



Faculty of Veterinary Science

Master in Food Safety

**Assessment of Microbiological Safety of Grated Coconut Sold in Maputo
Markets**

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July 30, 2026

DECLARATION

I declare that my dissertation title, Assessment of microbiological safety of grated coconut sold in Maputo markets, has not been submitted to any institution for any degree or qualification before, nor has it been submitted to any university for any academic purpose. This dissertation is presented as part of the requirement for obtaining my master's degree in food safety at the Faculty of Veterinary Medicine of Eduardo Mondlane University.

Date...../...../.....

Signature _____

Naserah Fatmata Koroma

APPROVAL

This is to certify that the dissertation titled “Assessment of Microbiological Safety of Grated Coconut Sold in Maputo Markets” submitted by Naserah Fatmata Koroma with student ID 20235552 was prepared under my supervision. I hereby confirm that I have reviewed the work and approved it for submission.

I, Prof Belisario Tomé Moiane (DVM, Lic. Phil., PhD)

Department of Food Safety

Faculty of Veterinary Medicine

Signature

Date

DEDICATION

I dedicate this great achievement to my parents, Mr. Mario and Zainab Koroma, to my beloved daddy, Mr. Umoro Bundu, and Marian Bundu, who never let me down and always want me to pursue my academic potential. I appreciate their moral support, and I will forever be grateful to them. My lovely and caring Husband (Mr. Kamara), who inspired me to reach this far; my uncle, Mr. Edwin Conteh; my brothers Kalilu and Abdul Bundu; Professor Father Joe Turay; Mummy Verio, Mrs. Cecilia Barrie, Supervisors (Prof Belisário Moiane and Charmila Mussagy Sineque, Dr), Kadiatu Rosetta Koroma, Mary Maltilda Seasy, Aminata Abudul Kamara, Saccoh Marrah, Musa Milton Fornah, Memuna Sesay, and Sister Fatmata Kamara, for their unwavering support, and also to my uncles, Mr. Kanu, Mr. and Mrs. Davide Kanu. The Mansaray and Kamara Family, Friends, and Families of Mozambique, for their motivation, patience, inspiration, and understanding, without which I would not have successfully completed my dissertation.

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TABLE OF CONTENTS

DECLARATION	ii
ACKNOWLEDGEMENTS	iv
DEDICATION	iv
LIST OF APPENDICES	ix
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xii
ABSTRACT	xiii
RESUMO	xiv
1. INTRODUCTION	1
1.1 Motivation.....	1
1.2 Problem statement.....	3
1.3 Objectives	4
1.3.1 General objective	4
1.3.2 Specific objectives	4
1.3.3 Research questions.....	4
1.4 Contribution of the study	4
2. LITERATURE REVIEW	6
2.1 Grated coconut overview	6
2.2 Foodborne Pathogens.....	8
2.3 Foodborne diseases in sub-Saharan Africa	9
2. 3 Indicators of food safety	9
2.3.1 Mesophilic bacteria	9
2.3.2 Staphylococcus aureus	10
2.3.3 Escherichia coli	10
2.4 Common sources of contamination in grated coconut.....	11
2.5 Legal and Normative aspects related to Grated coconut	12
2.5.1 Grated coconut safety standards	12
2.5.2 Mozambique food safety regulations.....	12
2.6 Theoretical framework.....	14
3. MATERIALS AND METHODS	15

3.1 Study area.....	15
3.2 Study design and participants	16
3.2.1 Inclusion criteria	16
3.2.2 Exclusion criteria	16
3.2.3 Participant consent and safety	16
3.2.4 Sample size determination	16
3.3 Data collection tools	17
3.4 Sample collection and handling	17
3.5 Laboratory analysis of microbiological contaminants	17
3.5.1 Sample preparation	18
3.5.2 Staphylococcus aureus isolation and confirmation	18
3.5.3 Total mesophilic bacteria count (TMBC)	19
3.5.4 Escherichia coli isolation and enumeration	19
3.6.1 Analysis for Objective 1: verification of hygienic conditions	20
3.6.2 Analysis for Objective 2: Identification of Staphylococcus aureus and E coli on grated coconut and potential surfaces.	20
3.6.3 Analysis for objective 3: Quantification of microbial contaminants	20
3.6.4 Analysis for Objective 4: Classification Against Food Safety Standards	20
3.7 Ethical consideration.....	21
4.1 Socio-demographic profile of grated coconut vendors	22
4.2 Hygienic conditions of grated coconut in selected markets.....	22
4.2.1 Personal Hygiene Practices.....	22
4.2.2 Facility hygiene and sanitation conditions.....	23
4.2.3. Personal hygiene practices by market.....	24
4.2.4. Facility hygiene conditions by markets.	24
4.2.5. Overall hygiene compliance scores by markets.....	25
4.2.6. Overall Hygiene Compliance Categories by Markets	25
4.2.7 Prevalence of S. aureus by markets and sample type	26
4.2.8. Prevalence of E. coli in coconut and contact surface by markets	26
4.4. Quantification of TMBC and E. coli.....	27
4.4.1 Mesophilic Bacteria and E. coli Counts.....	27
4.4.2. E. coli count by market and sample type	28
4.5 Classification of contamination levels against food safety standards.....	29

5. DISCUSSION	30
5.1 Hygienic Conditions of Grated Coconut Vendors	30
5.3 Identification of <i>S. aureus</i> and <i>E. coli</i> on contaminated surfaces and grated coconut	31
5.2.1 Prevalence of <i>S. aureus</i>	31
5.2.2 Prevalence of <i>E. coli</i>	32
5.3 Indicators count.....	34
5.3.1 Total mesophilic bacteria count	34
5.3.2 <i>E. coli</i> counts.....	35
5.4 Classification of Microbial load against food safety standards	35
6. CONCLUSION, RECOMMENDATIONS, AND LIMITATIONS	38
6.1 Conclusions.....	38
6.2 Recommendations	38
6.2.1 Recommendations for future research	38
6.2.2 Recommendations for vendors	39
6.2.3 Recommendations for governments	39
6.3 Limitations	39
7.0 REFERENCES.....	41
8. APPENDICES	49
Appendix 8.....	56

LIST OF APPENDICES

Appendix 1: Checklist for food hygiene and safety	49
Appendix 2: Human-informed consent forms	51
Appendix 3: Personal Hygiene Practices by Market Yes Responses.....	52
Appendix 4: Facility hygiene and sanitation conditions of grated coconut vendors	53
Appendix 5: Facility Hygiene Conditions by Market Yes Responses	54
Appendix 6: Kruskal-Wallis Test Comparing Hygiene Compliance Scores Across Markets.....	55

LIST OF TABLES

Table 1: Socio-demographic characteristics of grated coconut vendors	21
Table 2: Hygiene compliance scores by market.....	24
Table 3: Distribution of Overall Hygiene Compliance Categories by Market	24
Table 4: Classification of Grated Coconut Samples by Market.....	29

LIST OF FIGURES

Figure 1: Showing coconuts (<i>Cocos nucifera</i> sp.), a, b, c, and d show the whole coconut fruit, e shows de-husked and broken coconut, and f, grated coconut	7
Figure 2: Study Area	15
Figure 3: Assessment of the personal hygiene practices	23
Figure 4: Facility hygiene practice by markets.....	23
Figure 5: Prevalence of <i>S.aureus</i> by market and sample type	26
Figure 6: Total Mesophilic Bacteria count in grated coconut and contaminated surfaces by market	27
Figure 7: <i>E. coli</i> count by market and sample type.....	28
Figure 8: Prevalence of <i>E. coli</i> on coconut and contaminated surfaces by market	29

LIST OF ABBREVIATIONS

CAC	Codex Alimentarius Commission
CDC	Centers for Disease Control and Prevention
EHEC	Enterohemorrhagic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
FAO	Food and Agriculture Organization
FSI	Food Safety Index
GHP	Good Hygiene Practices
HUS	Haemolytic Uremic Syndrome
LMIC	Low- and Middle-income countries
MCFAs	Medium-chain fatty acids
MCTs	Medium chain triglycerides
RTE	Ready-to-eat
SEA	Staphylococcal Enterotoxin A
SEB	Staphylococcal Enterotoxin B
STEC	Shiga toxin-producing <i>E. coli</i>
WHO	World Health Organization
FBDs	Foodborne diseases
ME	Ministério da Economia
INNOQ	Instituto Nacional de Normalização e Qualidade

ABSTRACT

Grated coconut is a widely consumed, minimally processed food product in Mozambican cuisine, yet empirical data on its microbiological safety remain scarce. The informal market conditions under which grated coconut is prepared and sold raise concerns about microbial contamination, which may pose public health risks to consumers. This study assessed the microbiological safety and quality of grated coconut sold from 24 vendors across four municipal markets (Xipamanine, Janet, Malanga, and Adelina) in Maputo City, Southern Mozambique. A total of 71 samples were collected, comprising grated coconut (24), swabs from grating machines (24), and swabs from vendors' hands (23). Hygiene conditions were assessed using structured observational *checklists* administered via Kobo Collect. Microbial analysis was performed using microbiological standard methods for Total Mesophilic Bacteria count (TMBC), *Staphylococcus aureus*, and *Escherichia coli* identification. The overall hygiene compliance score was $48.6 \pm 8.3\%$, with facility hygiene scores differing significantly across markets ($p = 0.040$). The prevalence of *E. coli* on grated coconut and contaminated surfaces was 12.5% and 14.9%, respectively. The prevalence of *S. aureus* in grated coconut and contact surfaces was 29.2 % and 42.6%, respectively. The mean of TMBC in coconut was highest at Malanga and Adelina (3.74 log [CFU/g]) and lowest at Janet (3.47 log [CFU/g]), the mean of TMBC was highest for hands at Janet (3.42log CFU/g and lowest at Xipamanine (3.19 log [CFU/g]) while for machines at Malanga it was highest (3.61 log [CFU/g]) and lowest at Xipamanine (3.30log [CFU/g]). The TMBC for 79.2% of coconut samples was above the limit. All samples contaminated with *E. coli* and *S. aureus* did not meet the safety standards. Hygiene practices among grated coconut vendors in Maputo markets were inadequate, carrying microbial contamination risks primarily from *S. aureus*, TMBC, and faecal contamination. Good Hygiene Practices and regular machine sanitation by the vendors can prevent coconut contamination. Also, the development of national microbiological standards for minimally processed coconut products and routine market-level microbiological surveillance can reduce microbial contamination across the market in Maputo.

Keywords: grated coconut, *Staphylococcus aureus*, *Escherichia coli*, mesophilic bacteria, Mozambique, informal markets

RESUMO

O coco ralado é um produto alimentar minimamente processado e muito consumido na cozinha moçambicana; no entanto, os dados empíricos sobre a sua segurança microbiológica são ainda escassos. As condições dos mercados informais onde o coco ralado é preparado e vendido levantam preocupações quanto à contaminação microbiana, o que pode representar riscos para a saúde pública dos consumidores. Este estudo avaliou a segurança e a qualidade microbiológica do coco ralado vendido por 24 vendedores em quatro mercados municipais (Xipamanine, Janet, Malanga e Adelina) na cidade de Maputo, sul de Moçambique. Foram recolhidas 71 amostras, incluindo coco ralado (24), esfregaços de máquinas de ralar (24) e esfregaços das mãos dos vendedores (23). As condições de higiene foram avaliadas através de listas de verificação observacionais estruturadas, administradas através do Kobo Collect tool. A análise microbiológica foi realizada utilizando métodos microbiológicos padrão para a Contagem Total de Bactérias Mesófilas (TMBC) e identificação de *Staphylococcus aureus* e *Escherichia coli*. A pontuação global de conformidade com as normas de higiene foi de $48,6 \pm 8,3\%$, com as probabilidades de higiene das instalações a diferirem significativamente entre os mercados ($p = 0,040$). A prevalência de *E. coli* no coco ralado e nas superfícies contaminadas foi de 12,5% e 14,9%, respetivamente. A prevalência de *S. aureus* no coco ralado e nas superfícies de contacto foi de 29,2% e 42,6%, respetivamente. A média (CMTM) no coco ralado foi mais elevada na Malanga e na Adelina (3,74 log [UFC/g]) e mais baixa na Janet (3,47 log [UFC/g]). A de bactérias (MTBC) foi mais elevada nas mãos em Janet (3,42 log [UFC/g]) e mais baixa em Xipamanina (3,19 log [UFC/g]), enquanto nas máquinas foi mais elevada em Malanga (3,61 log [UFC/g]) e mais baixa em Xipamanina (3,30 log [UFC/g]). A contagem total de bactérias (CTB) em 79,2% das amostras de coco estava acima do limite. Todas as amostras contaminadas com *E. coli* e *S. aureus* não cumpriram as normas de segurança. As práticas de higiene entre os vendedores de coco ralado nos mercados de Maputo foram inadequadas, levando a riscos de contaminação microbiana, principalmente por *S. aureus*, CTB e contaminação fecal. As boas práticas de higiene e a higienização regular das máquinas pelos vendedores podem prevenir a contaminação do coco. Além disso, o desenvolvimento de normas microbiológicas nacionais para produtos de coco minimamente processados e a vigilância microbiológica de rotina nos mercados podem reduzir a contaminação microbiana em todo o mercado de Maputo.

Palavras-chave: coco ralado, *Staphylococcus aureus*, *Escherichia coli*, bactérias mesófilas, Moçambique, mercados informais

1. INTRODUCTION

1.1 Motivation

Coconut (*Cocos nucifera* L.) is a vital perennial crop widely cultivated in tropical and subtropical regions (Chan & Elevitch, 2006). Known as the "tree of life," it provides essential resources such as food, oil, fiber, and timber (Nulu *et al.*, 2024). The coconut industry significantly contributes to rural economies and global trade, supporting approximately 20 million livelihoods (Alouw *et al.*, 2025). The leading coconut-producing nations include the Philippines, Indonesia, India, Sri Lanka, Thailand, Malaysia, and Papua New Guinea, which account for 80% of global cultivation (Zainol *et al.*, 2023). In 2021, the global market for coconuts (fresh or dried) reached a value of up to \$1.92 billion (WITS, 2021).

In Africa, leading producers of coconut are Ghana, Tanzania, Mozambique, Nigeria, and Kenya. Mozambique produces 60,000 tons annually, with half consumed domestically and the rest processed for local sales or export (Trade, 2004). In 2022, its copra and processed coconut exports were valued at \$1,428,150, respectively (World Integrated trade solution, 2022). Coconut is a commercial and subsistence crop in Mozambique; it is particularly significant in coastal provinces like Zambezia, Inhambane, Nampula, and Cabo Delgado, where it greatly enhances household income, food security, and nutrition (FAO, 2016; Zainol *et al.*, 2023).

