UNIVERSIDADE
EDUARDO
MONDLANE

Faculty of Veterinary Science

Master in Food Safety

Assessment of Microbiological Safety and Antidiarrheal Effect of Cassava-Based Mahewu (Non-Alcoholic Fermented Beverage) in Maputo Province, Mozambique

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MAPUTO

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MAPUTO

July 30,2026

DECLARATION

I, Aminata Abdul Kamara declare that this dissertation titled: "Assessment of Microbiological Safety and Antidiarrheal Effect of Cassava-Based Mahewu (Non-Alcoholic Fermented Beverage) in Maputo Province, Mozambique" 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 of Eduardo Mondlane University.

Signature: ________________________ **Date: ________/________/__________**

Aminata Abdul Kamara

RECOMMENDATION

This dissertation has been submitted for examination with our approval as University and Technical supervisors.

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Faculty of Science, Department of Biological Sciences

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Prof. Doutor Belisário Tomé Moiane

Veterinary Faculty, Department of Animal Production and Food Technology

Signature __________________________ **Date: ________/________/__________**

ACKNOWLEDGEMENTS

I would like to appreciate God Almighty for granting me strength, wisdom, perseverance, and guidance throughout this journey and for making the successful completion of this work possible. My deepest gratitude to my parents, Mr. Abdul Kamara and Mrs. Hannah Josephine Kamara, siblings, and family for their unconditional love, prayers, encouragement, and unwavering support throughout my studies. Their belief in me has always been a great source of strength and inspiration.

I would like to express my sincere appreciation to my supervisors, Prof. Blisario Tome Moane and Prof. Elsa Maria Salvador, for their invaluable guidance, mentorship, patience, and continuous encouragement throughout this study. Their revision and constructive feedback, and academic support, greatly contributed to the successful completion of this research.

Special appreciation to the Food Technology Laboratory, where the laboratory analyses for this research were conducted. Special thanks go to Eng. Joaquim for his technical assistance, guidance, and support during the laboratory work. His contribution was instrumental to the successful completion of this study.

Additionally, I would like to acknowledge my colleagues in the MSc. Food Safety program, especially Marie, Maria, Fatimata, Abel, and Goliath, for their academic support, collaboration, and shared experiences throughout this journey. Their encouragement and teamwork created a supportive and inspiring learning environment. Also, my dear friends, Kolawole, Martha, Gloria, Simpson and Stephen, for their constant encouragement, motivation, and unwavering support throughout my academic journey. Their friendship and support helped me remain focused and resilient during challenging moments.

Finally, I want to thank the Center of Excellence in Agri-Food System and Nutrition (CE-ASFN) who made this programme achievable, and I am truly grateful for the opportunity given me to complete my master’s programme via this initiative. My heartfelt appreciation also goes to the World Bank for their support, funding this project.

DEDICATION

I dedicate this thesis to my beloved parents, Mr. Abdul Kamara and Mrs. Hannah Josephine Kamara, whose unwavering love, sacrifices, prayers, and encouragement have been the foundation of my academic journey. Your support and belief in me have been my greatest source of strength and inspiration.

To my dear brothers Foday Kamara, Alhaji Dukuray, and Mamudu Dukuray thank you for your constant support, motivation, and belief in my ability to succeed.

To my beloved sisters Abibatu Kamara, Isha Kamara, Anita Kamara, Isatu Kamara, Haja Dukuray, Dankay Sesay, and Mariama Sesay your love, encouragement, and prayers have continually uplifted me throughout this journey.

I also dedicate this work to my beloved children, Jamelatu Karina and Tetty Hannah Josephine, who have been a constant source of joy, hope, and motivation. Your presence in my life inspires me to strive for excellence and perseverance in all that I do.

This achievement is dedicated to all of you with profound love, gratitude, and appreciation. Your support has made this accomplishment possible.

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LIST OF ABBREVIATIONS AND ACRONYMS

BPW	Buffered Peptone Water
CFU/mL	Colony-Forming Units Per Millilitre
CIBS	Comité Institucional de Bioética em Saúde
CSV	Comma-Separated Values
DCA	Animal Science Directorate
EMB	Eosin Methylene Blue
FAO	Food and Agriculture Organization
IIAM	National Institute of Agrarian Research
INAE	National Inspectorate of Economic Activities
INNOQ	*Instituto Nacional de Normalização e Qualidade*
LAB	Lactic Acid Bacteria
MSA	Mannitol Salt Agar
NCG	Negative Control Group
PCA	Plate Count Agar
PCG	Positive Control Group
PDA	Potato Dextrose Agar
RVS	Rappaport–Vassiliadis Soya
SDG	Standard Drug Group
SS	Salmonella–Shigella
TAMC	Total Aerobic Mesophilic Bacteria
TG	Treatment Group
TSI	Triple Sugar Iron
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

ABSTRACT

Cassava-based mahewu is a traditional non-alcoholic fermented beverage produced and consumed, primarily in informal settings. Despite its growing consumption, no study has investigated its microbiological safety and antidiarrheal effect in Maputo province of Mozambique from one production unit. This study aims to assess the microbial safety and antidiarrheal properties of cassava-based mahewu produced in Maputo, Mozambique. Experimental laboratory-based design employed comprising two complementary components. For the microbiological assessment, twenty-five samples of cassava-based mahewu (250 mL each) were analyzed for *E. coli, Salmonella*, yeast and mould, *Staphylococcus aureus,* and total mesophilic bacteria count following ISO-referenced methods. On the other hand, antidiarrheal evaluation comprised of three independent experiments, each using thirty adult male BALB/c mice randomly allocated to six different groups: treatment group 1 (TG1) receiving castor oil and 3ml/kg mahewu, treatment group 2 (TG2) receiving castor oil and 5ml/kg mahewu, treatment group 3 (TG3) receiving castor oil and 10ml/kg mahewu, standard group (SDG) receiving castor oil and loperamide, positive control group (PCG) receiving castor oil and food and water, and negative control receiving food and water only to check for diarrhoea onset latency, stool frequency and consistency, body weight changes, and food and water intake. Data were analyzed using Stata version 17.0, using one-way ANOVA, two-way ANOVA, and non-parametric Kruskal-Wallis tests. Results show that cassava-based mahewu was free from *Salmonella* s*pp*, *E. coli* and *S. aureus* across all 25 samples. The overall mean total aerobic mesophilic count was 2.88 log_{10} CFU/mL and the overall mean of yeast and mould count was 3.7 log_{10} CFU/mL. The antidiarrheal evaluation showed that cassava-based mahewu at 10 mL/kg demonstrated the highest antidiarrheal property. There was significant difference in antidiarrheal effects between TG2, TG3, and SDG compared with PCG ($p<0.001$) and no significant difference observed between TG1 and PCG ($p=0.1$). Overall, cassava-based mahewu showed a good microbiological safety profile, with 88 % of the samples fulfilling bacterial pathogen regulations. The product demonstrated antidiarrheal effects at the doses tested. Food safety regulators must develop product-specific microbiological standards and producers must adopt standardized hygiene protocols during production. Future research should investigate higher doses to further evaluate the functional health potential of this beverage.

Keywords: *Antidiarrheal effect, cassava-base mahewu, Maputo, microbial safety, Mozambique.*

RESUMO

O mahewu à base de mandioca é uma bebida tradicional fermentada não alcoólica, produzida e consumida principalmente em contextos informais. Apesar do seu consumo crescente, nenhum estudo avaliou a sua segurança microbiológica e o seu efeito antidiarreico na província de Maputo, em Moçambique, a partir de uma única unidade de produção. Este estudo teve como objetivo avaliar a segurança microbiológica e as propriedades antidiarreicas do mahewu à base de mandioca produzido em Maputo, Moçambique. Foi utilizado um desenho experimental laboratorial, constituído por dois componentes complementares. Para a avaliação microbiológica, foram analisadas vinte e cinco amostras de mahewu à base de mandioca, com 250 mL cada, quanto à presença de E. coli, Salmonella, leveduras e bolores, Staphylococcus aureus e à contagem total de bactérias mesófilas, de acordo com métodos de referência da ISO. Por outro lado, a avaliação do efeito antidiarreico compreendeu três experiências independentes, cada uma utilizando trinta ratos BALB/c machos adultos, distribuídos aleatoriamente por seis grupos: grupo de tratamento 1 (TG1), que recebeu óleo de rícino e 3 mL/kg de mahewu; grupo de tratamento 2 (TG2), que recebeu óleo de rícino e 5 mL/kg de mahewu; grupo de tratamento 3 (TG3), que recebeu óleo de rícino e 10 mL/kg de mahewu; grupo padrão (SDG), que recebeu óleo de rícino e loperamida; grupo de controlo positivo (PCG), que recebeu óleo de rícino, alimentos e água; e grupo de controlo negativo, que recebeu apenas alimentos e água. Foram avaliados o tempo até ao início da diarreia, a frequência e a consistência das fezes, as alterações do peso corporal e o consumo de alimentos e água. Os dados foram analisados utilizando o Stata versão 17.0, recorrendo à ANOVA de uma via, ANOVA de duas vias e ao teste não paramétrico de Kruskal-Wallis. Os resultados mostraram que o mahewu à base de mandioca estava isento de Salmonella spp., E. coli e S. aureus em todas as 25 amostras. A contagem média global de bactérias mesófilas aeróbias totais foi de 2,88 log_{10} UFC/mL, enquanto a contagem média global de leveduras e bolores foi de 3,7 log_{10} UFC/mL. A avaliação do efeito antidiarreico mostrou que o mahewu à base de mandioca, na dose de 10 mL/kg, apresentou a maior propriedade antidiarreica. Foram observadas diferenças significativas nos efeitos antidiarreicos entre os grupos TG2, TG3 e SDG em comparação com o PCG ($p<0,001$), enquanto não foi observada diferença significativa entre TG1 e PCG ($p=0,1$). De forma geral, o mahewu à base de mandioca apresentou um bom perfil de segurança microbiológica, com 88 % das amostras a cumprirem os requisitos relativos aos agentes patogénicos bacterianos. O produto demonstrou efeitos antidiarreicos nas doses testadas. As autoridades responsáveis pela segurança alimentar devem desenvolver normas microbiológicas específicas para este produto, e os produtores devem adotar protocolos de higiene padronizados durante a produção. Estudos futuros deverão investigar doses mais elevadas para avaliar melhor o potencial funcional desta bebida.

Palavras-chave: Efeito antidiarreico, mahewu à base de mandioca, Maputo, segurança microbiológica, Moçambique.

1. INTRODUCTION

1.1 Motivation

Mahewu is an indigenous non-alcoholic fermented beverage widely consumed as meal across Southern African countries, including Mozambique, Zimbabwe, South Africa, and Zambia as well as in parts of the Arabian Gulf region(Daji et al., 2023; Olusanya et al., 2021). Its popularity stems from its refreshing taste, affordability, and deep cultural significance (Daji et al., 2023). The beverage serves multiple nutritional roles: it is commonly used as a weaning food for infants owing to its favorable nutritional composition such as iron, calcium and zinc(Armistice & Tafadzwa, 2021; Kudita et al., 2024), as a mahewu is also used in social gatherings, and its cost-effective meal replacement for students, employees, and other individuals with limited time or financial resources (Daji et al., 2023).

The production of mahewu involves the use of a lactic acid fermentation process, typically initiated using starter culture of grains such as sorghum, maize, wheat, and millet. This fermentation process enhances the digestibility of the beverage while simultaneously improving its nutritional profile, thereby making it a dietary staple in many households, schools, and informal markets throughout the region(Anyiam et al., 2023; Halake & Chinthapalli, 2020). Production of traditional mahewu is done by boiling'; maize flour to form a porridge, cooling the porridge to an optimal fermentation temperature at ambient conditions, and subsequently allowing it to undergo spontaneous fermentation for approximately 48 hours (Daji *et al*., 2022).

