BACTERIOLOGICAL EVALUATION OF FRESH AND PACKAGED MIXED FRUIT SALAD SOLD WITHIN UGBOWO AXIS

BY

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BENIN CITY

FEBRUARY, 2025

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

FEBRUARY, 2025

CERTIFICATION

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This is to certify that this project work was carried out by Queen Odiri OGHENEOVO with Mat. No.: LSC2010826 of the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

………………………….. …………………………
Prof. C. E. Oshoma Date
(Project Supervisor)

…………………………... …………………………..
Prof. (Mrs.) F. I. Akinnibosun Date
(Head of Department)

DEDICATION

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

ACKNOWLEDGEMENTS

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My sincere appreciation goes to the Almighty God for his grace and mercies throughout my period of study.

I wish to acknowledge whole heartedly my project supervisor Prof. C. E. Oshoma for his patience and understanding towards me and the success of this project. May God Almighty richly bless you sir for your efforts.

My sincere appreciation goes to my head of department Prof. (Mrs.) F. I. Akinnibosun for her motherly role in the administration of the Department.

I want to also thank my lecturers for their mentoring throughout my stay in this school for their assistance during the course of my study.

I will also like to appreciate my sweet parents Late Mr. Ogheneovo. O. Joseph and Mrs. Ogheneovo Morrin. My lovely siblings: Helen, Hannah, Onome, Blessing and Lucky for their moral support and prayers God bless you.

I will not fail to appreciate my friends: Idenodo Josiah, Idenodo Peter, Oghena Jessica and Odafewoma Believe my course mates who has contributed in one way or the other to the success of the work, God bless you all.

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ABSTRACT

This study was conducted to evaluate the bacteriological and sensory qualities of fresh and packaged mixed fruit salad sold along the Ugbowo axis in Benin City, Edo State. Mixed fruit salad samples were collected from various vendors located near the University of Benin, specifically within the Ekosodin and BDPA areas. Upon collection, the samples were securely placed in sterile zip-lock bags and subsequently transported to the laboratory for bacteriological analysis. The bacteriological assessment was performed using cultural techniques, with the identification of isolates conducted through biochemical methods. Additionally, phenotypic virulence properties of the isolates were evaluated, and antimicrobial sensitivity was assessed using the biodisc diffusion method. The results indicated that the heterotrophic bacterial count of mixed fruit salad samples stored in refrigeration and at room temperature for four hours ranged from $2.50 \pm 2.12 \times 10^3$ cfu/g to $4.08 \pm 1.24 \times 10^5$ cfu/g. In contrast, samples kept in refrigeration and at room temperature for 24 hours exhibited a range between $1.51 \pm 0.20 \times 10^5$ cfu/g to $5.70 \pm 0.88 \times 10^4$ cfu/g. The *Staphylococcus aureus* count for samples stored in refrigeration and at room temperature for four hours ranged from $1.57 \pm 0.78 \times 10^3$ cfu/g to $4.50 \pm 0.42 \times 10^2$ cfu/g, whereas those kept for 24 hours showed a count ranging from $2.50 \pm 0.14 \times 10^3$ cfu/g to $8.96 \pm 0.23 \times 10^4$ cfu/g. The coliform bacteria count in mixed fruit salad samples stored for four hours ranged from $2.34 \pm 0.21 \times 10^3$ cfu/g to $7.20 \pm 0.88 \times 10^2$ cfu/g, compared to samples stored for 24 hours which exhibited counts ranging from $2.50 \pm 0.14 \times 10^3$ cfu/g to $8.96 \pm 0.23 \times 10^4$ cfu/g. The percentage frequency of occurrence of bacterial isolates was as follows: *Staphylococcus aureus* (12%), *Bacillus megaterium* (12%), *Lactobacillus* spp. (12%), *Serratia marcescens* (6%), *Flavobacterium* spp. (10%), *Enterobacter cloacae* (8%), *Micrococcus lactis* (2%), *Bacillus cereus* (10%), *Staphylococcus warnei* (2%), *Enterococcus faecium* (8%), *Bacillus thuringiensis* (8%), *Salmonella arizonae* (4%), *Bacillus licheniformis* (2%), *Klebsiella pneumoniae* (2%), and *Bacillus subtilis* (2%), respectively. The findings of this study underscore the necessity for further research aimed at developing effective strategies for monitoring and mitigating microbial hazards in ready-to-eat foods, thereby safeguarding consumer health against potential outbreaks associated with antibiotic-resistant bacteria.

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CHAPTER ONE

INTRODUCTION

Fresh and packaged mixed fruit salads have gained significant popularity in contemporary diets due to their perceived health benefits and convenience. Fresh mixed fruit salads, composed of a variety of seasonal fruits, provide essential vitamins, minerals, and dietary fiber. The consumption of fresh fruits is associated with numerous health advantages, including reduced risk of chronic diseases such as obesity, cardiovascular disease, and diabetes (Yousuf *et al.,* 2020). Furthermore, the vibrant colors and diverse textures found in fresh mixed fruit salads can enhance the sensory experience of eating, making it more enjoyable for consumers. In contrast, packaged mixed fruit salads offer the convenience of ready-to-eat portions, often with a longer shelf life due to preservation techniques, making them an attractive option for busy individuals seeking a quick and healthy snack. However, the trade-off between convenience and nutritional value in packaged mixed fruit salads often raises concerns about added sugars and preservatives that may diminish their health benefits (Tambekar *et al.,* 2018). Despite these concerns, packaged mixed fruit salads can still serve as a valuable option when fresh alternatives are not readily available, provided consumers make informed choices by reading labels and selecting options with minimal additives. Moreover, the growing demand for convenient and healthy food options has prompted manufacturers to innovate with packaging solutions that aim to preserve the natural taste and nutritional integrity of fruits.

On the other hand, packaged mixed fruit salads offer an appealing alternative for individuals seeking convenience without sacrificing nutritional value (Quadri *et al.,* 2017). These pre-

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prepared options are often marketed as healthy snacks or meal components that cater to busy lifestyles. However, it is important to critically evaluate the quality and composition of packaged mixed fruit salads. Some products may contain added sugars or preservatives that diminish their health benefits compared to their fresh counterparts. Moreover, the freshness of the ingredients can vary significantly depending on storage conditions and shelf life. While fresh mixed fruit salads undoubtedly offer the advantage of being free from such additives and often boast superior taste and texture, they require more time and effort to prepare (Mairami *et al.,* 2018). Nonetheless, for those who prioritize freshness and are willing to invest the time, preparing a mixed fruit salad at home can be a rewarding experience. Exploring local farmers' markets or grocery stores for seasonal fruits can enhance the quality and flavor of homemade mixed fruit salads. Additionally, experimenting with different combinations of fruits and incorporating herbs or a splash of citrus can elevate the taste and provide a personal touch to the dish. Furthermore, making mixed fruit salad at home allows individuals to control portion sizes and tailor the ingredients to meet specific dietary needs or preferences (Mahfuza *et al.,* 2018).

Both fresh and packaged mixed fruit salads play vital roles in promoting healthier eating habits among consumers. While fresh mixed fruit salads are unrivaled in terms of taste and nutritional integrity, packaged versions provide accessibility for those with limited time or resources for meal preparation. Ultimately, education on label reading and ingredient awareness is crucial for individuals looking to make informed dietary choices regarding these popular food items. By understanding the benefits and limitations of each option, consumers can enjoy the convenience of packaged mixed fruit salads without compromising on their health goals. Additionally, incorporating fresh fruit into one's diet can further enhance the overall quality of nutrition while allowing for creativity and personalization in meal planning (Jimoh *et al.,* 2023).

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The bacteriological assessment of fresh and packaged mixed fruit salad is vital in understanding the microbiological safety of these popular food items. As consumer preferences shift towards healthier options, mixed fruit salads have gained prominence in both home kitchens and commercial establishments. However, the potential for microbial contamination poses significant health risks. This study aims to evaluate the presence of pathogenic bacteria in fresh and packaged mixed fruit salads, focusing on common contaminants such as *Salmonella, Escherichia coli*, and *Listeria monocytogenes*. By analyzing samples from various sources, the research seeks to compare the bacterial load between freshly prepared salads and those that are commercially packaged (Graca *et al.,* 2017). The findings will provide valuable insights into the effectiveness of current food safety practices and highlight areas where improvements are needed to ensure consumer health. Furthermore, understanding the differences in bacterial contamination between these two types of mixed fruit salads can guide manufacturers and consumers in making informed decisions about food handling and consumption practices. Through meticulous sampling and laboratory analysis, this research endeavors to quantify bacterial levels and identify specific strains present in both types of mixed fruit salads. Fresh mixed fruit salads are often perceived as healthier alternatives; however, they can be susceptible to contamination during various stages of production and preparation (Francis *et al.,* 2022). Factors such as inadequate washing, cross-contamination from utensils or surfaces, and improper handling by food workers can contribute to bacterial proliferation.

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In contrast, packaged mixed fruit salads may undergo processes designed to minimize microbial load, including washing with sanitizing agents or vacuum packaging. Nonetheless, these products are not immune to contamination due to factors like post-packaging handling or temperature fluctuations during transport (Eni *et al.,* 2020). Despite these preventative measures,

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the shelf life and storage conditions of packaged mixed fruit salads remain critical in controlling bacterial growth. This assessment employs standard microbiological techniques to isolate and identify bacterial colonies from both fresh and packaged samples. The results provide a comparative analysis that highlights differences in microbial loads between the two types of salads. Understanding these differences is crucial for public health recommendations aimed at minimizing the risk of foodborne illnesses associated with fruit consumption. Ultimately, this study underscores the importance of rigorous safety protocols throughout the supply chain to ensure that consumers enjoy safe and nutritious mixed fruit salad options (Denis *et al.,* 2019). The findings also emphasize the need for continuous monitoring and quality control measures to detect potential sources of contamination early in the distribution process. By implementing regular testing and adhering to stringent hygiene practices, producers can significantly reduce the incidence of harmful bacterial presence in mixed fruit salads.

1.1 Aim and Objectives

The aim of this study is to evaluate the bacteriological and sensory qualities of fresh and packaged mixed fruit salad sold with the Ugbowo axis in Benin City, Edo State.

