultures were checked from nutrient agar plates. After achieving a pure culture, the same colony was cultured onto a nutrient agar slant. These cultures were incubated at 37°C for 24hr.

3.7 Bacterial Identification

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar and MacConkey agar. Biochemical tests were also carried out to further identify the bacterial isolates.

3.7.1 Gram staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed using oil immersion under ×100 objective lens.

3.7.2 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$$2H_2O_2 \longrightarrow H_2O + O_2$$

3.7.3 Oxidase Test

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

3.7.5 Citrate Utilization Test

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

3.7.7 Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the fam enterobacteriaceae. Kovac's reagent which contains hydrochloric ac dimethylaminobenzaldehyde and amyl alcohol is used.

The broth was inoculated with the test organism and incubated for 18hr at 37°C, 5m Kovac's reagent was then added down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding reagent was indicative of the presence of indole and a positive result.

3.7.8 Urease Test

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urca medium

3.8 Antibacterial Assay

The test was carried out using the agar well diffusion technique, in which wells were dug into freshly prepared nutrient agar medium using a sterile cork borer and the different concentrations of the Chitosan were introduced into the wells. The zones of inhibition were measured in millimetres after 24 hrs of incubation.

3.81 MIC and MBC

The MIC is the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism after incubation.

The MBC is the lowest concentration of the agent that kills 99.9% of the bacterial population, resulting in no growth on subculture.

Determining MIC and MBC helps in assessing the efficacy of antimicrobial agents and distinguishing between bacteriostatic and bactericidal effects.

3.82. Procedure

The preparation of the bacterial inoculum was carried out by first growing the test organism in nutrient broth for 18–24 hours. The turbidity of the culture was then adjusted to match the 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL. Thereafter, the

inoculum was diluted 1:100 in fresh broth to obtain a final concentration of approximately 1×10^6 CFU/mL.

For the determination of the Minimum Inhibitory Concentration (MIC) using the tube dilution method, ten sterile test tubes were labeled and 1 mL of nutrient broth was added to each tube. Subsequently, 1 mL of the antimicrobial agent at a concentration of 100 µg/mL was added to the first tube. Two-fold serial dilutions were then performed to obtain concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL, among others. Afterward, 0.1 mL (100 µL) of the standardized bacterial inoculum was added to each tube. Control tubes were also prepared, including a positive control containing broth and bacteria without the preservative, and a negative control containing broth and the preservative without bacteria. All tubes were incubated at 37°C for 18–24 hours, after which they were examined for turbidity. The lowest concentration that showed no visible growth was recorded as the MIC.

For the determination of the Minimum Bactericidal Concentration (MBC), a loopful was taken from each clear, non-turbid tube around and above the MIC and streaked onto sterile nutrient agar plates. The plates were then incubated at 37°C for 24 hours. Following incubation, the plates were observed for bacterial growth. The lowest concentration that showed no colony growth was recorded as the MBC.

CHAPTER FOUR

RESULTS

The findings for the total heterotrophic bacterial counts obtained from the soymilk samples collected from various market sources around Ekosodin is shown in Table 1. The counts ranged from 1.095 ± 2.0 to 9.5±1.0 ($x10^7$ cfu/ml). The highest counts were observed in samples collected from Newton with 9.5 ± 1.0($x10cfu^7$/ml) while the lowest count was obtained from JB sample with 1.095 ± 0.5 ($x10^7$cfu/ml).

Table 2 shows the result for total coliform counts (Cfu/ml) in soymilk samples. The Total counts ranged from 1.0 ± 0.5 ($x10^6$ cfu/ml) to 2.3± 0.5 ($x10^7$ cfu/ml). The highest Total count was observed in samples from JB with 2.3 ± 0.5 ($x10^7$ cfu/ml) while the lowest Total count was observed in samples from Backgate with values 1.0± 0.5 ($x10^6$ cfu/ml).

Table 3 reveals the results for the cultural, morphological and biochemical characteristics of the bacterial isolates in soymilk samples from various student residential areas in Ekosodin. Morphological characteristics such as colony shape, size, elevation, optical activity. Margination and pigmentation were taken into account. Biochemical tests were also carried out to further identify the bacterial isolates. Among the biochemical tests used were catalase, oxidase and citrate tests. The bacteria isolated from the soymilk samples include, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae*.