The employment of lactic acid bacteria (LAB), species of Lactobacillus and Pediococcus, which decrease the pH of the medium and create an environment that is inhibitory to pathogenic microorganisms, is primarily responsible for the microbial ecology of the fermentation process (Sionek et al., 2023). Additionally, LAB converts carbohydrates to lactic acid. Additionally, other microbes including *Salmonella* spp. and *E. coli* are prevented from growing in medium by the lactic acid (Armistice & Tafadzwa, 2021),

Although maize is the major ingredient used to make mahewu, cassava has gained popularity as a substitute base ingredient, especially in Mozambique, where it is the second most popular staple after maize (Salvador et al., 2016). Although lactic acid bacteria fermentation has been shown to have antimicrobial properties, microbial contamination is still a concern, especially in conventional production settings (Obayomi & Edo, 2025). Pathogenic bacteria, such as *Salmonella* spp., *Escherichia* coli, and *Listeria monocytogenes*, can be introduced by poor hygiene, insufficient temperature control, and production, all of which pose serious dangers to public health(Mainardi & Bidoia, 2024). Furthermore, improper storage conditions may promote the growth of yeast and moulds, some molds such as *Aspergillus* and *Fusarium*, which can lead to aflatoxin contamination, a serious food safety concern across sub-Saharan Africa (Mashau et al., 2024). Small-scale operations require rigorous scrutiny to identify recurring bacterial pathogens, mycotoxin-producing fungi and also its antidiarrheal properties.

It is within this context that the present study was developed, with the specific aim of evaluating the batch-to-batch microbiological safety and antidiarrheal property of cassava-based mahewu from selected local producer in Maputo Province. By monitoring key microbial metrics including total anaerobic mesophilic bacteria counts, total yeast and mold, *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and comparing them directly to regulatory thresholds and evaluate antidiarrheal efficacy of the beverage using an animal (mice) model. It will help to know the safety of cassava-based mahewu produced and come up with cassava-based mahewu safety guidelines in order to protect consumers taking Mahewu.

1.2 Problem Statement

Cassava-based mahewu is a relatively novel traditional fermented beverage consumed in Maputo City and Maputo Province and select regions across Mozambique. It is widely consumed while its scientific evidence regarding its microbiological safety and consistency is limited, particularly under small-scale informal production conditions. However, the conventional production process of Cassava-based mahewu as described by Salvador et al, (2016), yields a preservative-free liquid product characterized by high moisture content and a rapidly degrading shelf life. Previous research on cassava-based mahewu have primarily focused on regional production patterns, instant powder formulation variations, fortification techniques, the optimization of fermentation dynamics, and baseline nutritional profiles (Olusanya et al., 2021; Anyiam et al., 2023).

A thorough safety assessment of the beverage format is critically lacking, especially with regard to regional microbiological contamination profiles and the beverage's antidiarrheal properties. Even while traditional lactic acid fermentation has been shown to limit microbial overgrowth, home-brewed goods are still very vulnerable to hazardous contamination if they are made in an unsanitary or informal manner (Adesulu-dahunsi & Dahunsi, 2022). In sub-Saharan Africa, pathogenic and spoiling microorganisms such as *Salmonella* spp., *Escherichia coli*, *Staphylococcus* aureus, moulds and yeasts have been regularly isolated from traditionally processed cereal and root-based beverages(Hawaz et al., 2025; Njolke et al., 2024).

Salmonella spp. are extremely acid-tolerant pathogens that can withstand the low pH environment of fermented products. Their presence in traditional beverages has been linked to poor post-fermentation handling hygiene, and they pose serious risks to public health due to their association with foodborne outbreaks and gastrointestinal infections (Bainotti et al., 2025). Similar to this, *Escherichia coli* such as O157:H7 is a sign of fecal contamination and can cause diarrhoeal illness in extremely susceptible consumers like children and people with impaired immune system (Sinyawa, 2024).

Additionally, Staphylococcus aureus strains that produce heat-stable enterotoxins that are physiologically active even after pasteurization can be introduced via improper food handling procedures (Ernesto & Neto, 2022). In addition to bacterial pathogens, spoilage microorganisms like yeasts and molds, including *Aspergillus, Penicillium,* and Candida species, can affect the safety and flavor of fermented foods. While unchecked yeast growth results in off-flavors, and some mold species run the danger of producing harmful mycotoxins (Njolke et al., 2024).

Traditional fermented food products contain lactic acid bacteria (LAB), which are well-known for their significance in preventing gastrointestinal infections and exhibiting probiotic-mediated antidiarrheal properties. However, the Mozambican cassava-based mahewu has not had these qualities pharmacologically confirmed. A thorough microbiological safety evaluation and antidiarrheal effect validations are needed as this beverage continues to acquire commercial popularity in Maputo City and Maputo Province. It is essential to assess local processing hygiene benchmarks against set regulatory norms and to systematically identify microbiological contaminants. Concurrently, this beverage's can be validated by assessing its *in-vivo* antidiarrheal efficacy. When taken as a whole, these results are critical for protecting consumer health, setting quality standards, and improving conventional production and storage methods.

1.3 Research Objectives

1.3.1 General

The overall objective of this study is to assess the microbiological safety and antidiarrheal effect of cassava-based mahewu obtained from a local producer in Maputo Province, Mozambique.

1.3.2 Specific

Specifically, the study aims:

i. To assess the microbiological safety of cassava-based mahewu across five distinct production cycles by quantifying total bacteria, yeasts and moulds and detecting key pathogens (*Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*), and comparing the results against established regulatory standards
ii. To evaluate the antidiarrheal effect of cassava-based mahewu on castor oil-induced acute diarrhoea in a BALB/c mouse model.

1.4 Research Questions

i. What are the quantitative levels of total bacteria mesophilic count, total yeasts and moulds, and key pathogens (*Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*) in cassava-based mahewu and do they comply with established regulatory standards?
ii. Does cassava-based mahewu have an antidiarrheal effect on castor oil-induced acute diarrhea in a BALB/c mouse model.

1.5 Significance of the Study

The research provides evidence on the microbiological safety and antidiarrheal potential of cassava-based mahewu produced by a small-scale processor in Maputo province, Mozambique. By assessing samples collected over five production cycles, the study generates empirical data on the microbial quality and consistency of this traditional fermented beverage. The findings will contribute to a better understanding of potential food safety risks associated with cassava-based mahewu and support the development or revision of food safety guidelines and quality standards for traditionally fermented beverages. Furthermore, the study contributes to the limited body of literature on the microbiological safety of cassava-based mahewu in Mozambique and other sub-Saharan African countries. The results will serve as a valuable based reference for researchers investigating traditional fermented foods and may encourage further studies on fermentation practices, product quality, and public health benefits. In addition, evidence on the beverage's antidiarrheal properties may support efforts to promote safe, nutritious, and functional traditional foods, thereby contributing to improved food safety, nutrition, and public health in local communities.

2. LITERATURE REVIEW

2.1 Mahewu and Its Cultural Significance

Mahewu is an indigenous non-alcoholic fermented beverage that is sometimes described as a thin, somewhat sour gruel or porridge widely consumed in southern Africa. Many ethnic and linguistic groups refer to it by different names because it is an indigenous food product. These include *mageu* (Setswana), *mahewu* (Shona/Chewa/Nyanja), *mahleu* (Sesotho), *magau* (Khoikhoi), *madleke* (Tsonga), *mabundu* (Tshivenda), *amarhewu* (Xhosa), and *amahewu* (Zulu and Northern Ndebele) (Daji et al., 2023). The beverage that is mostly made from sorghum and maize, is prized for its nutritious value, mild acidity, and pleasant flavor (Mashau et al., 2024). Mahewu is conventionally used as a weaning food for infants and as an low-cost, high-energy drink for adults, especially laborers and scholars who need a quick source of nourishment (Daji et al., 2023).

Natural fermentation has conventionally been used in African native food processing and preservation methods to improve food products' flavor, usefulness, and shelf life(Tura et al., 2025). Most of the traditional foods and drinks, including mahewu, have been made by spontaneous fermentation mediated by naturally existing lactic acid bacteria (LAB)(Bell et al., 2023). With the tremendous improvement of food science knowledge and changing consumer choices, novel varieties of mahewu have arisen, incorporating alternative cereals and tubers, such as cassava to broaden accessibility and improve nutritional composition.

A key element of mahewu manufacturing is lactic acid fermentation, LAB drives this process by converting carbs into organic acids, which lowers pH and increases product stability. As such, enhancing nutritional quality through the elimination of contaminants and antinutritional elements better flavor development, longer shelf life, and increased digestibility through the breakdown of complex polysaccharides are just a few advantages of fermentation along with yeasts and other microorganisms that contribute to the beverage's taste and nutritional qualities, LAB, primarily species of Lactobacillus, dominate the microbial ecology of mahewu fermentation (Dhiman et al., 2025). Despite yeasts may add more taste compounds through secondary metabolic pathways, LAB produce lactic acid, which lowers the medium's pH(Pswarayi, 2019).

2.2 Cassava as a Substrate for Mahewu Production

Cassava is a most important staple food in many sub-Saharan African nations including Mozambique. The crop's high carbohydrate content contrasts with its fairly poor protein and vitamin density. Although cassava is a major source of energy for food, its nutritional deficiencies require processing techniques such as fermentation to add macronutrients to derived goods (Salvador et al., 2016). In addition, cassava is recognized as a key drought-resilient crops and has the potential to contribute significantly to food and nutrition security across the African continent(Mutanda et al., 2025). Cassava offers significant economic benefits because it is widely cultivated and is a relatively economical crop (Salvador et al., 2016). Mozambique is the fifth-largest cassava-producing country in Africa and the eighth-largest in the worldwide(Salvador et al., 2014). It is second crop mostly consumed in Mozambique.

Recurrent drought-related maize crop failures and post-conflict rehabilitation initiatives targeted at bolstering regional food supply chains have contributed to the growth of cassava production (Salvador et al., 2014). The consumption of cassava is limited by the presence of cyanogenic glycosides and nutritional elements, which offer health hazards if not sufficiently reduced by processing, despite the crop's agronomic resilience and affordability. In order to guarantee the detoxification of cassava and the elimination of antinutritional compounds, traditional processing methods, such as fermentation, have been used(Dankwa et al., 2025) (Dankwa et al., 2025).

A variety of cassava-derived products, such as fresh boiled and roasted cassava, cassava flour, roasted flour products, cassava-based mixed dishes, porridge, and fermented products like karakata, rale, mahewu, ottaca, and oteka, have been developed in Mozambique to satisfy the dietary needs of an expanding population (Salvador et al., 2014). In Maputo, Mozambique, where cassava is a staple food, a relatively new fermented beverage called mahewu is made and enjoyed. It has been acknowledged as a functional diet with potential advantages for groups at risk for malnutrition, especially schoolchildren and expectant mothers (Salvador et al., 2014).

In order to gelatinize the starch, cassava flour and water are combined and boiled for around 10 minutes. After cooling to room temperature, the gelatinized slurry is either inoculated by back-slopping (using a part of previously fermented product as inoculum) or allowed to ferment spontaneously for 24 hours at around 45°C, stabilizing the pH at about 4.5 (Salvador et al., 2014). Fruit flavorings, sweeteners, or fortification agents may be added as optional post-fermentation treatments to improve nutritional value and sensory appeal. (Salvador et al., 2016).

2.3 Nutritional and Functional Properties of Mahewu

Mahewu is a well-known fermented beverage that contains vitamins, minerals, proteins, fats, and carbohydrates. However, the substrate used, the fermentation conditions, and any fortification additives added during processing all affect its nutritional makeup (Daji et al., 2023). Because cassava has a naturally high carbohydrate content and comparatively low amounts of protein and minerals, cassava-based mahewu differs significantly from cereal-based formulations, producing a product that is essentially an energy source (Salvador et al., 2014). Complex carbohydrates are hydrolyzed into simpler sugars and metabolites during fermentation by microbial and enzymatic activity, which improves digestibility and may increase nutritional bioavailability (Daji et al., 2023). This change could improve the gastrointestinal tract capability to absorb and use nutrients. The elimination of poisonous and antinutritional substances including phytates, tannins, and cyanogenic glycosides is a major nutritional benefit of fermentation. Fermentation is essential for detoxification in cassava-based systems as it helps break down cyanogenic chemicals like linamarin into less toxic metabolites. During fermentation, when pH drops and organic acids are produced, which encourages cyanide reduction and enhances product safety. Additionally, the inactivation of antinutritional factors improves the absorption of vital minerals, including zinc and iron (Daji et al., 2022).

Fermentation also helps to improve nutrition by producing advantageous metabolites like organic acids like lactic and acetic acids, vitamins, enzymes, and other bioactive substances (Daji et al., 2023). These organic acids give mahewu its distinctively sour flavor while also serving as organic preservatives by preventing the growth of harmful bacteria and spoiling (Pswarayi et al., 2019). Because lactic acid bacteria (LAB) dominate the fermentation process and may have probiotic properties, fermented mahewu shows increased shelf constancy and microbiological safety. These microbes may increase food absorption, digestion, gut microbial balance, and immunological regulation (Sionek et al., 2023).

