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FACULTY OF SCIENCE AND EDUCATION

DEPARTMENT OF BIOLOGY

COMPARATIVE MICROBIAL SPOILAGE ANALYSIS OF HASS AND FUERTE AVOCADO CULTIVARS, A CASE SYUDY OF NAGONGERA, TORORO DISTRICT, UGANDA

BY

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THIS RESEARCH REPORT IS SUBMITTED TO THE DEPARTMENT OF BIOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF THE BACHELOR OF SCIENCE WITH EDUCATION (BIOLOGICAL) OF BUSITEMA UNIVERSITY

AUGUST, 2026

DECLARATION

I **Odeke Joseph**, declare that this research project is my original work and has not been submitted to any other institution for academic credit.

Signature: ……………………

Date: 08/9/2026…………………

APPROVAL

This research project has been subjected to the Department of Biology and with approval of the University Supervisor.

Supervisor's Name: DR. NATUKUNDA FLAVIA

Signature: ....

Date: ...8TH SEPTEMBER, 2026...

DEDICATION

I dedicate this work to my brother, Oteko Alfred and my entire family who have been my inspiration and have supported my education amidst all financial milestones.

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ABSTRACT

This study presents a comparative microbiological analysis of postharvest internal pulp spoilage between Hass and Fuerte avocado cultivars sold at Nagongera Town Council, Tororo District, Uganda. While avocados are vital to both the local and national economy, approximately 25% of harvest is lost with significant losses driven by microbial decay yet there is limited empirical data regarding the microbial load and susceptibility difference between the Hass and Fuerte cultivars in tropical local markets. This research investigated the prevalence of three common bacterial microflora- *Escherichia coli, Salmonella spp* and *Staphylococcus spp* and compared microbial loads between two varieties over a fixed 7-day room-temperature storage period to determine cultivar postharvest resistance. Six fresh, ripe and healthy avocados (three from each cultivar) were collected. Following homogenization and serial dilution, bacteria were isolated using the pour plate technique on Nutrient agar and identified via Gram staining and biochemical testing including indole, catalase, citrate and urease tests. The findings revealed that all three target bacterial species (*Escherichia coli, Salmonella spp* and *Staphylococcus spp*) were present in all six examined samples demonstrating widespread bacterial contamination likely originating from local market environments or handling practices. Total bacterial counts across samples ranged from $3.8X10^5$ CFU/g to $6.8X10^5$ CFU/g. Fuerte cultivar exhibited a significantly higher mean microbial load of $6.3X10^5$ CFU/g compared to $4.4X10^5$ CFU/g in Hass cultivar establishing that Hass cultivar possesses greater postharvest resilience to microbial decay than Fuerte cultivar within tropical local markets, a trait strongly attributed to its thick, leathery exocarp mechanical barrier. These empirical insights offer evidence-based guidance for farmers and retailers to prioritize more resilient cultivars and adopt rigorous sanitation and postharvest preservation practices to reduce financial losses.

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FAO: Food and Agriculture Organization.

USA: United States of America.

LDL: Low Density Lipoproteins.

1 CHAPTER ONE

1.1 INTRODUCTION

1.1.1 BACKGROUND

Avocados (*Persea americana*) are highly susceptible to postharvest wastage, primarily due to spoilage microflora, the damage to the avocado pulp (edible tissue) always remains unseen until the avocado fruit is cut, (Umeoduagu, 2025). Globally, the majority of postharvest losses are attributed to microbial activity, with fungal and bacterial spoilage being the most widely reported causes, (Demisie & Melese, 2024; Umeoduagu, 2025; Wogu, M.D. & Ighile, 2014). Because of their high water content nutrient-dense composition, and relatively low P^H, avocados are classified as highly perishable and particularly vulnerable to microbial invasion.(Suleiman, 2024). Pathogenic microorganisms typically enter the fruit through physical wounds such as bruises and cuts, natural openings or via insect infestation. Visible signs of this spoilage include formation of spots and pits, moulds growth, internal discoloration and eventual rotting, (Hendrik & Schifferstein, 2024; Umeoduagu, 2025). A member of Lauraceae family, *Persea americana* originated in Mexico and Central America, (Sseruwagi *et al*., 2025). The global demand for the fruit has surged due to its extensive nutritional benefits and its application in industrial sectors like natural cosmetics and skincare, (Huang *et al*., 2023; Sseruwagi *et al*., 2025). In developing nations like Uganda, avocados serve as an affordable and vital source of essential nutrients including healthy fats, proteins, vitamins, fibers, and antioxidants, this helps to address the food challenge in such nations, (Bhore *et al*., 2021; Chaves & Borges, 2016; Coetzee *et al*., 2017). The production and marketing statistics reflect this growing demand; between 2012 and 2022, global production grew at a Compound Annual Growth Rate (CAGR) of 7%, reaching 8.5 million metric tons, (Sseruwagi *et al*., 2025). The projections suggest that by 2030, production will triple the levels seen in 2010, (OECD & FAO, 2021). The global market being provided by mainly USA and Canada(27%), followed by Europe(22%); Asia and Africa(4%); Egypt, Morocco and South Africa,(Sseruwagi *et al*., 2025). While Mexico remains the dominant global producer, Kenya has emerged as Africa's leading commercial exporter, producing roughly 518, 500 metric tons in 2023, (Rabobank, 2024; Racaud, 2026; Sseruwagi *et al*., 2025). Uganda has recently transitioned into a significant player in the avocado market, ranking among the top ten largest avocado-exporting countries in Africa as of 2023, (Racaud, 2026; Sseruwagi *et al*., 2025). Annual export volumes now exceed 15,000 metric tons, with primary markets located in Europe and the Middle East. Despite its status as "green gold," the fruit faces severe threats from microbial decay, (Sseruwagi *et al.*, 2025).While general research exists regarding avocado spoilage, there are limited local context-specific and comparative data regarding the microbial susceptibility of the Hass versus Fuerte cultivars within the Ugandan local market context. This study seeks to address these gaps by analyzing common specific bacterial microflora responsible for decay in these two economically vital varieties.