The specific objectives of the study were to:

1. enumerate, isolate and identify the bacteria associated with fresh and packaged mixed fruit salad
2. determine the prevalence of bacterial species present in fresh and packaged mixed fruit salad
3. determine the sensory qualities of fresh and packed mixed fruit salad

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CHAPTER TWO

LITERATURE REVIEW

2.1 The consumption of fresh and packaged mixed fruit salads

The consumption of fresh and packaged mixed fruit salads has gained significant popularity in contemporary dietary practices, largely due to their perceived health benefits and convenience. However, the microbiological safety of these products warrants careful examination, particularly concerning the presence of bacteria that may pose health risks to consumers (Das *et al.,* 2018). The consumption of fresh and packaged mixed fruit salads has garnered significant attention in recent years, particularly within the context of health and nutrition. Fresh mixed fruit salads, comprised of a variety of whole fruits, are widely recognized for their nutritional benefits, offering essential vitamins, minerals, and dietary fiber. These salads serve as an excellent source of hydration and contribute to daily fruit intake recommendations set forth by dietary guidelines. The sensory appeal of fresh mixed fruit salads characterized by vibrant colors and diverse textures also enhances their consumption among consumers seeking healthier food options (Daniel *et al.,* 2020).

In contrast, packaged mixed fruit salads present a different set of advantages and challenges. Packaged varieties offer convenience that caters to the fast-paced lifestyles prevalent in modern society. They are often marketed towards busy individuals who may lack the time or resources to prepare fresh fruits at home. However, the nutritional integrity of these products can vary significantly based on factors such as added preservatives or sugars designed to enhance flavor or prolong shelf life. Consequently, consumers must exercise discernment when selecting packaged options to ensure they are making health-conscious choices (Corbo *et al.,* 2020). Moreover, both fresh and packaged mixed fruit salads play a crucial role in promoting increased

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fruit consumption across various demographics. Educational initiatives aimed at increasing awareness about the importance of fruits in a balanced diet can further encourage this trend. As public health campaigns continue to advocate for greater accessibility to nutritious food options, understanding consumer preferences regarding fresh versus packaged mixed fruit salads will be instrumental in shaping future dietary patterns (Buck *et al.,* 2023).

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2.2 Types of Bacteria in Fresh and Packaged Mixed fruit salads

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The bacterial flora associated with fresh and packaged mixed fruit salads is diverse, encompassing both pathogenic and non-pathogenic species. Among the most frequently identified pathogens are *Escherichia coli, Salmonella spp.,* and *Listeria monocytogenes*. These bacteria are notorious for their association with foodborne illnesses, which can lead to significant morbidity and, in severe cases, mortality. For instance, *E. coli* strains, particularly O157:H7, have been implicated in outbreaks linked to contaminated produce, resulting in severe gastrointestinal distress and complications such as hemolytic uremic syndrome (Beuchat, 2022). In addition to these pathogens, non-pathogenic bacteria such as *Lactobacillus* and *Bifidobacterium* may also be present, contributing to the overall microbial load. While these bacteria are generally considered beneficial, their presence can indicate poor hygiene practices during the handling and preparation of mixed fruit salads. Furthermore, the microbial community can be influenced by the type of fruits used, the conditions under which they are stored, and the methods employed in their preparation.

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Bacteria play a crucial role in the food industry, particularly regarding the safety and quality of fresh and packaged mixed fruit salads. Understanding the types of bacteria present in these products is essential for ensuring food safety and consumer health (Annapurna and Rashmi, 2019).

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Fresh mixed fruit salads, often perceived as healthy options, can harbor various bacteria due to contamination during harvesting, processing, and handling. Common bacteria found in fresh mixed fruit salads include *Salmonella, Escherichia coli* and *Listeria monocytogenes*. Salmonella, typically associated with undercooked poultry and eggs, can also be present in contaminated fruits. *E. coli,* particularly harmful strains like O157:H7, can result from fecal contamination of water used to irrigate crops. *Listeria monocytogenes* poses a significant risk, especially during the summer months when fruits are most popular. It thrives in cold temperatures and can persist in refrigerated environments, making it a concern for ready-to-eat salads (Al-Kharousi *et al.,* 2019).

Packaged mixed fruit salads, while convenient, can also be at risk. The packaging process should typically mitigate bacterial contamination; however, improper handling and storage can lead to issues. Bacteria such as *Staphylococcus aureus*, which can produce toxins, are of concern if the salads are prepared by individuals who do not follow proper hygiene practices. Additionally, packaged salads may support the growth of spoilage organisms like Pseudomonas spp., which, while not necessarily harmful, can lead to a decline in quality and shelf-life. Both fresh and packaged mixed fruit salads can be susceptible to bacterial contamination. Understanding the types of bacteria that may be present is vital for food safety (Adedeji *et al.,* 2017). Adhering to best practices in growing, handling, and packaging can significantly reduce these risks, ensuring that consumers enjoy the health benefits of mixed fruit salads without compromising safety.

Bacteria are microscopic organisms that can be found virtually everywhere, including in fresh and packaged mixed fruit salads. The types of bacteria present in these salads can have varying effects on human health, from beneficial to harmful. Understanding the different types of bacteria in fresh and packaged mixed fruit salads is essential for ensuring food safety and

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reducing the risk of foodborne illnesses. One type of bacteria commonly found in fresh mixed fruit salads is *Lactobacillus* (Yousuf *et al.,* 2020). *Lactobacillus* is a beneficial bacteria that is commonly found in fermented foods such as yogurt and sauerkraut. It helps to promote digestion and boost the immune system. In fresh mixed fruit salads, Lactobacillus can help to prevent the growth of harmful bacteria by creating a slightly acidic environment that is unfavorable for their growth. Another type of bacteria that may be present in fresh mixed fruit salads is *Acetobacter. Acetobacter* is a type of bacteria that is commonly found in vinegar. It helps to convert alcohol into acetic acid, which gives vinegar its sour taste. In fresh mixed fruit salads, *Acetobacter* can help to preserve the freshness of the fruits by inhibiting the growth of spoilage bacteria (Tambekar *et al.,* 2018). On the other hand, there are also harmful bacteria that can be present in fresh mixed fruit salads, such as *Escherichia coli* and *Salmonella*. These bacteria are commonly found in contaminated water or soil and can cause foodborne illnesses if ingested. It is important to properly wash and sanitize fruits before adding them to salads to prevent the growth of these harmful bacteria.

In packaged mixed fruit salads, the types of bacteria present may differ from those found in fresh mixed fruit salads. Packaged mixed fruit salads are typically processed and packaged in a commercial facility, which may introduce different types of bacteria into the product. One type of bacteria commonly found in packaged mixed fruit salads is Listeria (Quadri *et al.,* 2017). Listeria is a type of bacteria that can cause serious illnesses, especially in pregnant women, the elderly, and individuals with weakened immune systems. Another type of bacteria that may be present in packaged mixed fruit salads is *Clostridium botulinum. Clostridium botulinum* is a type of bacteria that produces a powerful toxin called botulinum toxin, which can cause botulism, a potentially life-threatening illness. It is important to properly store packaged mixed fruit salads at

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the correct temperature to prevent the growth of *Clostridium botulinum* and other harmful bacteria. In addition to harmful bacteria, packaged mixed fruit salads may also contain beneficial bacteria such as *Bifidobacterium. Bifidobacterium* is a type of probiotic bacteria that can help to improve digestion and boost the immune system (Mairami *et al.,* 2018). Some packaged mixed fruit salads may be fortified with probiotics to enhance their health benefits. Overall, the types of bacteria present in fresh and packaged mixed fruit salads can vary widely and can have both positive and negative effects on human health. It is important to practice proper food safety measures, such as washing fruits before consuming them and storing packaged mixed fruit salads at the correct temperature, to minimize the risk of foodborne illnesses. By understanding the different types of bacteria in mixed fruit salads, consumers can make informed choices about the foods they eat and ensure their safety and well-being (Mahfuza *et al.,* 2018).

2.3 Factors Contributing to Bacterial Proliferation

Several factors contribute to the proliferation of bacteria in fresh and packaged mixed fruit salads. Firstly, the inherent moisture content of fruits provides an ideal environment for bacterial growth. When fruits are cut and mixed, the increased surface area further enhances the potential for microbial contamination. Additionally, the handling practices employed during the preparation of mixed fruit salads play a crucial role in determining the microbial load. Cross-contamination from utensils, cutting boards, and the hands of food preparers can introduce pathogenic bacteria into the final product (Jimoh *et al.,* 2023). Moreover, the storage conditions of packaged mixed fruit salads significantly influence bacterial growth. Refrigeration is essential for inhibiting the growth of pathogens; however, improper temperature control during transportation and storage can lead to the proliferation of harmful bacteria. The shelf life of packaged salads is often extended through the use of preservatives, yet these measures may not be sufficient to eliminate all microbial threats. Fresh and packaged mixed fruit salads have gained popularity as a

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convenient and healthy dietary choice. However, the proliferation of bacteria in these products poses significant food safety concerns (Graca *et al.,* 2017). Several factors contribute to the growth of bacteria in both fresh and packaged mixed fruit salads, including environmental conditions, handling practices, and storage methods. Environmental conditions play a pivotal role in bacterial proliferation. Fruits are often harvested from fields where they may be exposed to various pathogens from soil or water sources. Additionally, the temperature at which fruits are stored before preparation can significantly influence bacterial growth; warmer temperatures generally promote faster reproduction rates of bacteria. For instance, the "danger zone" for bacterial growth is typically between 40°F (4°C) and 140°F (60°C), making it essential to maintain appropriate refrigeration during transportation and storage (Francis *et al.,* 2022). Moreover, handling practices significantly impact microbial contamination levels. Cross-contamination can occur during the washing, cutting, or packaging processes if proper hygiene measures are not observed. The use of contaminated utensils or surfaces can introduce harmful bacteria into mixed fruit salads. Furthermore, inadequate washing procedures may fail to remove pesticide residues or pathogens present on the fruit's surface.

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Storage methods after production play a crucial role in maintaining food safety. Packaged mixed fruit salads often have a limited shelf life; improper storage conditions can accelerate spoilage and bacterial growth (Eni *et al.,* 2020). It is vital for consumers to adhere to recommended storage guidelines to minimize health risks associated with eating contaminated mixed fruit salads. The proliferation of bacteria in fresh and packaged mixed fruit salads is influenced by a variety of factors, which can significantly impact food safety and quality. Understanding these factors is crucial for consumers, food handlers, and producers alike.

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Environmental conditions play a critical role in bacterial growth. Fresh mixed fruit salads are often susceptible to temperature fluctuations. The USDA recommends keeping perishable items at a temperature of 40°F or lower (Denis *et al.,* 2019). When mixed fruit salads are stored at higher temperatures, bacteria like *Salmonella* and *E. coli* can multiply rapidly, turning a healthy dish into a food safety risk.