Consuming fermented foods with viable LAB on a systematic basis has been linked to better gastrointestinal health and a lower risk of intestinal infections. Also, protein breakdown during fermentation might result in the production of bioactive peptides. These peptides may have antihypertensive, antibacterial, and antioxidant qualities that contribute to more extensive functional health advantages (Anyiam et al., 2023). While antimicrobial peptides can block harmful bacteria, improving intestinal health and food safety, antioxidant peptides may assist reduce oxidative stress linked to long-term problems including cardiovascular diseases(Adesulu-dahunsi & Dahunsi, 2022).

Despite the significant nutritional and functional advantages of mahewu, formulations based on cassava are nevertheless constrained by the low protein and micronutrient content of cassava(Anyiam et al., 2023). Fortification techniques have been investigated as a solution to these constraints. For instance, protein enrichment utilizing edible insects has been utilized to improve amino acid profiles and overall nutritional quality, while iron fortification using ferrous sulphate has been demonstrated to dramatically increase bioavailable iron content in cassava mahewu (Salvador et al., 2016).

2.4 Microbiological Safety of Fermented Foods and Beverages

Fermented foods have been eaten for generations because of their improved nutritional profiles, preservation qualities, and microbial advantages. However, the microbiological safety of these goods continues to be a major worry, especially in informal and home-based production environments where industrial quality control procedures are lacking (Bell et al., 2023). A terrible event in Mozambique in 2015 that resulted in at least 75 deaths and 235 severe intoxications after ingesting tainted pombe a traditional fermented beverage made from sorghum, millet, maize, and sugar highlighted the seriousness of this issue(Gudo et al., 2018). The causative agent was identified as *Burkholderia gladioli*, which produced the highly toxic bongkrekic acid (Bell et al., 2023). This event emphasized the need for thorough microbiological testing and brought attention to the potentially lethal effects of microbial contamination in conventionally fermented drinks. As a traditional fermented beverage from Africa, mahewu is naturally prone to microbial contamination, which could present serious health risks if improperly handled.

Although the safety of mahewu made from sorghum and maize has been thoroughly studied, little is known about the microbiological safety of mahewu made from cassava

Beneficial and potentially hazardous bacteria interact intricately during the fermentation of mahewu. By producing organic acids that lessen the pH level of the medium, naturally occurring LAB control the fermentation process and prevent the growth of pathogens and spoiling organisms (Madilo et al., 2024). However, pathogens like *Escherichia coli, Salmonella spp., Listeria monocytogenes*, and mycotoxigenic molds, such as Aspergillus and Fusarium species, can survive and spread in unsanitary environments (Adesulu-dahunsi & Dahunsi, 2022).

The lack of controlled fermentation conditions in home-made cassava mahewu preparation may allow toxin-producing fungus to continue growing, which could result in mycotoxin contamination with substances like aflatoxins and fumonisins, which pose serious risks to food safety (Mashau et al., 2024). Ochratoxin A was found in kunun-zaki, a fermented beverage made from non-alcoholic cereals, according to a recent study done in Nigeria. According to the same study, the product's distinctive short shelf life was caused by high levels of spoilage and pathogenic Bacillus species (Bell et al., 2023). These findings are particularly relevant to cassava-based mahewu, as similar risks may be expected under comparable informal production conditions. Therefore, to reduce these risks, proper processing procedures like peeling, soaking, and controlled fermentation are crucial (Mashau et al., 2024). Similar study was done by Massamby et al., (2025), found out that *Staphylococcus aureus* and Bacillus species were in *rale* (roasted cassava flour) produced in Mozambique, suggesting that cassava-derived ingredients may serve as vehicles for pathogenic organisms if used without adequate quality assurance. Several factors influence the microbial quality of mahewu, including fermentation duration, temperature, and substrate composition.

By enabling LAB to take control of the microbiota and lowering the pH to levels that inhibit spoiling organisms, longer fermentation periods typically improve microbiological safety. Overfermentation, however, might result in over-acidification, which could cause unfavorable sensory alterations and even customer rejection (Mashau et al., 2024). .The variety of cassava used, whether bitter or sweet, also plays a determinative role, as bitter cassava cultivars require additional processing to adequately eliminate toxic cyanogenic compounds (Salvador et al., 2016). Post-fermentation storage circumstances have an additional impact on mahewu safety. Storage at room temperature can lead to secondary contamination, especially in mahewu made from cassava, where leftover sugars could encourage the growth of mold and yeast. Although pasteurization or refrigeration can prolong the shelf life of mahewu, these measures are sometimes impractical in rural areas, leading to a product with a very short shelf life, ongoing fermentation, increasing over-souring, and an unclear microbiological composition (Mashau et al., 2024).

In a cross-sectional study conducted in south-west Nigeria and South Africa between 2015 and 2016, Adekoya et al., (2019) reported that a significant proportion of fermented foods(ogiri, ugba, iru, ogi and ogi baba) and beverages (mahewu and umqombothi) from harbored elevated bacterial loads, including *Sphingomonas paucimobilis*, *Cronobacter sakazakii*, and *Acinetobacter haemolyticus*. Total aerobic plate counts in the samples ranged between 5.50×10^5 and 6.59×10^{10} CFU/g, surpassing internationally accepted microbiological limits for fermented foods. Notably, 73% of mahewu samples were found to contain deoxynivalenol (DON) at concentrations ranging from 18 to 32 μg/L, exceeding acceptable safety thresholds.

Additionally, ogiri samples had aflatoxin B1 levels above the 2 μg/kg threshold set by the Joint FAO/WHO Expert Committee on Food Additives(WHO and FAO, 2017). The risk of foodborne illness linked with traditionally fermented goods was further highlighted by the discovery of *Vibrio spp*. and *Aeromonas haemolyticus* in fermented foods. Together, these results show how urgently more sanitary processing techniques, quality assurance procedures, and regulatory supervision are needed to protect consumer health(Adekoya et al., 2019).

Research on cassava-based mahewu has mostly concentrated on its nutrients, standardizing fermentation procedures, creating quick mahewu powder, and fortifying it with iron, beetroot, and edible insects (Anyiam et al., 2023). However, there is still a still no information on microbiological safety, especially in the Maputo where cassava is commonly consumed. Cassava-based mahewu has not been thoroughly studied in terms of , possible contaminant profiles, or the effectiveness of toxin reduction during fermentation, in contrast to formulations based on maize and sorghum. Therefore, to ensure the safety of mahewu cooked at home, it is imperative to establish regulated fermentation conditions, use clean water, and execute appropriate sanitary methods (Daji et al., 2023).

2.5 Regulatory Framework and Food Safety Standards

In order to guarantee microbiological safety and safeguard public health, non-alcoholic beverages, including traditional fermented drinks like mahewu made from cassava, are subject to both national and international regulations. In Mozambique, ministry of health and the *Instituto Nacional de Normalção e Qualidade (INNOQ)* play big role in implementing food safety standard. Microbiological limits for different food and beverage categories are covered by standard, which include requirements for total plate count, total coliforms, *Escherichia coli, Salmonella* spp., *Listeria monocytogenes,* and *Staphylococcus aureus.*

The general criteria that apply to non-alcoholic fermented beverages serve as the pertinent regulatory baseline, even if there isn't yet a product-specific standard for mahewu made from cassava. International food safety standards, such as the *Codex Alimentarius*, which was co-created by the Food and Agriculture Organization of the United Nations and the World Health Organization (WHO), play important role for food safety standards. *Codex* rules state that ready-to-drink non-alcoholic beverages should not have more than 1 Salmonella in 25 milliliters of beverages and should not have higher than permissible levels of indicator organisms such *E. coli* and total coliforms (Codex. Alimentarius, 2025). The European Union microbiological criteria for foodstuffs, set forth in Regulation (EC) No. 2073/2005, also mandate the absence of *Salmonella* in 25 mL and stipulate low or undetectable levels of *E. coli* and *S. aureus* in products not subjected to post-process heat treatment(Regulation (EU) 2019/4, 2019). These standards are intended to minimize the risk of foodborne illness and to ensure hygienic production and storage practices.

The National Inspectorate of Economic Activities in Mozambique (INAE) conducts monitoring and inspections of product interms of price and registration. However, the informal food sector often works beyond the regular purview of official food safety monitoring, including home and small-scale manufacture of beverages like mahewu made from cassava. There is an urgent need for empirical study to determine if these goods meet defined microbiological safety limits because of this regulatory gap. There is a dearth of published information on cassava-based mahewu, especially in the Mozambican setting, even though numerous research has assessed the microbiological profiles of mahewu made from maize or sorghum. Considering this beverage's increasing appeal as a traditional fermented beverage and the fact that it is mostly produced informally in cities like Maputo, it is essential to look at whether.

2.6 Castor Oil-Induced Diarrhea Model in Mice

The castor oil is mostly used to induce diarrhea in animal model as it is cheap, safe and widely accepted in animal experimen (Ibrahim et al., 2025). This model's pharmacological foundation is ricinoleic acid, which is liberated from castor oil in the small intestine via lipase-mediated hydrolysis. The pathophysiology of acute secretory diarrhea in humans is well mimicked by ricinoleic acid, which then activates prostaglandin E2 (PGE2) receptors, namely the EP3 subtype, resulting in increased intestinal secretion, improved gut motility, and localized inflammation. The castor oil-induced paradigm reduces immunological variability among experimental subjects and avoids biosafety hazards when compared to alternative models that include bacterial pathogens as *E. coli* or *Salmonella spp* (Hu & Yang, 2023). The paradigm is especially useful for short-term, high-throughput screening of food-based therapies since diarrhea is usually caused within one to four hours of castor oil administration (Anyiam et al., 2023). The model permits quantitative evaluation through several well-established parameters, including stool frequency, onset time of diarrhea, body weight changes, and stool consistency scoring.

Stool frequency, onset time of diarrhea, body weight changes, and stool consistency scoring. make it particularly suitable for the initial assessment of traditional products, like mahewu made from cassava, which are thought to have antidiarrheal qualities mediated by their organic acid composition and probiotic LAB concentration. The current study's use of this model is justified by the known link between gastrointestinal health and LAB-fermented drinks. Fermented mahewu contains organic acids, bioactive peptides, and probiotic organisms that may work together to control intestinal secretion, decrease gut motility, and strengthen epithelial barrier function all of which are important processes in the reduction of diarrheal episodes(Bigi et al., 2023). A systematic and morally acceptable approach for producing initial evidence on the functional health advantages of cassava-based mahewu is provided by evaluating these effects using the castor oil-induced diarrhea paradigm.

3. THEORETICAL FRAMEWORK

The theoretical of this study is drawn on hurdle technology and microbial safety, Competitive Exclusion and Microbial Ecology of Fermentation and Probiotic Functionality and the Antidiarrheal Hypothesis

3.1 Hurdle Technology and the Microbial Safety of Fermented Foods

The idea of hurdle technology offers a helpful theoretical framework for comprehending the microbiological safety of fermented foods (Bigi et al., 2023). This concept holds that a variety of antimicrobial barriers, or "hurdles," work in concert to inhibit the growth and survival of pathogenic and spoilage microbes, rather than a single factor. These obstacles include the production of antimicrobial metabolites like bacteriocins and hydrogen peroxide, competition for nutrients by dominant LAB populations, pH reduction through organic acid accumulation, and decreased water activity due to solute concentration in lactic acid-fermented beverages like mahewu (Daji, 2017; Daji et al., 2022).

LAB, which metabolize available carbohydrates and produce lactic acid as the primary end product of fermentation, are the main microorganisms responsible for the fermentation of cassava-based mahewu. The medium's pH is lowered by this acid buildup, usually to levels between 3.5 and 4.5, which inhibits the growth of many harmful microbes (Daji et al., 2022). From the hurdle technology perspective, pH reduction constitutes the dominant hurdle in mahewu. The hurdle technology paradigm emphasizes how crucial extrinsic elements are to preserving the integrity of antimicrobial barriers, especially processing hygiene, post-fermentation handling, and storage conditions. These extrinsic obstacles are frequently compromised in the informal and home-based production contexts that define Maputo's cassava mahewu production. Inadequate hygiene during process permits the introduction and proliferation of *Salmonella spp., Escherichia coli, Staphylococcus aureus*, and toxin-producing moulds such as *Aspergillus* and *Fusarium* species (Mashau et al., 2024).