1.1.2 THE PROBLEM STATEMENT.

Sustainable community development requires the minimization of postharvest food loss within both commercial markets and consumer households. Despite this necessity, global food waste remains a staggering issue, with approximately 900 million tons of food , valued at nearly one trillion dollars is wasted annually before reaching the point of consumption, (Cromwell *et al.*, 2025; Hendrik & Schifferstein, 2024; Tian *et al.*, 2026). Avocados are among the most nutritionally significant fruits however, their short life and high susceptibility to microbial decay result to massive economic losses. This perishability often leads to a market oversupply of spoiled fruit, evident from the large heaps of decomposing avocados in both village and urban marketplaces, (Hendrik & Schifferstein, 2024; Okuthe, 2024; Wogu, M.D. & Ighile, 2014). The decay process, characterized by pitting, mold growth, and internal discoloration, is primarily driven by pathogenic bacteria and fungi that degrade the fruit's quality and alter flavor, (Hendrik & Schifferstein, 2024). Such microbial actions create a dual crisis, causes a shortage of consumable, nutrient-dense fruit for the population and leads to significant revenue losses for farmers and industries that rely on avocados as a primary raw material. In Nagongera Town Council, Tororo District, where small-scale avocado trade is a vital economic lifeline for many, particularly women, this spoilage acts as a critical barrier to financial stability. Despite these clear economic impacts, there is limited context-specific research regarding the exact microorganisms responsible for avocado decay in this region. Furthermore, while general identification methods exist, limited comprehensive data is available regarding the microbial load and susceptibility difference between the Hass and Fuerte cultivars in tropical local markets. This study therefore seeks to investigate the prevalence of three common bacterial microflora, (*E. coli, Salmonella spp and Staphylococcus*) involved in the decay process and compare the spoilage rates of these two economically important cultivars to provide evidence-based guidance on postharvest resistance.

1.1.3 OBJECTIVES.

1.1.3.1 The main objective.

The main objective of this study is to assess the prevalence of specific common spoilage bacterial microflora of the edible pulp of the ripe avocado and compare microbial load between Hass and Fuerte cultivars sold in Nagongera Town council market, Tororo district, during the 7-day storage period.

1.1.3.2 Specific objectives.

1. To investigate the prevalence of specific common bacterial species associated with the spoilage of ripe avocado fruits during the 7-day storage period.
2. To quantify and compare microbial load between the Hass and Fuerte cultivars to determine which is more susceptible to post-harvest spoilage during the 7-day storage period.

1.1.4 HYPOTHESIS.

H_0: There are no identifiable pathogenic bacteria associated with the decay of the edible pulp of ripe avocados during a 7-day storage period.

H_A: There are identifiable pathogenic bacteria responsible for the observable spoilage and degradation of the avocado pulp.

H_0: There is no statistically significant difference in the microbial load between the Hass and Fuerte avocado cultivars during the 7-day storage period.

H_A: There is a significant difference in microbial load between the two avocado cultivars, indicating that one race is more susceptible to postharvest spoilage than other.

1.1.5 SIGNIFICANCE OF THE STUDY

This research provides essential empirical data that directly benefits avocado farmers, fruit processing industries and general consumers. By isolating and identifying specific bacterial pathogens and their degradation effects, the findings offer a scientific basis for developing targeted disease control strategies and improving preservation methods to extend avocado shelf-life. Furthermore, this study contributes to the existing body of literature concerning *Persea americana*, (avocado fruit), acting as a valuable technical resource for students and researchers in microbiology and agricultural science. It also provides data for commercial bodies such as cosmetics and food manufacturers utilizing avocados as a raw material. Generally, improving the understanding of local microbial spoilage helps support selection of more resilient cultivars for both local and international markets.

1.1.6 THE LIMITATIONS OF THE STUDY

The study focuses exclusively on fruit samples collected from Nagongera Town Council in Tororo District, Uganda, hence the findings regarding microbial load and spoilage are highly context-specific to the tropical climate of Eastern Uganda and may not be fully representative of other regions with different environmental conditions. The presence of microbes like *E. coli* and *Salmonella spp* suggest contamination likely occurs through local market surfaces or shared handling practices in Nagongera. These specific patterns may differ in more regulated or industrial supply chains. The research is strictly centered on bacteriological isolation and characterization and while it identifies three bacterial species (*E. coli, Salmonella spp* and *Staphylococcus spp*) it does not provide a primary focus on fungal pathogens that are also known to cause significant quality loss like Anthracnose. The comparative analysis is limited to only two economically significant varieties (Hass and Fuerte) while excluding other varieties grown in Uganda like Reed, Bacon or Jumbo. The study evaluates spoilage trends over a fixed 7-day period which may not account for long-term storage effects or accelerated decay during international shipping and expanded supply chains.