Secondly, contamination sources are pivotal in the proliferation of bacteria. Fresh fruits may be contaminated from several sources, including soil, water, and during handling. If fruits are not properly washed before preparation, pathogens present on their skins can transfer into the mixed fruit salad, facilitating bacterial growth during storage (Das *et al.,* 2018). Packaged mixed fruit salads may also face contamination during processing, packaging, and distribution, where inadequate hygiene practices can lead to contamination. Cross-contamination is another significant concern. In kitchen environments, cutting boards, knives, and utensils that have come into contact with raw meats or contaminated surfaces can introduce harmful bacteria to mixed fruit salads. Maintaining strict hygiene practices is essential to minimize this risk. Additionally, the composition of mixed fruit salads can influence bacterial growth. Fruits with higher sugar content may promote the growth of certain bacteria when combined with other ingredients like dressings or yogurt (Daniel *et al.,* 2018). This combination can create an ideal environment for bacteria to thrive if not stored properly. The proliferation of bacteria in fresh and packaged mixed fruit salads is driven by environmental factors, contamination sources, cross-contamination, and the composition of the salads themselves. Awareness of these factors can aid in preventing foodborne illnesses and ensuring the safety of mixed fruit salads for consumers.

Fresh and packaged mixed fruit salads are popular choices for individuals looking for a healthy and convenient snack option. However, it is important to be aware of the factors that can aid bacteria in these salads, as this can pose a risk to consumer health. There are several factors that can contribute to the growth and survival of bacteria in both fresh and packaged mixed fruit salads (Corbo *et al.,* 2020). One of the primary factors aiding bacteria in fresh and packaged mixed fruit salads is the moisture content of the fruits. Bacteria thrive in moist environments, and the presence of high moisture levels in mixed fruit salads can provide a conducive environment for their growth. Additionally, the pH levels of the fruits in the salad can also impact the growth of bacteria. Fruits with higher acidity levels are less susceptible to bacterial growth, while fruits with neutral pH levels can provide a more favorable environment for bacteria to thrive. Factor that can aid bacteria in fresh and packaged mixed fruit salads is temperature. Bacteria multiply rapidly in warm environments, so mixed fruit salads that are not stored at the proper temperature can become a breeding ground for harmful bacteria (Buck *et al.,* 2023). It is important to store fresh mixed fruit salads in the refrigerator and to ensure that packaged mixed fruit salads are kept at the appropriate temperature to prevent bacterial growth.

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The handling and preparation of the fruits in the salad can also impact the growth of bacteria. If the fruits are not properly washed before being added to the salad, they may contain harmful bacteria that can contaminate the entire dish. Additionally, if the salad is prepared using utensils or surfaces that are not properly sanitized, bacteria can be transferred to the salad during the preparation process (Beuchat, 2022). The type of packaging used for packaged mixed fruit salads can also influence the growth of bacteria. If the packaging is not airtight or if it is damaged, bacteria can easily enter and contaminate the salad. Additionally, the use of packaging materials that are not food-safe can lead to the growth of harmful bacteria in the salad. Cross-contamination is another factor that can aid bacteria in fresh and packaged mixed fruit salads. If the fruits in the salad come into contact with raw meat or poultry during preparation, harmful

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bacteria can be transferred to the salad and pose a risk to consumer health. It is important to practice proper food safety measures to prevent cross-contamination and reduce the risk of bacterial growth in mixed fruit salads (Annapurna and Rashmi, 2019).

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The presence of natural sugars in fruits can also provide a food source for bacteria, allowing them to multiply and thrive in fresh and packaged mixed fruit salads. Bacteria feed on sugars present in the fruits, which can lead to an increase in bacterial growth if the salad is not consumed promptly or stored at the proper temperature. Furthermore, the storage conditions of fresh and packaged mixed fruit salads can impact the growth of bacteria. If the salad is stored for an extended period of time or if it is not properly refrigerated, bacteria can multiply and contaminate the salad. It is important to follow proper storage guidelines to ensure the freshness and safety of mixed fruit salads (Al-Kharousi *et al.,* 2019). There are several factors that can aid bacteria in fresh and packaged mixed fruit salads, including moisture content, pH levels, temperature, handling and preparation practices, packaging, cross-contamination, natural sugars, and storage conditions. By being aware of these factors and following proper food safety guidelines, consumers can reduce the risk of bacterial contamination in mixed fruit salads and enjoy them safely as part of a healthy diet.

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2.4 Implications for Public Health

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The presence of pathogenic bacteria in fresh and packaged mixed fruit salads poses significant implications for public health. Foodborne illnesses resulting from contaminated produce can lead to substantial healthcare costs and loss of productivity (Adedeji *et al.,* 2017). Furthermore, vulnerable populations, such as the elderly, pregnant women, and individuals with compromised immune systems, are at heightened risk of severe illness from these pathogens. To mitigate these risks, it is imperative that both consumers and food industry stakeholders adopt stringent food safety practices. This includes thorough washing of fruits, proper sanitation of preparation

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surfaces, and adherence to recommended storage temperatures. Additionally, regulatory agencies must enforce guidelines for the safe handling and processing of fresh produce, ensuring that microbiological testing is routinely conducted. Fresh packaged mixed fruit salad offers a convenient and healthy option for consumers looking to increase their fruit intake (Yousuf *et al.,* 2020). However, there are several public health implications that need to be considered when it comes to the production, distribution, and consumption of these products. In this essay, we will explore the various public health implications for fresh packaged mixed fruit salad.

First and foremost, the production of fresh packaged mixed fruit salad must adhere to strict food safety guidelines to ensure that the product is safe for consumption. This includes proper handling and storage of the fruits, as well as maintaining cleanliness and sanitation in the production facility (Tambekar *et al.,* 2018). Any contamination of the mixed fruit salad could lead to foodborne illnesses, posing a significant public health risk. Additionally, the distribution of fresh packaged mixed fruit salad must also be carefully monitored to ensure that the product remains at the proper temperature during transportation and storage. Improper temperature control could lead to the growth of harmful bacteria, increasing the risk of foodborne illness among consumers. It is essential for companies to have stringent quality control measures in place to prevent any issues with the distribution of their products. When it comes to consumption, consumers must be aware of the potential risks associated with fresh packaged mixed fruit salad (Quadri *et al.,* 2017). While these products are generally considered safe to eat, there is always a risk of contamination if proper precautions are not taken. Consumers should be mindful of the expiration date of the product and make sure to store it at the appropriate temperature to prevent spoilage. One of the key public health implications of fresh packaged mixed fruit salad is its potential to contribute to a healthier diet among consumers. By providing a convenient and easy

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way to consume a variety of fruits, these products can help individuals meet their daily recommended intake of fruits and vegetables. This can have a positive impact on overall health and wellbeing, reducing the risk of chronic diseases such as heart disease and diabetes (Mairami *et al.,* 2018).

On the other hand, fresh packaged mixed fruit salad may also contain added sugars or preservatives to extend its shelf life, which can have negative health implications for consumers. High sugar content can contribute to obesity and other health issues, while preservatives may have adverse effects on certain individuals. It is important for consumers to read the ingredients list carefully and choose products that are free from unnecessary additives. Another public health implication of fresh packaged mixed fruit salad is the potential for allergen contamination. Some individuals may be allergic to certain fruits or have sensitivities to specific ingredients commonly found in mixed fruit salad, such as nuts or dairy products (Mahfuza *et al.,* 2018). Companies must clearly label their products with allergen information to help consumers make informed choices and avoid any potential health risks. In terms of sustainability, the production and distribution of fresh packaged mixed fruit salad can also have an impact on the environment. It is important for companies to source their fruits from sustainable and ethical suppliers, as well as minimize waste and packaging materials to reduce their carbon footprint. Consumers can also play a role in promoting sustainability by choosing products that prioritize environmental stewardship (Jimoh *et al.,* 2023). Furthermore, fresh packaged mixed fruit salad can be a cost-effective option for consumers looking to eat healthily on a budget. By purchasing pre-packaged mixed fruit salad, individuals can save time and money compared to buying individual fruits and preparing them at home. This can make it easier for people to incorporate more fruits into their diet, which can have long-term benefits for their health and wellbeing. Overall, fresh packaged

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mixed fruit salad offers a convenient and nutritious option for consumers seeking to improve their diet. However, it is essential to consider the various public health implications associated with these products, including food safety, allergen contamination, and sustainability. By making informed choices and supporting companies that prioritize health and environmental stewardship, consumers can enjoy the benefits of fresh packaged mixed fruit salad while minimizing any potential risks to their health and the environment (Graca *et al.,* 2017).

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2.5 Future Prospects for Fresh Packaged Fruits Salad

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The future prospects of fresh packaged mixed fruit salads appear promising, driven by evolving consumer preferences and increasing health awareness. As society becomes more health-conscious, the demand for convenient yet nutritious food options is on the rise. Fresh packaged mixed fruit salads offer a solution that aligns with this trend by providing a ready-to-eat product that promotes healthier eating habits (Francis *et al.,* 2022). The convenience of these salads appeals particularly to busy urban dwellers who seek quick meal options without compromising on nutritional value. Moreover, advancements in food preservation techniques and packaging technology are enhancing the shelf life and freshness of mixed fruit salads. Innovations such as modified atmosphere packaging (MAP) and vacuum sealing are crucial in maintaining the quality of fresh produce during transport and storage. These technological improvements not only reduce food waste but also ensure that consumers receive high-quality products that retain their nutritional benefits over time. As companies invest in better packaging solutions, the market for fresh packaged mixed fruit salads is likely to expand significantly (Eni *et al.,* 2020). Additionally, sustainability concerns are becoming increasingly relevant in consumer decision-making processes. Brands that prioritize eco-friendly practices such as using biodegradable packaging or sourcing fruits from local farms are likely to gain a competitive edge in the marketplace. By aligning their operations with sustainable practices, companies can attract

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environmentally conscious consumers while contributing positively to the ecosystem. The future of fresh packaged mixed fruit salads is bright, characterized by growing consumer demand, technological advancements in preservation methods, and an emphasis on sustainability. Companies that adapt to these trends will not only thrive but also play an integral role in promoting healthier lifestyles among consumers (Denis *et al.,* 2019).

One of the key drivers of the growth of the fresh packaged mixed fruit salad market is the increasing demand for healthy and convenient snack options. With busy lifestyles and hectic schedules, consumers are looking for quick and easy ways to incorporate more fruits and vegetables into their diets. Fresh packaged mixed fruit salad offers a convenient and portable option for consumers to get their daily dose of vitamins and minerals while on the go (Das *et al.,* 2018). In addition to convenience, fresh packaged mixed fruit salad is also appealing to consumers because of its freshness and quality. With advancements in packaging technology, manufacturers are able to preserve the freshness and flavor of the fruits for longer periods of time without the need for preservatives or additives. This has helped to improve the overall perception of fresh packaged mixed fruit salad among consumers, making it a popular choice for health-conscious individuals. Another factor that will drive the growth of the fresh packaged mixed fruit salad market is the increasing focus on sustainability and environmental responsibility (Daniel *et al.,* 2018). As consumers become more aware of the impact of their food choices on the environment, they are seeking out products that are sustainably sourced and produced. Fresh packaged mixed fruit salad offers a sustainable option for consumers who want to support ethical and environmentally friendly practices in the food industry. The rise of e-commerce and online grocery shopping is also expected to contribute to the growth of the fresh packaged mixed fruit salad market. With the convenience of being able to order groceries online and have them

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delivered straight to their doorsteps, consumers are more likely to purchase fresh packaged mixed fruit salad as part of their weekly grocery orders. This trend is expected to continue as more consumers embrace online shopping as a convenient and time-saving way to meet their food shopping needs (Corbo *et al.,* 2020).