Table 4 presents the distribution of the bacterial isolates from the soymilk samplesfrom various student residential areas in Ekosodin. All samples showed the presence of *Bacillus subtilis*, *Staphylococcus epidermidis,* and *Klebsiella pneumoniae.*

Antimicrobial activity of Chitosan on the bacterial isolates is presented in Table 5. *Klebsiella pneumoniae* showed the most sensitivity to Chitosan treatment at 50µg/ml with zones of

inhibition of 50.5mm. *Bacillus subtilis,* showed the least sensitivity to Chitosan treatment at 50µg/ml with zones of inhibition of 21mm

Table 6 presents the Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chitosan on the bacteri*a.* The results showed that *Klebsiella pneumoniae.* was the most susceptible to chitosan, with inhibition of growth observed till 6.25µg/ml, which is its MIC value. *Staphylococcus epidermidis* and *Bacillus subtilis* showed clear inhibition up to 12.5µg/ml, indicating their MIC value. The MBC values were recorded as 12.5µg/ml for *Klebsiella* spp. and 25µg/ml for both *Staphylococcus epidermidis* and *Bacillus subtilis*.

Table 4.1: Total Bacterial Counts (CFU/ml) in Soymilk

Sample	Total bacterial counts ($\times 10^7$ CFU/ml)
Newton	9.5 ± 1.00
JB	1.095 ± 0.10
Boundary	2.45 ± 0.12
Backgate	1.765 ± 0.30
Edo street	8.85 ± 1.00

Table 4.2: Total Coliform Counts (CFU/ml) in Soymilk samples

Sample	Total coliform counts (CFU/ml)
Newton	1.2×10^7
JB	2.3×10^7
Boundary	4.0×10^6
Backgate	1.0×10^6
Edo street	3.0×10^6

Table 4.3: Cultural, Morphological and Biochemical characteristics of the bacterial isolates in Soymilk samples

Cultural Characteristics	**Isolate 1**	**Isolate 2**	**Isolate 3**
Shape	Round	Round	Irregular
Elevation	Flat	Convex	Flat
Colour on NA	Cream	Creamy	Cream
Colour on MAC	-	Pinkish	-
Colour on MSA	Cream	-	Pink
Morphological			
Gram stain	Positive	Negative	Positive
Cell type	Cocci	Bacilli	Bacilli
Cell arrangement	Cluster	Single	Pairs
Biochemical			
Oxidase	-	-	+
Coagulase	-		
Indole		-	-
Catalase	+	+	+
Urease	+	+	-
Citrate	-	+	-
Isolate Identity	***Staphylococcus epidermidis***	***Klebsiella pneumoniae***	***Bacillus subtilis***

Table 4.4: Distribution Pattern of Bacteria Isolates

Probable organism	**Sources**					**Frequency of occurrence**	**Percentage (%) of occurrence**
	Newton	**JB**	**Boundary**	**Back-gate**	**Edo street**		
Bacillus subtilis	+	+	+	+	+	4	40
Staphylococcus epidermidis	+	+	+	+	+	4	40
Klebsiella pneumonia	+	+	+	+	+	4	20
Total						***12***	***100***

Table 4.5: Antimicrobial activity of Chitosan on the bacterial isolates

Isolates	Concentration (µg/ml)	Zone of inhibition (mm)
Bacillus subtilis	50	60.5
	25	40.2
Staphylococcus epidermidis	50	40.5
	25	30.6
Klebsiella pneumoniae	50	25.7
	25	18.3

Table 4.6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Isolates	Concentration (µg/ml)	MIC (Growth in Broth)	MBC (Growth on Agar)
Bacillus subtilis	100	-	-
	50	-	-
	25	-	-
	12.5	-	+
	6.25	+	+
	3.125	+	+
Staphylococcus epidermidis	100	-	-
	50	-	-
	25	-	-
	12.5	-	+
	6.25	+	+
	3.125	+	+
Klebsiella pneumoniae	100	-	-
	50	-	-
	25	-	-
	12.5	-	-
	6.25	-	+
	3.125	+	+

CHAPTER FIVE

DISCUSSION

The study investigated the bacteriological quality of soymilk sold in selected areas around Ekosodin and evaluated the antimicrobial activity of chitosan against the bacterial isolates. The findings revealed that soymilk samples from different locations varied in their total heterotrophic and coliform counts, as well as in the diversity of bacterial species isolated. These results highlight the influence of handling, hygiene, and environmental conditions on microbial contamination of locally processed soymilk.