In traditional Mozambican cuisine, coconuts are used in various ways, such as oil extraction, grated pulp, fresh kernels, and coconut milk. Generally, grated coconut is frequently used in various dishes such as rice, sauces, custards, porridge, and in making milk and oil (Ramesh Praveen, 2024). The national cuisine includes dishes like xima and coconut milk peanut soup (chicken with coconut), matapa (cassava leaves fried with ground peanuts and coconut milk), and porridges made with coconut milk (FAO, 2008). Coconut milk and grated coconut are also used in sauces accompanying staple foods like rice and maize flour (*xima*), highlighting their central role in the Mozambican diet (FAO, 2008).

Grated coconut is a popular product in Maputo, Mozambique, commonly purchased with minimal processing and categorized as a market food from the municipal markets. It is made from the finely grated edible pulp of fresh coconuts, which are grated at the point of sale using either a grater or a food processor, and consumers purchase it in small plastic bags for use in household dishes (Adalima, 2022). The processing of coconut can lead to microbial contamination due to exposure to unsanitary environmental conditions. This includes contaminated surfaces, unclean equipment, unwashed hands, and exposure to airborne particles

or insects during handling (Balali *et al.*, 2020; Ray *et al.*, 2024). Grated coconut, in particular, provides a moist and nutrient-rich medium that supports the survival and growth of both spoilage and pathogenic microorganisms, including *Mesophilic bacteria*, *Escherichia coli*, and total coliforms, under typical tropical conditions, especially when grated coconut is stored without refrigeration, these microorganisms can survive and multiply rapidly (Lamdande *et al.*, 2018)

Moreover, the survival of these organisms in grated coconut and potential contaminant surfaces without preventive measures can lead to poor hygiene, highlighting the public health risks associated with poor hygiene practices during coconut handling and processing. These factors can adversely affect the quality of grated coconuts and potentially lead to foodborne illnesses (Centers for Disease Control and Prevention, 2018). Several foodborne illness outbreaks have been linked to contaminated coconut products, particularly grated or processed forms; 19 cases of *Salmonella* were found in pre-cut coconut pieces in Canada (Luna *et al.*, 2018). Four positive samples of *S. aureus* from coconut milk drink; only one sample did not meet the requirements of the Indonesian National Standard (Sari & Muhlisin, 2019).

Inadequate vendor hygienic practices, such as handling coconuts and money with bare hands, washing utensils with non-potable water, and keeping the machine uncovered, may increase the risk of grated coconut contamination and/or cross-contamination during processing (Lues *et al.*, 2006). According to WHO. (2024) long-term health consequences, mostly affecting vulnerable groups such as children under five years, pregnant women, immunocompromised people, and the elderly.

Foodborne diseases affect approximately one in ten people worldwide; around 600 million individuals fall ill due to consuming unsafe food (WHO, 2024). Coconut products, especially grated or processed coconut and its milk, sold under unhygienic conditions, have been linked to several foodborne diseases that occurred as a result of eating coconut milk dessert made in an elementary school, 50 of 485 students (10%) were affected with *Shigella dysenteriae* diarrheal outbreak, through from the preparation involved kneading the grated coconut by hand without the use of plastic gloves, and the food handler also had diarrhea shortly before the outbreak and did not follow a handwashing policy before making the dessert (Hoge *et al.*, 1995). Also, *E. coli* was found in 34% out of 17 samples of coconut milk vendors in Kota Bharu, which exceeded the standard limit (Ramakarishnan, 2016).

FBD illnesses are responsible for approximately 420,000 deaths each year. Children under five years account for about 125,000 of these fatalities, with the impact being more severe in developing countries. In Africa, foodborne illnesses lead to approximately 91 million cases of illness annually and 137,000 deaths (WHO, 2024a). In 2018, over 500 000 diarrhea cases were attributed to foodborne pathogens, with children under five years showing a high disease burden during that period in the urban setting of Maputo, where most of the cases were related to infection with foodborne *E. coli* and *Enterobacter* (Faife *et al.*, 2024). However, foodborne data resulting from coconuts is missing in Mozambique. There is still limited data on microbial safety related to grated coconuts sold in the municipal market of Mozambique. Therefore, this study assessed the microbial safety of grated coconut sold in Maputo City markets, in order to provide data-driven insights to ensure the health and well-being of the consumers. This provides valuable insights for food safety regulators to implement favorable measures to guide consumer well-being and prevent foodborne illness.

1.2 Problem statement

Foodborne disease remains a heavy burden in Mozambique, and the informal markets where most ready-to-eat foods are sold are a recognized point of exposure. Across the country, cases of diarrhoea have been attributed to contaminated food and water, which affected young children and the immunocompromised population (Faife *et al.*, 2024; Salamandane, Vila-Boa, *et al.*, 2021). Surveys of street foods sold in Maputo have repeatedly revealed that *S. aureus* and *E. coli* are prevalent, confirming that the indicator and pathogenic organisms relevant to grated coconut are already circulating in the city's food trade (Salamandane *et al.*, 2021). A study in Maputo, which evaluated good hygienic practices (GHP) of food establishments in several food markets, observed that 67% of vendors handled food and money with their bare hands simultaneously, which can be a source of cross-contamination of food. (Glória *et al.*, 2019). These poor hygiene and sanitation practices among food manipulators can lead to exposure to foodborne illness and public health concerns.

Lack of information on the foodborne pathogens associated with grated coconut is a major concern owing to the large population who consume the product in Mozambique. The prevalence of illness related to this pathogen shows that this product can be a host for the pathogen. Since grated coconut is sold in the open market, there is a high risk of contamination and dissemination among people within a short period of time. Also, the pathogen has been found to thrive and reproduce under moist conditions; this contributes to the spread of the

disease, leading to infection. Therefore, there was a need to understand the microbiological contamination level and the associated risk to consumers.

1.3 Objectives

1.3.1 General objective

To assess the microbiological safety and quality of grated coconut sold in Maputo markets

1.3.2 Specific objectives

1. To verify the hygienic conditions of grated coconut in selected markets of Maputo.
2. To identify *Staphylococcus aureus* and *Escherichia coli* in grated coconut, machines and hands
3. To quantify Mesophilic bacteria and *E. coli* in grated coconut and potential contamination surfaces
4. To compare the grated coconut count with standards for Mesophilic and *E. coli*.

1.3.3 Research questions

- i. What are the hygiene conditions under which grated coconut is handled and sold in selected markets in Maputo?
- ii. Are *Staphylococcus aureus* and *E. coli* present in surfaces associated with its handling?
- iii. What is the concentration of mesophilic bacteria in grated coconut and potential surfaces of contamination?
- iv. How do contamination levels detected in grated coconut samples compare with international standards?

1.4 Contribution of the study

This study is expected to raise public awareness about the microbiological safety of grated coconut, a common food product in Maputo markets. These insights might help in behavioral change among vendors, such as improved hygiene practices, leading to safer food in local markets. Additionally, the study shall advocate for the establishment and enforcement of regulatory standards for grated coconut in Mozambique, benefiting both policymakers and researchers.

In alignment with Sustainable Development Goals, this study will significantly contribute to SDG 3 (Good health and well-being). The reduction of foodborne diseases directly supports

the goal of ensuring healthy lives and promoting well-being. Identifying and addressing food safety hazards aligns with target 3.9 for the Sustainable Development Goals (SDGs), which aims to substantially reduce illnesses and deaths caused by hazardous contaminants.

2. LITERATURE REVIEW

2.1 Grated coconut overview

In tropical and subtropical regions, including Mozambique, grated coconut, which comes from the mature kernel of the coconut palm, *Cocos nucifera* (Figure 1), is an important food that can be eaten raw or as an ingredient in various meals (Adalima, 2022). Its creamy texture makes it essential in traditional recipes such as matapa, a traditional meal prepared with peanuts, coconut, cassava leaves, and shrimps (FAO, 2008). Grated coconut is culturally important and usually seen in festival meals, religious events, and community gatherings. It's a traditional food of Mozambique coastal areas, where coconut palms grow well.

Grated coconut has a lot of nutrients; it has a unique high content of medium-chain fatty acids (MCFAs), particularly lauric acid and medium-chain triglycerides (MCTs), which are easily metabolized for energy and linked to potential alleviation of non-communicable diseases such as cardiovascular and Alzheimer (Sandupama *et al.*, 2022). It has a lot of dietary fiber, which helps with digestion, and vital minerals like iron and copper, which support immunological function, and manganese, which is important for bone health (Ramesh, Praveen, 2024). The macronutrient composition has been reported to be 7.5 % protein, 37% fat, 12.3% carbohydrates, 14.3% crude fiber, 1.0% ash content, and 42.2% moisture (DebMandal & Mandal, 2011; Rajamohan & Archana, 2019).

Coconut is also utilized in other functions, such as coconut oils and husks, which are used as biofuel or handicraft materials (Vieira *et al.*, 2024). Grated coconut has become highly recognized worldwide as a dairy substitute in vegan and health-conscious diets (Lu *et al.*, 2024). Essential ingredients for plant-based cheeses, gluten-free baking, coconut flour, milk, and oil made from processed coconut, as well as organic skincare products (Lu *et al.*, 2024; Pandiselvam *et al.*, 2024).

Maputo's small-scale food establishments make grated coconut, which supports local livelihoods and household income (Adalima, 2022). Mature coconuts are harvested first, then their husks are removed, and the coconuts are broken open to reveal the white kernel. Traditionally, grating is done by hand, using basic tools such as a metal rod or knives; however, electrical coconut graters are now widely used in Maputo markets. Freshly grated coconuts are often sold in market stalls, such as those at Malanga and Xipamanine markets. This sector sustains local food systems and enhances the livelihoods of the community, from coconut farmers, processors, and food vendors.

Coconut that has been grated is extremely perishable and susceptible to microbial contamination. Its perishability results from a high-water activity of more than 0.9, which promotes the fast growth of bacteria and fungi, especially mesophilic bacteria (Lamdande *et al.*, 2018). Surface area increases when the coconut is grated, exposing interior tissues to contaminants from the surrounding environment, food handlers, or equipment used during the process (Lamdande *et al.*, 2018).

Poor observation of hygienic practices, such as the use of unclean grating machines, bare hand contact, and improper storage, increases the risk of microbial contamination. Studies in comparable settings have linked these factors to higher microbial contamination in perishable foods, which pose a risk of foodborne illnesses (Yap *et al.*, 2019).

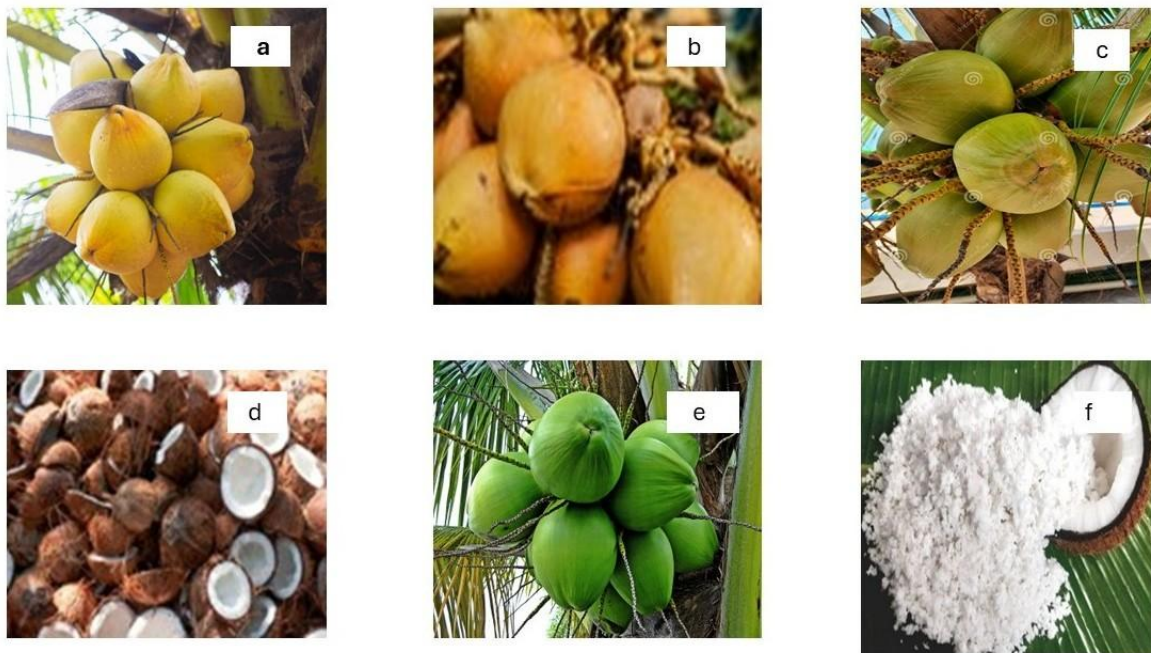


Figure 1: Coconut a, b, c showing coconuts (*Cocos nucifera* sp.) and d shows the whole coconut fruit, e shows de-husked and broken coconut, and f, grated coconut (Nulu *et al.*, 2024).

2.2 Foodborne Pathogens

Food safety remains a cornerstone of public health, especially in developing countries/regions where foodborne diseases impose significant socioeconomic burdens. Microbial contamination of food is a leading cause of such illnesses, contributing to an estimated 600 million cases of foodborne diseases and 420,000 deaths (WHO, 2024).

Bacteria, viruses, parasites, or chemicals that enter the body through contaminated food are the main causes of foodborne illnesses, which are typically toxic or infectious (Adley & Ryan, 2016). Chronic (Long-term) illnesses like infections or acute (short) poisoning can result from chemical pollution. Several foodborne illnesses can cause death and permanent disability. Bacteria are the most prominent causes of foodborne illness globally (Gupta, 2017).

Bacteria such as *Salmonella*, *Campylobacter*, and enterohaemorrhagic *E. coli* are some of the most common foodborne pathogens that affect millions of people annually, sometimes with severe and fatal outcomes (Adley & Ryan, 2016). Symptoms can include fever, headache, nausea, vomiting, abdominal pain, and diarrhea (Adley & Ryan, 2016).