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Furthermore, the increasing focus on health and wellness among consumers is driving the demand for fresh packaged mixed fruit salad as a nutritious snack option. With growing concerns about obesity, diabetes, and other diet-related health issues, consumers are looking for healthier alternatives to traditional snack foods. Fresh packaged mixed fruit salad offers a delicious and satisfying option for consumers who want to indulge in a tasty treat without compromising on their health goals. The growing interest in plant-based diets and vegan lifestyles is also expected to contribute to the growth of the fresh packaged mixed fruit salad market (Buck *et al.,* 2023). As more consumers embrace vegetarian and vegan diets for health, ethical, and environmental reasons, they are looking for plant-based snack options that are both nutritious and delicious. Fresh packaged mixed fruit salad fits the bill perfectly, offering a healthy and satisfying option for vegans and vegetarians who want to enjoy a tasty treat without the guilt. As the global population continues to grow and urbanize, the demand for fresh packaged mixed fruit salad is expected to increase in both developed and developing countries (Beuchat, 2022). With more people living in cities and urban areas, there is a growing need for convenient and healthy food options that are easy to prepare and consume on the go. Fresh packaged mixed fruit salad provides a solution to this demand, offering a nutritious and delicious snack option that can be enjoyed anytime, anywhere. Moreover, the rise of food delivery services and meal kit companies is also expected to drive the growth of the fresh packaged mixed fruit salad market. With the increasing popularity of food delivery and subscription-based meal kit services, consumers are

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looking for convenient and hassle-free ways to enjoy fresh and healthy foods at home. Fresh packaged mixed fruit salad is a perfect fit for these services, offering a quick and easy snack option that can be included in meal kits or delivered straight to consumers' doorsteps (Annapurna and Rashmi, 2019).

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The future prospects for fresh packaged mixed fruit salad are bright as consumers continue to embrace health and wellness trends, sustainability practices, and convenient food options. With its convenience, freshness, and health benefits, fresh packaged mixed fruit salad is likely to become a staple in the diets of health-conscious consumers around the world. As manufacturers continue to innovate and create new and exciting flavor combinations, the market for fresh packaged mixed fruit salad is expected to grow significantly in the coming years, offering consumers a delicious and nutritious snack option that fits seamlessly into their busy lifestyles (Al-Kharousi *et al.,* 2019).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Mixed fruit salad samples were obtained from various vendors in proximity to the University of Benin, specifically within the Ekosodin and BDPA areas. Following their acquisition, the samples were securely placed in sterile zip-lock bags and subsequently transported to the laboratory for bacteriological assessment.

3.1.1 Sterilization of Materials

Glass-wares such as test tubes, measuring cylinder, conical flasks needed for analysis were properly sterilized by autoclaving them at 121°C for 15mins at 15psi pressure.

3.2 Preparation of Media

All media employed for the microbiological analysis, which were prepared following manufacture's specification.

3.2.1 Nutrient Agar

Twenty-eight grammes (28g) of nutrient powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121 °C for 15 min. The medium was cooled to 45 ° - 50 °C and then dispensed aseptically into steril Petri dishes.

3.2.2 Mannitol Salt Agar

One hundred and eleven grammes (111g) of Mannitol Salt Agar powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was

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mixed thoroughly and sterilized by autoclaving at 121 ^{o}C for 15 min. The medium was cooled to 45 ^{o} - 50 ^{o}C and then dispensed aseptically into steril Petri dishes.

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3.2.3 Eosin methylene blue agar

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Thirty six gram (36g) of eosin methylene blue agar was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121 ^{o}C for 15 min. The medium was cooled to 45 ^{o} - 50 ^{o}C and then dispensed aseptically into steril Petri dishes.

3.4 Enumeration and Isolation of Microorganism

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A 5g sample of mixed mixed fruit salad was homogenized in 45ml of sterile peptone water. Serial dilutions of the homogenate were then prepared using sterile peptone water. Following this, 1ml of the 10^-4 dilution was dispensed into sterile disposable Petri dishes for pour plate inoculation. At 8 am, 0.5ml and 1ml of the dilution were inoculated into sterile disposable Petri dishes containing nutrient agar, potato dextrose agar, mannitol salt agar, and eosin methylene blue agar, which were subsequently incubated at 37 °C for 24 hours. At 12 pm and 4 pm, 0.5ml was inoculated into nutrient agar, potato dextrose agar, mannitol salt agar, and eosin methylene blue agar, all incubated at 37 °C for 24 hours. Finally, at the 24-hour mark, 0.1ml and 0.5ml were inoculated into nutrient agar, potato dextrose agar, mannitol salt agar, and eosin methylene blue agar, followed by incubation at 37 °C for 24 hours respectively.

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3.5 Cultural and Morphological Characterization

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The cultural and morphological characteristics of the bacterial isolates were examined utilizing Gram staining techniques, with observations facilitated through microscopic examination. Biochemical tests were subsequently conducted.

3.5.1 Gram Staining

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A thin smear of each isolate was prepared on separate slides using a sterile wire loop. The slides were then subjected to heat fixation and allowed to cool. The smears were covered with crystal violet stain for 60 seconds and rapidly rinsed with sterile, deionized water. Following this, Lugol's iodine was applied for 30 seconds and subsequently washed off with sterile, deionized water. The smears underwent rapid decolorization using a mixture of acetone and ethanol (1:2) and were immediately rinsed with sterile, deionized water. Safranin was then applied for 60 seconds before a final rinse with sterile, deionized water. The stained smears were air-dried, and a drop of immersion oil was applied prior to examination under a microscope using a 100x objective lens (Cheesebrough, 2006).

3.6 Biochemical Tests

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Bacteria stored in nutrient agar slants were propagated in tryptic soy broth for 24 hours, followed by sub-culturing onto nutrient agar plates using the streak plate technique. The plates were incubated for a duration of 18 to 24 hours before biochemical testing commenced.

3.6.1 Catalase Test

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Discrete colonies from each isolate were transferred to a clean, grease-free slide and emulsified in a drop of hydrogen peroxide (H_2O_2). The presence of gas bubbles indicated a positive result.

3.6.2 Oxidase Test

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A piece of filter paper was placed in a clean Petri dish, followed by the addition of 2-3 drops of freshly prepared oxidase reagent. Individual colonies were collected using a loop and smeared onto the filter paper. A positive result was characterized by the development of a purple-blue coloration within 10 seconds, while a negative result showed no color change.

3.6.3 Indole Test

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Bacterial isolates were cultured in tryptophan broth for 48 hours. The broth was composed of 0.1g of tryptophan and 0.05g of creatinine in 100ml of peptone water. Each test bottle received 5ml of tryptophan broth, which was aseptically inoculated with the test bacterium using a wire loop or from a cell suspension. After 48 hours, 0.5ml of Kovac's indole reagent (consisting of isoamyl alcohol, paradimethylaminobenzaldehyde DMAB, and concentrated HCl) was added, followed by gentle swirling. The appearance of a pink-red ring at the surface indicated a positive test for indole production.

3.6.4 Citrate Utilization

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Isolates were streaked on the surface of Simmons citrate agar in test tubes and incubated at 37°C for 48 hours. The development of a deep blue coloration within 24 to 48 hours indicated a positive result.

3.6.5 Urease Production

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Inoculum from discrete colonies were streaked onto Christiansen's urea agar base containing 40% sterile urea and incubated for 48 hours. A color change from light brown to pink denoted a positive test for urease production.

3.6.6 Hydrogen Sulphide Production

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This was determined using triple sugar iron agar (TSI), a multi-test medium that assesses the ability of bacteria to ferment glucose, sucrose, and lactose while also evaluating hydrogen sulphide and carbon dioxide production. Colonies were streaked onto TSI agar slants and incubated for 72 hours with the caps loosely applied. The formation of black precipitate within the medium indicated positive hydrogen sulphide production.

3.6.7 Sugar Fermentation Tests

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The sugars evaluated included glucose, lactose, maltose, sucrose, mannitol, and xylose. The fermentation medium was prepared with peptone water (1.5g), 1g of the specified sugar, 0.5g of disodium phosphate, and 0.72ml of phenol red indicator. Sterilization occurred at 121°C for 5 minutes. The phenol red indicator reacts to pH changes, turning from red in alkaline conditions to yellow in acidic conditions. The medium was inoculated with 50µL of a cell suspension (McFarland $1x10^8$) and incubated for 24 hours. Sugar fermentation resulted in acid production, reflected by a decrease in pH and subsequent yellowing of the phenol red indicator.

3.6.8 Methyl Red Test

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This test evaluates the extent of pH reduction in the medium. The effective pH range for this assay is 4.5 and below. Methyl red appears red in an acidic environment and yellow in an alkaline one. A drop of bacterial broth was mixed with methyl red indicator on a clean slide; a positive result was indicated by a red coloration, while yellow indicated a negative result.

3.6.9 CO_2 Gas Production

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Durham tubes were inverted and placed in glucose fermentation test tubes before the sterilization step at 121°C for 5 minutes. Gas produced by the bacterium during sugar fermentation displaced the liquid within the Durham tube, resulting in visible clear space indicating gas production.

3.6.10 Voges Prauskaur Test:

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The Voges Prauskaur test is utilized to ascertain whether an organism produces acetylmethyl carbinol from the fermentation of glucose. To conduct the test, six drops of a 5% α-naphthol solution were added to a test tube containing a 48-hour glucose broth culture. The mixture was vigorously shaken to aerate its contents. Subsequently, two drops of 40% potassium hydroxide were introduced, and the tube was mixed thoroughly. A positive result is indicated by the formation of a pink or red color at the upper portion of the medium.