1.1.7 SCOPE OF THE STUDY

The scope of this study is centered on bacteriological isolation and characterization of microorganisms associated with spoilage of ripe avocado pulp (*Persea americana*). Specifically, the study examines the prevalence and role of common bacterial microbes, (*E. coli, Salmonella spp and Staphylococcus*) in Hass and Fuerte. It also describes mechanisms of avocado contamination and provides effective postharvest handling recommendations of avocados. This research integrates principles from Microbiology, Food science (food microbiology), Botany (plant pathology), ecology and Environmental science. The study focuses on fruit samples collected from Nagongera Town Council market, Tororo District, Uganda. While localized in its sampling, the findings contribute to a broader global understanding of developing spoilage-resistant varieties to benefit the entire avocado value chain.

1.1.8 JUSTIFICATION OF THE STUDY

Avocados are a vital nutritional resource, rich in vitamins, fibers, and essential minerals, (Ana F. Vinha, 2020; Huang *et al*., 2023). Given their high economic value and perishability, understanding their specific spoilage rates is matter of urgent necessity for both producers and consumers. This study addresses local context gaps existing in comparative microbial susceptibility of economically important Hass and Fuerte cultivars within the tropical climate of Eastern Uganda, a case study of Nagongera town council, Tororo District. By identifying postharvest-resistant varieties, the study provides actionable insights for farmers and market authorities countrywide to reduce on financial losses. This work fulfills a critical need for context-specific microbiological research, advancing the field of food science within the Ugandan academic landscape.

2 CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 INTRODUCTION

The consumption of avocados is integral to human health and well-being, as they serve an essential source of vitamins, fibers, minerals, and health-promoting phytochemicals. This high nutrient density, coupled with diverse industrial applications, has driven a global surge in demand for high-quality avocados, (Ana F. Vinha, 2020; Huang *et al.*, 2023). However, the same nutritional richness that benefits humans also makes avocado highly susceptible to microbial attack. Pathogenic bacterial and fungal species are primarily responsible for fruit decay, leading to significant yield losses during transit and storage, (Cromwell *et al.*, 2025; Hendrik & Schifferstein, 2024). This spoilage presents a formidable barrier to reliable availability of avocados for both end-consumers and processing industries, resulting in substantial revenue loss for farmers, (Cromwell *et al.*, 2025). Despite the clear economic and nutritional implications, there remains a lack of context-specific research regarding microbial fruit spoilage within Nagongera Town Council, Tororo District. To address this gap, this review explores the botanical origin of avocado fruit, its multifaceted uses, the common preservation techniques, and specific pathways of microbial contamination. Particular emphasis is placed on the biochemical characteristics of bacterial isolates to support the study's objective of identifying the common bacterial microflorae responsible for the decay of the Hass and Fuerte cultivars.

2.1.2 ORIGIN OF AVOCADO FRUIT

The avocado, *Persea americana* is a prominent member of the Lauraceae family , which comprises over 2,500 species dispersed across tropical and subtropical regions, (Arzate-Ferna´ndez, 2008; Sseruwagi *et al.*, 2025). Historically, the species is believed to have originated in Mexico and Central America, (Huang *et al.*, 2023; Sseruwagi *et al.*, 2025), with evidence suggesting it was among the first trees or shrubs cultivated in these regions as early as 6,400BC, (Arzate-Ferna´ndez, 2008). Within the agricultural industry, approximately 500 varieties exist, varying in morphology and colour, (Ana F. Vinha, 2020). Among these the Hass and Fuerte cultivars are the most economically significant, dominating the international trade, (Dong *et al*., 2024a; Exporters, 2026; Racaud, 2026). Modern production statistics highlight the rapid global expansion of the crop. Between 2012 and 2022, global production grew at a compound annual rate of 7% reaching 8.5 million metric tons, (Sseruwagi *et al*., 2025). While Mexico remains the world's leading producer, Kenya has emerged as a top African exporter, facilitating a transition in neighboring countries, including Uganda; from subsistence farming to significant global participation, (Rabobank, 2024; Racaud, 2026; Sseruwagi *et al*., 2025). In Uganda, avocado production is expanding rapidly, with over ten cultivars grown nationwide, including the Hass, Fuerte, Reed. Bacon, and Jumbo varieties, (Exporters, 2026; Sseruwagi *et al*., 2025). Understanding the distinct biological origins and physical characteristics of these cultivars is essential for analyzing their varying susceptibility to postharvest microbial spoilage.

2.1.3 MORPHOLOGY AND PHYSIOLOGY OF THE HASS VERSUS FUERTE CULTIVARS

The anatomical structure of the avocado, comprising the epicarp(skin), mesocarp(edible pulp), and the endocarp(seed), (Ana F. Vinha, 2020), determines it's inherent resistance to microbial invasion. The physical and chemical variations between Hass and Fuerte cultivars significantly influence their postharvest stability. The exocarp(skin) serves as the primary mechanical barrier against pathogenic entry. The Hass cultivar is distinguished by a thick, pebbly and leathery skin that transitions from green to purplish-black upon ripening. This robust exterior provides superior protection against mechanical injuries like bruises, cuts, which often serve as primary entry points for pathogens, (Exporters, 2026; Ponce *et al.*, 2024). Fuerte cultivar is characterized by a relatively thin, smooth green skin that remains green even at full maturity. Due to its thinner profile, the Fuerte is significantly vulnerable to physical damage and subsequent microbial colonization, (Ponce *et al*., 2024). Generally, the Hass is oval-shaped and genetically smaller than a typically pear-shaped Fuerte cultivar, (Beiro-valenzuela *et al*., 2025; Ponce *et al*., 2024). Hass is recognized for more uniform ripening and a longer shelf-life particularly in cold climates, (Ponce *et al*., 2024). While both are rich in C_7 sugars (mannoheptulose and perseitol), Fuerte exhibits a more rapid conversion to C_6 sugars during the ripening phase. These sugars serve as respiratory substrates that not only influence the fruit's buttery texture and aroma but also affect the rate of tissue degradation, (Beiro-valenzuela *et al*., 2025; Ponce *et al*., 2024). Hass cultivar generally possesses a high oil content in the creamy mesocarp (edible part), while Fuerte is noted for its high unsaturated fatty acid profile. This high nutrient density in both cultivars makes them an ideal medium for the proliferation of spoilage microflora once the skin barrier is breached, (Beiro-valenzuela *et al*., 2025; Ponce *et al*., 2024).