The burden of FBDs on public health and economies has often been underestimated due to underreporting and difficulty in establishing causal relationships between food contamination and resulting illness or death (WHO, 2024). According to (World Bank, 2019) report on the economic burden of FBDs indicated that the total productivity loss associated with foodborne disease in low- and middle-income countries (LMIC) was estimated at US\$ 95.2 billion per year, and the annual cost of treating foodborne illnesses is estimated at US\$ 15 billion, and trade disruptions, undermining progress toward poverty reduction and food security (Grace, 2023). The contaminated and unsafe food perpetuates a cycle of malnutrition and disease, particularly in low- and middle-income countries.

The Codex Alimentarius Commission (CAC), jointly managed by WHO and FAO, plays a pivotal role in mitigating these risks by establishing international food safety standards. Codex guidelines aim to harmonize global trade and reduce contamination (Godefroy *et al.*, 2016). However, implementation gaps persist, particularly in informal sectors where regulatory oversight is minimal. For instance, vended foods in urban Africa and Asia frequently exceed Codex-recommended thresholds for pathogens like *Staphylococcus aureus* and faecal coliforms, highlighting the disconnect between policy and practice (Akhtar *et al.*, 2014; Salamandane, Silva, *et al.*, 2021). This report reveals the immediate need for targeted interventions, broad surveillance systems, and cross-sectoral collaboration to align global food

safety standards with on-the-ground realities, particularly in developing countries, such as Mozambique.

2.3 Foodborne diseases in sub-Saharan Africa

Sub-Saharan Africa bears a disproportionately high burden of foodborne illnesses, driven by systemic challenges such as weak enforcement of food safety regulations, limited access to clean water, and poor hygiene practices (WHO, 2024). The region accounts for nearly one-third of global foodborne disease deaths, with children under five and individuals with weak immunity facing the highest risks (WHO, 2024a).

Informal food markets, which dominate urban and rural food systems, often operate without regulatory approval and increase the risk of contamination. For example, a study in Nigeria showed 60% *E. coli* 0157:H7 contamination in food such as meat pie, cabbage, cucumber, bread, chicken, apple, salad, and pawpaw, and other harmful bacteria such as *S. aureus* linked to contaminated water and dirty preparation surfaces (Makinde *et al.*, 2020; Odo *et al.*, 2021).

Common bacterial pathogens in the region include *E. coli*, *Salmonella* spp, and faecal coliforms, all of which grow and multiply in environments with poor sanitation (Isara *et al.*, 2010; Rosiane *et al.*, 2018). *E. coli* is frequently detected in ready-to-eat foods like salads and dairy products, indicating faecal contamination from water used or food handlers (Salamandane, Vila-Boa, *et al.*, 2021a). *Staphylococcus aureus*, transmitted through bare-hand contact or nasal secretions, poses unique risks due to its heat-stable enterotoxins, which survive during the cooking process. A study conducted in Tanzania shows 67.1% of food samples from Morogoro market were contaminated by *E. coli* (49.2%) and *S. aureus* (19.7%), all associated with poor hygiene of vendors, unsafe food handling practices, and use of contaminated water in food preparation (Ndunguru & Ndossi, 2020).

2. 3 Indicators of food safety

2.3.1 Mesophilic bacteria

Mesophilic bacteria are bacteria with optimal growth at moderate temperatures between 20°C and 45°C, and their presence indicates the overall microbial quality in food products, including grated coconut (Roberts & Greenwood, 2023). Their presence provides insight into hygiene practices during processing, handling, and storage. Their high counts often show poor sanitation of equipment, handler hygiene, or temperature abuse during processing and storage (Glória *et al.*, 2019). Mesophilic bacteria have a lot of consequences, ranging from food spoilage to health risks. Their proliferation can degrade food quality, leading to texture changes,

off-flavors, and nutrient loss. More critically, high counts may indicate the presence of pathogenic bacteria that can cause gastrointestinal infections (Salamandane, *et al.*, 2021). Metabolism from these microbes in contaminated foods can produce biogenic amines or endotoxins, which can cause intoxication even in well-cooked food.

2.3.2 *Staphylococcus aureus*

Staphylococcus aureus is one of the leading causes of FBDs globally (Mairi *et al.*, 2025), primarily due to its ability to produce heat-stable enterotoxins that cause acute gastrointestinal disease with symptoms such as nausea, vomiting, abdominal cramps, and diarrhea (Adams & Moss, 2008). Compared to other foodborne pathogens, the consequences of *S. aureus* infection depend not only on the ingestion of viable bacteria but also on the persistence of its toxins, which cannot be degraded by normal cooking methods (Kadariya *et al.*, 2014). Enterotoxins such as SEA (Staphylococcal Enterotoxin A) and SEB (Staphylococcal Enterotoxin B) are particularly resistant to high temperatures and proteolytic enzymes, enabling them to survive in a wide range of food matrices (Adams & Moss, 2008) including grated coconut. The primary route of contamination is through direct human contact, as *S. aureus* colonizes the skin, nasal passages, and throats of approximately 20–50% of healthy individuals (González-garcía *et al.*, 2021).

In street food settings, poor hygiene practices such as neglecting handwashing during food preparation or handling money and raw ingredients without sanitization facilitate the transfer of these bacteria to food (Ndunguru & Ndossi, 2020). Therefore, to prevent *S. aureus* contamination in food like grated coconuts, food vendors must observe good hygienic practices. (Glória *et al.*, 2019). Temperature control is important to limit the time food, such as grated coconut, spends in danger zone, such as room temperature, where *S. aureus* can multiply and produce toxins (Adams & Moss, 2008).

2.3.3 *Escherichia coli*

E. coli contains both pathogenic and non-pathogenic strains. Non-pathogenic strains of *E. coli* are natural inhabitants of the human and animal intestinal microbiota and typically pose no health risks (Adams & Moss, 2008). Pathogenic strains, such as enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), and shiga toxin-producing *E. coli* (STEC), cause gastrointestinal illnesses. For instance, STEC O157:H7 can lead to haemorrhagic colitis, haemolytic uremic syndrome (HUS), and renal failure (Adams & Moss, 2008). Though *E. coli* serves as a faecal coliform, a vital microbiological indicator for assessing sanitation and

hygiene in food and food processing conditions (Adams & Moss, 2008) *E. coli* contamination can occur during different stages of food processing, such as transportation, washing, processing, packaging, or vending, due to a lack of clean water, inadequate waste disposal, and poor food handling practices, which can contribute significantly to microbial contamination (Adeleke *et al.*, 2020).

In minimal processing of fruits and vegetables, such as peeling, slicing, cutting, shredding, washing, and packaging, they can be highly susceptible to microbial contamination, like *E. coli*, because fruits and vegetables are often consumed without further heat treatment, and consumers are convenience to consume because of their freshness and nutritional value (Dos Santos *et al.*, 2021). Their presence in food products shows the potential for noncompliance with Good Hygienic Practices (GHP) during processing, handling, or storage (Membré, 2016).

2.4 Common sources of contamination in grated coconut

Both environmental and human factors are the main sources of microbial contamination in grated coconut, which can occur at various points during grating and handling. Raw coconut shells that have been contaminated act as a first reservoir for microbes (Coutinho & Neves, 2024). According to studies, coconut shells are colonized by *Acinetobacter*, *Enterobacteriaceae*, and *Micrococcus species* (KAJS *et al.*, 1976; Limtong *et al.*, 2020). Pathogens from these shells may spread to the edible pulp during processing by contact with handlers or poorly cleaned grating instruments as reported by Glória *et al.*, (2019) , who indicated that pathogens can spread during processing.

Poor hygiene practices among food vendors further increase contamination risks. Observations in Maputo markets reveal that handlers frequently neglect handwashing, glove use, or protective clothing (Glória *et al.*, 2019). Cross-contamination is also prevalent when vendors handle money, raw ingredients, or unclean surfaces before touching the food (Acácio *et al.* 2021),. When coconut grater equipment and utensils are not cleaned properly, they create biofilms or residual organic matter that help in the proliferation of microbes (Carrascosa *et al.*, 2021). Cracks or rust on equipment surfaces trap moisture and food particles, creating a favorable environment for bacterial growth (Moraes, 2023).

Storage containers and packaging materials that are not properly cleaned can introduce microbes like *E. coli* or other faecal coliforms (Glória *et al.*, 2019). Microbial contamination and growth are significantly influenced by the conditions during processing and storage. A few

hours of storage at temperatures higher than 25°C reduces product safety by speeding up the growth of contaminant microorganisms (Zhao *et al.*, 2022).

2.5 Legal and Normative aspects related to Grated coconut

2.5.1 Grated coconut safety standards

Codex Alimentarius doesn't have direct standard for ready-to-eat and minimally processed fresh grated coconut, but it has a standard for desiccated coconut, the Code of Hygienic Practice for Desiccated Coconut (CAC/RCP 4-1971 & FAO.WHO, 1971). However, Codex has put forward a general code of practice which can be adapted for grated coconut to ensure hygienic practice and acceptable levels of microbial presence, such as General Principles of Food Hygiene according to the CXC 1-1969 (FAO and WHO, 1969) and Principles and Guidelines for the Establishment, Application of Microbiological Criteria Related to Foods (CXG 21-1997 (FAO .WHO, 1997) and Regional Guidelines for the Design of Control Measures for Street-vended Foods (Africa) CAC/GL 22R (WHO. FAO, 1997).

The European Union (EU) also doesn't have regulation for ready-to-eat grated coconut or minimally processed coconut; it might relate to Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs under processed fruits and vegetables, which specifies, for instance, *E. coli*: to be ≤ 100 CFU/g as a hygiene indicator (European.Union, 2007).

Codex standards provide baseline recommendations such as personal and machine hygiene, and microbial contaminants standards in foods; national regulations may adapt these limits based on regional risks (Codex. Alimentarius, 2025). Adopting these international standards reduces the incidence of foodborne illnesses and increases consumers' confidence and compliance with global trade requirements.

2.5.2 Mozambique food safety regulations

Mozambique's food safety framework is based on and works under four Ministries and four Institutions. Ministry of Health (Ministério da Saúde, MISAU), Ministry of Agriculture, Environment and Fisheries (Ministério da Agricultura Ambiente e Pescas, MAAP), with its National Directorate of Agriculture (Direcção Nacional de Agricultura, DINA). In Mozambique, there is no standard for grated coconut that has been laid out. Ministry of Economy (Ministério da Economia, ME) with the National Institute for Standardization and Quality (Instituto Nacional de Normalização e Qualidade, INNOQ) and the General Inspection of Food Safety and Economics (Inspeção Geral de Segurança Alimentar e Económica,

IGSAE). Despite having defined tasks and responsibilities, these Ministries and Institutions lack coordination in practice, which causes fragmentation and makes it difficult to implement food safety laws.

The regulatory framework for all processed food and food products intended for consumption within the country is established by MISAU. This involves monitoring both imported and domestically produced goods and determining their hygiene and quality standards. In addition, the Ministry issues operation permits and establishes hygiene standards for businesses that handle and sell ready-to-eat (RTE) food. Food hygiene guidelines are one of the specific food safety regulations that fall under the jurisdiction of Ministério da Saúde (MISAU)

Products exported are supervised by MIC; in Mozambique, these mostly consist of primary agricultural products. The primary organization that oversees creating and carrying out quality policy and organizing all national standardization is INNOQ. To develop the food safety technical regulations and conformance evaluation procedures, INNOQ should work closely with the other food safety agencies, particularly MISAU. Lastly, the only entity responsible for ensuring compliance and implementing the food safety regulatory framework is the INAE (World Bank, 2020)).

Three main obstacles and challenges facing Mozambique's food safety system include inadequate coordination and communication among agencies within the food safety regulatory framework, insufficient technical skills at the surveillance and enforcement levels, and inadequate analytical infrastructure(Akademiya, 2019)At the monitoring, surveillance, and enforcement levels, lack of communication becomes particularly critical.

The founding of the former Inspeção Nacional das Actividade Económicas (INAE), currently IGSAE, offered a workable solution for the overwhelming workload caused by several audits from various regulators that were examining food enterprises. It was effective in addressing that specific issue and promoting an integrative approach. However, its development was not accompanied by the development of competence of agents to carry out risk-based audits of a high technical level (World Bank, 2020).

Therefore, many vendors are still operating without formal registrations and are rarely inspected for hygienic practices.

2.6 Theoretical framework

This study evaluates the microbiological safety of grated coconut in four Maputo markets by using principles of microbiology, food hygiene, and food safety management systems. Microbial theories explain how grated coconut has high water activity, and its high nutritional content creates an ideal environment for mesophilic bacteria. It highlights contamination routes from handlers, surfaces, and equipment. Good Hygienic Practices (GHP) frameworks provide a preventive approach to mitigating these risks by identifying critical control points and following hygienic practices (Menon, 2017).

However, the gap between Codex Alimentarius standards and actual market practices reveals the challenges of implementing and enforcing formal food safety regulations, particularly for foods such as grated coconut and other minimally processed crops, like other high-moisture ready-to-eat plant foods (e.g., pineapple, mango, papaya, grated cassava) that are sold in informal markets under unhygienic conditions. These circumstances raise the possibility of microbiological contamination, foodborne pathogens, and indicator organisms such as *E. coli*, mesophilic bacteria and *S.aureus*, which can be detected by microbial analysis to evaluate the safety and hygienic quality of prepared food (Sunarti, 2024).

3. MATERIALS AND METHODS

3.1 Study area

The study was conducted in Maputo City, Mozambique. The study area was selected using a purposive sampling technique due to its high population density and urban nature, which creates a concentrated environment for grated coconut vendors. Samples were collected from four municipal markets: Xipamanine, Malanga, Janet, and Adelina (

Figure 2: Study area

Map of Maputo Province, highlighting Maputo City (green square), with the study area (in green color), where the markets are located. Map was drawn using ArcGIS 10.8 Version (Esrri California)

). Maputo is the capital city of Mozambique, located in the southern part of the country along the Indian Ocean coast, with an area of 347.69 km² and a population of approximately 1.11 million people according to the 2017 census (INE, 2025).