3.2 Competitive Exclusion and Microbial Ecology of Fermentation

This principle point that when two or more microbial species compete for the same ecological niche and resources, the species with the superior competitive advantage will ultimately dominate, suppressing or eliminating competing populations (Holt, 2017). In the context of fermented foods, LAB occupies the dominant ecological niche during fermentation by virtue of their rapid growth rate, efficient substrate utilization, and production of antimicrobial metabolites (Bell et al., 2023).

The competitive exclusion exerted by LAB in mahewu operates through multiple mechanisms. First, LAB rapidly consumes available fermentable sugars, depleting the carbon sources required by competing organisms. Second, the production of lactic acid and other organic acids lowers the environmental pH below the growth thresholds of most pathogenic bacteria. Third, many LAB strains produce bacteriocins that are ribosomally synthesized antimicrobial peptides with targeted activity against closely related Gram-positive organisms and, in some cases, against Gram-negative pathogens (Daji, 2017; Daji et al., 2022). Fourth, certain LAB strains produce hydrogen peroxide, diacetyl, and other inhibitory compounds that further restrict the growth of undesirable microorganisms(Daji, 2017; Daji et al., 2022).

The competitive exclusion principle is predicated on the assumption that LAB are present in sufficient numbers and diversity to dominate the fermentation microbiota. In spontaneous fermentation, which is the predominant mode of mahewu production in informal settings, the initial microbial inoculum is unpredictable and dependent on the environmental microflora associated with raw materials, water, equipment, and the hands of producers. If the initial LAB population is insufficient or if the fermentation conditions do not favor LAB proliferation, competing pathogenic or spoilage organisms may establish themselves before competitive exclusion takes effect. This situation is especially pertinent to cassava-based mahewu, which, in contrast to the more well-known formulations based on maize and sorghum, is a relatively new product for which optimum starting cultures have not been generated (Mashau et al., 2024). Diverse LAB communities, including species of Lactobacillus, Pediococcus, Leuconostoc, and Weissella, have been reported in earlier research on mahewu made from maize and sorghum. which collectively establish the competitive exclusion dynamics that underpin microbial safety (Daji et al., 2022).

However, nothing is known about the population dynamics and LAB diversity in cassava-based mahewu(Daji et al., 2022).. Since the microbial ecology of fermentation is greatly influenced by the composition of the substrate, the carbohydrate profile of cassava, which is high in starch but low in proteins and amino acids when compared to cereal substrates, may either promote or inhibit the growth of LAB and the production of bacteriocin. This knowledge gap reinforces the rationale for objective 1 of the present study, as the quantification of pathogenic organisms provides an indirect measure of the efficacy of competitive exclusion during cassava mahewu fermentation since the presence of LAB prevent growth of other pathogens such as *E. coli*, *Salmonella* spp and *S.aureus.*

3.3 Probiotic Functionality and the Antidiarrheal Hypothesis

The second objective of this work, which aims to assess the antidiarrheal activity of cassava-based mahewu using a murine model, is theoretically supported by the probiotic functioning paradigm. The FAO/WHO defines probiotics as live bacteria that, when given in sufficient quantities, improve the host's health. The probiotic qualities of the LAB strains frequently linked to fermented foods, such as Lactobacillus and Pediococcus species, have been well investigated, especially their ability to regulate gastrointestinal health (Adekoya et al., 2019).

The antidiarrheal potential of probiotic LAB is theorized to operate through several interconnected mechanisms. First, probiotic organisms can competitively exclude diarrhea-causing pathogens from intestinal adhesion sites, thereby preventing colonization and infection. Second, LAB produce organic acids, predominantly lactic acid, which lower the intestinal pH and create conditions unfavorable for the growth of enteropathogenic bacteria. Third, certain LAB strains stimulate the production of mucins and strengthen tight junctions between intestinal epithelial cells, reinforcing the mucosal barrier against pathogen translocation (Daji et al., 2023).

Fourth, the bioactive peptides such as antimicrobial and antioxidant, and bacteriocins such as nisin, pediosin, and subtilosin produced during fermentation may exert direct antimicrobial activity against diarrhea-causing organisms within the intestinal lumen (Daji et al., 2022). Collectively, these mechanisms provide a plausible theoretical basis for the hypothesis that cassava-based mahewu may possess antidiarrheal properties. A critical consideration within this theoretical framework, however, is the question of probiotic viability. For a food product to confer probiotic health benefits, the beneficial microorganisms must survive gastric acidity and bile salt exposure and arrive at the intestinal mucosa in viable and metabolically active form.

The viability and functioning of LAB in cassava-based mahewu are still mostly unknown, despite research on maize- and sorghum-based mahewu confirming the presence of LAB strains with probiotic potential, including tolerance to simulated gastrointestinal conditions (Mashau et al., 2024). The nutritional composition of cassava, particularly its lower protein and amino acid content relative to cereal substrates, may influence LAB metabolic activity and, consequently, the production of bioactive compounds such as bacteriocins and exopolysaccharides that contribute to probiotic functionality. This represents a significant gap in the current knowledge base and provides scientific justification for Objective 2 of this study.

This study used the castor oil-induced diarrhea paradigm, which provides a standardized pharmacological method for assessing antidiarrheal efficacy. Ricinoleic acid released from castor oil activates prostaglandin E^2 (PGE^2) receptors, inducing intestinal hypersecretion and hypermotility that closely mimic acute secretory diarrhoea (BenchChem Technical Support Team, 2026). Within the probiotic functionality framework, if the LAB and their metabolites present in cassava mahewu are functionally active, they would be expected to attenuate the diarrheal response through one or more of the mechanisms described above: competitive exclusion of inflammatory mediators, reinforcement of the epithelial barrier, or direct modulation of intestinal secretion through organic acid and bioactive peptide activity. The murine model thus serves as a critical experimental test of the probiotic functionality hypothesis as applied to cassava-based mahewu.

4. MATERIALS AND METHODS

4.1 Study Area

The study was conducted both in Maputo city and Maputo province. Cassava-based Mahewu is produced in Maputo Province but it is consumerd in both Maputo city and Province. Cassava-based mahewu is actively consumed at the household level, informal market, formal market and urban area. The cassava mahewu samples were collected directly from a representative small-scale household producer operating within Maputo Province. Following data collection, all laboratory-based processing and analytical workflows were distributed across specialized research facilities in Maputo city:

a. **Microbiological Analyses:** Performed at the Food Technology Laboratory, Faculty of Veterinary, Universidade Eduardo Mondlane (UEM), Maputo.

***In-Vivo* Antidiarrheal Experiments:** Conducted at the Animal Research Facility at the Faculty of Veterinary.

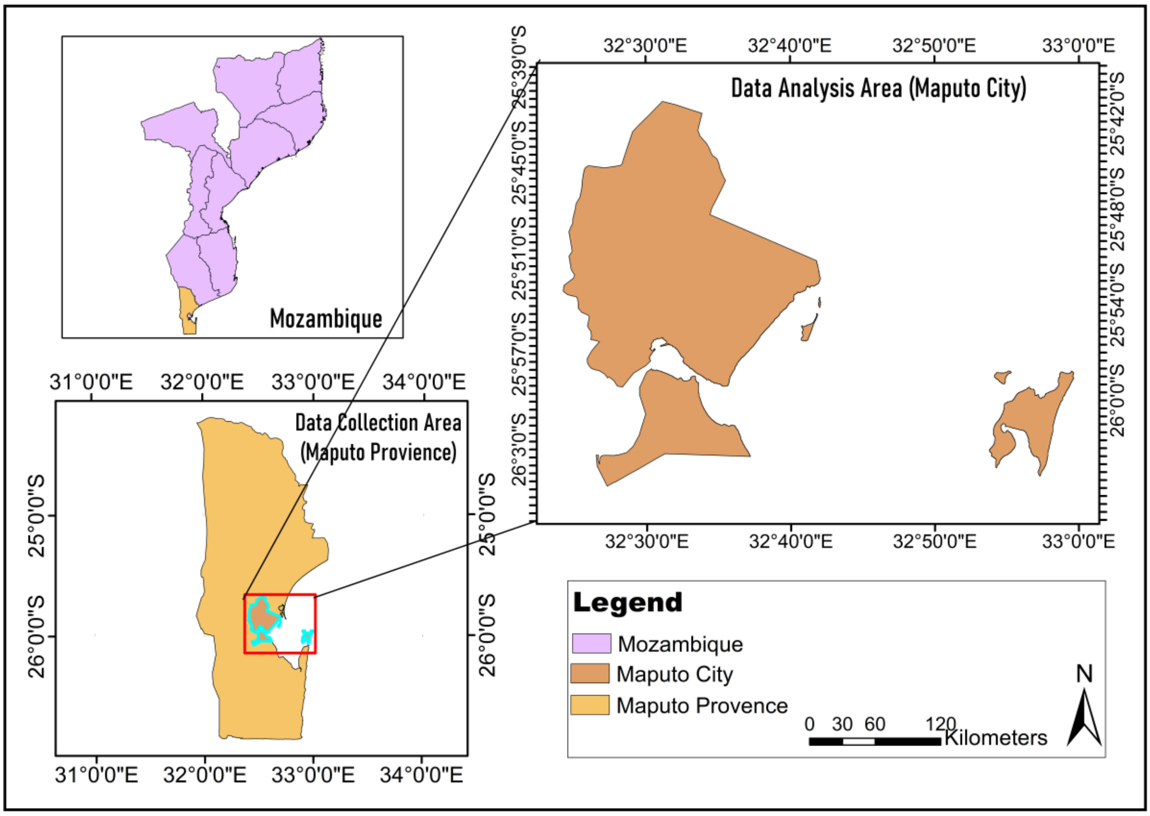

Figure 1: Map Showing Maputo province (Cassava mahewu production site) and Maputo city (laboratory analysis area). This map was generated using ArcGIS Software (version 10.8).

4.2 Study Design

The microbiological safety and in vivo therapeutic antidiarrheal qualities of cassava-based mahewu obtained from a local producer in Maputo Province, Mozambique, were assessed in this study using an experimental, laboratory-based design. There were two interconnected components to the study. Microbiological safety assessment was the first part. Samples of cassava-based mahewu were methodically gathered from the chosen producer throughout the course of five separate, consecutive production cycles. To determine the overall microbial counts and check for the presence of important indicators and harmful organisms. These samples were put through established culture-based microbiological assays. The second part involved a castor oil-induced acute diarrhoea paradigm in BALB/c mice for antidiarrheal assessment. This model evaluated the cassava-based mahewu batches' antidiarrheal efficacy at three different dose levels (3 mg/kg, 5 mg/kg, and 10 mg/kg).

4.3 Microbiological Safety Assessment

4.3.1 Sample Collection and Transport

Twenty-five (25) bottles of mahewu made from cassava (250 mL each) were collected aseptically from a single small-scale household producer in the province of Maputo. Five separate batches of five replicate bottles each were used to collect the samples ($n = 5$ bottles × 5 batches). Animal model experiments and animal-house logistics determined the collection schedule, particularly to synchronize the successive execution of the in-vivo experiments with newly obtained beverage inputs. As a result, there were irregular sample intervals. Batches 2, 4 and 5 were gathered at nearly 14-day intervals, whereas Batches 1 and 3 were taken at roughly 7-day intervals. This adaptive sampling schedule reflected the practical need to balance sample freshness with the timely completion of successive animal cohorts while accommodating laboratory workflow and animal-house operational constraints.

4.3.2 Sample preparation

Serial dilutions were prepared following standard microbiological protocols as outlined in ISO 6887-1:2017 (ISO, 2017b). A 10 mL aliquot of liquid cassava mahewu was aseptically transferred into a sterile stomacher bag and combined with 90 mL of sterile Buffered Peptone Water (BPW), yielding the initial 10^{-1} dilution (mother solution). The mixture was homogenized using a Stomacher® laboratory blender (Seward Ltd, UK) at 230 rpm for 2 minutes to ensure a uniform microbial suspension. From this mother solution, further serial dilutions were prepared by transferring 1 mL of each preceding dilution into 9 mL of sterile peptone water contained in labeled, sterile test tubes to achieve 10^{-2} and 10^{-3} dilutions, with thorough vortex mixing executed between each successive transfer. A fresh sterile pipette was used for each distinct dilution step to eliminate the risk of cross-contamination.