2.1.4 IMPORTANCE OF AVOCADO FRUITS

Avocados (*Persea americana*) are often referred to as "green gold" and are highly profitable due to their nutritional, medicinal and cosmetic values, (Sseruwagi *et al*., 2025). The edible portion (edible pulp) is rich in fatty acids, proteins, bioactive compounds (carotenoids and phenolics), vitamins (K, E, B1, B2, D, C) as well as minerals like potassium and iron, (Ana F. Vinha, 2020; Bhore *et al*., 2021; Chaves & Borges, 2016). This justifies why avocado is very essential to human diet and in helping developing countries improve their nutritional standards. Organizations like "The Heart Foundation" in Australia recognize avocados as heart-healthy due to high levels of oleic acid, which helps reduce "bad" LDL cholesterol, (Ana F. Vinha, 2020). Alpha-carotenes have antioxidant properties which prevent oxidation of LDL (bad) cholesterol hence reducing on the occurrence of atherosclerosis, High fiber content (7% of total mass) aids in weight loss and digestion while its alkaline properties benefit individuals with gastric ulcers, (Bhore *et al*., 2021). Due to antioxidant and anti-inflammatory effects, avocado fruits are recommended as anti-aging foods, (Ana F. Vinha, 2020; Bhore *et al.*, 2021). In cosmetics avocado oil is used in skincare, soaps, and lotions for its moisturizing and vitamin-rich profile, (Bhore *et al*., 2021). Metabolites from the mature unripe avocado pulp, leaves and seeds have shown significant antibacterial and antifungal activity, (Ana F. Vinha, 2020; Bhore *et al*., 2021; Puspitasari *et al*., 2023).

2.1.5 PRESERVATION OF AVOCADO FRUITS

Avocados are exceptionally perishable often suffering a 30% damage rate due to poor postharvest handling, (Puspitasari *et al.*, 2023). As climacteric fruits, their metabolic rate increases with maturity speeding up the aging process, (Dong *et al*., 2024b; Puspitasari *et al*., 2023). The treatments given to avocados after harvesting are cleaning, sorting(basing size, quality), storing, packaging and transporting regarded as postharvest handling techniques, aimed at retaining its quality, (Dong *et al.*, 2024b; Puspitasari *et al*., 2023). Storage in refrigerated rooms decreases respiration rates and slows the decay process. The nature of the storage environment greatly influences the ripening and decay processes of avocados, (Dong *et al*., 2024b; Puspitasari *et al*., 2023; Spalding, 1976). Cold-shock treatment with ice-cold water(<4^0C) for 30 minutes can delay ripening indicators, such as softening and skin colour change by several days, (Dong *et al*., 2024b; Puspitasari *et al*., 2023). It's cheap, effective and can be used as a practical alternative in fruit life extension. In Ethiopia, (Berihu, 2021) demonstrated that edible coating using Aloe vera gel or corn starch creates a barrier against mechanical damage and oxygen while providing antimicrobial protection, (Puspitasari *et al.*, 2023). This keeps avocados in their normal condition (colour) due to delayed ripening and slowed chlorophyll degradation. The use of corrugated cardboard with partitions or dry banana leaves as filters reduces vibration-induced mechanical damage during transport maintaining the fruits safe until they reach desired places, (Puspitasari *et al*., 2023). Cultivars like Hass can remain on the tree up to six months after maturity, (Spalding, 1976), though this is unsuitable for fruits intended for long-distance shipping because of quick ripening making transport difficult.

2.1.6 WAYS AVOCADO FRUIT UNDERGOES CONTAMINATION

To maintain the high market demand, it's vital to preserve the fruit's taste, appearance, and aroma through the chain. Even though avocados naturally harbor microflora, pathogens are introduced during, pre-harvest, harvest and postharvest handling processes hence contamination occurs at any stage from a farm to storage places, (Puspitasari *et al*., 2023). Contamination in the farm through untreated water, contaminated soils or animal fecal matter are a source of disease spread to avocados, (Puspitasari *et al*., 2023). During harvesting and postharvest periods, high human contact and unsterilized equipment (bins, picking sticks) increases the risk of pathogen introduction. Storing fruits in improperly cleaned rooms or dirty packaging (dirty boxes) exposes them to environmental contaminants, (Demisie & Melese, 2024; Dong *et al*., 2024a; Puspitasari *et al*., 2023). Skin breaks, bruises, and lesions; indicators of mechanical damage caused by improper handling serve as primary entry points for bacteria and fungi, (Dong *et al*., 2024b; Puspitasari *et al*., 2023).