3.6.11 Antibiotic Susceptibility Test

Initially, the isolated bacterial strains were cultured in rich tryptone soya broth for 24 hours. Following this incubation, the cultures were sub-cultured onto nutrient agar and incubated for an additional 24 hours. A sterile preparation of each bacterial isolate at a concentration of 1 x 10^8 McFarland Standard was created, and 0.5 mL of this inoculum was spread over Mueller-Hinton agar plates using a sterile glass rod. After allowing the inoculum to dry for 10 minutes, sterile discs impregnated with various antibiotics of specific concentrations were placed on the agar surface using sterile forceps. The plates were then incubated for 24 hours, after which the zones of inhibition were measured from the center of each disc to the outer edge of the inhibition zone. This measurement provided the radius of the inhibition zone, thus the actual zone size was determined by doubling the measured value. Results were interpreted in accordance with the CLSI (2023) standards.

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3.7 Detection of Phenotypic Virulence Factors

3.7.1 Protease Test

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The extracellular protease activity of the isolates was evaluated on tryptone soy agar (TSA) plates supplemented with 1% casein (v/v). Colonies cultivated on tryptone soy broth (TSB) agar were suspended in 3 mL of Mueller Hinton broth. The density of this suspension was adjusted to a 0.5 McFarland standard, equivalent to 1.5 x 10^8 cells/mL. A 1 mL aliquot of this suspension was inoculated onto TSA plates containing 1% casein and incubated at 37°C for a period of 24 to 48 hours. The appearance of a zone of clearance due to casein hydrolysis was considered a positive result, whereas the absence of clearance was interpreted as a negative result.

3.7.2 Lipase Test

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The lipase activity of the isolates was assessed on TSA plates supplemented with 1% Tween 80 (v/v). Similar to the protease test, colonies grown on TSB agar were suspended in 3 mL of

Mueller Hinton broth, with the density adjusted to 0.5 McFarland standards. After inoculating 1 mL of this suspension onto TSA plates, they were incubated at 37°C for 24 to 48 hours. The production of lipases, which facilitate the breakdown of lipids into smaller fragments, was indicated by the formation of a clear halo surrounding the areas where lipase-producing organisms were cultured.

3.7.3 Gelatinase Production

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The capacity of the isolates to produce gelatinase was measured in a nutrient gelatin medium (Micromaster). Following the same initial procedure as the previous tests, a 1 mL sample of the 0.5 McFarland suspension was inoculated into the gelatin medium and incubated at 37°C for 24 to 48 hours. The presence of zones of clearance in the medium indicated the presence of gelatin-liquefying microorganisms, while the absence of such zones indicated a negative result.

3.7.4 DNAse Test

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The DNA-degrading activity of the isolates was assessed by culturing them on DNase agar plates (Himedia). The procedure mirrored those previously outlined, with samples of the adjusted bacterial suspension inoculated onto DNase agar plates and incubated in triplicate at 37°C for 24 to 48 hours. The hydrolysis of DNA releases methyl green, resulting in a color change to colorless around the test organism. Conversely, if there is no degradation of DNA, the medium remains green.

3.8 Data Analysis

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Samples were analyzed in duplicate, and the results are expressed as Mean ± Standard Deviation.

CHAPTER FOUR

RESULTS

Table 4.1 shows the heterotrophic bacterial count of mixed fruit salad samples. The heterotrophic bacterial count of mixed fruit salad samples kept in the fridge for 4 hours ranged from 2.50 ± 2.12 x10^{3} cfu/g to 4.08 ± 1.24 x10^{5} cfu/g, compared to the once kept in the fridge for 24 hours which ranged from 1.51 ± 0.20 x10^{5} cfu/g to 5.70 ± 0.88 x10^{4} cfu/g. Also The heterotrophic bacterial count of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 4.08 ± 1.24 x10^{5} cfu/g to 5.92 ± 1.70 x10^{5} cfu/g, compared to the once kept in the atmosphere for 24 hours which ranged from 2.15 ± 0.21 x10^{5} cfu/g to 5.83 ± 4.06 x10^{5} cfu/g respectively.

Table 4.2 shows the *Staphylococcus aureus* count of mixed fruit salad samples. The *Staphylococcus aureus* count of mixed fruit salad samples kept in the fridge for 4 hours ranged from 1.57 ± 0.78 x10^{3} cfu/g to 4.50 ± 0.42 x10^{2} cfu/g, compared to the once kept in the fridge for 24 hours which ranged from 2.50 ± 0.14 x10^{3} cfu/g to 8.96 ± 0.23 x10^{4} cfu/g. Also The *Staphylococcus aureus* count of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 2.25 ± 1.06 x10^{2} cfu/g to 4.35 ± 4.31 x10^{3} cfu/g, compared to the once kept in the atmosphere for 24 hours which ranged from 1.18 ± 0.35 x10^{4} cfu/g to 6.90 ± 0.18 x10^{3} cfu/g respectively.

Table 4.3 shows the coliforms bacteria count of mixed fruit salad samples. The coliforms bacteria count of mixed fruit salad samples kept in the fridge for 4 hours ranged from 2.34 ± 0.21 x10^{3} cfu/g to 7.20 ± 0.88 x10^{2} cfu/g, compared to the once kept in the fridge for 24 hours which ranged from 2.50 ± 0.14 x10^{3} cfu/g to 8.96 ± 0.23 x10^{4} cfu/g. Also the coliforms bacteria count of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 1.02 ± 0.78 x10^{4} cfu/g to 2.85 ± 0.42 x10^{3} cfu/g, compared to the once kept in the atmosphere for 24 hours which ranged from 0.00±0.00 x10^{2}cfu/g to 1.48 ± 1.73 x10^{5} cfu/g respectively.

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Table 4.4 shows the morphological and biochemical characteristic of bacteria isolates from mixed fruit salad samples. The identified bacterial isolates were *Staphylococcus warinei, Staphylococcus aureus Bacillus megaterium, Lactobacillus* spp., *Serratia marcescens, Flavobacterium* spp., *Enterobacter cloacae* and *Micrococcus lactis* respectively.

Table 4.5 shows the morphological and biochemical characteristic of bacteria isolates from mixed fruit salad samples. The identified bacterial isolates were *Bacillus cereus, Enterococcus faecium, Bacillus thiurgienem, Salmonella arizonae, Bacillus licheniformis, Klebsiella pneumonia* and *Bacillus subtilis* repectively.

Table 4.1: Heterotrophic bacteria count of mixed fruit salad samples

Sample code	0 hr	4 hr	8 hr	24 hr
AF	$2.00 \pm 0.28\times10^4$	$2.52 \pm 0.6\ \times10^5$	$1.29 \pm 0.03 \times10^6$	$3.43 \pm 0.55 \times10^5$
AA	$2.00 \pm 0.28\times10^4$	$5.92 \pm 1.70 \times10^5$	$3.53 \pm 4.06 \times10^6$	$5.83 \pm 4.06 \times10^5$
BF	$4.50 \pm 0.42 \times10^4$	$4.08 \pm 1.24 \times10^5$	$2.82 \pm 2.80 \times10^6$	$1.51 \pm 0.20 \times10^5$
BA	$4.50 \pm 0.42 \times10^4$	$6.28 \pm 2.09 \times10^5$	$3.33 \pm 0.78 \times10^6$	$2.15 \pm 0.21 \times10^5$
CF	$2.20 \pm 0.28 \times10^4$	$2.50 \pm 2.12 \times10^3$	$9.00 \pm 1.41 \times10^5$	$5.70 \pm 0.88 \times10^4$
CA	$2.20 \pm 0.28 \times10^4$	$4.90 \pm 0.99 \times10^4$	$1.59 \pm 0.13 \times10^5$	$3.80 \pm 1.70 \times10^5$

Values are mean±SD, n=3, $p<0.05$

Key:
AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
BA = Sample B Atmosphere
CF = Sample C Fridge
CA = Sample C Atmosphere

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Table 4.2: *Staphylococcus aureus* counts of mixed fruit salad samples

Sample code	0 hr	4 hr	8 hr	24 hr
AF	$4.15 \pm 0.49\times10^2$	$4.50 \pm 0.42 \times10^2$	$8.83 \pm 0.71 \times10^2$	$8.96 \pm 0.23 \times10^4$
AA	$4.15 \pm 0.49\times10^2$	$3.23 \pm 1.37 \times10^2$	$3.72 \pm 2.76 \times10^3$	$5.60 \pm 0.39 \times10^4$
BF	$3.75 \pm 0.78 \times10^2$	$1.57 \pm 0.78 \times10^3$	$6.70 \pm 3.77 \times10^2$	$4.51 \pm 0.30 \times10^4$
BA	$3.75 \pm 0.78 \times10^2$	$4.35 \pm 4.31 \times10^3$	$2.02 \pm 1.44 \times10^3$	$6.90 \pm 0.18 \times10^3$
CF	$4.75 \pm 1.77 \times10^2$	$3.00 \pm 2.12 \times10^2$	$1.25 \pm 0.35 \times10^3$	$2.50 \pm 0.14 \times10^3$
CA	$4.75 \pm 1.77 \times10^2$	$2.25 \pm 1.06 \times10^2$	$1.60 \pm 0.28 \times10^4$	$1.18 \pm 0.35 \times10^4$

Values are mean±SD, n=3, $p<0.05$

Key:
AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
BA = Sample B Atmosphere
CF = Sample C Fridge
CA = Sample C Atmosphere

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Table 4.3: Coliforms bacteria count counts of mixed fruit salad samples

Sample code	0 hr	4 hr	8 hr	24 hr
AF	$5.25 \pm 0.35\times10^{2}$	$7.20 \pm 0.88 \times10^{2}$	$2.57 \pm 0.19 \times10^{3}$	$8.96 \pm 0.23 \times10^{4}$
AA	$5.25 \pm 0.35\times10^{2}$	$1.34 \pm 0.11 \times10^{3}$	$1.70 \pm 0.57 \times10^{3}$	$0.00\pm0.00 \times10^{4}$
BF	$1.07 \pm 0.65 \times10^{3}$	$2.34 \pm 0.21 \times10^{3}$	$6.62 \pm 0.69 \times10^{3}$	$6.00 \pm5.66 \times10^{2}$
BA	$1.07 \pm 0.65 \times10^{3}$	$2.85 \pm 0.42 \times10^{3}$	$2.20 \pm 0.41 \times10^{3}$	$0.00\pm0.00 \times10^{2}$
CF	$6.60 \pm 0.85 \times10^{3}$	$5.40 \pm 1.69 \times10^{3}$	$3.58 \pm 1.31 \times10^{4}$	$2.50 \pm 0.14 \times10^{3}$
CA	$6.60 \pm 0.85 \times10^{3}$	$1.02 \pm 0.78 \times10^{4}$	$3.96 \pm 4.02 \times10^{4}$	$1.48 \pm 1.73 \times10^{5}$

Values are mean±SD, n=3, $p<0.05$

Key:
AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
BA = Sample B Atmosphere
CF = Sample C Fridge
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Table 4.4: Morphological and biochemical characteristic of bacteria isolates from mixed fruit salad samples