4.3.3 Microbiological Analysis

The cassava-based mahewu samples' microbiological contaminants were quantified and isolated using Codex-referenced techniques outlined in the pertinent international standard (ISO) frameworks. The study methodically evaluated three major bacterial pathogens, namely: *Escherichia coli, Salmonella spp., and Staphylococcus aureus;* yeasts and molds as indications of spoiling kinetics and mycotoxin hazard risk; and total aerobic mesophilic bacteria as general indicators of overall microbial food hygiene and sanitation. For every dilution series, every analysis was carried out twice.

A. Quantification of total aerobic mesophilic Plate Count

In compliance with ISO 4833-1:2013, total aerobic mesophilic bacteria were counted using the pour plate method on Plate Count Agar (PCA). After dispensing one milliliter (1 mL) of each dilution (10^{-1}, 10^{-2}, and 10^{-3}) onto sterile Petri dishes, around 15 mL of molten PCA that had been cooled to 45–47°C was added. After allowing the inoculum to settle and gently swirling the plates to achieve equal distribution, they were incubated at 37°C for 48 ± 2 hours under aerobic conditions. Six plates were utilized for every sample (two plates per dilution). Colonies were counted on plates with 15–300 colony-forming units (CFU) after incubation, and the results were reported as CFU/mL. In other cases, colonies were too many to count, especially at the 10^{-1} dilution (TNTC).

B. Quantification of total yeasts and moulds

Yeasts and moulds were enumerated using the spread plate technique on Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/L) to suppress bacterial growth, in accordance with ISO 21527-1:2008. Approximately 15 mL of molten PDA was poured into sterile Petri dishes and allowed to solidify at room temperature. Subsequently, 0.1 mL of the appropriate dilution (10^{-1}, 10^{-2}, and 10^{-3}) was transferred onto the surface of the pre-dried PDA plates and spread uniformly using a sterile glass spreader to ensure even distribution of the inoculum across the agar surface. For each sample, six plates were prepared (two plates per dilution).

The inoculated plates were incubated at 25 ± 1°C for 5 days under aerobic conditions. The plates were examined daily from day 2 onward for the development of characteristic yeast and mould colonies. The results were expressed as log CFU/ml. The overall count was taken as fungal load. Following incubation, colonies were counted on plates containing between 15 and 150 colony-forming units. Results were expressed as CFU/mL, calculated as: CFU/mL = mean colony count × dilution factor × 10 (the multiplication by 10 accounted for the 0.1 mL inoculum volume used in the spread plate technique). In instances where colonies at the 10^{-1} dilution were too numerous to count (TNTC), the calculation relied on counts from higher dilution plates.

C. Isolation and identification of *Escherichia coli*

Escherichia coli was quantified and isolated using Eosin Methylene Blue (EMB) agar, which served as a selective and differential medium for Gram-negative enteric bacteria. After dispensing one milliliter (1 mL) of the suitable dilution onto sterile Petri dishes in duplicate, around 15 mL of molten EMB agar was added. The plates were aseptically mixed, allowed to harden, and then incubated aerobically for 24 ± 2 hours at 37°C. Six plates were used per sample.

Following incubation, the plates were examined for colonies exhibiting the characteristic green metallic sheen with dark centers, which would indicate presumptive *E. coli*. The presumptive were subjected to gram staining and then observed on microscope, the gram-negative bacteria were then subjected to biochemical confirmation tests (indole test, methyl red, Voges–Proskauer).

D. Isolation and quantification of *Staphylococcus aureus*

Staphylococcus aureus was quantified using the surface spread plate method on Mannitol Salt Agar (MSA), which is selective for *staphylococci* due to its high sodium chloride concentration (7.5%) and differential based on mannitol fermentation. Approximately 15 mL of MSA was poured into sterile Petri dishes and allowed to solidify. An aliquot of 0.1 mL from the 10^{-1} dilution was dispensed onto the surface of pre-solidified MSA plates and spread uniformly using a sterile glass spreader. Plates were prepared in duplicate and incubated in an inverted position at 37°C for 48 ± 2 hours. Yellow colonies were subjected to gram staining and then observed under microscope to determine whether they were gram-positive cocci. They were then subjected to coagulase test to determine whether they were coagulase-positive or not.

E. Detection of *Salmonella* spp.

Detection of *Salmonella* spp. followed the standard four-stage procedure described in ISO 6579-1:2017 (ISO, 2017a). Twenty-five milliliters (25 mL) of the cassava mahewu sample were homogenized in 225 mL of Buffered Peptone Water (BPW) for 3 minutes and incubated at 37 ± 1°C for 18–24 hours to permit resuscitation of sub-lethally injured Salmonella cells. Following pre-enrichment, 0.1 mL of the incubated BPW culture was transferred into 10 mL of Rappaport–Vassiliadis Soya (RVS) broth and incubated at 41.5°C for 24 hours. Simultaneously, a separate 1 mL aliquot was inoculated into 10 mL of Tetrathionate (TT) Broth and incubated at 37°C for 24 hours.

From each enrichment broth, a sterile loopful of culture was streaked onto Salmonella–Shigella (SS) agar and Xylose Lysine Deoxycholate (XLD) agar, followed by incubation at 37°C for 24–48 hours. (pink colonies with black centers indicating hydrogen sulfide production) appeared on the XLD agar and SS agars in plates samples presumptive Presumptive Salmonella colonies were observed on microscope as shown in **Erreur ! Source du renvoi introuvable.** to confirm whether are gram positive or not then sub-cultured onto nutrient agar slants and subjected to a differential biochemical testing namely Triple Sugar Iron (TSI) agar slants, Lysine Iron Agar (LIA) slants, urea broth hydrolysis, and indole production tests. Positive colonies on TSI, LIA, Urea and indole could would indicate Salmonella spp.

4.4 *In-Vivo* Antidiarrheal Evaluation

4.4.1 Selection of a Microbiologically Safe Batch for the Animal Experiment

All batches which were not contaminated by *E. coli*, *Salmonella* spp and *Staphylococus* spp and the total TAMC and Total yeast and moulds within the standards were selected for animal experiments. The chronological allocation of the evaluated production batches was executed as follows: Batch 2 was used for Experiment 1, Batch 4 was used for Experiment 2, and Batch 5 was used for Experiment 3 since these batches the bacteria account were within the limit meaning there were safe.

4.4.2 Study Animals

The study protocol initially specified the utilization use of ninety (90) adult male BALB/c mice (Mus musculus), aged 7–8 weeks and weighing between 21.7 and 40.3 g at the time of arrival, distributed across three independent experiments of 30 mice each (6 groups × 5 mice per group). The Animal Science Directorate's (DCA) specialist breeding unit at the National Institute of Agrarian Research (IIAM) in Maputo provided all of the experimental animals.

In order to minimize the confusing physiological effects of hormonal changes related to the estrous cycle on baseline gastrointestinal motility and mucosal secretory capabilities, it was methodologically essential to employ only male mice as reported by Anyiam et al. (2023).

Additionally, the BALB/c strain was specifically chosen because of its well-defined immunological profile and significant historical validation in toxicological and pharmacological screening paradigms, including standardized in vivo antidiarrheal evaluations. Each mouse was placed in a standard polycarbonate cage with stainless steel wire lids upon arrival at the testing laboratory, provided with clean wood-shaving bedding (Figure 3), and kept under closely monitored environmental conditions: 22 ± 2°C, 50–60% relative humidity, and a 12-hour light/dark cycle. All mice had libitum access to standard commercial rodent pellet feed and clean drinking water throughout the study.

An acclimatization period of seven (7) days was observed prior to each experiment to allow the animals to adapt to the laboratory environment and to reduce stress-related physiological variability (OECD, 2025). All animals were assessed as healthy on arrival based on looking clinical records, physical examination and weight (see Figure 2).

Figure 2. Specimen arrival and weighing (Each mouse was kept individually in a confinement).

4.4.3 Experimental Grouping and Treatment Design

In each of the three experiments, mice were weighed and randomly allocated to six (6) experimental groups of five (5) animals each (n = 5 per group; N = 30 per experiment; total N = 90 across three experiments). Group sizes of five animals were consistent with the recommendations of the OECD Guidelines for Testing of Chemicals (OECD, 2008) and the 3Rs principle (Replacement, Reduction, Refinement) of minimizing animal use while maintaining sufficient statistical power (Lauwereyns et al., 2010; Azkona, 2023). The experimental grouping and treatment design are presented in Table 1.

Table 1. Experimental Grouping and Treatment Design

Group	Designation	Castor oil Induction	Treatment	Purpose
1	NCG	No	Normal diet and water only	Baseline reference
2	PCG	Yes	Normal diet and water only	Untreated diarrhoea control
3	TG1	Yes	Cassava mahewu at 3 mL/kg body weight	Low-dose evaluation
4	TG2	Yes	Cassava mahewu at 5 mL/kg body weight	Mid-dose evaluation
5	TG3	Yes	Cassava mahewu at 10 mL/kg body weight	High-dose evaluation
6	SDG	Yes	Loperamide 5 mg/kg body weight	Positive pharmacological benchmark

Note: NCG = Negative Control Group; PCG = Positive Control Group; TG = Treatment Group; SDG = Standard Drug Group. n = 5 mice per group per experiment (intended).

To determine a clear dose-response relationship for the beverage's antidiarrheal activity, three distinct dose levels of cassava mahewu:3, 5, and 10 mL/kg body weight—were chosen. The usual physiological tolerance limits for oral gavage procedures in murine models, which specify a maximum acceptable single-gavage volume of roughly 10 mL/kg body weight to prevent stomach distension or aspiration risk, were closely adhered to while calibrating these target volumes.

4.4.3 Experimental Protocol

The 3Rs concepts of replacement, reduction, and refinement were strictly adhered to in all animal procedures (Azkona, 2023) and adhered to the ARRIVE criteria for animal research reporting (Kilkenny et al., 2010). All mice were fasted for 18 hours prior to each experiment with free access to water, to standardize gastrointestinal transit and ensure consistent baseline conditions across groups as reported by (Jensen et al., 2013). Post-fast weights were recorded before the start of each experiment. Diarrhea induction was induced in Groups 2–6 (PCG, TG1, TG2, TG3, and SDG) by oral administration of 0.5 mL of castor oil (analytical grade) via oral gavage. Group 1 (NCG) received 0.5 mL of normal saline as a vehicle control.

The castor oil was administered in the morning by a vet (typically between 07:30 and 10:30) and the onset of diarrhea was monitored by recording the appearance of the first loose or watery stool. In most cases, the first loose stool appeared within 1 to 4 hrs of castor oil administration. Diarrhea was confirmed when at least three loose or watery stools were observed within the first to four hours and post-induction. Since this study employed a antidiarrheic design rather than a preventive (prophylactic) design, treatments were administered only after diarrhea had been confirmed in each animal. Once diarrhea onset was confirmed, the respective treatments were administered to Groups 3–6 by oral gavage. The treatment groups (TG1, TG2, TG3) received a single dose of cassava mahewu at 3, 5, and 10 mL/kg body weight, respectively. The standard drug group (SDG) received a single oral dose of loperamide at 5 mg/kg body weight. The time of treatment administration was recorded for each animal. The primary temporal endpoint in this antidiarrheal design was the resolution time, defined as the time interval (in minutes) between the administration of treatment and the passage of the last watery or loose stool, after which normal stool consistency was restored.

This measure captured the therapeutic efficacy of each treatment in resolving established diarrhea, rather than the onset latency (time from castor oil administration to first loose stool), which would be more appropriate for a preventive study design.

Stool frequency was monitored at hourly intervals during the first four hours post-treatment (0–1 h, 1–2 h, 2–3 h, 3–4 h). Stool consistency was assessed at each monitoring time point using a three-point scoring system: 0 = normal, well-formed pellets; 1 = soft, semi-formed stools; 2 = watery, liquid diarrhea. The cumulative consistency score over the 72-hour observation period was calculated for each mouse. Improvement in stool consistency (i.e., a progressive shift from scores of 2 toward scores of 0) following treatment administration was considered evidence of an antidiarrheal effect. The resolution time was also calculated as the time interval (in minutes) between the administration of treatment and the passage of the last watery (score = 2) or loose (score = 1) stool, after which only normal stools (score = 0) were observed for the remainder of the 72-hour observation period. Body weight, food and water intake recovery were recorded at 24, 48, and 72-hours post-treatment for each mouse. All animals were observed for clinical signs of distress at each monitoring time point throughout the 72-hour observation period.