2.1.7 AVOCADO FRUIT SPOILAGE BY MICROORGANISMS

Avocado spoilage is a major issue to all buyers and consumers as it accounts for huge losses and decreased market demand. Approximately one-quarter of harvested avocados spoil before reaching the consumer, (Malick Bill1 *et al*., 2022; Mbah-Omeje Kelechi N., 2023). Spoilage is characterized by spotting, rotting, pitting, and internal discoloration,(Hendrik & Schifferstein, 2024; Shiferaw & Kibret, 2021). The pathogens penetrate the cellulose and pectin of the cell walls, altering the fruit's physical structure and aroma. Related studies, (Coetzee *et al*., 2017; Demisie & Melese, 2024; Frances *et al*., 2022; Shiferaw & Kibret, 2021; Umeoduagu, 2025; Wogu, M.D. & Ighile, 2014) have identified various bacteria such as *Salmonella spp,* *Staphylococcus spp, Escherichia coli, Bacillus spp* etc. along with fungi like *mucor spp*, *Geotrichum spp, Saccharomyces spp* from spoilt avocados. Pathogenic microbes produce lytic enzymes (pectinases, cellulases) that digest the protective outer layers of the fruit. While true pathogens actively break down cell walls, opportunistic pathogens enter through wounds caused by insects or mechanical injury, (Demisie & Melese, 2024; Gupta, 2006; Lidia Dorantes, *et al*., 2004).

2.1.8 AVOCADO FRUIT DISEASES AND CONTROL STRATEGIES

Despite the fact that avocados are good health promoting foods with worldwide acceptance and increased daily market demands, postharvest diseases like Anthracnose (caused by *Colletotrichum gloeosporioides*), and stem-end rot are leading causes of quality loss, (Coates *et al*., 2001; Granada *et al.*, 2020; Malick *et al*.,, 2022). Pepper spot is another specific fungal disease that targets the stem and tissues of the Hass cultivar, (Coates *et al*., 2001). To ensure production of high-quality avocados it's important to reduce disease development and spread throughout avocado chain. On field activities like pruning thick canopies reduces humidity and removes infected materials, creating an environment less conducive to fungal growth. While the use of fungicides like Prochloraz are effective, health concerns have shifted interest towards biological control agents and bioactive components from avocado leaves, roots or bark have antimicrobial effects against pathogens of avocados, (Granada *et al*., 2020; Malick *et al*., 2022). Research done by (Berihu, 2021; Hafeez, 2024) , both demonstrated that the use of bioactive components as biological control agents in surface coating of avocados helps extend its shelf life. However, there is inconsistency in this method since various regions have different soil and climatic conditions which may reduce their effectiveness. Storing fruits in cold conditions (<4^0C) as they approach ripening, preventing pathogen proliferation and slowing the metabolic rate, (Coates *et al.*, 2001; Tenea *et al*., 2024). Use of clean irrigation water and balanced nutrient application in the orchard is crucial for reducing initial disease outbreaks, ensuring high quality avocados are produced.

3 CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1.1 STUDY DESIGN

This study centered on a laboratory-based experimental design to assess the microbial internal spoilage of Hass and Fuerte avocado fruits (*Persea americana*). The fruits were collected from a selected local market (Nagongera Town Council market). The study involved visual assessment of internal spoilage and microbial isolation, identification, as well as quantification.

3.1.2 SAMPLE COLLECTION AND PREPARATION

Six avocado fruits comprising three from each avocado variety, (Hass & Fuerte) were purchased from sellers in Nagongera Town Council market in Tororo District. The avocado samples collected were fresh, unblemished, ripe and healthy. They were placed in a clean polyethylene bag and brought to the biology laboratory, Nagongera campus, Busitema University for microbiological analysis. The avocado samples were then thoroughly washed with clean water to ensure they were dust free, allowed to dry and were stored at room temperature for one week(7days) to allow them undergo natural spoilage before being used in the study. Following the methods of (Wogu, M.D. & Ighile, 2014), 10g of each avocado pulp sample was extracted using a sterile scalpel blade and blending with 100mL of distilled(sterile) water, . The mixture was homogenized using a sterile blender and serial dilutions of 10^{-1} to 10^{-5} were made in sterile test tubes by transferring 1mL of previous dilutions into 9mL of sterile water and the tubes marked appropriately.

3.1.3 PREPARATION AND STERILIZATION OF MEDIA AND MATERIALS

Nutrient Agar (NA) which was used for general growth in the study was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121^0C for 15minutes. All laboratory apparatus including test tubes, petri dishes, scalpel blade, blender, measuring cylinders and pipettes were sterilized under the same conditions to prevent contamination. After sterilization, the agar was allowed to cool down to 40^0C and poured into petri dishes under aseptic conditions to solidify.

3.1.4 BACTERIAL CULTURING AND ISOLATION FROM AVOCADO FRUIT

The pour plate technique according to (Asoso *et al.*, 2022; Suleiman, 2024) was used for inoculation by transferring 0.1mL of the serially diluted samples, (10^{-1} to 10^{-3}) into NA plates. The plates were incubated at 37^0C for 24hours. Selective media (MacConkey agar for *E. coli* and Hektoen Enteric agar for *Salmonella spp*) were used for pure cultures using the streaking method. The plates were incubated at 37^0C for 24hours. The Colony Forming Units (CFUs) were counted using direct count method and results recorded in CFU/mL

3.1.5 BACTERIAL ISOLATION AND IDENTIFICATION

Bacterial isolates were characterized by colour, shape, opacity, elevation and margin, and identified using Hans Christian Gram's staining technique as described by (Tripathi *et al*., 2025) and biochemical tests including catalase, indole, citrate, and urease tests.