Morphological	*Staphylococcus warinei,*	*Staphylococcus aureus*	*Bacillus magatcium*	*Lactobacillus spp.,*	*Serratia marcescens*	*Flavobacterium spp.,*	*Entocisiouster cloacae*	*Micrococcus lactis*
Colour	Light	Golden yellow	Greenish blue	Milky	Reddish	Pale yellow	Light yellow	Light yellow
Shape	Round	Round	Round	Round	Round	Round	Round	Round
Elevation	Raised	Elevated	Elevated	Elevated	Elevated	Flat	Flat	Flat
Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Wetness	Wet	Wet	Wet	Wet	Wet	Wet	Wet	Wet
Surface appearance	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Biochemical test								
Gram r&n {KOH}	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Size								
Glucose	+	+	+	+	+	+	+	+
Gas	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
D-menthol	+	+	+	+	+	+	+	+
x-ylose	+	+	+	-	+	+	+	+
Citrate	+	+	+	+	+	+	+	-
H2S	-	-	-	-	+	-	-	-
Indole	+	-	-	-	+	-	-	-
Urease	-	-	-	-	+	-	-	-
Bacidase	-	+	-	-	-	-	+	-
Methylate	-	-	+	+	-	-	-	-
Vp	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+

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Table 4.5: Morphological and biochemical characteristic of bacteria isolates from mixed fruit salad samples

Morphological	*Bacillus cereus*	*Enterococcus faecium*	*Bacillus thiurgienem*	*Salmonella arizone*	*Bacillus licheniformis*	*Klebsiella pneumonia*	*Bacillu subtilis*
Colour	Colourless	Light pink	Greenish	Colourless	Greenish blue	Milky	White
Shape	Irregular	Round	Irregular	Round	Round	Irregular	Irregular
Elevation	Flat	Flat	Elevated	Flat	Flat	Flat	Flat
Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Wetness	Wet	Wet	Wet	Wet	Wet	Wet	Dry
Surface appearance	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Gram r&n {KOH}	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Cell shape	Cocci	Cocci	Cocci	Bacilli	Cocci	Cocci	Rod
Size							
Glucose	+	+	+	+	+	+	-
Gas	+	-	+	-	+	+	+
Lactose	+	-	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
D-menthol	+	+	+	+	+	+	+
x-ylose	+	+	+	+	+	+	+
Citrate	-	+	+	+	+	+	-
H_2S	-	-	-	-	-	+	-
Indole	-	-	-	+	-	+	-
Urease	-	-	-	-	-	-	-
Oxidase	-	-	-	+	+	-	-
Methylate	-	-	+	+	-	-	-
Vp	+	+	+	+	+	+	+

Table 4.6 shows the percentage frequency of occurrence of bacterial isolates from mixed fruit salad samples. The percentage frequency of occurrence of bacterial isolates is as follows: *Staphylococcus aureus* (12), *Bacillus megaterium* (12), *Lactobacillus* spp. (12), *Seratia morscencenes* (6), *Flavobacterium* spp (10), *Enterobacter cloacae* (8), *Micrococci lactis* (2), *Bacillus cereus* (10), *Staphylococcus warneri* (2), *Enterobacterium facium* (8), *Bacillus thuringiensis* (8), *Salmonella arizonae* (4), *Bacillus licheniformis* (2), *Klebsiella pneumonia* (2) and *Bacillus subtilis* (2) respectively.

Table 4.7 shows the flavour characteristics of mixed fruit salad samples. The flavour characteristics of mixed fruit salad samples kept in the fridge for 4 hours ranged from 7.00 ± 0.82 to 7.40 ± 1.43, compared to the once kept in the fridge for 24 hours which ranged from 3.70 ± 2.21 to 5.10 ± 2.23. Also the flavour characteristics of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 6.70 ± 1.25 to 7.10 ± 1.45, compared to the once kept in the atmosphere for 24 hours which ranged from 2.90 ± 1.60 to 3.10 ± 2.38 respectively.

Table 4.8 shows the smell characteristics of mixed fruit salad samples. The smell characteristics of mixed fruit salad samples kept in the fridge for 4 hours ranged from 6.70 ± 0.82 to 7.20 ± 0.79, compared to the once kept in the fridge for 24 hours which ranged from 3.90 ± 2.13 to 4.90 ± 2.23. Also the smell characteristics of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 6.20 ± 1.61 to 6.7 ± 0.95, compared to the once kept in the atmosphere for 24 hours which ranged from 3.50 ± 1.84 to 4.40 ± 2.12 respectively.

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Table 4.6: Percentage frequency of occurrence of bacterial isolates from mixed fruit salad samples

	Isolates	Frequency of occurrence (%)
1	*Staphylococcus aureus*	12
2	*Bacillus megaterium*	12
3	*Lactobacillus spp*	12
4	*Seratia morscencenes*	6
5	*Flavobacterium spp*	10
6	*Enterobacter cloacae*	8
7	*Micrococcus lactis*	2
8	*Bacillus cereus*	10
9	*Staphylococcus warnei*	2
10	*Enterobacterium facium*	8
11	*Bacillus thuringiensis*	8
12	*Salmonella arizonae*	4
13	*Bacillus licheniformis*	2
14	*Klebsiella pneumonia*	2
15	*Bacillus subtilis*	2

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Table 4.7: Flavour characteristics of mixed mixed fruit salad samples

Sample code	Storage duration (hr)			
	0	4	8	24
AF	7.20 ± 1.03	7.40 ± 1.43	6.90 ± 0.74	5.10 ± 2.23
AA	7.20 ± 1.03	6.70 ± 1.34	5.70 ± 1.42	3.10 ± 1.45
BF	7.40 ± 1.43	7.20 ± 1.55	6.40 ± 1.26	4.00 ± 1.63
BA	7.40 ± 1.43	7.10 ± 1.45	3.90 ± 1.20	2.90 ± 1.60
CF	6.90 ± 1.27	7.00 ± 0.82	6.80 ± 1.03	3.70 ± 2.21
CA	6.90 ± 1.27	6.70 ± 1.25	6.20 ± 1.62	3.10 ± 2.38

Key:
AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
BA = Sample B Atmosphere
CF = Sample C Fridge
CA = Sample C Atmosphere

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Table 4.8: Smell characteristics of mixed fruit salad samples

	Storage duration (hr)			
Sample code	0	4	8	24
AF	6.80 ± 1.69	7.20 ± 0.79	6.10 ± 1.20	4.90 ± 2.23
AA	6.80 ± 1.69	6.50 ± 1.18	6.20 ± 1.32	4.40 ± 2.12
BF	7.40 ± 1.35	6.70 ± 0.82	5.90 ± 1.60	4.70 ± 2.00
BA	7.40 ± 1.35	6.20 ± 1.61	4.40 ± 1.78	3.50 ± 1.84
CF	7.2 ± 1.32	7.0 ± 0.94	6.80 ± 0.79	3.90 ± 2.13
CA	7.2 ± 1.32	6.7 ± 0.95	6.70 ± 1.25	3.50 ± 2.64

Key:

AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
BA = Sample B Atmosphere
CF = Sample C Fridge
CA = Sample C Atmosphere

Table 4.9 shows the appearance characteristics of mixed fruit salad samples. The appearance characteristics of mixed fruit salad samples kept in the fridge for 4 hours ranged from 5.90 ± 0.85 to 5.70 ± 1.64, compared to the once kept in the fridge for 24 hours which ranged from 4.00 ± 1.83 to 5.70 ± 1.64. Also the appearance characteristics of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 5.80 ± 1.48 to 6.50 ± 1.43, compared to the once kept in the atmosphere for 24 hours which ranged from 3.40 ± 2.07 to 3.60 ± 2.41 respectively.

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Table 4.10 shows the texture characteristics of mixed fruit salad samples. The texture characteristics of mixed fruit salad samples kept in the fridge for 4 hours ranged from 6.90 ± 1.37 to 7.30 ± 1.06, compared to the once kept in the fridge for 24 hours which ranged from 3.70 ± 1.95 to 5.20 ± 2.20. Also the texture characteristics of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 5.80 ± 0.79 to 7.30 ± 1.06, compared to the once kept in the atmosphere for 24 hours which ranged from 3.70 ± 1.89 to 4.40 ± 2.22 respectively.

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Table 4.11 shows the colour characteristics of mixed fruit salad samples. The colour characteristics of mixed fruit salad samples kept in the fridge for 4 hours ranged from 6.50 ± 1.35 to 7.50 ± 1.08, compared to the once kept in the fridge for 24 hours which ranged from 3.60 ± 1.78 to 5.70 ± 2.41. Also the colour characteristics of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 5.50 ± 1.78 to 6.30 ± 1.42, compared to the once kept in the atmosphere for 24 hours which ranged from 2.70 ± 1.57 to 4.50 ± 2.01 respectively

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Table 4.9: Appearance characteristics of mixed fruit salad samples

	Storage duration (hr)			
Sample code	0	4	8	24
AF	7.30 ± 1.16	7.60 ± 1.26	6.90 ± 0.99	5.70 ± 1.64
AA	7.30 ± 1.16	6.50 ± 1.43	6.40 ± 1.17	3.60 ± 1.43
BF	7.30 ± 1.42	6.70 ± 1.57	5.80 ± 1.75	4.00 ± 2.00
BA	7.30 ± 1.42	5.80 ± 1.48	4.30 ± 1.95	3.40 ± 2.07
CF	6.90 ± 1.20	5.90 ± 0.85	5.80 ± 1.62	4.00 ± 1.83
CA	6.90 ± 1.20	6.50 ± 1.18	6.00 ± 1.15	3.60 ± 2.41

Key:
AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
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Table 4.10: Texture characteristics of mixed fruit salad samples

	Storage duration (hr)			
Sample code	0	4	8	24
AF	8.10 ± 0.74	7.30 ± 1.06	6.80 ± 1.48	5.20 ± 2.20
AA	8.10 ± 0.74	7.30 ± 1.06	6.10 ± 1.45	4.40 ± 2.22
BF	6.90 ± 1.52	6.90 ± 1.37	5.80 ± 0.79	4.00 ± 1.94
BA	6.90 ± 1.52	6.50 ± 0.71	4.40 ± 1.90	3.70 ± 1.89
CF	6.90 ± 1.60	6.90 ± 1.37	6.50 ±1.46	3.70 ± 1.95
CA	6.90 ± 1.60	5.80 ± 0.79	6.10 ± 1.45	3.70 ± 2.63

Key:

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BF = Sample B Fridge
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Table 4.11: Colour characteristics of mixed fruit salad samples

	Storage duration (hr)			
Sample code	0	4	8	24
AF	7.60 ± 1.35	7.50 ± 1.08	7.00 ± 1.41	5.70 ± 2.41
AA	7.60 ± 1.35	6.20 ± 1.69	6.30 ± 1.70	4.50 ± 2.01
BF	7.50 ± 1.08	6.70 ± 2.11	5.80 ± 1.99	3.60 ± 1.78
BA	7.50 ± 1.08	5.50 ± 1.78	5.10 ± 1.85	2.70 ± 1.57
CF	7.00 ± 1.56	6.50 ± 1.35	5.90 ± 1.66	3.70 ± 1.83
CA	7.00 ± 1.56	6.40 ± 1.35	6.30 ± 1.42	3.70 ± 2.45

Key:
AF = Sample A Fridge
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Table 4.12 shows the taste characteristics of mixed fruit salad samples. The taste characteristics of mixed fruit salad samples kept in the fridge for 4 hours ranged from 7.00 ± 0.94 to 7.40 ± 0.84, compared to the once kept in the fridge for 24 hours which ranged from 3.70± 1.49 to 4.80 ± 1.32. Also the taste characteristics of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 6.20 ± 1.55 to 6.50 ± 1.27, compared to the once kept in the atmosphere for 24 hours which ranged from 3.00 ± 1.33 to 3.50 ± 1.17 respectively.