4.5 Statistical Analysis

All data were entered into Microsoft Excel, screened for entry errors, and analyzed in Stata version 17 (StataCorp, 2021, USA). Descriptive statistics were computed for every variable. Continuous measurements such as microbial counts, body weight, feed and water intake are reported as the mean and standard deviation, while count-based stool variables are reported as the mean and, where appropriate, the median with the interquartile range. Microbial counts were transformed to base-ten logarithms ($\log_{10}$ CFU/mL) before analysis to stabilize the variance, and a value of one was added to zero counts to permit logarithmic transformation.

For the microbiological objective, the assumptions of normality and homogeneity of variance were examined using the Shapiro–Wilk test and Levene's test. Differences in mean $\log_{10}$ counts across the five production batches were assessed using one-way analysis of variance (ANOVA) when the assumptions held, and the Kruskal–Wallis test was applied when they did not. Compliance with regulatory limits was assessed descriptively by calculating the proportion of samples within each batch and across all batches that fell below the regulatory thresholds of 5.0 $\log_{10}$ CFU/mL for total aerobic mesophilic count and 4.0 $\log_{10}$ CFU/mL for the combined yeast and mould count. This descriptive approach was appropriate because regulatory standards are applied to individual product units rather than to the mean value of all samples.

For the evaluation of antidiarrheal property of cassava mahewu objective, baseline body weights were compared across groups by one-way ANOVA to confirm that randomization produced comparable starting conditions. Group differences in stool frequency, the cumulative stool consistency score, and the time to resolution of diarrhoea were evaluated using the Kruskal–Wallis test, because these outcomes were ordinal or did not satisfy the normality assumption (Shapiro–Wilk, all $p < 0.05$). The percentage change in body weight and the food and water intake was compared using one-way ANOVA. Where an overall test was significant, pairwise comparisons against the untreated positive control were performed using Dunn's test (for Kruskal-Wallis) or Tukey's HSD test (for ANOVA), with probabilities adjusted for multiple comparisons using the Bonferroni correction. Statistical significance was set at $p < 0.05$. The progression of stool consistency over the 72-hour observation window was examined with a mixed-effects model that included group, time, and interactions as fixed effects and the individual mouse as a random effect.

A dose–response relationship across the three mahewu doses was assessed by regressing the cumulative consistency score, the resolution time, and the percentage weight change on the administered dose. Consistency of the treatment effects across the three independent experiments was examined by including the experiment as a factor and testing the group-by-experiment interaction. A probability value below 0.05 was treated as statistically significant throughout.

4.6 Ethical Considerations

This study involved the use of live animals and therefore required ethical approval prior to commencement. Ethical clearance was obtained from the *Comité Institucional de Bioética em Saúde* (CIBS) (Approval Reference: Ref.0016/CEI-UEM/2026)

5.0 RESULTS

5.1 Microbiological Safety of Cassava-Based Mahewu

5.1.1 Detection of Foodborne Pathogens

None of the twenty-five mahewu samples were positive for *Escherichia coli, Salmonella* and *Staphylococcus aureus*.

5.1.2 Total aerobic microbial count (TAMC) and total "yeast and moulds" Across Production Batches

The total aerobic mesophilic count (TAMC) and the yeast and moulds count were enumerated for every sample and expressed as $\log_{10}$ CFU/mL (

Figure). The mean TAMC across all samples was 2.88log [CFU/mL], Batch 5 had highest mean TAMC (3.01log [CFU/mL] and Batch 1 to Batch 4 had lowest TAM. The overall mean yeast and mould was 3.70 log [CFU/mL], Batch 1 had the highest mean value of yeast and mold 4.2 log $_{10}$ [CFU/ml] and batch 4 had lowest mean yeast and mold (3.61 log [CFU/ml]. The mean log of TAMC was not significant across the batch ($p = 0.086$) and total yeast, and mold count varies across the batches.

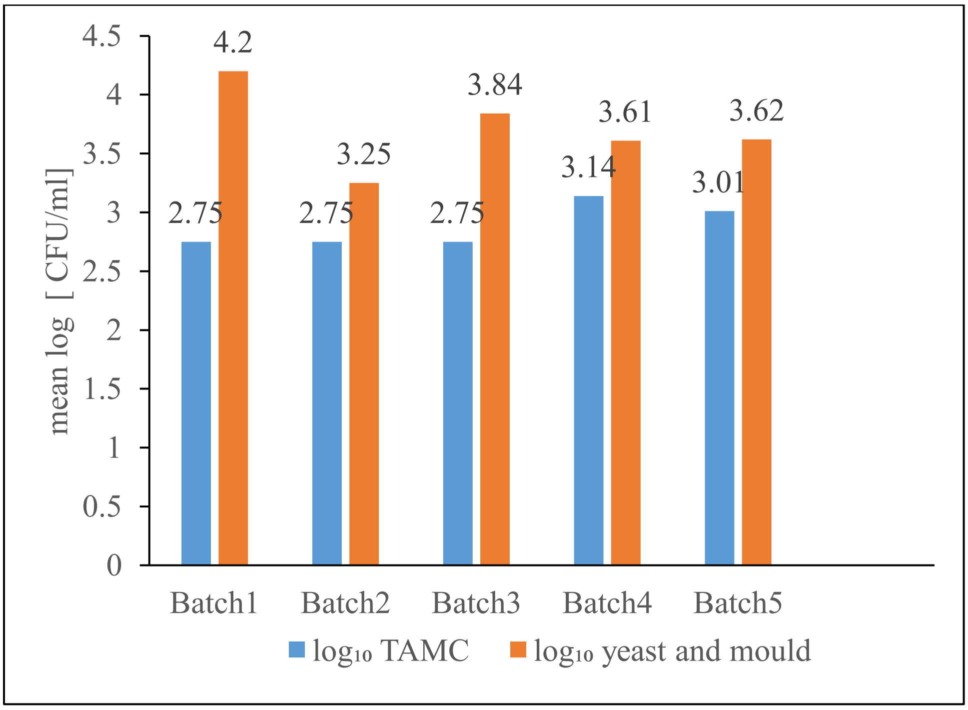

Figure 3: Mean of TAMC and Yeast and mould count

5.1.3 Compliance with Regulatory Limits

All 25 samples (100%) had TAMC values below the 5.0 log_{10} CFU/mL limit. For the combined yeast and mould count, 21 of 25 samples (84%) were within the 4.0 log_{10} CFU/mL limit (**Table 2**). Batches 1 (3 samples) and 3 (1 sample) had mean total log yeast and moulds were above the standard. All samples met the zero-tolerance criteria for *E. coli*, *Salmonella* spp., and coagulase-positive *S. aureus*

Table 2: Microbial limit of cassava-based mahewu across the five production batches

Parameter	Regulatory Limit	Samples Compliant	Non-Compliant Samples	Compliance Rate (%)
TAMC	≤ 5.0 log_{10} CFU/mL	25/25	0	100%
Combined Yeasts and moulds	≤ 4.0 log_{10} CFU/mL	21/25	4 (B1: 3, B3: 1)	84%
E. coli	Not detected	25/25	0	100%
***Salmonella* spp.**	Not detected in 25 mL	25/25	0	100%
***S. aureus* (coagulase-positive)**	Not detected	25/25	0	100%

5.2 The Antidiarrheal Effect of Cassava-Based Mahewu on Castor Oil-Induced Acute Diarrhea in A BALB/C Mouse Model

5.2.1 Baseline Comparability and Animal Survival

During the course of the three experiments, a total of 4 mice died and were excluded from the analysis. In Experiment 1, two mice died: TG1-01 died during the castor oil induction and TG2-04 died during the acclimatization period prior to the start of the experiment. In Experiment 2, two mice died: TG2-05 and SDG-02 both died during fasting. None were attributable to the experimental treatments (cassava mahewu or loperamide). These deaths reduced the effective sample size in some groups below the intended n = 5, but the conduct of three independent experiments partially compensated for this attrition.

Baseline body weights did not differ significantly between the groups (one-way ANOVA, F = 1.21, p = 0.312), which confirms that randomization produced comparable starting conditions. Confirmed diarrhea developed in 15 of the 15 animals in the PCG, 14 of 14 in TG1, 13 of 13 in TG2, 15 of 15 in TG3, and 14 of 14 in the SDG which received castor oil whereas no animal in the NCG, which received no castor oil, developed diarrhea. The PCG therefore experienced diarrhea comparable to that of the treated groups but received food and water, serving as the untreated reference.

5.2.2 Stool Frequency and consistency during the first four hours 'and over 72 Hours post-treatment

As shown in Table 3, diarrheal severity varied across the experimental groups during the first four hours following treatment. The positive control group (PCG) exhibited the highest stool output, and watery stool frequency indicating the greatest severity of diarrhea. In contrast, the normal control group (NCG) did not produce any watery stools and maintained a cumulative score of zero. Among the treatment groups, TG1 showed relatively higher diarrhoeal activity, whereas TG2, TG3, and the standard drug group (SDG) demonstrated lower frequencies of watery stools compared with the PCG. The reduction was most evident in TG3 and SDG, which recorded the lowest watery stool frequencies among the treated groups.

Table 3. Stool Frequency during the first 4 hours Post-Treatment by Group for 3 experinments

Group	N	**Mean Total Stools (0–4h)**	**Mean Watery Stools**
NCG	15	3.6±2.3	0.0±0.0
PCG	15	4.9±3.5	3.2±5.8
TG1	14	4.0±2.0	2.4±2.6
TG2	13	2.8±2.9	1.0±2.1
TG3	15	3.5±2.6	0.7±2.7
SDG	14	2.4±2.4	0.7±1.5

SDG=Standard drug group, TG3=treatment group 3, TG2=treatment group, TG1=treatment group 1, PCG=positive control group, NCG=negative control group.

The total number of stools passed over the seventy-two-hour observation period did not differ significantly between the groups (Kruskal–Wallis $\chi^2 = 2.61$, p = 0.760), and the early stool output during the first four hours was likewise comparable across the groups (χ^2 = 9.97, p = 0.076). The cumulative stool consistency score, which increased with the severity and persistence of watery stools, differed markedly between the groups (χ^2 = 48.94, p < 0.001). The untreated Positive Control Group recorded the highest score (10.1), whereas the Negative Control Group recorded a score of zero. The three mahewu groups recorded progressively lower scores as the dose increased, from 7.0 in Treatment Group 1 to 3.8 in Treatment Group 2 and 3.4 in Treatment Group 3, approaching the score of 2.0 observed in the loperamide-treated Standard Drug Group. The group summary is presented in

.

Among the groups in which diarrhoea was induced and treated, the time to resolution of watery stools differed significantly (Kruskal–Wallis χ^2 = 9.50, p = 0.023). Resolution was slowest in the low-dose Treatment Group 1 at 9.9 hours and became faster as the dose increased, falling to 6.6 hours in Treatment Group 2 and 4.5 hours in Treatment Group 3. The loperamide-treated Standard drug group resolved most rapidly, at 1.1 hours. The standard deviations for the mahewu groups were large

The percentage change in body weight over the observation period differed significantly between the groups (one-way ANOVA, F = 7.95, p < 0.001). The Negative Control Group gained weight (+4.05%), as expected for healthy animals, whereas all groups that received castor oil lost weight (Table 4). The loss ranged from 1.82% in the Standard Drug Group and 1.88% in Treatment Group 1 to 4.40% in Treatment Group 3. The food intake (F = 0.78, p = 0.567) and water intake (F = 0.56, p = 0.730) did not differ significantly between the groups, which indicates that the treatments did not suppress feeding or drinking behavior during recovery.

Table 4: Antidiarrhoeal outcomes by treatment group in the castor oil-induced mouse model

Group	N	Baseline wt (g)	Consistency score	Resolution time (h)	Weight change (%)
NCG	15	29.6	0.0	—	+4.05
PCG	15	29.9	10.1	—	−2.88
TG1 (3 mL/kg)	14	30.1	7.0	9.9	−1.88
TG2 (5 mL/kg)	13	29.7	3.8	6.6	−2.35
TG3 (10 mL/kg)	15	30.0	3.4	4.5	−4.40

Group	N	Baseline wt (g)	Consistency score	Resolution time (h)	Weight change (%)
SDG (loperamide)	14	29.8	2.0	1.1	−1.82

Note: NCG, Negative Control Group; PCG, Positive Control Group; TG1–TG3, mahewu Treatment Groups; SDG, Standard Drug Group. The cumulative consistency score increases with diarrhoea severity. Resolution time is not defined for the NCG and PCG because diarrhoea was absent. Overall group differences: consistency score (Kruskal–Wallis $\chi^2 = 48.94$, $p < 0.001$); resolution time ($\chi^2 = 9.50$, $p = 0.023$); weight change (ANOVA $F = 7.95$, $p < 0.001$).

