DSpace Institution

DSpace Repository **http://dspace.org**

Biology Thesis

2026-09

Assessment of Microbiological Quality and Physicochemical Properties of Locally Prepared Juices and Ready-to-Eat Chopped Watermelon in Dessie Town

Mohammed, Aragaw

http://ir.bdu.edu.et/handle/123456789/17512

Downloaded from DSpace Repository, DSpace Institution's institutional repository

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

Assessment of Microbiological Quality and Physicochemical Properties of Locally Prepared Juices and Ready-to-Eat Chopped Watermelon in Dessie Town

By:

Mohammed Aragaw

SEPTEMBER, 2026

BAHIR DAR, ETHIOPIA

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

Assessment of Microbiological Quality and Physicochemical properties of Locally Prepared Juices and Ready-to-Eat Chopped Watermelon in Dessie Town

By:

Mohammed Aragaw

Main Advisor: Negash Kabtimer (PhD)

Co-advisor:-Habtamu Mekonnen (PhD)

A thesis submitted to the College of Science, School of Graduate Studies

Bahir Dar University

In Partial Fulfilment of the Requirements for the Degree of Masters of Science in Applied Microbiology

SEPTEMBER, 2026

BAHIR DAR, ETHIOPIA

DECLARATION

This is to certify that the thesis entitled "**Assessment of Microbiological Quality and Physicochemical Properties of Locally Prepared Juices and Ready-to-Eat Chopped Watermelon in Dessie Town**", submitted in partial fulfilment of the requirements for the degree of Master of Science in Applied Microbiology of Department of Biology, Bahir Dar University, is a record of original work carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

Name of the candidate …….Date…….. ……. Place…….

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

Approval of Thesis for Defense

As a thesis research advisor, I hereby certify that I have supervised, read, and evaluated this thesis titled "**Assessment of Microbiological Quality and Physicochemical Properties of Locally Prepared Juices and Ready-to-Eat Chopped Watermelon in Dessie Town"** by Mohammed Aragaw, prepared under my guidance. I recommend the thesis to be submitted as fulfilling the requirement for the Degree of Master of Science in Applied Microbiology.

Advisor's name	Signature	Date
_____	_____	_____
Co-Advisor's name	Signature	Date
_____	_____	_____
Department Head	Signature	Date
_____	_____	_____

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

Approval of thesis for defence result

As members of the board of examiners for the MSc thesis open defense examination, we examined this thesis entitled "**Assessment of Microbiological Quality and Physicochemical Properties of Locally Prepared Juices and Ready-to-Eat Chopped Watermelon in Dessie Town**" prepared by Mohammed Aragaw. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the Degree of Master of Science in Applied Microbiology

Board of Examiners

Chair person's name _____ Signature _____ Date _____

Internal examiner name _____Signature _____ Date _____

External examiner name _____ Signature _____ Date _____

ACKNOWLEDGEMENT

First and foremost, I would like to express my heartfelt appreciation to my wife for her continuous support, patience, encouragement, and understanding throughout my study. Her emotional and moral support was invaluable throughout the completion of this thesis.

I would also like to extend my deepest gratitude to my advisors, Dr Negash Kabtimer and Dr Habtamu Mekonnen, for their careful guidance and constructive comments throughout the development of proposal writing, laboratory work, and writing this research, which have been invaluable in helping me to develop my research skills.

I would also like to express my sincere appreciation to Wollo University for providing me with financial support and sponsorship to pursue my MSc studies, which enabled me to advance my academic and professional development. I also extend my heartfelt gratitude to Bahir Dar University for providing me with a valuable and conducive teaching environment that helped me to complete my MSc program.

Finally, I would like to thank Dessie City Administration Health Office for giving me permission, information, and a support letter.