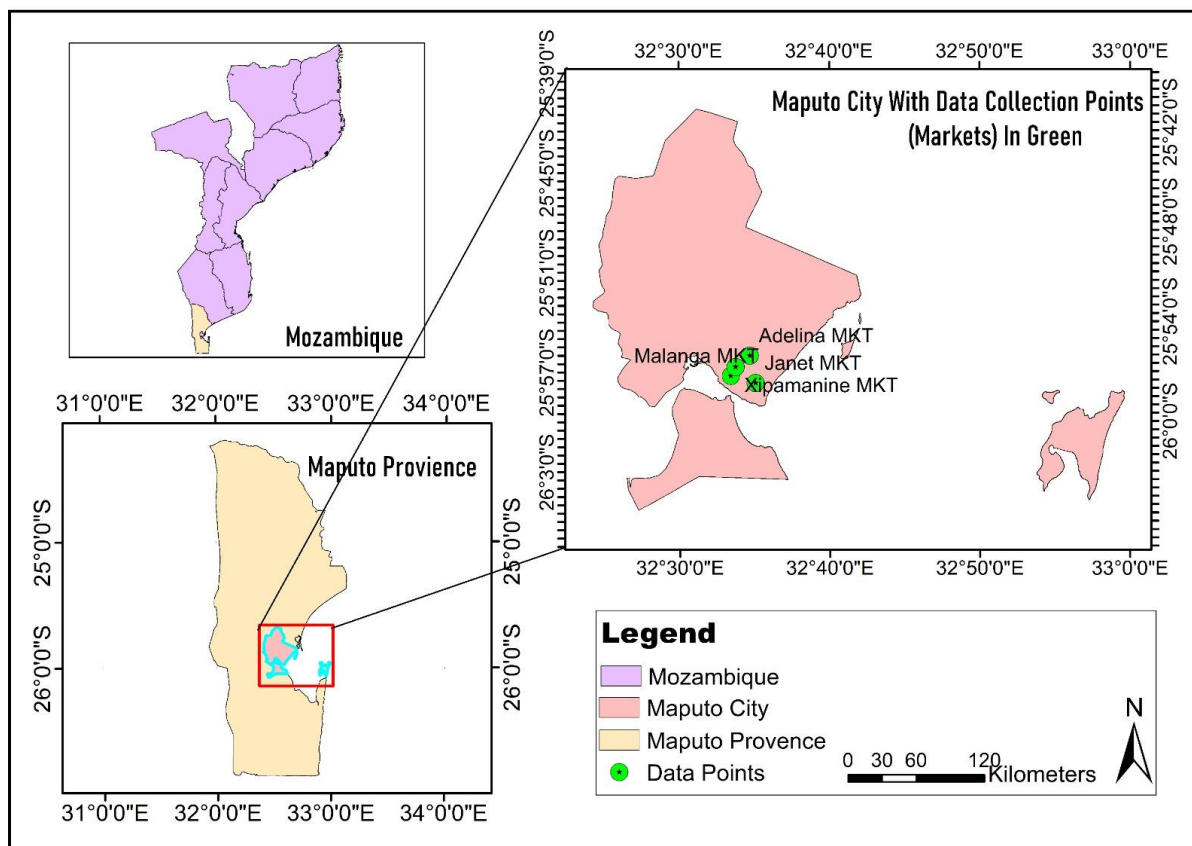


Figure 2: Study area

Map of Maputo Province, highlighting Maputo City (green square), with the study area (in green color), where the markets are located. Map was drawn using ArcGIS 10.8 Version (Esrri California)

3.2 Study design and participants

A cross-sectional study design was employed from March 3 to April 13, 2026, and a simple random sampling technique was adopted to collect samples from grated coconut vendors across the four selected markets in Maputo.

3.2.1 Inclusion criteria

Included coconut vendors who were at least eighteen years old, had grating equipment at the market or location where they sold grated coconut, and voluntarily signed a consent form.

3.2.2 Exclusion criteria

Vendors under the age of eighteen were excluded. Additionally, vendors who prepared grated coconut at home and sold it already grated at the market were also excluded.

3.2.3 Participant consent and safety

There was no potential risk associated with the study. All information collected was kept confidential and used for academic and research purposes only. Participation was completely voluntary and anonymous. Participants who decided to participate were given a detailed explanation about the study and were free to stop their participation at any stage of the interview or sample collection at any time. Furthermore, they were free not to answer any specific question if they did not feel comfortable to reply. A written consent was given to each participant before the observation of hygienic practices and sample collection.

3.2.4 Sample size determination

A pre-survey of the four markets identified a total of 25 grated coconut vendors distributed as follows: Xipamanine (10 vendors), Adelina (5 vendors), Janet (5 vendors), and Malanga (4 vendors). The sample size was estimated using the OpenEpi calculator with the following parameters:

$$n = \frac{DEFF * N * p(1 - p)}{\frac{d^2}{Z_{1-\alpha/2}^2} * (N - 1) + p(1 - p)}$$

Where:

- n = required sample size
- DEFF = design effect (1)
- N = population size (25)
- p = estimated proportion of the attribute in the population (0.5)
- d = margin of error (precision) (0.05)
- $Z_{1-\alpha/2}$ = Z-score corresponding to the desired confidence level (1.96)

$n \approx 24$

The calculation yielded a required sample of 24 vendors.

Three types of samples were collected from each vendor: grated coconut, swabs from the grating machine, and swabs from the vendor's hands during processing. A total of 72 samples were targeted across all four markets, comprising 30 samples from Xipamanine (3 samples from each 10 vendors), 15 from Janet (3 samples from each 5 vendors), 12 from Malanga (3 samples from 4 vendors), and 15 from Adelina (3 samples from 5 vendors).

3.3 Data collection tools

Two observational checklists (Annex 1) were used to assess: (a) the personal hygiene of the vendors (13 items), and (b) the coconut grating facilities, storage, and general hygiene (22 items). The checklists were adopted with modification from Glória *et al.* (2019) and Jan Mei (2019). Data was collected using the Kobo Collect Tool, a digital survey platform. After sample collection, each vendor's practices were observed for close to 20 mins during three consecutive processing cycles of coconut grating, swabbing, and packaging the grated coconut in sterilized plastics.

3.4 Sample collection and handling

A 60 g portion of freshly grated coconut was purchased from each randomly selected vendor and collected using a sterile spatula into sterilized plastic bags. Swab samples were collected from the coconut grating machines (on 1 cm × 1 cm) and the hands of vendors (1 cm × 1 cm), using sterile cotton swabs moistened in 10 mL of Buffered peptone water (2%) contained in sterilized tubes. All samples were labelled in the field, placed in an isothermal box maintained at 4 °C, and immediately transported to the Food Technology Laboratory at Veterinary Faculty, Eduardo Mondlane University (UEM-FAVET) for microbiological analysis.

3.5 Laboratory analysis of microbiological contaminants

Laboratory analysis focused on isolating and quantifying mesophilic bacteria and *E. coli* and detecting *Staphylococcus aureus* in grated coconut samples, swabs from the coconut grating machines, and swabs from the vendors' hands. Were performed following the Food and Water Hygiene Laboratory, Ministry of Health of Mozambique (LNHAA, 1997)

3.5.1 Sample preparation

For coconut samples, 10 g of each was weighed using a spatula to transfer the sample on to sterilized stomacher plastic bag, then added 90 mL of buffered peptone water 2% (BPW), followed by homogenization for 3 minutes to obtain the mother dilution (10^{-1} dilution). Serial dilutions were then prepared, from the mother dilution up to 10^{-3} using sterile peptone water. For swab samples, the transport tubes containing 10 mL of buffered peptone water (BPW) were vortexed for 10 seconds; this was the mother solution, and 1 mL was transferred from the mother solution to 9 mL of sterile peptone water to prepare the 10^{-2} , 10^{-3} dilutions. For swabs, the samples were analyzed at the 10^{-1} , 10^{-2} dilution.

3.5.2 *Staphylococcus aureus* isolation and confirmation

S. aureus was assessed qualitatively (presence or absence) rather than quantitatively. For isolation, 0.1 mL of the prepared sample from the 10^{-1} dilution was spread onto Mannitol Salt Agar (MSA) plates in duplicate, and streaking was done and incubated at 37 °C for 24 hours. Yellow colonies (indicating mannitol fermentation) and creamy or yellow colonies were selected for further confirmation. Presumptive colonies were sub-cultured onto nutrient agar at 37 °C for 18–24 hours to ensure purity.

Confirmation was performed through three sequential tests:

(a) Gram stain: A drop of sterilized water was placed on a microscopic slide, and purified colonies were transferred using a sterile loop. After gentle heat fixation, the slides were stained sequentially with crystal violet (1 minute), Gram's iodine (1 minute), decolorized with alcohol (30 seconds), and counter-stained with safranin (1 minute), with rinsing under running water between each step. The slides were air-dried and examined under a optic microscope with immersion oil. Gram-positive cocci appearing in clusters with purple staining were recorded as positive for *Staphylococcus*.

(b) Catalase test: A drop of hydrogen peroxide (H_2O_2) was placed on a microscopic slide and purified colonies from nutrient agar were transferred using a sterile loop. The production of gas bubbles was recorded as a positive catalase result, consistent with *Staphylococcus* species.

(c) Coagulase test: Purified colonies from nutrient agar sub-culture were suspended in three drops of rabbit plasma coagulase reagent (Pro-Lab Diagnostics, UK) in a small tube and incubated for 4–24 hours at 37 °C. The formation of a clot indicated coagulase enzyme activity, confirming the presence of coagulase-positive *S. aureus* (Katz, 2016).

3.5.3 Total mesophilic bacteria count (TMBC)

The Plate Count method was used to enumerate total mesophilic bacteria. For each dilution, 1 mL of the prepared sample was inoculated onto Plate Count Agar (PCA) in duplicate. Coconut samples were plated at three dilutions (10^1 , 10^2 , 10^3), resulting in six plates per sample. Swab samples were plated at one or two dilutions, depending on the analytical batch. All plates were incubated for 24 hours at 37 °C. Following incubation, colonies were counted using a colony counter (Counter, USA), and results were expressed as colony-forming units per gram (CFU/g) for coconut samples or CFU/mL for swab samples, using microbial count formulae (Roberts & Greenwood 2003).

$$n = \frac{C}{v * (n_1 + 0.1 \times n_2) \times d}$$

Where: N = number of CFU per g or mL; C = total sum of colonies counted on all plates; v = volume of diluted sample applied to each plate (mL); n_1 = number of plates counted at the first dilution; n_2 = number of plates counted at the second dilution; d = dilution factor of the first dilution from which colonies were counted.

3.5.4 *Escherichia coli* isolation and enumeration

E. coli was isolated using Eosin Methylene Blue (EMB) agar as a selective and differential medium. For each dilution, 1 mL of the prepared sample was spread onto EMB agar plates in duplicate (six plates per coconut sample across three dilutions; two to four plates per swab sample). Plates were incubated for 24 hours at 37 °C. Colonies exhibiting the characteristic metallic green sheen were considered presumptive *E. coli* and were counted. Pink colonies without a metallic green sheen were considered negative for *E. coli*.

For confirmation, presumptive *E. coli* colonies were purified by sub-culturing onto PCA media and incubated at 37 °C for 24 hours. Creamy white colonies were then inoculated into tryptone broth containing 4 mL per tube and incubated for 24 hours at 37 °C. Three drops of Kovacs reagent were added to each tube. The formation of a pinkish-red ring on the top layer of the broth was considered a positive indole reaction, confirming the prevalence of *E. coli*.

3.6 Data processing, Analysis, and Interpretation

Data from the field observations and laboratory analyses were processed, coded, and entered into Microsoft Excel for initial organization. All statistical analyses were subsequently performed using Stata version 17 (Stata Corp, College Station, TX, USA 2021). The Kruskal-Wallis test was used to compare microbial counts among the different markets because the data was not normally distributed. Fisher's Exact Test was used to examine associations between categorical variables, including contamination status and market. Statistical significance was set as a threshold of $p < 0.05$. The analytical approach was structured around the four specific objectives of the study, as described below:

3.6.1 Analysis for Objective 1: verification of hygienic conditions

Checklist data on personal hygiene (13 items) and facility hygiene (22 items) were coded as binary variables (1 = Yes, 0 = No). Descriptive statistics, including frequencies and percentages, were calculated for each vendor as the proportion of items meeting acceptable standards, expressed as a percentage. An overall hygiene score was derived as the arithmetic mean of both component scores. Differences in hygiene compliance across the four markets were assessed using the Kruskal-Wallis test since the data were not normally distributed and did not fit heterogeneity. Fisher's exact test was used for individual checklist items to determine whether specific hygiene practices varied significantly by market location.

3.6.2 Analysis for Objective 2: Identification of *Staphylococcus aureus* and *E coli* on grated coconut and potential surfaces.

All positive *S. aureus* samples and *E. coli* were reported as frequencies and percentages for each surface type (vendor hands and grating machines) and grated coconut across the four markets. Fisher's exact test was used to compare positive samples across markets.

3.6.3 Analysis for objective 3: Quantification of microbial contaminants

For total mesophilic bacteria count (TMBC), descriptive statistics (mean, standard deviation, median, and range) were reported for both raw CFU values. The Shapiro-Wilk test was applied to assess normality, because the data were not normally distributed. The Kruskal-Wallis test was used to compare TMBC across the four markets, stratified by sample type. For *E. coli*, frequency was used for positive samples. The frequency of positive *S. aureus* was used and compared across markets using Fisher's exact test.

3.6.4 Analysis for Objective 4: Classification Against Food Safety Standards

Grated coconut samples were classified against applicable food safety thresholds. For TMBC, samples were categorized as acceptable in comparison with 3.70 log CFU/g for coconut water processing according to or *E. coli*, the EU Commission Regulation (EC) No. 2073/2005 (EU, 2007) threshold for processed fruits and vegetables (≤ 100 CFU/g as satisfactory) was applied. For *S. aureus*, samples were classified as present or absent. A composite “Overall Safe” classification was derived, defined as simultaneously meeting all three criteria: TMBC acceptable, *E. coli* not unsatisfactory, and *S. aureus* absent.

3.7 Ethical consideration

This study got ethical approval from the Postgraduate Council of Veterinary Faculty, reference No: (09 CC-FAVET/2025) and the Municipal Human Resources Service of Maputo, reference No: (MHRS# 1287/SG/217) dated 21 April 2025.

4. RESULTS

4.1 Socio-demographic profile of grated coconut vendors

The socio-demographic characteristics of the grated coconut vendors surveyed across the four markets based on sex, age, and education level (**Table 1**). From all markets, grated coconut was sold mainly by female vendors. Most vendors who took part on study were aged 18-25 years, and lowest was aged 34-45 years were more predominant, most of whom had a secondary school education level.