3.1.6 GRAM STAINING TECHNIQUE FOR BACTERIAL ISOLATES.

The Gram staining technique as applied by (Wogu, M.D. & Ighile, 2014) was used for bacterial identification of one day cultured samples. The Gram staining process, (Tripathi *et al*., 2025) employs various steps designed to distinguish between bacterial species based on their cell wall features. It therefore helps in the classification and identification of bacteria hence enabling diagnosis or treatment decisions. A smear slide of each bacterial isolates was made on a clean slide and heat-fixed using flames or allowed to air-dry at room temperature in a sterile environment. Crystal violet stain was added to the fixed culture and left to stand for 60seconds and then later rinsed with distilled water to wash off excess stain without removing the fixed culture. The iodine solution which acts as a mordant was added and left to stand for 60seconds before it was washed off with distilled water, this step is called dye fixing. The fixed culture was then treated with 95% ethanol which acts as a decolorizer when added and left to stand for 30seconds before it was rinsed off with distilled water for 5seconds. The smear was then counter stained with another stain (secondary stain), Safranin and was also allowed to stand for 60seconds before excess stain was washed off with distilled water and the slide allowed to air-dry after excess water was shaken off. The stained smear was subjected to microscopic examination with oil immersion using a X100 objective lens.

3.1.7 BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIAL ISOLATES

To confirm the identity of the bacterial species, several biochemical tests were performed, including catalase, citrate, and urease tests. Specifically, for Indole Test;

As used by (Wogu, M.D. & Ighile, 2014), this test was also applied for confirmation of bacterial species. A bacterial isolate was introduced in to a tryptophan broth and incubated for 2days at 37^0C. 2mL of the broth was then transferred to another test-tube under sterile conditions and 0.5mL of Kovacs' reagent was added. The mixture was then agitated to ensure thorough mixing and was later observed for colour change. The appearance of the pink-colored ring around the interface between the broth suspension, and alcohol reagent that rose to the top indicated a positive result.

4 CHAPTER FOUR

4.1 RESULTS AND DISCUSSION

4.1.1 INTRODUCTION

The primary focus of this chapter is to present and interpret the findings obtained from the microbiological analysis of Hass and Fuerte avocado cultivars. The study specifically evaluates the prevalence of common spoilage bacteria including *E. coli, Salmonella spp* and *Staphylococcus spp* and compares the total microbial load between two varieties after a 7-day storage period. By analyzing these results, the research aims to determine which cultivar exhibits greater postharvest resilience in a tropical local market context.

4.1.2 PRESENTATION OF RESULTS

The results of the total bacterial counts of the spoilt avocado cultivars that were collected in Nagongera Town Council, Tororo District are shown in the table 1. The total bacterial count ranged between 3.8-6.8X10^5 CFU/g in all the six avocado samples examined. For the Hass avocado cultivar, the total bacterial count ranged from 3.8-4.8X10^5 CFU/g while it ranged between 5.9-6.7X10^5 CFU/g in Fuerte avocado cultivar.

To validate these observations, an independent Samples t-test was conducted. The analysis showed a statistically significant difference in the microbial load between the two cultivars, the SPSS determined t-value, $t_{(4)} = -5.21$, p=0.006 hence the p-value, (0.006) is $p<0.05$.

4.1.2.1 Total Bacterial Count (Microbial Load Quantification).

Table 1: The total bacterial counts (microbial load quantification) of Hass and Fuerte avocado cultivars. The mean total bacterial count from the two avocado cultivars was higher in Fuerte cultivar at 6.3X10^5 CFU/g and lower in Hass cultivar at 4.4X10^5 CFU/g.

Table 1.

Sample code	Dilution factor used	Number of colonies	Total Bacterial count (CFU/g)	Mean CFU/g per Cultivar
H1	1000	48	4.8X10^5	4.4X10^5
H2	1000	45	4.5X10^5	
H3	1000	38	3.8X10^5	
F1	1000	64	6.4X10^5	6.3X10^5
F2	1000	59	5.9X10^5	
F3	1000	67	6.7X10^5	

Key: CFU/g= Colony Forming Units per gram, H1=Hass avocado sample 1, H2= Hass sample 2, H3= Hass sample 3, F1 = Fuerte avocado sample 1, F2 and F3 are Fuerte samples 2 and 3 respectively.

4.1.2.2 Morphological and Gram Staining Characteristics.

Table 2: The cultural, morphological and Gram staining characteristics of bacterial isolates from two avocado cultivars. All the three target bacterial species, *E. coli, Salmonella spp* and *Staphylococcus spp* were suspected bacterial isolates present in all samples.

Table 2.

Isolate ID	Colony Morphology (Colour, Form, Edge, Elevation opacity) on NA agar plate	On MacConkey agar plate (Lactose Ferm.)	On Hektoen Enteric agar (H_2S)	Gram stain (+/-)	Cell shape (Cocci/Rod)
ISO-01	Creamy-white, circular, entire, raised, semi-translucent	Pink/Red colonies (Lactose +)	Orange (Fermenter)	−	Rod-shaped
ISO-02	Grey-white, circular, entire, convex, transparent	Colourless (Lactose -)	Blue-green with a black center (H2S +)	−	Rod-shaped
ISO-03	Golden-yellow, circular, entire, convex, opaque	No growth	No growth	+	Spherical (round)

Key: ISO-01(Suspected Isolate-01) = *E. coli*, ISO-02 = *Salmonella spp*, ISO-03 =*Staphylococcus spp*; **+ =** Gram positive**, -** = Gram negative.

4.1.2.3 Summary of Bacterial Isolates and Biochemical Characteristics.

Table 3.