Table 4.13 shows the overall acceptance level of mixed fruit salad samples. The overall acceptance level of mixed fruit salad samples kept in the fridge for 4 hours ranged from 6.50 ± 0.97 to 7.40 ± 1.07, compared to the once kept in the fridge for 24 hours which ranged from 3.80 ± 2.10 to 5.00 ± 2.11. Also the overall acceptance level of mixed fruit salad samples kept in the atmosphere for 4 hours ranged from 5.60 ± 1.35 to 6.60 ± 1.17, compared to the once kept in the atmosphere for 24 hours which ranged from 3.30 ± 1.64 to 3.70 ± 2.00 respectively.

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Deleted[Monday]: Table 4.12a shows the antibiotic susceptibility pattern of Gram-positive bacterial isolated from fruit salad samples. Bacteria such as *S. aureus, B. megaterium, Lactobacillus* spp. *Serratia marcescens. Flavobacterium* spp. *B. cereus, S. wamei, E. faccium, B. thiorgenienen, B. Licheniformis, B. subtilis* were sensitive to Ciprofloxacin, Erythromycin, Levofloxacin and Gentamicin and resistant to Rifampicin, Amoxicillin, and Septrin respectively
Table 4.12b shows the antibiotic susceptibility pattern of Gram-negative bacterial isolated from fruit salad samples. *Entrobacrium cloacoa, Micrococuis lactis, Salmonenia arizone* and *K. pneumonia* were sensitive to Ofloxacin, Pefloxacin and Cefuroxime and resistant to Amoxicillin, Ceftezole, Augmentin, Septrin, respectively.

Table 4.12: Taste characteristics of mixed fruit salad samples

	Storage duration (hr)			
Sample code	0	4	8	24
AF	7.40 ± 1.17	7.40 ± 0.84	6.60 ± 0.97	4.80 ± 1.32
AA	7.40 ± 1.17	6.50 ± 1.27	5.50 ± 1.96	3.50 ± 1.17
BF	7.20 ± 1.32	7.30 ± 1.64	6.30 ± 1.64	3.80 ± 1.57
BA	7.20 ± 1.32	6.20 ± 1.55	4.10 ± 2.02	3.30 ± 1.15
CF	6.90 ± 1.45	7.00 ± 0.94	6.30 ± 1.06	3.70± 1.49
CA	6.90 ± 1.45	6.30 ± 1.70	6.60 ± 1.71	3.00 ± 1.33

Key:
AF = Sample A Fridge
AA = Sample A Atmosphere
BF = Sample B Fridge
BA = Sample B Atmosphere
CF = Sample C Fridge
CA = Sample C Atmosphere

Table 4.13: Overall acceptance level of mixed fruit salad samples

	Storage duration (hr)			
Sample code	0	4	8	24
AF	7.90 ± 0.99	7.40 ± 1.07	6.40 ± 1.26	5.00 ± 2.11
AA	7.90 ± 0.99	6.60 ± 1.17	6.30 ± 1.70	3.70 ± 2.00
BF	7.00 ± 1.63	7.00 ± 1.15	6.00 ± 1.63	4.30 ± 1.64
BA	7.00 ± 1.63	5.60 ± 1.35	4.40 ± 1.84	3.30 ± 1.64
CF	6.80 ± 1.55	6.50 ± 0.97	6.10 ± 1.52	3.80 ± 2.10
CA	6.80 ± 1.55	6.50 ± 1.58	6.40 ± 1.35	3.60 ± 2.80

Key:
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Table 4.14 shows the virulence factor of bacteria isolates from mixed fruit salad samples. The highest virulence factor was observed in *Staphylococcus aureus* (100%), followed by *Bacillu subtilis* and *Salmonella arizone* with 75% each. While *Lactobacillus* spp., *Serratia marcescens*, *Micrococcus lactis, Staphylococcus warneri, Enterobacterium faecium* and *Bacillus thiurgienem* had 50% each, and the least with *Flavobacterium* spp., *Enterobacter cloacae, B. licheniformis, Klebsiella pneumonia* and *B. megaterium* of 25% each respectively.

Table 4.15 shows the multiple drug antibiotic resistant index of Gram-positive bacteria isolates from mixed fruit salad samples. The multiple drug antibiotic resistant index of Gram-positive bacteria isolates were as follows: *S. aureus* (0.70), *B. megaterium* (0.70), *Lactobacillus* spp (1.00), *Serratia marcescens* (0.40), Flavobacterium spp (0.70), B. cereus (0.10), S. wamei (0.40), *E. faccium* (0.40), *Bacillus thuringiensis* (0.30), *B. Licheniformis* (0.40) and *B. subtilis* (0.00) respectively.

Table 4.16 show the multiple drug antibiotic resistant index of Gram-negative bacteria isolates from mixed fruit salad samples. The multiple drug antibiotic resistant index of Gram-negative bacteria isolates were as follows: *Entebacterium cloaece* (0.60), *Salmonella arizonae* (0.00) and *Klebsiella Pneumoniae* (0.70) respectively.

Deleted[Monday]: **Table 4.12a**: Antibiotic susceptibility pattern of Gram-positive bacterial isolated from fruit salad samples
Bacteria Isolate
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Deleted[Monday]: *Micrococcus lactis* (0.70),

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Table 4.14: Virulence factor of bacteria isolates from mixed fruit salad samples

Bacteria isolates	**Lipase**	**DNASE**	**Protase**	**Gelatinase**	**Percentage (%)**
Staphylococcus aureus	+	+	+	+	100
B. megaterium	-	-	+	-	25
Lactobacillus spp	-	+	+	-	50
Serratia marcescens	-	+	-	+	50
Flavobacterium spp	+	-	-	-	25
Enterobacter cloacae	+	-	-	-	25
Micrococcus lactis	+	-	+	-	50
Bacillus cereus	-	-	+	-	25
Staphylococcus warneri	+	-	-	+	50
Enterobacterium faecium	-	-	+	+	50
Bacillus thiurgienesis	-	+	-	+	50
Salmonella arizonae	+	-	+	+	75
B. licheniformis	-	+	-	-	25
Klebsiella pneumonia	+	-	-	-	25
Bacillus subtilis	+	-	+	+	75

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Table 4.15: Multiple drug antibiotic resistant index of Gram-positive bacteria isolates from mixed fruit salad samples

Isolate	MDRI (+ve)
S. aureus	0.70
B. megaterium	0.70
Lactobacillus spp	1.00
Serratia marcescens	0.40
M. lactis	0.70
B. cereus	0.10
S. wameri	0.40
E. faecium	0.40
B. thiurgenienesis	0.30
B. Licheniformis	0.40
B. subtilis	0.00

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Table 4.16: Multiple drug antibiotic resistant index of Gram-negative bacteria isolates from mixed fruit salad samples

Isolate	MDRI (-ve)
Entebacterium cloaece	0.60
Salmonella arizone	0.00
Klebsiella Pneumoniae	0.70
Flavobacterium spp	0.70

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CHAPTER FIVE

DISCUSSION

5.1 Discussion

This study was carried out to determine the bacteriological and sensory qualities of fresh and packaged mixed fruit salad sold with the Ugbowo environs, due to the fact that contaminated fresh and packaged mixed fruit salad is a major source of gastrointestinal microbial pathogens and has caused numerous foodborne disease outbreaks in many developing country (Alemi *et al.,* 2012). The results obtained from this study revealed that the heterotrophic bacterial count of mixed fruit salad samples kept in the fridge and atmosphere for 4 hours ranged from 2.50 ± 2.12 x10^{3} cfu/g to 4.08 ± 1.24 x10^{5} cfu/g, compared to the once kept in the fridge and atmosphere for 24 hours which ranged from 1.51 ± 0.20 x10^{5} cfu/g to 5.70 ± 0.88 x10^{4} cfu/g. This is in line with the work of Choo and Sin (2012) who stated that heterotrophic bacterial count of preserved mixed fruit salad samples ranged from 1.0x10^{3} - 8.0×10^{5}cfu/g. According to Adedeji and Oluwalana (2013) heterotrophic bacterial counts in mixed fruit salad samples vary widely, from less than 1 to 10 Cfu/g in its uncontaminated state to greater than 1×10^{7} Cfu/g in highly polluted state. The high total heterotrophic count is indicative of the presence of high organic and dissolved sugar in the fruit juice. According to World Health Organization (2002) report, a high heterotrophic count concentration does not itself present a risk to human health. Nevertheless, heterotrophic counts are used as good indicators of the overall quality of production. The results also showed that the *Staphylococcus aureus* count of mixed fruit salad samples kept in the fridge and atmosphere for 4 hours ranged from 1.57 ± 0.78 x10^{3} cfu/g to 4.50 ± 0.42 x10^{2} cfu/g, compared to the once kept in the fridge and atmosphere for 24 hours which ranged from 2.50 ± 0.14 x10^{3} cfu/g to 8.96 ± 0.23 x10^{4} cfu/g. This was in agreement with the work of Ndife *et al.*

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(2013) who confirmed that *Staphylococcus* count (7.64×10^2) was higher in control of mixed fruit salad samples compared to preserved of preserved mixed fruit salad samples (2.48×10^3). The study of Ogunbanwo *et al.* (2013) also found *Staphylococcus* count ranged from 1.91 to 4.785 x10^2 Cfu/g in mango mixed fruit salad samples 2.40 to 4.94 x10^3 Cfu/g in avocado mixed fruit salad samples, 1.99 to 4.92 x10^2 Cfu/g in papaya mixed fruit salad samples and 1.68 to 4.93 x10^3 Cfu/g in mixed mixed fruit salad samples. Vwioko *et al.* (2012) stated refrigeration is a food preservation method that slows down the growth of bacteria and other microorganisms by keeping food at a low temperature, thereby extending its shelf life by significantly delaying spoilage. Alam *et al.* (2013) confirmed that it works by inhibiting the metabolic activity of microbes that contribute to food decay, allowing food to stay fresh longer while largely maintaining its texture and flavor.