LIST OF ACRONYMS

AMR: Anti-Microbial Resistance

AMS: Anti-Microbial Susceptibility

AMBC: Aerobic Mesophilic Bacterial Count

CDC: Centers for Disease Control

CFU: Colony-Forming Unit

EMB: Eosin Methyl Blue Agar

FDA: Food and Drug Administration

FC: Fecal Coliform

ISO: International Standard Organization

MPF: Minimally Processed Food

MSA: Mannitol Salt Agar

SDA: Sabour Dextrose Agar

SS: Salmonella-Shigella Agar

TC: Total Coliform

TAMBC: Total Aerobic Mesophilic Bacterial Count

TA; Titratable Acidity

UKHSA: United Kingdom Health Security Agency

XLD: Xylose Lysine Deoxycholate Agar

YMDC: Yeast and Mold Count

WHO: World Health Organization

TABLE OF CONTENTS

Contents **Page**

ACKNOWLEDGEMENT.....iv

LIST OF ACRONYMS.....v

TABLE OF CONTENTS.....vi

LIST OF FIGURES.....xi

ABSTRACT.....xii

1. INTRODUCTION.....1

1.1 Background of the Study.....1

1.2 Statement of the Problem.....4

1.3 Objective of the Study.....6

1.3.1 General Objective.....6

1.3.2 Specific Objectives.....6

1.4 Significance of the Study.....6

2. LITERATURE REVIEW.....7

2.1 Microbiological Quality of Fresh Juices and Foodborne Diseases.....7

2.1.1 Fruit juice microbial contamination and foodborne diseases in Africa.....7

2.1.2 Fruit juice microbial contamination and foodborne diseases in Ethiopia.....8

2.1.3 Antibiotic- resistance bacterial pathogens in freshly prepared juices.....8

2.2 Source of Microbial Contamination of Fresh Fruit Juice.....9

2.3 The Common Microorganisms in Fruit Juices.....10

2.3.1 Aerobic mesophilic bacteria.....10

2.3.2 Coliforms.....10

2.3.3 Yeast and mold.....11

2.3.4 *Staphylococcus aureus*.....11

2.3.5 *Escherichia coli* O157:H7.....11

2.3.6 *Salmonella* 12
2.4 Physicochemical Properties and their Effects on Microbial Contamination..... 12
2.4.1 pH and Titratable acidity 13
2.5 International Guidelines for Examination of Fruit Juice..... 14
3. MATERIALS AND METHODS 15
3.1 Description of the Study Area 15
3.2 Study Design 16
3.3 Source of Sample 16
3.4 Sample Size Determination 16
3.5 Sampling Technique..... 16
3.6 Study Variables 17
3.6.1 Dependent variables 17
3.6.2 Independent variables 17
3.7 Sample Collection and Preparation 17
3.8 Data Quality Management 17
3.9 Microbiological Analysis 18
3.9.1 Aerobic Mesophilic Bacterial Count 18
3.9.2 Enumeration of coliform 18
3.9.3 *Staphylococcus aureus* count..... 18
3.9.4 Yeast and Mold Count..... 18
3.9.5 Detection of *Escherichia coli* 19
3.9.6 Detection of *Salmonella* 20
3.9.7 Antimicrobial susceptibility test for isolated *E.coli* and *Salmonella*..... 20
3.10 Physicochemical Analysis..... 21
3.11 Socio-Demographic and Handling practices of the study participants 21
3.12 Data Analysis 21
3.13 Microbiological Risk Assessment..... 22

3.14 Ethical Consideration 22
4. RESULTS AND DISCUSSION 23
4.1 Results 23
4.1.1 Aerobic Mesophilic Bacterial Count 23
4.1.2 Coliform Count 25
4.1.3 Fecal coliform 25
4.1.4 *Staphylococcus aureus* Count 25
4.1.5 Yeast and Mold Count 26
4.1.6 Isolation and characterization of *E. coli* and *Salmonella* from sample Juices and chopped watermelon 27
4.1.6.1 Morphological characteristics of bacterial pathogen isolates 27
4.1.6.2 Biochemical characteristics of bacterial pathogens 28
4.1.6.3 Detection of *Escherichia coli* 30
4.1.6.4 Detection of *Salmonella* 30
4.1.7 Antimicrobial Susceptibility profile of bacterial isolates 30
4.1.7.1 Antimicrobial Susceptibility pattern of *Escherichia coli* isolates 30
4.1.7.2 Antimicrobial Susceptibility profile of *Salmonella* isolates 31
4.1.8 Determination of pH and Titratable Acidity 32
4.1.9 Socio-demographic characteristics and Handling practices 34
4.1.9.1 Sociodemographic characteristics of the respondents 34
4.1.9.2 Sources of fruits, spoilage assessment and addition of additives 35
4.1.9.3 Hygienic practices 36
4.1.9.4 Juice preparation practices 37
4.1.9.5 Cleaning practices 38
4.1.9.6 Observation analysis 39
4.2 Discussion 40
4.2.1 Bacterial load 40

4.2.2 Coliform Count....42
4.2.3 *Staphylococcus aureus* Count....43
4.2.4 Yeast and Mold Counts....43
4.2.5 Detection of *Escherichia coli*....44
4.2.6 Detection of *Salmonella*....44
4.2.7 Antimicrobial Susceptibility profile of bacterial isolates....45
4.2.7.1 Antimicrobial Susceptibility pattern of *Escherichia coli i*solates....45
4.2.7.2 Antimicrobial Susceptibility profile of *Salmonella* isolates....45
4.2.8 Association of physicochemical characteristics of juices with microbial load....46
4.2.9 Personal hygiene and handling practices of the respondents....47
4.2.9.1 Hygienic practices of vendors....47
4.2.9.2 Fruit sources and processing practices....47
4.2.9.3 Equipment handling practices....48
4.2.9.4 Environmental sanitation and waste management practices....48
5. CONCLUSION AND RECOMMENDATIONS....49
5.1 Conclusion....49
5.2 Recommendations....50
6. REFERENCE....51
7. APPENDIX....61