Test	*E. coli*	*Salmonella spp*	*Staphylococcus spp*
Catalase	Positive	Positive	Positive
Indole	Positive	Negative	Negative
Citrate	Negative	Positive	Variable
Urease test	Negative	Negative	Variable

4.1.2.4 Prevalence of the three target Bacterial Pathogens in Hass and Fuerte Avocado Cultivars.

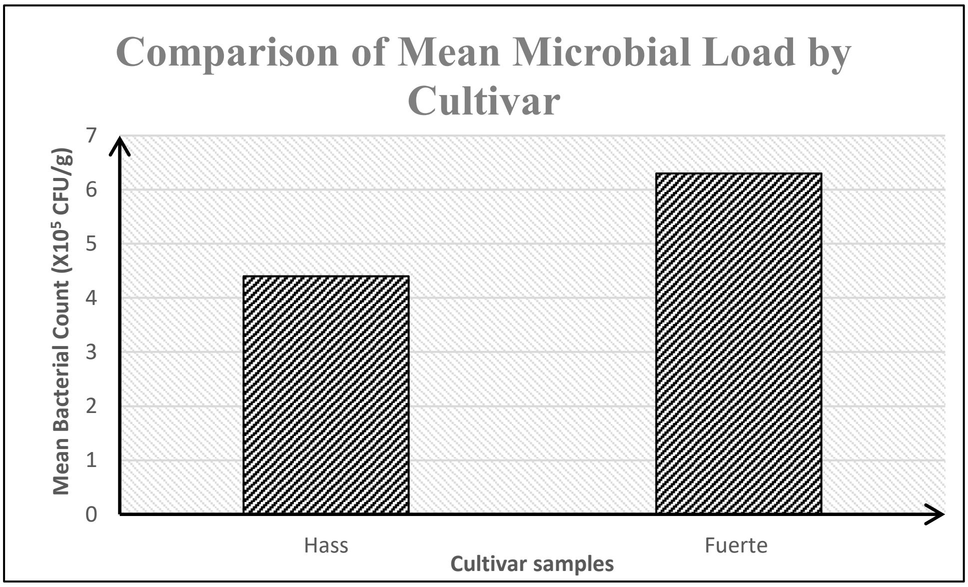
Table 4: The three specific bacterial species of interest, (*E. coli, Salmonella spp* and *Staphylococcus spp*) were all isolated from all the six avocado samples collected from Nagongera Town Council, Tororo District.

Table 4.

Bacterial species	**Hass samples (H1, H2, H3)**	**Fuerte samples (F1, F2, F3)**
E. coli	Present	Present
Salmonella spp	Present	Present
Staphylococcus spp	Present	Present

Figure 1: The graph demonstrates the comparative spoilage trends between the Hass and Fuerte avocado cultivars. The primary trend identified is a significantly higher mean microbial load in the Fuerte cultivar (approximately 20% higher) compared to the Hass cultivar.

Figure 1.



4.1.3 DATA ANALYSIS.

The quantitative data obtained from the Total Bacterial Counts (TBC) was processed and analyzed using **Microsoft Excel 2021** and **SPSS v27.0 (Statistical Package for the Social Sciences).** Microsoft Excel was utilized for initial data entry, calculation of mean Colony Forming Units per gram (CFU/g) and generation of the comparative bar chart shown in **figure 1.** For inferential statistics, an **independent Samples t-test** was conducted via SPSS to determine if the variation in microbial load between the Hass and Fuerte cultivars was statically significant at a 95% confidence interval level (p<0.05). The analysis revealed a statistically significant difference in the microbial load between the two varieties ($t_{(4)} = -5.21$, p=0.006).

4.1.4 DISCUSSION OF RESULTS.

The primary goal of this research was to assess the prevalence of specific spoilage bacteria and compare the microbial susceptibility of Hass and Fuerte avocado cultivars sold in Nagongera Town Council, Tororo District.

The study identified a 100% prevalence of *Escherichia coli, Salmonella spp* and *Staphylococcus spp* across all the samples of both cultivars. This consistent presence aligns with literature indicating that avocados are highly perishable and vulnerable to microbial invasion due to their nutrient-rich composition and relatively low P^H. The high prevalence of these microbes supports the rejection of the first Null Hypothesis (H_0) and Alternative Hypothesis (H_A) accepted as identifiable bacteria were indeed responsible for the observable degradation of the ripe avocado pulp. The isolation of these specific pathogens supports previous findings by Shiferaw & Kibret, (2021); Umeoduagu, (2025); Wogu, M.D. & Ighile, (2014), who also identified similar bacterial profiles in spoiled avocados. The identification of *E. coli* and *Salmonella spp* as Gram-negative rods and *Staphylococcus spp* as Gram-positive cocci, (as shown in **Table 2**) confirms the diverse microbial community involved in the decay process. The presence of these microbes in all samples suggests that contamination likely occurs through shared handling practices, contaminated market surfaces or environmental factors in Nagongera rather than being cultivar specific. These microbes likely enter the fruit through physical wounds, natural openings or during postharvest handling in the market environment, (Dong *et al*., 2024b; Puspitasari *et al*., 2023).