5.2.3 Comparison of groups with the Untreated Control

Pairwise comparisons against the untreated Positive Control Group are presented in Table **5**. The two higher mahewu doses produced significant improvements in stool consistency relative to the untreated control, with the cumulative score lower by 6.31 points in Treatment Group 2 ($p = 0.003$) and by 6.68 points in Treatment Group 3 ($p < 0.001$). The low-dose Treatment Group 1 improved by 3.08 points, but this did not reach significance ($p = 0.105$). The loperamide group showed the largest improvement (8.08 points, $p < 0.001$), and the Negative Control Group differed from the untreated control in the opposite direction, as expected for animals that never developed diarrhoea. For the percentage weight change, only the Negative Control Group differed significantly from the untreated control ($p < 0.001$), and the mahewu groups did not differ significantly from the untreated control on this measure.

Table 5: Pairwise comparison of each group against the untreated Positive Control Group (PCG) for stool consistency and weight change

Comparison vs PCG	Δ consistency	P	Δ weight (%)	P
NCG	−10.08	<0.001	+6.93	<0.001
TG1 (3 mL/kg)	+3.08	0.105	+1.00	-
TG2 (5 mL/kg)	+6.31	0.003	+0.53	-
TG3 (10 mL/kg)	+6.68	<0.001	−1.52	-
SDG (loperamide)	+8.08	<0.001	+1.06	-

A positive Δ consistency value indicates improvement (a lower cumulative score) relative to the untreated control. Probabilities were adjusted for multiple comparisons. The dose–response regression across TG1–TG3 was significant for the consistency score ($\beta = -0.430$, $p = 0.012$).

5.2.8 Relationship of animal model groups consistency across independent experiments

The mixed-effects model showed that neither the group effect ($\chi^2 = 4.45$, $p = 0.487$) nor the time effect ($\chi^2 = 2.99$, $p = 0.560$) was significant on its own, but the group-by-time interaction was highly significant ($\chi^2 = 70.43$, $p < 0.001$). A dose–response relationship was confirmed for the cumulative consistency score, which decreased significantly as the mahewu dose increased ($\beta = -0.430$, $t = -2.62$, $p = 0.012$). The corresponding trends for resolution time and weight change pointed in the same direction but did not reach significance ($p = 0.155$ and $p = 0.112$, respectively).

The group-by-experiment interaction was not significant for any of the outcomes. The repeatability of the dose-dependent increase in stool quality was confirmed by the cumulative consistency score, which did not change between experiments ($F = 0.44$, $p = 0.646$). The percentage weight change ($F = 4.07$, $p = 0.021$), stool frequency ($F = 6.28$, $p = 0.003$), and food intake ($F = 6.47$, $p = 0.002$) all showed some between-experiment variation. displays the cumulative consistency score by group together with the significance of each treatment relative to the untreated control over 72 hrs.

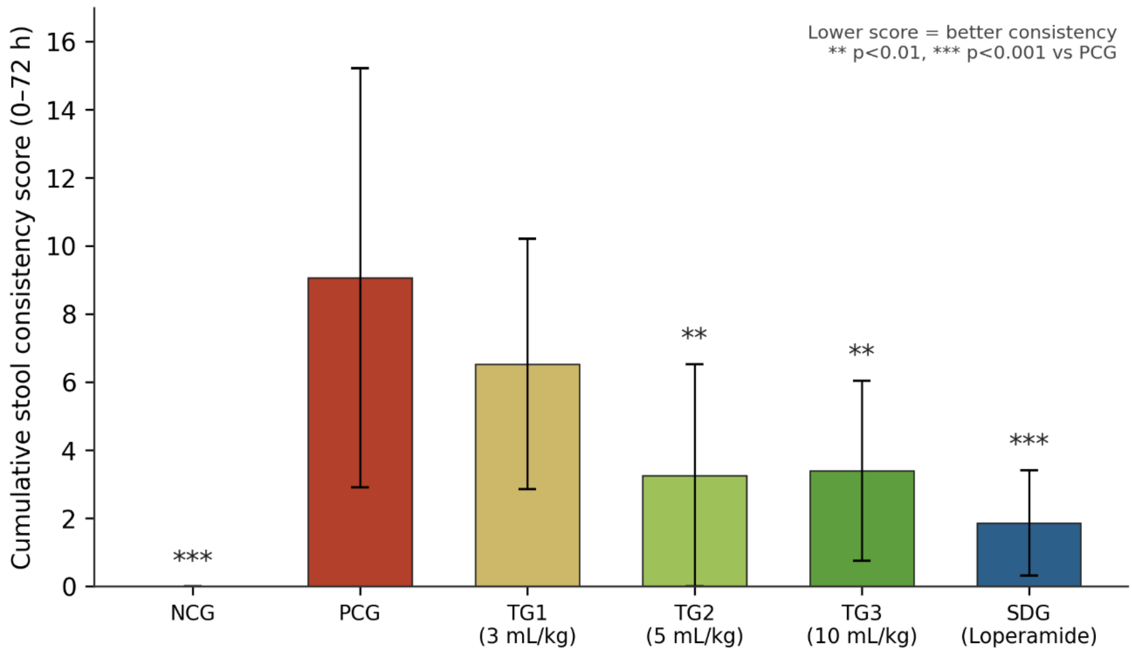

Figure *4*: The castor oil-induced mouse model's cumulative stool consistency score by treatment group.

Note: Asterisks above the bars denote a significant difference relative to the untreated Positive Control Group (two asterisks, $p < 0.01$; three asterisks, $p < 0.001$). *Lower scores indicate firmer stools and better diarrhoea control.*

6.0 DISCUSSION

6.1 Microbiological Safety Findings

6.1.1 Prevalence of *Escherichia coli*, *Salmonella* spp. and *S. aureus*

The absence of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* in all 25 samples is the study's most significant findings. Consumers get food-borne illnesses from these three microorganisms. Given the informal, household-based production context of cassava-based mahewu, where pathogen contamination is typically expected to be more prevalent due to the lack of standardized hygiene protocols, controlled water sources, and industrial-grade sanitation measures, their absence is especially notable.

The absence of *E. coli*, a recognized sign of fecal contamination, implies that there were no faecal-oral contamination pathways in the production environment. This finding contrasts with the results reported by Adekoya et al. (2019), who detected *E. coli* alongside other enteric pathogens, including *Sphingomonas paucimobilis* and *Cronobacter sakazakii*, in traditionally fermented foods sampled from Nigeria and South Africa. This discrepancy may reflect differences in the production environments, raw material quality, or water sources employed across the studies or the hygiene conditions among the producers. Additionally, lactic acid bacteria populations(LAB populations) might have suppressed competing organisms through substrate depletion, acid production, and bacteriocins activity, during the fermentation of cassava mahewu as reported in the study by Daji et al. (2022) on physicochemical properties and bacterial Community profiling of optimal Mahewu (A Fermented Food Product) Prepared Using White and Yellow Maize with Different Inoculum.

The non-detection of *Salmonella spp*. following the full four-stage ISO 6579-1:2017 protocol is equally significant. The absence of *Salmonella* spp. indicates that cassava mahewu is safe for human consumption. The absence of confirmed *Salmonella* is consistent with the regulatory requirements stipulated by (Codex. Alimentarius, 2025) which mandate the absence of *Salmonella* in 25 mL of ready-to-drink non-alcoholic beverages. The absence of confirmed *S. aureus* is particularly reassuring given that this organism is commonly introduced through manual handling during food preparation and is capable of producing heat-stable enterotoxins that persist even after thermal processing as reported by

Vincent et al. (2024). This shows that the product is safe for human consumption as the product can not cause any food borne illness associated with *Salmonella* spp, *E. coli* and *S.aureus* spp.

6.2 Total aerobic microbial count (TAMC), and total yeast and mould count

The level of log TAMC observed in this study is markedly low relative to the levels commonly reported in the literature for traditionally fermented beverages in sub-Saharan Africa. Adekoya et al., (2019), reported total aerobic plate counts ranging between 5.7 to 10.8 Log CFU/g in a cross-sectional survey of beverages(maheu) from Nigeria and South Africa which is greater than the findings in this study. The substantially lower TAMC recorded in this study suggests that the cassava mahewu produced in Maputo province might be produced at favorable production hygiene, with appropriate fermentation conditions, and effective cold chain management during sample transport.

The low log TAMC values are consistent with the predictions of the hurdle technology framework as reported by Bigi et al. (2023). According to hurdle technology, the cumulative antimicrobial barriers operating during lactic acid fermentation, particularly pH reduction through organic acid accumulation and nutrient competition by dominant lactic acid bacteria (LAB), collectively suppress the proliferation of spoilage and indicator organisms. The low aerobic plate counts observed across all five batches provide indirect evidence that the fermentation-derived hurdles were functioning effectively that high LAB contribute to low TAMC.

Contrary to the concerns noted in the literature for fermented beverages manufactured informally, the low TAMC values and the lack of significant between-batch variation further imply that the fermentation-derived obstacles were not weakened by flaws in production hygiene, handling, or storage (Bell et al., 2023; Varzakas, 2020). According to the competitive exclusion theory, dominant LAB populations will consistently inhibit rival pathogenic organisms by producing acid, depleting substrate, and developing antimicrobial metabolites. The fact that all 25 samples (100%) met this criterion shows that the producer's overall microbial load was continuously managed across all five manufacturing batches. This stability across batches is significant because it indicates that the basic hygiene and fermentation conditions used by this producer were adequate to maintain acceptable general microbiological quality even in the absence of consistent production processes. However, the role of competitive exclusion cannot be explicitly confirmed because the current investigation did not measure or characterize the LAB populations present in the mahewu samples. To give a more comprehensive view of the microbial community, future research should include species identification and LAB enumeration.

For lactic acid-fermented beverages, where yeasts are a natural part of the fermentation microbiota, the majority of samples' total yeast and mold counts were below the permitted level within the anticipated range. yeasts add to the sensory profile of mahewu by producing carbon dioxide and flavored chemicals. Their moderate presence is typically seen as a sign of spontaneous fermentation rather than a sign of contamination (Daji et al., 2023).

Given that the initial yeast/mold inoculum is determined by the environmental microflora associated with raw materials, fermentation vessels, and ambient conditions, the significant variation in the combined yeast and mold counts across samples, especially the wide range seen in Batches 1: 3 samples and Batch 3: 1 sample, probably reflects the inherent unpredictability of spontaneous fermentation (Daji et al., 2022; Mu & Zhang, 2023). This variability is consistent with the observation by Mashau et al. (2024), that non-industrial production environments lack controlled inoculation, and monitoring systems are necessary to ensure uniform microbial populations across production batches. Although the cassava mahewu's overall microbiological safety profile was favorable, the high detection of mold and yeast in 16% of samples calls for careful evaluation. In sub-Saharan Africa, where aflatoxin-producing Aspergillus species are common in the food supply, the presence of molds and yeast in a fermented beverage raises reasonable worries about mycotoxin contamination as reported by Mashau et al. (2024).

The sporadic nature of elevated mould and yeast counts, occurring in isolated samples across different batches, may indicate intermittent environmental contamination rather than a systemic production hygiene failure. Potential sources might be due to airborne spores during the cooling phase of mahewu preparation, contaminated fermentation vessels, or the use of cassava flour with pre-existing fungal colonization as reported by Mashau et al. (2024) and Molnar et al. (2023). This concern is amplified by the findings of Adekoya et al. (2019), who reported that 73% of mahewu samples in their study contained deoxynivalenol (DON) at concentrations exceeding acceptable safety thresholds. While the present study the high limit was observed only in 16 % of samples and did not test for mycotoxins, as this was beyond the scope of the study, the high mould and yeast detection rate observed here in 4 samples suggests that mycotoxin screening should be a priority in future investigations of cassava-based mahewu. However, in this study since dried flour was used in the process of cassava mahewu, and the mycotoxins level is outlawed.