Table 1: Socio-demographic characteristics of grated coconut vendors

Variable / Category	Frequency (n)	Percentage (%)
<i>Sex</i>		
Female	14	58.3
Male	10	41.7
<i>Age group</i>		
18-25	10	41.7
26-35	9	37.5
36-45	5	20.8
<i>Education level</i>		
No formal education	1	4.2
Primary	8	33.3
Secondary	15	62.5

4.2 Hygienic conditions of grated coconut in selected markets

4.2.1 Personal Hygiene Practices

The assessment of personal hygiene shows variations across the vendors. Completely 100% of vendors were not following hand hygiene. Personal cleanliness and protective clothing were below 50%; however, only 76% and 39% of vendors were following good hygiene behavior practices when handling coconuts for customers and following personal cleanliness, respectively, as seen in Figure 3 and Appendix 4.

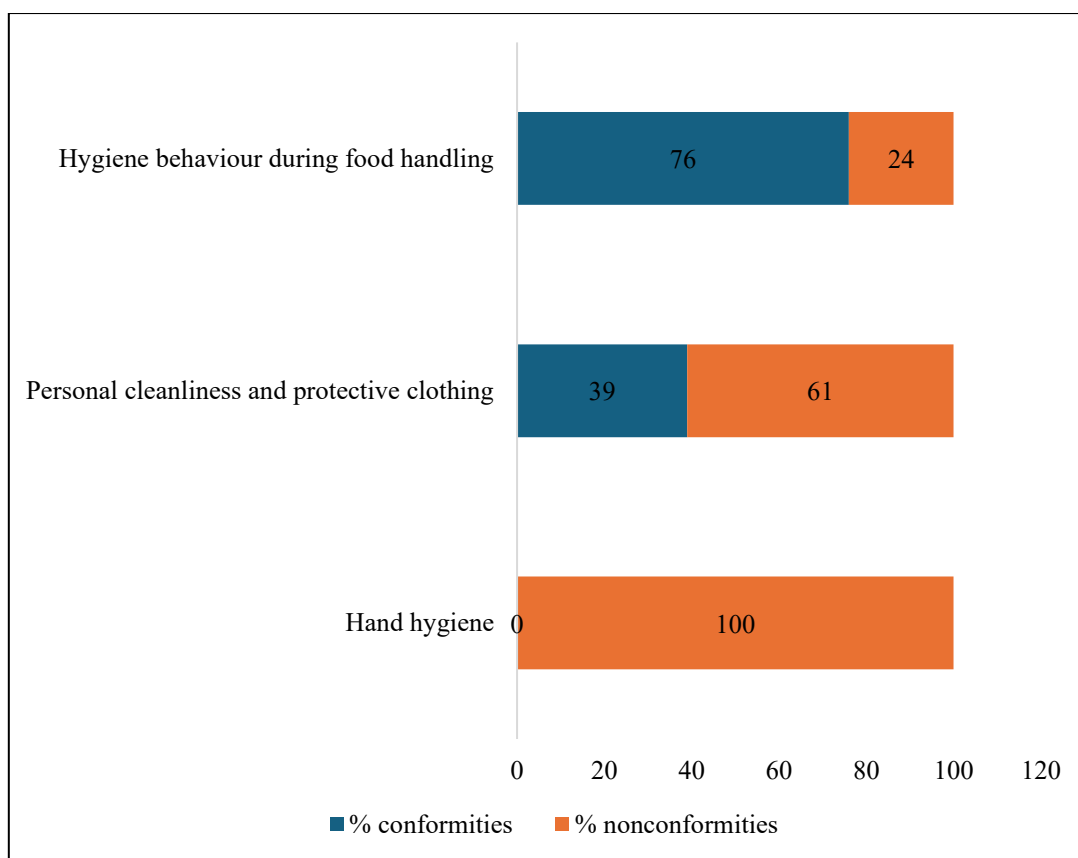


Figure 3: Assessment of personal hygiene (n= 24)

4.2.2 Facility hygiene and sanitation conditions

The results of facility hygiene and sanitation practices show that several positive practices were identified among the vendors. It was observed that grated coconut is served directly to the consumer in a clean plastic bag across all the vendors (98%). Also, hygiene conditions and management practices of the machine showed good compliance among the vendors (60% and 70%), respectively. However, waste water management, water hygiene, equipment hygiene and coconut storage and handling showed noncompliance (**Figure 4**)

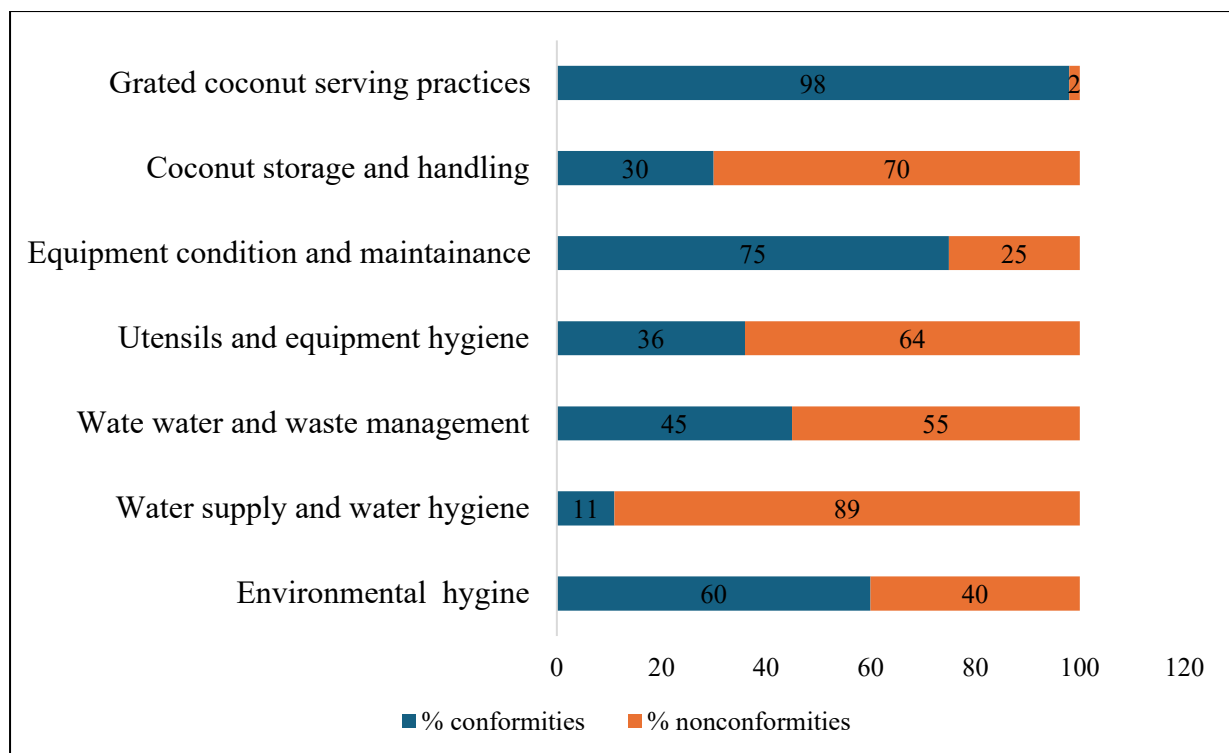


Figure 4. Facility hygiene practices by markets (n=24)

4.2.3. Personal hygiene practices by market

When comparing personal hygiene practices among vendors across markets for personal hygiene indicators (**Error! Reference source not found.3**), the majority of vendors (60%) at Xipamanine Market used hairnets during processing, whereas none of the vendors from Adelina and Janet markets protected their hair ($p = 0.029$). Regarding all other items, no differences were observed across the markets as shown in Appendix 4

4.2.4. Facility hygiene conditions by markets.

To compare facility hygiene practices among all markets, for seven of the twenty-two facility hygiene indicators: pest and fly control ($p = 0.015$), proper wastewater disposal ($p = 0.008$), sewage system condition ($p = 0.015$), cleanliness and orderliness of the coconut area ($p = 0.044$), use of clean water for utensil washing ($p = 0.003$), equipment storage safety ($p = 0.047$), and regular garbage disposal ($p = 0.009$). The rest of the indicators show no difference among all markets with a p-value greater than 0.05 (**Error! Reference source not found. 5**). To compare hygiene scores across markets, all overall hygiene practices differ among all markets($p=0.020$), while personal hygiene practices did not differ among all markets (0.282).

4.2.5. Overall hygiene compliance scores by markets

The overall mean hygiene compliance score across all 24 vendors was $48.6 \pm 8.3\%$ (table 2). The highest mean score was observed in Xipamanine ($50.0 \pm 6.5\%$), while the lowest was recorded in Malanga ($42.3 \pm 4.4\%$). These results indicate that hygiene practices varied across the markets, with Adelina generally demonstrating better overall hygiene conditions and Malanga showing the poorest hygiene performance.

Table 2. Hygiene compliance score by market

Markets	n	Personal hygiene score (%) Mean +/- SD	Facility hygiene score (%) Mean +/- SD	Overall hygiene score (%) Mean +/- SD
Xipamanine	10	50.0 ± 6.5	45.0 ± 12.6	47.5 ± 6.9
Janet	5	46.2 ± 7.7	54.5 ± 15.7	50.3 ± 10.4
Malanga	4	42.3 ± 4.4	36.4 ± 0.0	39.3 ± 2.2
Adelina	5	49.2 ± 8.8	63.6 ± 5.6	56.4 ± 3.7
Overall	24	47.8 ± 7.2	49.4 ± 14.1	48.6 ± 8.3

4.2.6. Overall Hygiene Compliance Categories by Markets

In terms of personal hygiene compliance categories among all markets, only 16.7 % (4) demonstrated good hygiene compliance ($\geq 60\%$) the majority of vendors falls below 60%, only 40 % of vendors at Janet demonstrated good hygiene practices while $\geq 10\%$ vendors from Xipamanine, Janet and Malanga comply hygiene practices however overall compliance of the hygiene practices did not differ across the markets ($p=0.295$) (Table 3)

Table 3: Distribution of overall hygiene compliance categories by markets

Compliance Category	Xipamanine		Janet		Malanga		Adelina		Total	
	n	%	n	%	n	%	n	%	n	%
Poor ($<40\%$)	1	10.0	1	20.0	2	50.0	0	0.0	4	16.7
Moderate (40-59%)	8	80.0	2	40.0	2	50.0	4	80.0	16	66.7
Good ($\geq 60\%$)	1	10.0	2	40.0	0	0.0	1	20.0	4	16.7

4.2.7 Prevalence of *S. aureus* by markets and sample type

The results show that only 29.2 % (7/24) were positive for *S. aureus* from coconut, and 42.6% (n=20) were positive from contact surfaces. No significant differences in detection rates were observed for *S. aureus* ($p = 0.944$). Xipamanine market recorded the highest prevalence of *S. aureus*, with positive detection in 56% of vendors' hand samples and 50% of machine samples (Figure). Overall, *S. aureus* was detected in 38% (n=27) of the 71 samples analyzed.

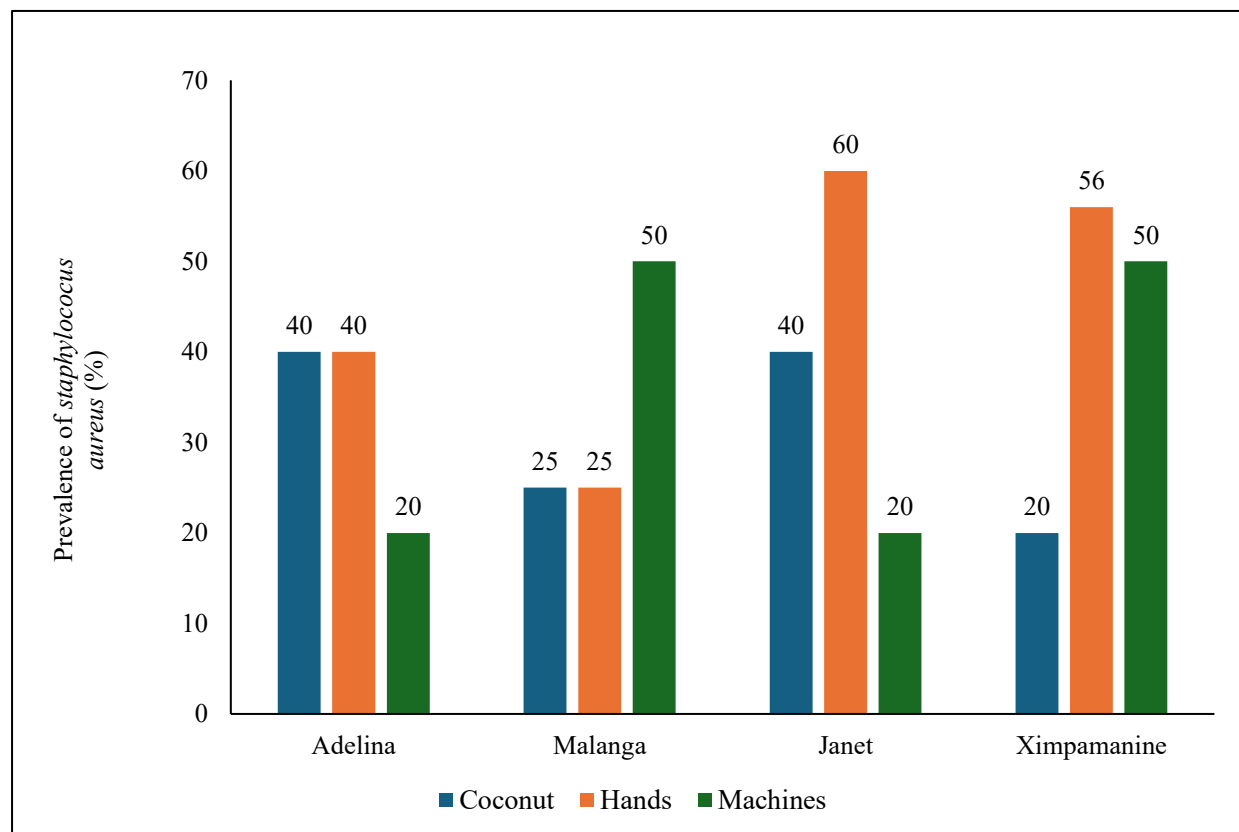


Figure 5: Prevalence of *Staphylococcus aureus* by market and sample type

4.2.8. Prevalence of *E. coli* in coconut and contact surface by markets

No coconut or surface samples from Xipamanine tested positive for *E. coli*. Only coconut samples from Adelina recorded *E. coli* prevalence of 60% (n=3) (Figure 6). The *E. coli* prevalence in coconut samples was 12.5% (3/24), and in contact surfaces was 14.9% (7/47). The inter-market variation was statistically significant ($p = 0.006$); 3 (60%) and 2 (50%) of the total machines sampled from Adelina and Malanga, respectively, were confirmed to be positive

for *E. coli*. Janet and Malanga recorded (20% and 25%, respectively) positive sample out of the total number of machines sampled.