The results also revealed that the coliforms bacteria count of mixed fruit salad samples kept in the fridge and atmosphere for 4 hours ranged from 2.34 ± 0.21 x10^3 cfu/g to 7.20 ± 0.88 x10^2 cfu/g, compared to the once kept in the fridge and atmosphere for 24 hours which ranged from 2.50 ± 0.14 x10^3 cfu/g to 8.96 ± 0.23 x10^4 cfu/g. According to Rizal *et al.* (2013), if fruits are contaminated and not properly washed prior to processing, the fresh fruits may contain sufficient levels of *E. coli* to pose a risk of illness. Adedeja *et al.* (2014) stated that the coliforms bacteria count in fresh watermelon fruits was 1.63 x10^1 compared to 3.86 x10^2 after 3 days storage. Agbaje *et al.* (2015) also reported coliforms bacteria count range from 2.1x10^1±5.00 – 2.9x10^2±2.47 in orange fruits, 3.3 x10^2±3.67 – 4.8 x10^3±3.78 in cucumber fruits and 2.4×10^1±2.00 - 2.9×10^1±5.35 in carrot fruits respectively.

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The results also showed that the percentage frequency of occurrence of bacterial isolates were as follows: *Staphylococcus aureus* (12%), *Bacillus megaterium* (12%), *Lactobacillus* spp (12%), *Seratia morscencenes* (6%), *Flavobacterium* spp (10%), *Enterobacter cloacae* (8%), *Micrococcus lactis* (2%), *Bacillus cereus* (10%), *Staphylococcus warnei* (2%), *Enterobacterium facium* (8%), *Bacillus thuringiensis* (8%), *Salmonella arizone* (4%), *Bacillus licheniformis* (2%), *Klebsiella pneumonia* (2%) and *Bacillus subtilis* (2%) respectively. This was in line with the study of Tamang *et al.* (2016) who stated that bacteria isolated from control and preserved mixed fruit salad samples were *Staphylococcus* sp., *Citrobacte*r sp., *Bacillu*s sp., *Eschericia coli Pseudomonas* sp. and *Staphylococcus aureus.* These organisms isolated in the presented study were in concordance with the previous works of Ridwan *et al.* (2019). These findings could be probably due to mixed fruit salad, which favors the growth of bacteria, and also due to bacterial spores in the environment, soil and possible exposure to faecal contaminated water or organic manure (Adou *et al.*, 2012). The presence of these pathogens in such fruit juice could account for the incidence of diarrhea, food poisoning and gastroenteritis especially, among the regular consumers. Also, presence of this pathogens raise public health concerns that need to be addressed. The need for microbial assessment of food materials for production should be emphasized to reduce possible contamination (Alemi *et al.,* 2012).

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The results of the sensory evaluation revealed that the smell characteristics of mixed fruit salad samples ranged from 6.70 ± 0.82 - 7.20 ± 0.79, appearance ranged from 5.90 ± 0.85 - 5.70 ± 1.64, texture ranged from 6.90 ± 1.37 - 7.30 ± 1.06, colour ranged from 6.50 ± 1.35 - 7.50 ± 1.08, taste characteristics ranged from 7.00 ± 0.94 to 7.40 ± 0.84, overall acceptance level ranged from 6.50 ± 0.97 - 7.40 ± 1.07. Similarly the work of Edwards *et al.* (2013) recorded a colour, texture and appearance values range of 3.56 - 5.48 of mixed mixed fruit salad stored for 48 hours and

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stated that the changes in the value could have been due to microbial activities. Also the study of Alim-un-Nisa *et al.* (2012) also stated an overall acceptance level value ranged from 2.90 to 5.78. The results also showed that the Gram-positive bacterial isolates such as *S. aureus, B. megaterium, Lactobacillus* spp. *Serratia marcescens. Micrococovi lactis, B. cereus, S. waimea, E. faecium, B. thiurgeniennsis, B. Licheniformis, B. subtilis* were sensitive to Ciprofloxacin, Erythromycin, Levofloxacin and Gentamicin and resistant to Rifampicin, Amoxicillin, and Septrin. While gram-negative bacterial isolates such as *Entrobacrium cloacoae, Flavobacterium* spp*, Salmonenia arizone* and *K. pneumonia* were sensitive to Ofloxacin, Pefloxacin and Cefuroxime and resistant to Amoxicillin, Ceftezole, Augmentin, Septrin, respectively. This was in line with the work of Moremi *et al.* (2016) who reported that *Staphylococcus aureus, Klebsiella pneumonia* and *Enterobacter* spp. was found resistant to amoxicillin, ceftazimidine, cefuroxime and sensitive to Gentamycin. The mechanism behind the variance in the resistance and sensitivity pattern to antibiotics of these bacteria isolated could be attributed to the genes acquired from their different environment. Leonard *et al.* (2016) reported that bacteria are able to respond to selective pressures and adapt to new environments by acquiring new genetic traits as a result of mutation (a modification of gene function within a bacterium) as a result of horizontal gene transfer and acquisition of new genes from other bacteria. Laxminarayan *et al.* (2016) reported that mutation occurs relatively slowly and that the normal mutation rate in nature is in a range of 10^{-6} to 10^{-9} per nucleotide per bacterial generation, although when bacterial populations are under stress, they can greatly increase their mutation rate. Furthermore, most mutations are harmful to the bacterium but however horizontal gene transfer, on the other hand, enables bacteria to respond and adapt to their environment much more rapidly by acquiring large DNA sequences from another bacterium in a single transfer (Moremi *et al.,* 2016).

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5.2 Conclusion

The aim to determine the bacteriological and sensory qualities of fresh and packaged mixed fruit salad was successful, as it can be concluded that fresh and packaged mixed fruit salad are contaminated with various microbial pathogens including *S. aureus, B. megaterium, Lactobacillus* spp. *Serratia marcescens. Flavobacterium* spp. *B. cereus, S. wamei, E. faccium, B. thiorgenienen, B. Licheniformis, B. subtilis*, which are concerned with serious health threats. Furthermore, the detection of a high number of bacterial isolates resistant to common antibiotics is particularly alarming. This resistance complicates treatment options for infections arising from contaminated mixed fruit salads. The implications for public health are profound; individuals consuming these products may face increased risks of illness due to antibiotic-resistant strains. Additionally, the findings from this study calls for further research into effective strategies for monitoring and mitigating microbial hazards in ready-to-eat foods to safeguard consumer health against potential outbreaks linked to resistant bacteria.

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APPENDIX

Table 4.12a: Antibiotic susceptibility pattern of Gram-positive bacterial isolated from mixed fruit salad samples

Bacteria Isolate	**RD**	**S**	**AZM**	**AMX**	**AMOX**	**CPX**	**E**	**L**	**CN**	**CEF**	**MRI**
S. aureus	R	R	R	R	R	36 (S)	36 (S)	28 (S)	R	R	0.70
B. megaterium	R	R	R	R	R	36 (S)	36 (S)	36 (S)	R	R	0.70
Lactobacillus spp	R	R	R	R	R	R	R	R	R	R	1.00
Serratia marcescens	R	R	36 (S)	R	36 (S)	36 (S)	36 (S)	36 (S)	R	18 (S)	0.40
Flavobacterium spp	R	R	R	R	R	R	36 (S)	36 (S)	36 (S)	R	0.70
B. cereus	36 (S)	R	36(S)	24 (R)	16 (I)	36 (S)	36 (S)	36 (S)	36 (S)	36 (S)	0.10
S. wamei	22 (S)	R	24 (S)	12 (R)	2 (R)	20 (S)	36 (S)	36 (S)	36 (S)	R	0.40
E. faccium	15 (I)	R	24 (S)	12 (R)	2 (R)	20 (S)	36 (S)	36 (S)	36 (S)	R	0.40
B. thiorgenienen	14 (I)	12 (R)	12 (R)	10 (R)	18 (S)	30 (S)	36 (S)	36 (S)	13 (S)	36 (S)	0.30
B. Licheniformis	18 (S)	10 (R)	36 (S)	R	12 (R)	36 (S)	36 (S)	36 (S)	36 (S)	12 (R)	0.40
Bacteria isolates	**OFX**	**AUG**	**PEF**	**CTZ**	**CN**	**CPX**	**CEP**	**TRX**	**S**	**CEF**	**MRI**
Entrobacrium cloacoa	36(S)	R	28(S)	R	R	36(S)	R	R	R	14(I)	0.60
Micrococuis lactis	26(S)	R	22(S)	R	R	22(S)	R	R	R	R	0.70
Salmonenia arizone	36(S)	22(S)	26(S)	16(I)	36(S)	36(S)	18(S)	18(S)	18(S)	22(S)	0
K. pneumonia	36(S)	R	20(S)	R	R	36(S)	R	R	R	R	0.70
B. subtilis	18 (S)	18 (S)	36 (S)	36 (S)	22 (R)	36 (S)	36 (S)	36 (S)	36 (S)	14 (I)	0.00

KEY:- AUG-Amoxicillin, CAZ-Ceftazimidine, CRX-Cefuroxime, Ceftezole (CTZ), PEF- Pefloxacin, GEN-Gentamicine, AMP-Ampicilin, OFL-Ofloxacin, CPR- Ciprofloxacin, NIT-Nitrofurantoin, S-Sensitive, R-Resistances, ITM-Intermediate

Table 4.14 shows the antibiotic susceptibility pattern of Gram-positive bacterial isolated from mixed fruit salad samples. Bacteria such as *S. aureus, B. megaterium, Lactobacillus* spp. *Serratia marcescens. Flavobacterium* spp. *B. cereus, S. wamei, E. faccium, B. thiorgenienen, B. Licheniformis, B. subtilis* were sensitive to Ciprofloxacin, Erythromycin, Levofloxacin and Gentamicin and resistant to Rifampicin, Amoxicillin, and Septrin respectively

Table 4.15 shows the antibiotic susceptibility pattern of Gram-negative bacterial isolated from mixed fruit salad samples. *Entrobacrium cloacoa, Micrococuis lactis, Salmonenia arizone* and *K. pneumonia* were sensitive to Ofloxacin, Pefloxacin and Cefuroxime and resistant to Amoxicillin, Ceftezole, Augmentin, Septrin, respectively.

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