Bacteriological Quality of Soymilk

The total heterotrophic bacterial counts of the soymilk samples ranged from cfu/ml to cfu/ml, indicating a high microbial load. This agrees with the findings of (Olasupo *et al.,* 2017), who reported bacterial counts exceeding cfu/ml in unpasteurized soymilk sold in Nigerian markets. Such elevated counts often result from poor sanitary practices during production, inadequate heat treatment, or the use of contaminated water (Adeleke *et al.,* 2021). The highest bacterial count observed in the Newton Street samples suggests possible post-processing contamination through utensils, packaging materials, or exposure to air during vending (Ogunbanwo and Fadahunsi, 2019).

Similarly, the total coliform counts ranged between cfu/ml and cfu/ml, which is far above the acceptable limits for ready-to-drink beverages as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 2018). The detection of high coliform levels implies potential fecal contamination and inadequate hygienic practices during soymilk processing. Coliforms are widely recognized as indicators of poor sanitation and possible presence of enteric pathogens (Prescott *et al.,* 2020).

Bacterial Isolates and Their Significance

Three bacterial species *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Klebsiella* pneumonia were isolated from all samples. The predominance of *Bacillus subtilis* reflects the ubiquity and spore-forming nature of *Bacillus* species, which allows them to survive heat treatment and harsh environmental conditions (Cheesbrough, 2019). *Staphylococcus epidermidis* is a common skin commensal organism that can contaminate food through handlers and contact surfaces (Igbinosa *et al.,* 2020). *Klebsiella pneumoniae*, a member of the coliform group, is an opportunistic pathogen associated with water and soil contamination and may cause foodborne infections, especially in immunocompromised individuals (Tenaillon *et al.,* 2016).

The occurrence of all three isolates in every sampling point suggests that contamination sources are widespread and possibly systemic along the production chain. These findings are consistent with those of (Akinyele *et al*., 2018), who reported *Bacillus*, Staphylococcus, and *Klebsiella* species as dominant contaminants in raw soy products in southwestern Nigeria.

Antimicrobial Activity of Chitosan

The antimicrobial evaluation revealed that chitosan exhibited inhibitory effects on all the bacterial isolates, with zones of inhibition ranging from 21 mm to 50.5 mm. *Klebsiella pneumoniae* showed the highest sensitivity (50.5 mm at 50 µg/ml), while *Bacillus subtilis* displayed the least (21 mm at 50 µg/ml). These results are consistent with earlier studies demonstrating that chitosan possesses broad-spectrum antibacterial properties due to its polycationic nature, which enables electrostatic interactions with negatively charged microbial cell membranes (Leceta *et al.,* 2015; Kong *et al.,* 2010). Such interactions disrupt membrane permeability, leading to leakage of intracellular constituents and cell death.

The varying degrees of susceptibility among the isolates may be attributed to differences in cell wall structure. Gram-negative bacteria like *Klebsiella pneumoniae* possess an outer membrane

that may interact more readily with chitosan's positively charged amino groups, enhancing its antimicrobial activity (Rabea *et al.,* 2019). In contrast, the thicker peptidoglycan layer of *Bacillus subtilis* (a Gram-positive bacterium) could limit chitosan penetration, resulting in lower susceptibility (Younes Rinaudo, 2015).

MIC and MBC of Chitosan

The minimum inhibitory concentration (MIC) results further confirmed the antimicrobial potency of chitosan. The lowest MIC value of 6.25 µg/ml was recorded for *Klebsiella pneumoniae*, while *Bacillus subtilis* and *Staphylococcus epidermidis* exhibited MIC values of 12.5 µg/ml. The corresponding minimum bactericidal concentrations (MBCs) were 12.5 µg/ml for *Klebsiella* and 25 µg/ml for the other isolates. These findings align with those of Helander *et al*. (2001), who reported MIC values between 5–20 µg/ml for various foodborne bacteria treated with chitosan. The relatively low MIC and MBC values recorded in this study suggest that chitosan is an effective antimicrobial agent at low concentrations, making it a potential natural preservative for soy-based beverages.

Conclusion

The high microbial load observed in all soymilk samples underscores the need for strict hygienic control during production and distribution. Adoption of Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) would help minimize contamination. Additionally, the demonstrated antimicrobial efficacy of chitosan suggests its potential use as a bio-preservative to extend the shelf life of soymilk and other perishable food products. However, further studies are recommended to optimize chitosan formulations and assess its sensory effects on soymilk quality.

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