6.3 Compliance with regulatory limit

The low TAMC, yeast and mold microbial standards, and the absence of *S. aureus*, *E. coli* and *salmonella* spp. in all samples shows that cassava-based mahewu produced meet the international standards and it is safe for human conservation. From a public health standpoint, the compliance of cassava-based mahewu with established safety standards is an encouraging finding, particularly in a context where the informal food sector operates largely outside the purview of routine food safety monitoring.

INNOQ and the INAE are responsible for implementing and enforcing food safety standards in Mozambique, but the informal food sector, including household producers of traditional fermented beverages, is rarely subject to systematic inspection or surveillance as reported by Grwambi (2020). The present findings suggest that effective microbiological safety can be achieved even in small settings. provided that fundamental hygiene practices are observed and the fermentation process is conducted under reasonably controlled conditions. Nevertheless, these results should be interpreted with caution, as they derive from a single producer and cannot be generalized to all cassava mahewu producers in Maputo or elsewhere in Mozambique without further investigation.

6.2 Antidiarrheal property of cassava-based mahewu

6.2.1 Antidiarrheal Effect of Cassava Mahewu

The statistically significant impact of cassava mahewu on stool consistency, as determined by the cumulative consistency score, was the study's most reliable and consistent conclusion. SDG and TG3 had an antidiarhoeal effect in the first four hours after therapy, reducing water stools by up to mean of 0.7. Pairwise comparisons showed that TG2 (5 mL/kg) and TG3 (10 mL/kg) recorded considerably lower cumulative scores of fecal than the PCG ($p = 0.003$ and $p < 0.001$, respectively), and the Kruskal–Wallis test for the cumulative score over 72 hours was highly significant ($\chi^2 = 48.94$, $p < 0.001$). This conclusion was further corroborated by the dose response analysis, which showed a statistically significant negative linear connection between dosage and cumulative score (β

$= -0.430$, $p = 0.0123$), suggesting that greater doses of cassava mahewu were linked to reduce diarrhea within few hours. The reduction/stopping of diarrhea in mice support to a review by Raudhah et al., (2023), who reported that Cassava-based Maheu inhibits prostaglandin synthesis leading to decreased gastrointestinal motility and intestinal fluid accumulation. In another study by Sarma et al., (2023) , indicated that cassava-based mahewu contains bioactive compounds such as flavonoids, alkaloids and tannins which decreases fluid retention in the intestines. From this study, Cassava Mahewu has indicated that it has health benefits as long as it is safe.

The groups did not differ consistently throughout all time points, as indicated by the lack of a significant main effect for group. Stool consistency did not vary consistently over time in all groups, as indicated by the lack of a significant main effect for time. The substantial group-by-time interaction, however, shows that the groups' patterns of change over time varied, with the treated groups showing improvement while the untreated control group continued to exhibit symptoms. This suggests that mahewu, which is made from cassava, has significant antidiarrheal property over time. Treatment groups 2, 3, and the standard group demonstrated a substantial impact on stool consistency compared to TG1 and PCG, with a more significant improvement reported in SDG, according to a comparison of the treatment group and negative control group with the positive group.

This result is in line with the theoretical framework, which postulated that the lactic acid bacteria (LAB), organic acids, and fermentation metabolites found in cassava mahewu may influence intestinal function through a variety of mechanisms, such as direct modulation of intestinal secretion, reinforcement of the integrity of the epithelial barrier, and competitive exclusion of pathogenic organisms (Daji et al., 2022, 2023). The improvement in stool consistency, without a corresponding reduction in total stool frequency, suggests that cassava mahewu may exert its effect primarily through mechanisms that influence the water content and consistency of stools, potentially by reinforcing intestinal epithelial tight junctions and reducing secretory flux, rather than by inhibiting gut motility.

From a molecular perspective, this distinction is important because it separates the potential mechanism of action of cassava mahewu from that of loperamide, which primarily decreases intestinal motility through μ-opioid receptor agonism (Pannemans & Corsetti, 2018). Although the resolution time did not reach statistical significance in the dose–

response regression, the consistent downward trend across the three dose levels suggests a clinically meaningful dose-dependent pattern that may have failed to reach significance due to the substantial inter-individual variability observed within the mahewu treatment groups. Future research should consider the effects of each batch on animal groups and compare them among all batches.

The standard drug group (SDG), which received loperamide at 5 mg/kg body weight, consistently outperformed all three mahewu treatment groups across the key outcome measures. Loperamide produced the lowest cumulative consistency score (2.0 ± 1.5), the shortest mean resolution time (1.1 ± 0.8 hours), and the smallest mean weight loss (−1.82 ± 3.55%). These results validate the sensitivity of the experimental model in identifying antidiarrheal effects and are in line with the proven pharmacological potency of loperamide as an antidiarrheal drug (BenchChem Technical Support Team, 2026).

Crucially, the direction and statistical significance of the effect on cumulative consistency score were similar, even though the antidiarrheal effect of cassava-based mahewu at the higher doses (TG2 and TG3) was smaller than that of loperamide: both TG3 and the SDG showed highly significant reductions relative to the PCG ($p < 0.001$). This result implies that cassava mahewu has a real and antidiarrheal impact on stool consistency in this model at dosages of 5 mL/kg and higher. The potential of cassava mahewu as an easily accessible, food-based complementary intervention that could be used in conjunction with pharmaceutical treatments in resource-constrained settings where access to commercial antidiarrheal medications may be limited is what makes this finding health relevant (Anyiam et al., 2023).

There was difference in terms of weight gain and intake among the groups. The NCG exhibited consistent weight gain across all time points, reflecting normal physiological growth in the absence of disease. In contrast, the PCG exhibited an initial weight drop following fasting and diarrhoea induction, followed by incomplete recovery by 72 hours. Both the treated groups, SDG and PCG retain their normal weight due to their normal feeding and water drinking TG3 despite being given high dose of Mahewu, the body weight reduced despite normal feeding and water intake over 72 hours, such weight loss might be attributed by cassava fiber contained in high dose. High dose might have a lot of cassava which might reduce the body weight and this support to the study done by Yang et al.

(2025) on the effects of cassava fiber found that cassava mahewu significantly reduced body weight, body fat, triglycerides, and blood glucose in mic.

However, future research should consider why mice given a high dose of mahewu of weight decrease over 72 hours. The TG1 and SDG groups exhibited patterns of partial recovery that more closely approximated the NCG trajectory, suggesting that these treatments may have facilitated a faster return to normal physiological function. The absence of significant differences in these secondary outcomes after 4 hours may reflect the relatively mild and self-limiting nature of the castor oil-induced diarrhea model, in which the acute gastrointestinal disturbance resolves within the first 24 hours for most animals, irrespective of treatment. Similar findings have been reported in comparable studies, where food and water intake were relatively insensitive markers of treatment effect in short-duration diarrhoea models (Anyiam et al., 2023).

The one-way ANOVA across experiment revealed significant variability in several outcome measures, including percentage weight change and total food intake, indicating that uncontrolled environmental or procedural factors influenced the absolute values of these outcomes across the three experiments. This between-experiment variability is a common challenge in laboratory animal studies and may be attributed to several factors, including seasonal variation in ambient temperature, differences in the physiological state of mice from the breeding colony at different time points, and variation in the microbiological and biochemical composition of cassava mahewu across the three production batches used for the three experiments (Mashau et al., 2024).

7.0 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The study revealed that cassava-based mahewu produced by a household producer in Maputo Province is microbiologically safe and possesses antidiarrheal activity. None of the target foodborne pathogens (*Escherichia coli*, *Salmonella* spp., or coagulase-positive *Staphylococcus aureus*) were detected in any of the twenty-five samples analysed, while total aerobic mesophilic counts in all samples and total yeast and mould counts in indicated good microbiological quality. Furthermore, the in vivo evaluation using a castor oil-induced diarrhoea model in BALB/c mice demonstrated that cassava-based mahewu exhibited dose-dependent antidiarrheal activity, improving stool consistency compared with the untreated positive control group. Overall, the traditionally produced cassava-based mahewu is both microbiologically safe for consumption and has promising antidiarrheal potential, supporting its value as a probable functional fermented beverage and providing a scientific basis for further investigation into its therapeutic properties.

7.2 Recommendations

Based on the findings of this study, the following recommendations are proposed for producers, standards bodies, and researchers to improve the microbiological safety of cassava-based mahewu and advance research on its antidiarrheal potential.

7.2.1 For producers

a. Should follow good hygiene and fermentation practices so as to prevent mahewu from being produced.

7.2.2 For standards bodies

a. Should develop microbiological standards specific to cassava-based mahewu and similar non-alcoholic fermented beverages
b. Should introduce routine market-level microbiological monitoring of informally produced fermented beverages, giving particular attention to the yeast and mould count, which was the least stable safety parameter and the one most likely to drift out of compliance.

7.2.3 For researchers and academics

a. Future research should isolate and characterize the specific components and mechanisms responsible for the antidiarrheal effect, since the present work measured the functional outcome rather than its cause.

b. Extend the antidiarrheal evaluation to models of infectious diarrhoea, which more closely resemble the conditions under which the beverage is likely to be of practical benefit, and apply molecular typing to characterize any pathogen isolates together with their toxin and resistance profiles.

c. Adopt a longitudinal design that samples across seasons and across the shelf life of the product, so that the influence of temperature, humidity, and storage on contamination, which a single cross-sectional survey cannot capture, can be quantified, and broaden the assessment to other settings and larger samples to strengthen the evidence base.

d. Future research should investigate the antidiarrheal potential of cassava-based mahewu using higher doses, extended pre-treatment protocols, and repeated-dose antidiarrheal regimens

7.3 Limitations

The findings of this study provide valuable evidence on the microbiological safety and antidiarrheal potential of cassava-based mahewu. However, several limitations were considered, as they may have affected the interpretation and broader applicability of the results. These limitations are outlined below:

a. Four animals were lost during the in-vivo experiment: one following castor oil induction, one during the acclimatization period, and two during the fasting period. Although replacement animals were used where appropriate, these losses may have influenced the statistical power of the antidiarrheal evaluation.

b. The microbiological assessment used a cross-sectional design, with samples collected at the point of sale. Consequently, seasonal variations in environmental conditions, such as temperature and humidity, and changes in microbiological quality throughout the product's shelf life were not evaluated.

c. Samples were obtained from a single household producer in Maputo Province. Therefore, the findings may not be representative of cassava-based mahewu produced by other household producers or of the broader informal mahewu market.

d. The study evaluated the antidiarrheal effects of microbiologically acceptable cassava-based mahewu only. Consequently, it did not investigate the potential health effects associated with the consumption of microbiologically unsafe batches.

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9.0 APP ENDICES.

Appendix 1: Ethical approval letter

Comité de Ética em Investigação da UEM (CEI-UEM)

Exma. Aminata Abdul Kamara
Faculdade de Veterinária
Universidade Eduardo Mondlane

Ref.0016/CEI-UEM/2026 — 20 de Maio de 2026

Assunto: Avaliação do Comité de Ética do protocolo de estudo intitulado: *"Assessment of Microbiological Safety and Antidiarrheal Effect of Cassava-BasedMahewu(Non-Alcoholic Fermented Beverage) in Maputo City, Mozambique"*

O Comité de Ética em Investigação da UEM (CEI-UEM) avaliou o protocolo do estudo supracitado e que se encontra registado no CEI-UEM com o número 0016/CEI-UEM/2025. O estudo tem como objectivo geral avaliar a segurança microbiana e as propriedades antidiarreicas *in vivo* do mahewu à base de mandioca produzido e consumido na Cidade de Maputo, Moçambique. Um total de doze (12) garrafas de mahewu à base de mandioca, cada uma contendo 250 ml, será coletado de forma asséptica de um único produtor doméstico de pequeno porte na Cidade de Maputo.

Na avaliação feita na componente de ética, nos termos das atribuições definidas no Despacho No: 404/RT/2024, de 23 de Dezembro, o CEI-UEM concluiu que o protocolo cumpre com o recomendado e, por essa razão, **APROVA** o protocolo de pesquisa, conforme as atribuições definidas no Despacho acima mencionado.

De referir que esta aprovação tem a validade de 12 meses, a contar a partir da data acima indicada. Recomenda-se ainda que, no final do projecto, seja partilhado o Relatório para fins administrativos do CEI-UEM. Para eventuais dúvidas ou esclarecimentos, queira contactar o Gabinete do CEI-UEM.

O Presidente do CEI-UEM,

Humberto Muquingue

Prof. Doutor Humberto Muquingue
(Professor Associado)

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