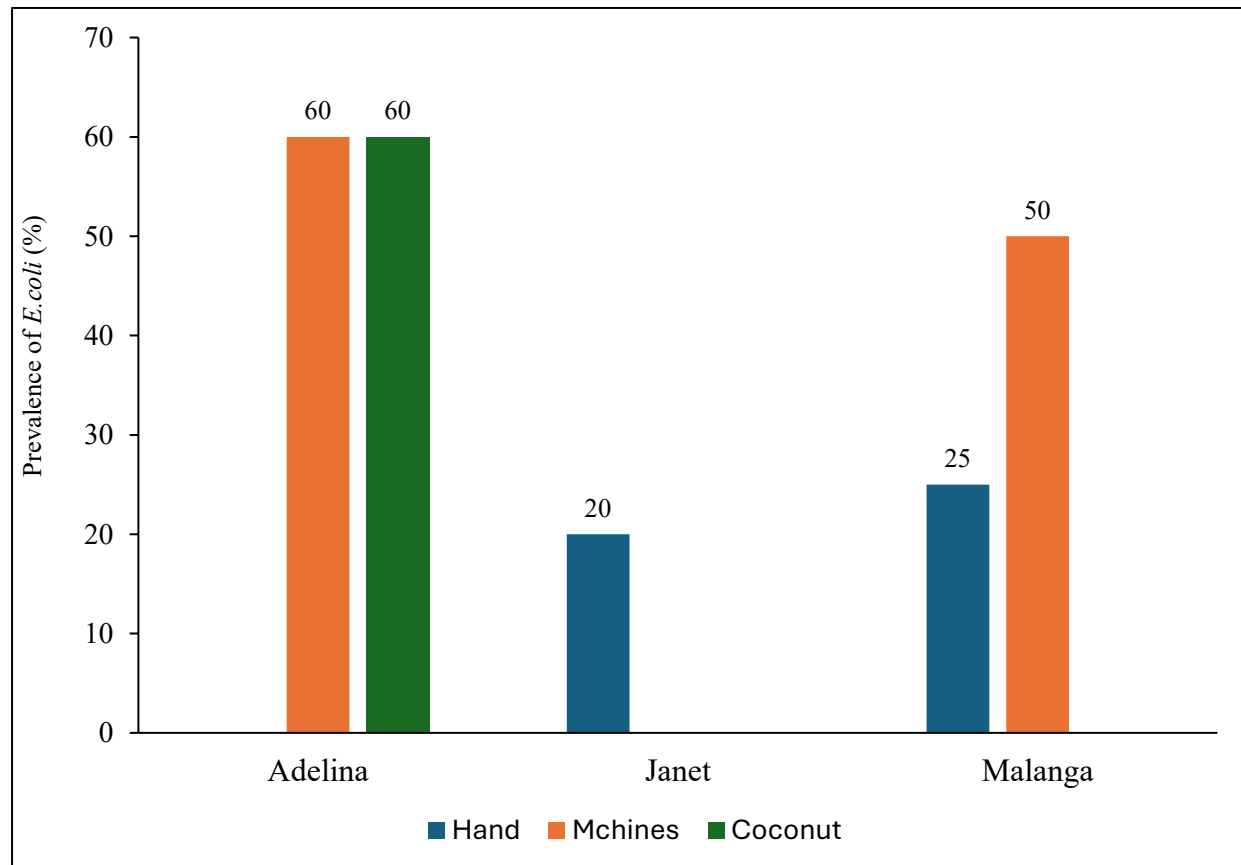


Figure 6: Prevalence of *E. coli* on coconut and contaminated surfaces by Market

4.4. Quantification of TMBC and *E. coli*

4.4.1 Mesophilic Bacteria and *E. coli* Counts

The results show that the Mesophilic bacteria were recovered from every one of the 71 samples. The Mesophilic Bacteria Counts levels of the grated coconut across the markets showed a mean of 3.67 log [CFU/g], with Janet having the lowest mean values, as seen in Appendix 7 and Figure 7. Vendors' hands and machines had mean values of 3.33 and 3.45 log [CFU/g], respectively, as compared to a standard mean of 2.0 log [CFU/g]. Xipamanine had the lowest mean of 3.19 and 3.30 log [CFU/g], for hand and machine, respectively. Generally, hand and machine have higher means above the standard limit, with machines having a higher mean in all the markets.

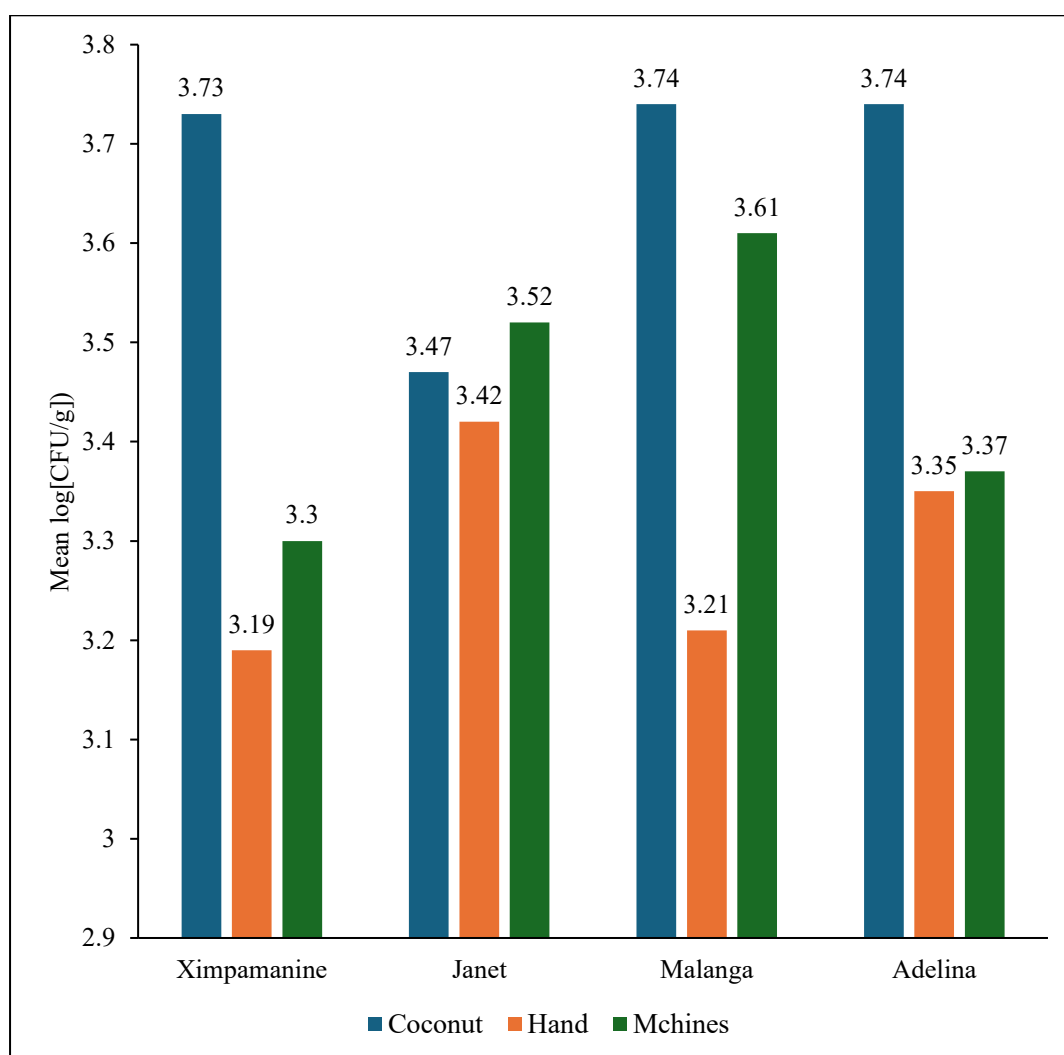


Figure 7: Total Mesophilic Bacteria Count in grated coconut and contact surfaces by market

4.4.2. *E. coli* count by market and sample type

To determine *E. coli* counts, only 10 of 71 samples (14.1%) were enumerated, as their colonies exhibited metallic-green on EMB agar. The occurrence of *E. coli* varied significantly across markets ($p = 0.001$). *Escherichia coli* was detected in coconut samples exclusively from Adelina market, with a mean count of 3.24 log [CFU/g]. Among environmental samples, the highest counts were recorded in machines and vendors' hands from Malanga market, reaching 2.74 log CFU/g, and 2.68 log CFU/g, respectively (Figure 8).

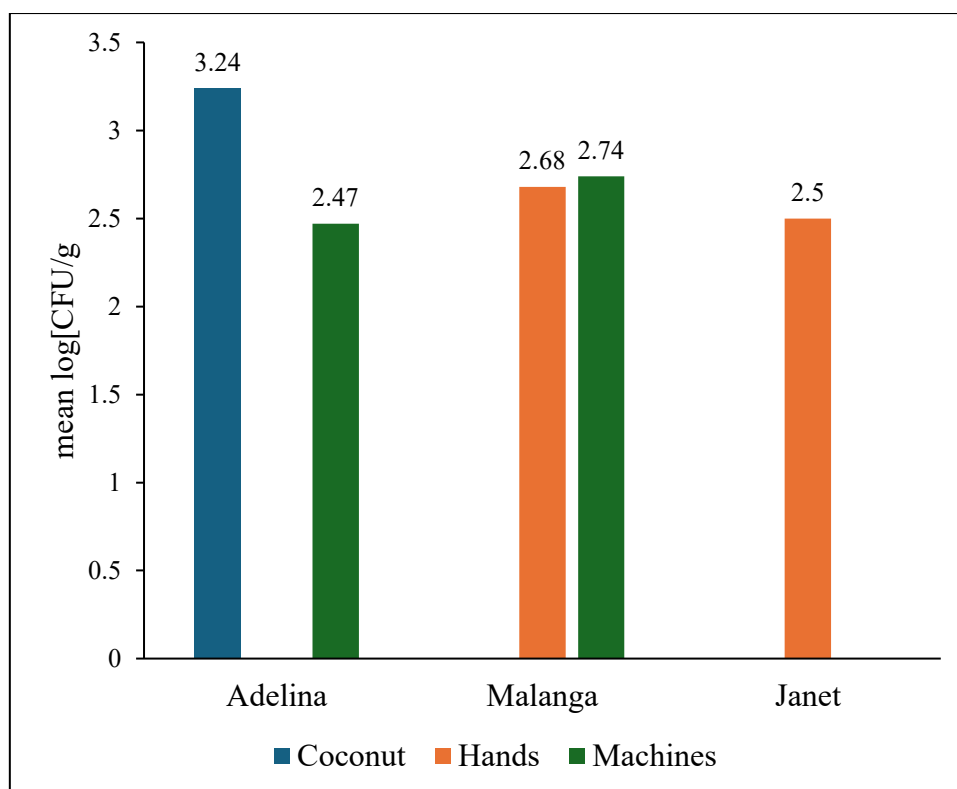


Figure 8: *E. coli* count by market and sample type

4.5 Classification of contamination levels against food safety standards

The 24 grated coconut samples were classified against 2 safety criteria: total mesophilic bacteria count (TMBC) using the standard for Coconut Water Processing, and *E. coli* levels per EU Commission Regulation (EC) No. 2073/2005, and *S. aureus* presence. To translate microbiological findings into actionable food safety assessments, the results showed that total mesophilic bacteria count falls within the acceptable category; only 5 samples were below the standard. For *E. coli*, only 3 samples (12.5%) exceeded the unsatisfactory limit, and for *S. aureus*, 7 samples did not meet the safety standard (**Error! Reference source not found.4**).

Table 4: Classification of grated coconut samples by market

Safety Criterion	Classification	N	Percentage (%)
TMBC	Acceptable ($\leq 3.7\log$ [CFU/g])	5	20.8
	Unsatisfactory ($> 3.7\log$ [CFU/g])	19	79.2
<i>E. coli</i>	Satisfactory ($\leq 2\log$ [CFU/g])	21	87.5
	Unsatisfactory ($> 2\log$ [CFU/g])	3	12.5
<i>S. aureus</i>	Satisfactory	17	70.8
	Unsatisfactory	7	29.2

5. DISCUSSION

5.1 Hygienic Conditions of Grated Coconut Vendors

The study found that the overall hygiene compliance among grated coconut vendors in the four Maputo markets was 48.6 %, indicating that vendors, on average, met fewer than half of the assessed hygiene criteria. The low mean hygiene score suggests that most vendors do not follow personal hygiene and sanitation practices, meaning they have not been trained in hygiene practices. This finding indicates that coconut is often processed (grated) under inadequate hygienic conditions, increasing the likelihood of bacterial contamination and potentially compromising the safety of the final product for humans.

The pattern of universal non-compliance with hand hygiene practices is particularly concerning, as direct hand contact with food is one of the major routes through which *S. aureus* is transferred to food products. This is especially relevant given that *S. aureus* colonizes the skin and nasal passages of approximately 20–50% of healthy individuals (Adams & Moss, 2008). The present finding correlates with a study done by Manhique *et al.*, (2019) in Maputo, who found that most of the vendors were not following hygiene practices and were not trained in hygiene. Similar observations have been documented in other sub-Saharan African settings. For example, Ndunguru & Ndossi (2020) found that poor hand hygiene among market vendors in Morogoro, Tanzania, was a primary contributor to *E. coli* and *S. aureus* contamination in ready-to-eat foods. *S. aureus* is a significant safety problem as it can cause skin infections such as boils, abscesses, cellulitis, impetigo, and folliculitis; it can be transmitted through the nose, hands, and the skin, etc. It is an enterotoxin which survives through heat stability and can cause the illness to the consumers.

The facility hygiene scores varied significantly across markets, whereas personal hygiene scores were comparable across all studied markets. This differential pattern suggests that while poor personal hygiene practices are uniformly pervasive across all four markets, reflecting a systemic lack of awareness or resources rather than market-specific failures, facility-level conditions vary considerably depending on the infrastructure and management of individual markets. Adelina market recorded the highest overall compliance ($56.4 \pm 3.7\%$), while Malanga recorded the lowest ($39.3 \pm 2.2\%$). This disparity aligns with observations made by World Bank (2020), which identified inadequate market infrastructure as a fundamental obstacle to food safety compliance in Mozambique's informal sector.