A significant finding of this study was the disparity in microbial load between the two cultivars after the 7-day storage period. The Fuerte cultivar showed a consistently higher microbial load, with a mean total bacterial count of $6.3X10^5$ CFU/g whereas the Hass cultivar recorded a significantly lower mean of $4.4X10^5$ CFU/g as shown in **Table 1.** This difference in susceptibility can be explained by the morphological variations discussed in the literature review. The Hass cultivar is distinguished by a thick, leathery and pebbly exocarp that serves as a robust mechanical barrier against pathogenic entry. In contrast, Fuerte cultivar possesses a relatively thin and smooth skin, making it vulnerable to physical damage and subsequent microbial colonization observed during the study. Furthermore, the rapid conversion of *C7* to

C6 sugars in the Fuerte variety during ripening may provide a more readily available respiratory substrate for bacterial proliferation compared to the Hass variety, (Beiro-valenzuela *et al*., 2025; Ponce *et al*., 2024).

Since the *p*-value, (0.006) is $p<0.05$, the second Null Hypothesis (H_0) which stated that there is no statistically significant difference in microbial load between the two cultivars is rejected and Alternative Hypothesis (H_A) which is strongly supported by these results is accepted, confirming that the Fuerte cultivar is significantly more susceptible to postharvest spoilage than the Hass cultivar in this tropical local market context. These results fulfill the study's objective to provide evidence-based guidance on postharvest resistance. For small scale traders in Nagongera, this data suggests that prioritizing the Hass cultivar could significantly reduce financial losses due to its inherent biological resilience to microbial decay. This data also successfully addresses the local context gap by providing empirical proof of cultivar performance in Eastern Uganda's tropical climate.

5 CHAPTER FIVE

5.1 CONCLUSION AND RECOMMENDATIONS

5.1.1 CONCLUSION

This study successfully assessed the prevalence of spoilage bacteria and compared the microbial susceptibility of Hass and Fuerte avocado cultivars. All the tested fruit samples consistently showed the presence of *E. coli, Salmonella spp* and *Staphylococcus spp*. This proves that these specific pathogens are primary contributors to the observable degradation of avocado pulp during the storage period. There is a statistically significant difference in microbial resistance between the two cultivars. Fuerte cultivar exhibited a higher microbial load ($6.3X10^5$ CFU/g) compared to the Hass ($4.4X10^5$ CFU/g). The superior resilience of the Hass cultivar is attributed to its thick, leathery exocarp which acts as a more effective mechanical barrier against pathogen entry than the thinner skin of the Fuerte variety. Based on the ***p*-value** of **0.006** ($p<0.05$), the Null hypotheses were rejected. The study concludes that identifiable pathogenic bacteria are responsible for spoilage and that cultivar type significantly impacts the rates of this decay.

5.1.2 RECOMMENDATIONS

Small-scale traders and farmers should prioritize the Hass cultivar for commercial trade as its inherent biological resilience significantly reduces financial losses during the transportation and its storage period. To minimize contamination, stakeholders should avoid using unsterilized equipment and ensure that storage environments (bins and packaging) are properly cleaned to prevent introduction of microbes like *E. coli*. Given that skin breaks are primary entry points for pathogens, traders and farmers (harvesters) should use protective materials like dry banana leaves or corrugated cardboard to reduce vibration-induced mechanical damage during transportation. Consumers are advised to use cold-storage methods (below 4^0C) where possible to slow down the metabolic rate and prevent rapid pathogen proliferation. Market authorities in Nagongera Town Council should improve general hygiene to reduce the environmental microbial load that contributes to fruit contamination.

While this study focused on bacterial microflora, further research should be conducted to isolate and identify fungal pathogens such as *Colletotrichum spp* responsible for anthracnose in these cultivars. Also, investigation into the antimicrobial efficacy of local botanical extracts (such as onion or Aloe vera gel) as edible coatings could provide a low-cost preservation solution for local traders. Future studies should evaluate how seasonal changes (wet vs dry seasons) in Uganda affect the microbial load and spoilage rates of avocados in open-air markets.

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7 APPENDIX

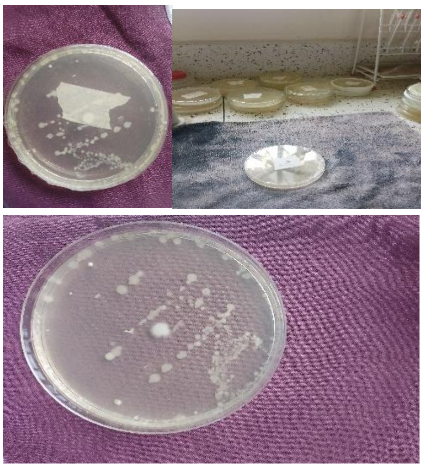
(a) Measurement of media.

(b) Pouring of sterilized media in sterilized petri dishes.

(c) Serial dilutions process.

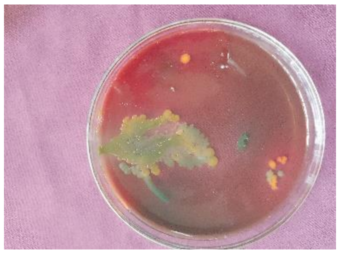
(d) 24hrs Incubation process.

(e) Bacterial growth on NA plates.

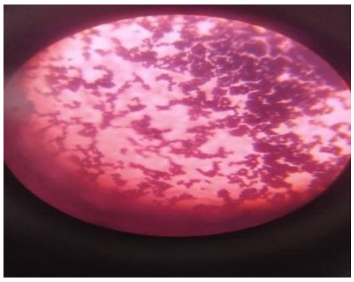


(f) Bacterial growth on McConkey plates for *E. coli*; pink/red colonies

(g) Bacterial growth on Hektoen Enteric acid for *Salmonella spp*; Blue-green with a black center



(h) Gram staining of the Bacterial isolates
(Gram positive; *Staphylococcus spp*).



(Gram negative; *E. coli* and *Salmonella spp)*