The absence of toilet facilities near all grating premises and the limited availability of adequate water facilities (89%) represent major structural barriers in maintaining proper hygiene. These deficiencies restrict vendors' ability to implement basic sanitation practices and may increase the risk of food contamination. In particular, lack of water or use of unsafe water to wash coconuts before grating can lead to contamination of coconuts with pathogens, which can cause foodborne diseases in humans. These findings are consistent with the study by Salamandane *et al.* (2022), who found high fecal contamination in different street water sources consumed in Maputo, which were associated with a lack of personal and environmental hygiene. The present study suggests that similar limitations in access to clean water and poor sanitation practices persist among grated coconut vendors, and this can create conditions that can facilitate fecal contamination of food products, as reported by Salamandane, Silva, Brito. Malfeito-Ferreira *et al.*, 2021) in Maputo city (Mozambique), and Okito *et al.* (2025) in Bukavi city (Democratic Republic of Congo).

One-third of vendors (33.3%) used grating machines with visible cracks, rust, or food residues, a condition that can promote biofilm formation and microbial proliferation whereby the pathogens such as *E.coli*, *Salmonella* and *Listeria monocytogenes* will attach themselves to moist areas or cracks of machine by forming extracellular matrix which prevent them from being cleaned and high possibility of causing cross-contamination, coconut spoilage during grating, expensive to be removed and at same time posing significant risks of food borne illness to consumers. This has also been discussed in the annual review of food science and technology biofilms in food processing environments, and a review of microbial biofilm in the food industry by Alvarez-Ordóñez *et al.*, (2019) and Carrascosa *et al.*, (2021) respectively, as a major factor that leads to microbial contamination and food spoilage during processing and storage. Also, processing machines have been found out to be reduced in efficiency as a result of biofilm formation, weakling its component parts. Furthermore, only 12.5% of vendors reported regular washing of their utensils and grating machines. These findings are of particular concern because food-contact surfaces represent critical points for cross-contamination and may act as persistent reservoirs of pathogenic microorganisms if not properly cleaned and maintained.

5.3 Identification of *S. aureus* and *E. coli* on contaminated surfaces and grated coconut

5.2.1 Prevalence of *S. aureus*

The prevalence of *S. aureus* is due to microbial contamination by vendors when handling food or contact surfaces. This is according to the study by (Moraes, 2023), who indicated that food and contact surfaces can be contaminated due to failure of hygiene practices by vendors. The

confirmed 29.2 % and 42.6% of *S. aureus* on coconuts and contact surfaces, respectively, indicated that nearly half of vendors' hands, grating machines, and a quarter of coconuts harbored the pathogen at the time of sampling. From the result, the presence of *S. aureus* in hand swabs, coconut samples, and machine swabs demonstrates that contamination is not limited to a single contact point but spans the entire processing chain (Moraes, 2023). Specifically, the detection of *S. aureus* on grating machines is of particular concern because machines are used continuously throughout the trading day and are rarely cleaned between use, as evidenced by the findings that only 12.5% of vendors washed their machines. Equipment surfaces with cracks or rust were observed during the study. In addition, the observed lack of mask use among vendors may have further increased the risk of contamination. This shows the existence of poor hygiene practices among vendors, which include poor personal hygiene, poor maintenance of the machines, and lack of sanitation facilities.

The detection of *S. aureus* in 38.0% of samples did not differ significantly across markets ($p = 0.944$). This means the risk of *Staphylococcus* contamination is widespread and not confined to a particular market setting. The pattern aligns with the uniform absence of personal hygiene practices observed across all four markets, as *S. aureus* is primarily transmitted through direct hand contact with food (Adams & Moss, 2008). The prevalence of *S. aureus* presented in this study is lower than the 58% reported by Ghosh *et al.* (2007) for coconut slices sold in India but higher than those reported by Ndunguru & Ndossi (2020) in Saudi Arabia (4.4%) and Tanzania (19.7%), respectively. The finding that *S. aureus* was more frequently detected on vendor hands (47.8%) than on coconut samples (29.2%) supports the assumption that hand-to-food transfer is a likely contamination pathway. This is consistent with the pathogen's known ecology as reported by Adams & Moss, (2008) and the report of Sari & Muhlisin (2019) on personal hygiene. In view of this, personal hygiene should be promoted among the vendors to reduce the extent of contamination among the fruit and transmission to the consumers.

5.2.2 Prevalence of *E. coli*

High prevalence of *E. coli* observed in 3 out of the 4 markets indicated that coconuts sold in these areas are not safe for consumption. The inter-market homogeneity is consistent with poor personal hygiene, poor machine maintenance, and lack of sanitation facilities, which support *E. coli* as a well-established indicator of faecal contamination. Its presence suggests compromised sanitary conditions, particularly involving water sources and vendors' hands (Coutinho & Neves, 2024; Salamandane, Vila-Boa, *et al.*, 2021). The presence of confirmed *E. coli* on vendor hands in Malanga and Janet markets suggests potential faecal transmission

through water. Similarly, Salamandane *et al.* (2022) found that 66% of 118 water samples from different sources, including street home-bottled water, municipal water distribution systems (tap water), and selected supply wells in several neighborhoods, were positive for *E. coli*. This can be a result of sewage contamination and could be associated with broad contamination of the coconut, machine, and vendors' hands as a result of poor water quality used by the vendors. Although the prevalence of *E. coli* in this study (14.1%) is lower than the report of Bukhari *et al.* (2021) in Saudi Arabia, who found that the prevalence of *E. coli* in contact surfaces was 17.7%. Still, there is a need for extensive analysis of the water sources across these markets to prevent contamination of hands, machines, and coconuts. Negative reports from Xipamanine and variation among the locations in terms of the prevalence of *E. coli* in the samples collected from the vendors' hands, coconuts, and machines do not justify the absence of *E. coli*. This is a result of poor personal hygiene, poor machine maintenance, and lack of sanitation facilities reported in all markets. Instead, this calls for intensive and thorough sampling techniques using an increased number of participants and variables (surfaces) to confirm the prevalence of the pathogen in the markets.

In this study, the presence of *E. coli* is different from the study done by (Fahim *et al.*, 2024) where they found that the prevalence of *E. coli* on contact surfaces and ready to eat foods was 9.38%. Hands, machines, and water are major sources of contamination, and this indicated that poor hygiene contributed to such prevalence (Giwa *et al.*, 2021). Also, this suggests that contact surfaces of various food processing machines can contribute to the prevalence of *E. coli* in foods. Differences reported between the markets in terms of the variables show that prevalence sometimes differs depending on the type of hygiene practices by different vendors and the food sample analyzed. A study done in Malaysia by Ramakarishnan (2016) reported a very high prevalence of *E. coli* in coconut milk. Although in this study, coconut milk samples were not analyzed, the prevalence of *E. coli* in grated coconut was low. This suggests future research should consider analyzing grated coconut and milk separately to compare and study the prevalence of *E. coli* in both samples. This is because coconut milk is made by adding water to grated coconut; this difference can be associated with the water used to make it and shows that coconut is a safe food naturally. The exposure to the environment and the processing facilities are the principal routes to the result observed in this study.

5.3 Indicators count

5.3.1 Total mesophilic bacteria count

Mesophilic bacteria are indicators that show poor hygiene, deterioration, sanitation failures, in processing methods, and safety and quality. All samples in this study were positive for total mesophilic bacteria count (TMBC), indicating widespread environmental contamination across the study sites because processing of grated coconut is done at temperatures favorable for mesophilic growth (Sibanyoni & Tabit, 2019). Adelina, Malanga, and Xipamanine markets recorded the highest microbial counts, whereas Janet market recorded the lowest TMBC. Although the overall mean value of the TMBC in coconut was found within the limit ($3.67 \log [\text{CFU/g}] \leq 3.70 \log [\text{CFU/g}]$) according to the standard documented by (Rolle, 2007), the highest mean observed in the 3 locations suggests high contamination levels of grated coconut.

In this study, all samples from hands and machines were above the standard limit, indicating unsatisfactory hygienic conditions during processing. These findings suggest that vendors' hands and grating machines may serve as the major sources of microbial contamination and could contribute to the elevated bacterial loads observed in grated coconut samples. This is similar to the findings of Giwa *et al.* (2021), where they reported high TMBC relative to high prevalence of pathogens in the samples collected from street-food vendors' hands and their processing equipment. Handling and processing equipment has the potential to harbor and spread pathogens in different cycles of processing. Therefore, it is essential to ensure personal hygiene and effective maintenance and sanitation of the handling/processing equipment.

Environmental conditions like relative humidity and temperature have been found to support pathogen survival and microbial contamination of food. High temperatures between 20°C to 45°C have been found to influence microbial growth, as documented in some studies (MaLak & Nevee Soliman Mohamed Soliman, 2021; Zhao *et al.*, 2022). This might also contribute to the spread of these pathogens, as coconuts were kept under the sun and in unhygienic conditions in the market studied in Maputo. Apart from temperature and unhygienic conditions, high microbial counts can also be associated with high moisture contents and fats in coconut, which provide a good environment for TMBC (Ebisintei *et al.*, 2024; Si Win, 2022). This is similar to the study by Lamdande *et al.* (2018) and Ramakrishnan (2016), who indicated that high TMBC is influenced by moisture content, making coconut vulnerable to bacteria. These factors were prevalent in the market and could contribute to the high TMBC.

The high microbial counts in grated coconuts and coconut surfaces have been associated with an unhygienic environment, lack of clean water, and sanitation facilities. Most markets had no

water to wash their hands and machines, and practiced open refuse disposal. An unhygienic environment promotes the growth of bacteria and supports flies as a vector that spreads and contaminates more surfaces used in food processing (Berihu *et al.*, 2024; Borrusso & Quinlan, 2017; Salamandane *et al.*, 2023). This can lead to longitudinal spread of the pathogens in the market within a short period of time, hence influencing the high TMBC in grated coconut. Similar associations between poor hygienic conditions and elevated microbial loads in informal markets and street-vended foods have been reported in a global review of antimicrobial-resistant *Staphylococcus spp.*, *Escherichia coli*, and *Salmonella spp.* in food handlers (Viana *et al.*, 2025; Zerabruk *et al.*, 2019). This shows that microbial counts in food are a result of both personal and environmental hygiene, which acts as a significant step in the prevention and control of foodborne disease.

5.3.2 *E. coli* counts

The confirmed *E. coli* counts were found to be high in the samples across the markets. This shows that there is a high prevalence of faecal contamination across the markets. *E. coli* is widely recognized as an indicator of faecal contamination arising from the gastrointestinal tract (Govindarajan *et al.*, 2020; Okeke, 2009). Its transmission is a result of poor personal hygiene and a lack of sanitation facilities in the environment (Mugampoza *et al.*, 2013; Okito *et al.*, 2025). The mean of 3.24 log CFU/g reported in this study is above the 3.0 log CFU/g of the maximum limit recommended by Commission Regulation (EU, 2007). In another study done by Nurul *et al.* (2017) found that the samples of coconut milk contaminated with *E. coli* ranged from 2.4 to 10.7 MPN/ml and were attributed to lack of hygiene as well. From the present study, it was observed that most vendors do not wash their hands regularly, cut their nails, or wear face masks when handling the coconut. Washing and maintenance of the machine were found to be on the lower side as well, making the machine a transmission channel for the pathogen. All these conditions are due to poor personal hygiene, lack of quality water, and poor sanitation facilities for the vendors. These gaps have been reported by some studies (Giwa *et al.*, 2021; Moraes, 2023; Nurul *et al.*, 2017), which implies that, even when vendors wash their hands and machines regularly, there is a possibility of microbial contamination as a result of unclean water. Therefore, the high counts of *E. coli* suggest that grated coconut produced and handled in these markets could harbor foodborne diseases.

5.4 Classification of Microbial load against food safety standards

The classification of microbial load present in the grated coconut samples in comparison with food safety standards highlights substantial microbiological quality concerns. A larger

percentage of the analyzed samples, including grated coconut, vendor's hands, and the machines, do not comply with the acceptable limit for TMBC, indicating that the majority of coconut samples exceeded the standard limits reported by EU (2007) and (Rolle) 2007), that suggests poor personal hygiene and lack of good sanitation may have contributed to the high TMBC loads observed in the samples. The high prevalence of the pathogens and high count of TMBC and *E. coli* reported in this study is a significant public health concern, as it indicates that consumers purchasing grated coconut from certain vendors may be exposed to foodborne diseases. Unclean environment, low access to quality water, and faecal contamination are prominent across all these markets. These factors have been reported in many studies as major sources of foodborne disease (Salamandane, Vila-Boa, *et al.*, 2021; Salamandane *et al.*, 2021; Yeleliere *et al.*, 2017).

The prevalence of *S. aureus* in 29.2% of samples reduced the proportion of samples classified as “Overall Safe” to 62.5% when all three criteria were considered simultaneously. Generally, *S. aureus* is not supposed to be found in food, despite only 7 samples being positive for it. This finding underscores the advantages of using more than one microbiological indicator for safety assessment, as general hygiene indicators such as TMBC may mask the presence of specific pathogens (Fetsch *et al.*, 2026). Although this study does not estimate the count of *S. aureus*, its prevalence under different identification techniques is a starting point for further analyses on grated coconuts. Therefore, frequent microbial evaluation is needed to assess safety and the quantification of this pathogen in grated coconut.

The inter-market variation in overall safety classification was low due to a lack of basic facilities that ensure a healthy and safe environment for the vendors and the consumer. Xipamanine market achieved the highest proportion of safe samples (80.0%), despite having the largest number of vendors, while the Adelina market recorded the lowest (20.0%), largely due to *E. coli* contamination. This finding is somewhat contradictory, as Adelina market recorded the highest facility hygiene compliance score (56.4%). However, this apparent inconsistency may be explained by the fact that *E. coli* contamination is more strongly associated with water quality, sanitation conditions, and faecal contamination sources than with visible facility cleanliness (Kipelo *et al.*, 2025; Salamandane, Vila-Boa, *et al.*, 2021). Consequently, good surface-level cleanliness does not necessarily reflect the number of times the surface was cleaned; rather, it is the quality of the water and other materials used in cleaning during processing.

The absence of national microbiological standards for minimally processed coconut products in Mozambique represents a significant regulatory gap, as vendors can process coconut any how and sell grated coconut which are infected with food pathogens. The present study relied on the Coconut Water Processing for TMBC limit (Rolle, 2007) and the European Union Commission Regulation (EC) No. 2073/2005 as the reference standard for *E. coli*, and Codex Alimentarius guidance for general microbiological limits (CXS, 2023; EU Commission, 2007). This regulatory gap means that vendors are not held to any specific national and international standard, and enforcement agencies lack the appropriate benchmarks necessary for surveillance and compliance monitoring. The development of Mozambique-specific standards for minimally processed coconut products, informed by baseline data such as those generated by this study, would represent an essential step toward strengthening food safety governance and consumer protection within this informal food sector.

6. CONCLUSION, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusions

- a. The study established that hygiene compliance among grated coconut vendors in Maputo markets was inadequate.
- b. The total mesophilic bacteria count was high in most of the markets
- c. The prevalence of *E. coli* in grated coconut and contact surfaces was confirmed to be related to faecal contamination due to poor personal hygiene practices by the vendor, an unclean environment, and poor water quality used during coconut processing.
- d. The prevalence of *Staphylococcus aureus* in grated coconut and contact surfaces was also related to an unclean environment where the coconuts are kept and processed in all the markets.
- e. All TMBC from all contact surfaces were above the limit, posing a threat of foodborne-related disease outbreaks among consumers. This is due to widespread contamination and transmission of the pathogens by the vendors and machines used in the handling and processing of the coconut.

6.2 Recommendations

6.2.1 Recommendations for future research

- a. Extend this assessment to other Mozambican cities and to other minimally processed food products sold under similar informal market conditions to understand microbial contamination in those foods.
- b. Include yeast and mold and aflatoxin analysis, as the residues in machines can contribute to their presence,
- c. Also, molecular analysis to identify *E. coli* species that are found in coconut
- d. Assess seasonal variations in humidity, temperature, and market activity, which may influence microbial contamination levels.
- e. Evaluate the effectiveness of hygiene education in improving vendors' handling practices of grated coconut and in reducing microbial contamination of fresh grated coconut.

6.2.2 Recommendations for vendors

- a. Vendors should follow personal hygiene when handling coconut
- b. Vendors should be required to replace grating machine components that exhibit visible cracks, rust, or food residue accumulation, as such surfaces promote biofilm formation and resist routine cleaning

6.2.3 Recommendations for governments

- a. Municipal market authorities and the Ministry of Health (MISAU) should install permanent handwashing stations with soap and clean water at each grated coconut vending site within the four markets. Such action would thereby reduce hand-mediated pathogen transfer to food products.
- b. The National Inspectorate of Economic Activities (INAE) and MISAU should mandate periodic food safety training for all grated coconut vendors, focusing on Good Hygiene Practices that would strengthen the capacity of informal food vendors to meet basic regulatory expectations.
- c. The Ministry of Health and municipal water and sanitation authorities should investigate and improve the quality of water sources available to grated coconut vendors, to reduce faecal contamination of food products. The National Institute for Standardization and Quality (INNOQ), in collaboration with MISAU, should develop and promulgate national microbiological standards specifically for minimally processed coconut products, including maximum permissible limits for *E. coli* and *S. aureus*, for enforcement and routine surveillance.

6.3 Limitations

The following limitations were encountered during the study:

- a. One hand swab sample from Vendor 1 at Xipamanine market (first batch) could not be collected, reducing the total hand swab samples to 23 instead of the targeted 24. This reduced the total number of successfully collected samples from the intended 72 to 71.
- b. The qualitative assessment of *S. aureus* (present/absent based on coagulase testing) rather than quantitative enumeration limits the ability to compare contamination levels against numerical concentration limits. This presented a constraint in interpreting the *S. aureus* findings for objective 4 (classification against standards), since only mesophilic bacteria and *E. coli* counts could be compared with numerical food safety limits.

- c. Cross-sectional study design captured microbiological conditions at a single point in time. Seasonal variations in humidity, temperature, and market activity, which may influence microbial contamination levels, could not be assessed.
- d. This study could not employ a follow-up intervention in which vendors could be trained on good hygiene practices and subsequently reassessed to determine whether the training resulted in sustained improvements in hygiene and reductions in microbial contamination.

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8. APPENDICES

APPENDIX 1: CHECKLIST FOR FOOD HYGIENE AND SAFETY

CHECKLIST A: GRATED COCONUT HANDLERS/ PERSONAL HYGIENE

(The checklist was adapted with modifications from (Glória *et al.*, 2019; Jan Mei, 2019)

No	Criteria	Yes	No
1	Does the coconut vendor have any visible cuts, spots, or skin disease symptoms on their hands?		
2	Do coconut vendors have short and clean nails?		
3	Do vendors wear hairnets during the coconut processing?		
4	Do vendors wash their hands with soap and water before handling food?		
5	Do coconut vendors smoke while handling food?		
6	Do vendors wear clean clothing/uniform/apron during coconut handling (PPE)?		
7	Does coconut vendors chew gum while handling the products?		
8	Is hand sanitizer available and used by vendors?		
9	Does the vendor sneeze, cough, or touch their face while processing coconut?		
10	Does the vendors handle the products while he/she is ill?		
11	Does the vendor used towel or air dryers for drying hands while processing coconut (headscarf/turban)?		
12	Does the coconut vendor cover their hands with gloves?		

CHECKLIST B: COCONUT GRATING FACILITIES, STORAGE, AND GENERAL HYGIENE

(The checklist was adopted with modification from (Glória *et al.*, 2019; Jan Mei, 2019)

Name:

Code:

Gender: Male ☐

Female ☐

No	Criteria	Yes	No
1	Are the coconuts prevented from sun, wind, and dust?		
2	Is the environment free from pests and flies?		
3	Is the toilet nearby the premises of grating coconut?		
4	Are the coconut grating facility maintained in a clean condition?		
5	Are there any adequate water facilities in the market?		
6	Is processed water store in clean containers?		
7	Do the vendors dispose off wastewater properly without causing environment contamination?		
8	Are utensils free from visible dirt, grease, or damage?		
9	Are there visible cracks, rust, or food residues on the machine surfaces?		
10	Is the sewage system in good condition and free from contaminants?		
11	Is the coconut area kept clean and in good order?		
12	Does the vendor use clean water to wash utensils?		
13	Do the vendors wash utensil and machine regularly?		
14	Do the vendors stored equipment safely to prevent contaminants?		
15	Do the vendors stored the coconut in good condition to prevent spoilage?		
16	Is the machine well dried before being stored?		
17	Do the vendors prepared grated coconut and serve immediately?		
18	Do the vendors use clean plastics to serve the grated coconut?		
19	Does coconut grate from the shell?		
20	Are there any facilities to stored left-over grated coconut?		
21	Do the vendors dispose garbage regularly and stored away from food handling area?		
22	Is there any damage in the machine part during coconut processing?		

APPENDIX 2: HUMAN-INFORMED CONSENT FORMS

HUMAN INFORMED CONSENT FORM

Researcher: Nasserah Fatmata Koroma

Title of Project: Assessment of Microbiological Safety of Grated Coconut Sold in Maputo Markets.

Greetings, I am Nasserah Fatmata Koroma from Eduardo Mondlane University. I am researching on hygiene and safety practices on grated coconut. I kindly seek your voluntary participation in my research. I would appreciate the key insights that your participation would add to my study.

Purpose of the Research:

To assess the microbiological safety of grated coconut sold in Maputo markets with a focus on environmental assessment, safety and quality checks on hygiene practices by coconut vendors.

The data collection process is expected to take about 15-25 minutes

Potential Risks of the study:

I assure you that there is no potential risk related to this study from the procedures that will be handled in this study

Voluntary Participation:

All the records will be stored securely and confidentially.

Participation in this study is completely voluntary. Participants are at will to stop participating at any time, and they are free not to answer or respond to specific questions that they are not comfortable with.

Confidentiality

All participant identities will be kept anonymous, and the collected information will solely be used for the intended purpose of academics; the information collected will be stored safely with encryptions so ensure its safety.

By signing this form, you, the participant, consent that you have understood and are satisfied with the information provided and that you consent to taking part in this study.

Additionally, if you have any concerns, please do not hesitate to contact the lead researcher, Naserah Fatmata Koroma, at naserahfatmatakoroma@gmail.com or by telephone at 878629179.

Participant Name/ ID:.....

Signature/ Thumb print:.....

APPENDIX 3: PERSONAL HYGIENE PRACTICES BY MARKET YES RESPONSES N (%)

Item	Xipamanine (n=10)	Janet (n=5)	Malanga (n=4)	Adelina (n=5)	p-value
Visible cuts/skin disease on hands	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0.167
Short and clean nails	4 (40.0)	3 (60.0)	0 (0.0)	1 (20.0)	0.312
Wears hairnets during processing	6 (60.0)	0 (0.0)	1 (25.0)	0 (0.0)	0.029
Handwashing with soap before handling	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Smoking while handling coconut	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Clean clothing/uniform/apron (PPE)	6 (60.0)	1 (20.0)	2 (50.0)	4 (80.0)	0.308
Chewing gum while handling	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Hand sanitizer available and used	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Sneezing/coughing/touching face	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Handling products while ill	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Uses towel/air dryer for hands	0 (0.0)	1 (20.0)	0 (0.0)	2 (40.0)	0.130
Covers hands with gloves	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Covers hands with mask	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
<i>Note: p-values from Fisher's exact test. Significance at $p < 0.05$.</i>					
<i>Source: Field survey data, 2025</i>					

APPENDIX 4: FACILITY HYGIENE AND SANITATION CONDITIONS OF GRATED COCONUT VENDORS

Facility Hygiene Item	Yes n (%)	No n (%)
Coconuts protected from sun/wind/dust	12 (50.0)	12 (50.0)
Environment free from pests/flies	12 (50.0)	12 (50.0)
Toilet nearby grating premises	0 (0.0)	24 (100.0)
Grating facility clean	10 (42)	14 (58.3)
Adequate water facilities in market	1 (4.2)	23 (95.8)
Processed water in clean containers	2 (8.3)	22 (91.7)
Proper wastewater disposal	9 (37.5)	15 (62.5)
Utensils free from dirt/grease/damage	7 (29.2)	17 (70.8)
Visible cracks/rust on machine	8 (33.3)	16 (66.7)
Sewage system in good condition	12 (50.0)	12 (50.0)
Coconut area clean and orderly	14 (58.3)	10 (41.7)
Clean water used to wash utensils	5 (20.8)	19 (79.2)
Utensils and machines washed regularly	3 (12.5)	21 (87.5)
Equipment stored safely	16 (66.7)	8 (33.3)
Coconut stored properly	20 (83.3)	4 (16.7)
Machine dried before storage	22 (91.7)	2 (8.3)
Grated coconut served immediately	24 (100.0)	0 (0.0)
Clean plastics used for serving	23 (95.8)	1 (4.2)
Coconut grated from shell	24 (100.0)	0 (0.0)
Facilities for leftover storage	2 (8.3)	22 (91.7)
Garbage disposed regularly	11 (45.8)	13 (54.2)
Machine damage during processing	8 (33.3)	16 (66.7)

APPENDIX 5: FACILITY HYGIENE CONDITIONS BY MARKET YES RESPONSES [N (%)]

Item	Xipamanine (n=10)	Janet (n=5)	Malanga (n=4)	Adelina (n=5)	p-value
Coconuts protected from sun/wind/dust	2 (20.0)	4 (80.0)	3 (75.0)	3 (60.0)	0.107
Environment free from pests/flies	4 (40.0)	3 (60.0)	0 (0.0)	5 (100.0)	0.015
Toilet nearby grating premises	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	.
Grating facility clean	4 (40.0)	2 (40.0)	0 (0.0)	4 (80.0)	0.153
Adequate water facilities in market	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	0.583
Processed water in clean containers	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	0.094
Proper wastewater disposal	3 (30.0)	1 (20.0)	0 (0.0)	5 (100.0)	0.008
Utensils free from dirt/grease/damage	3 (30.0)	1 (20.0)	0 (0.0)	3 (60.0)	0.327
Visible cracks/rust on machine	4 (40.0)	3 (60.0)	0 (0.0)	1 (20.0)	0.312
Sewage system in good condition	4 (40.0)	3 (60.0)	0 (0.0)	5 (100.0)	0.015
Coconut area clean and orderly	4 (40.0)	4 (80.0)	1 (25.0)	5 (100.0)	0.044
Clean water used to wash utensils	0 (0.0)	4 (80.0)	0 (0.0)	1 (20.0)	0.003
Utensils and machines washed regularly	1 (10.0)	2 (40.0)	0 (0.0)	0 (0.0)	0.308
Equipment stored safely	9 (90.0)	2 (40.0)	1 (25.0)	4 (80.0)	0.047
Coconut stored properly	9 (90.0)	4 (80.0)	3 (75.0)	4 (80.0)	0.894
Machine dried before storage	9 (90.0)	4 (80.0)	4 (100.0)	5 (100.0)	1.000
Grated coconut served immediately	10 (100.0)	5 (100.0)	4 (100.0)	5 (100.0)	.
Clean plastics used for serving	10 (100.0)	5 (100.0)	4 (100.0)	4 (80.0)	0.583
Coconut grated from shell	10 (100.0)	5 (100.0)	4 (100.0)	5 (100.0)	.
Facilities for leftover storage	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	0.094
Garbage disposed regularly	3 (30.0)	3 (60.0)	0 (0.0)	5 (100.0)	0.009

Machine damage during processing	2 (20.0)	4 (80.0)	0 (0.0)	2 (40.0)	0.058
<i>Note: p-values from Fisher's exact test. Significance at $p < 0.05$.</i>					
<i>Source: Field survey data, 2025</i>					

APPENDIX 6: KRUSKAL-WALLIS TEST COMPARING HYGIENE COMPLIANCE SCORES ACROSS MARKETS

Hygiene Component	Chi-square (H)	Degree of freedom(df)	p-value
Personal Hygiene Score	3.81	3	0.282
Facility Hygiene Score	8.30	3	0.040
Overall Hygiene Score	9.86	3	0.020

Note: Kruskal-Wallis H test with ties adjustment. $df = 3$. Significance at $p < 0.05$.

APPENDIX 7: TMBC ACROSS THE MARKETS

Markets	Coconut (log [CFU/g])	Hand (log [CFU/g])	Machine (log [CFU/g])
Standard	3.70	2.00	2.00
Adelina	3.74	3.50	3.37
Janet	3.47	3.42	3.52
Malanga	3.74	3.21	3.61
Xipamanine	3.73	3.19	3.30
Mean	3.67	3.33	3.45

Appendix 8

The above figures a and b show the sample collection of grated coconut and swabs, and figures c and d show the laboratory analysis. Positive *E. coli* was confirmed by the Kovacs indole test, and *S. aureus* positive were confirmed by rabbit plasma for coagulase.