LIST OF TABLES

Table 1: The overall mean comparison of microbial count of freshly prepared pineapple, sugarcane juice, and ready-to-eat chopped watermelon (n=90.....23

Table 2:Model of multiple linear regression analysis as predictors of bacterial load (n=90).....**Error! Bookmark not defined.**

Table 3: Overall comparison of mean microbial load of sample juices with the Gulf Standard 2000 (n=90) 27

Table 4: Morphological characteristics of isolated bacteria in selective and differential media.....28

Table 5: Biochemical characterization of bacterial isolates from samples (n=18).....29

Table 6: Antimicrobial susceptibility patterns of bacterial isolates (n=18).....31

Table 7: Multiple antimicrobial resistance patterns of bacterial isolates (mm) (n=18) 31

Table 8: The mean value of pH and Titratable acidity (n=90) 32

Table 9: Correlation analysis between the pH and TA with microbial load (n=90)....33

Table 10: Sociodemographic characteristics of the respondents (n=30) 34

Table 11: Preliminary spoilage assessment, source fruits, and water (n=30).....35

Table 12: Hygienic practices of the respondents (n=30) 36

Table 13: Juice preparation practices of the respondents (n=30) 37

Table 14: Cleaning practices of the respondents (n=30) 38

Table 15:On-site observation analysis of handling practices in juice houses (n=30)..39

LIST OF FIGURES

Figure 2.1: Conceptual framework that shows the variables' effect on microbial contamination.....10

Figure 3.1:Map of the study area 15

Figure 4.1: The prevalence of bacterial isolates in sample juices (n=18).....30

Figure 4.2: The mean of pH and Titratable acidity among chopped watermelon, pineapple, and sugarcane juices (n=90) 32

ABSTRACT

Fresh juices and fresh-cut fruits are widely consumed worldwide, because of their health-promoting antioxidants. However, their microbial quality is still a concern. A cross-sectional laboratory-based study was conducted to assess the microbiological quality and physicochemical properties of chopped watermelon, pineapple, and sugarcane juices in Dessie Town. Eight sites were purposively selected, and a total of 90 samples (30 samples each) were collected from proportionally allocated juice houses using the lottery method. Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. The mean total aerobic mesophilic bacterial counts were higher in chopped watermelon (6.61±0.56 log CFU/g) than in sugarcane (5.75±0.76 log CFU/mL) and pineapple juice (5.89±0.53 log CFU/mL), with a significant difference among sample types ($p<0.001$). The mean *Staphylococcus aureus* counts did not differ significantly among chopped watermelon, sugarcane, and pineapple juices and were 4.94±1.79, 3.63±2.84, and 2.83±2.72, respectively, whereas the corresponding yeast and mold counts were 5.85± 0.75, 5.98±0.75, and 4.13±2.42 log CFU/mL ($p<0.001$). Coliform counts of chopped watermelon, sugarcane, and pineapple juice were 3.87±2.81, 3.89±1.85, and 3.59±2.61 log CFU/mL, respectively ($p>0.05$). The faecal coliform counts were 1.74±2.73, 0.73±1.66, and 1.88±2.53 log CFU/mL, respectively ($p>0.05$). *E. coli* was detected in 13(14.4%) samples, whereas *Salmonella* was detected in 5(5.6%) samples of chopped watermelon and pineapple, but absent in sugarcane juices. The chopped watermelon had a higher pH (5.23) than sugarcane (4.79) and pineapple juices (3.83), whereas pineapple juices had a higher TA (0.41%) than sugarcane juices (0.26%*)* and chopped watermelon (0.037%). Both isolates were resistant to ampicillin and amoxicillin, while many of them were susceptible to norfloxacin and tetracycline. The overall resistance rate was 31.1%. A multiple linear regression model showed a strong association among predictors and bacterial load ($R=0.826$) and explained 68.3% of the variation in bacterial load ($R^2=0.683$). In most juice houses, poor environmental sanitation and waste management, lack of hygienic practices, unsanitary equipment, and TA were the major predicted factors. Generally, most of the tested samples were unsatisfactory in microbial quality against Gulf Standard, indicating food safety risks to consumers. Therefore, improving hygiene and handling practices should be implemented to minimize foodborne diseases.

Keywords: Antimicrobial resistance, Fruit, Hygiene, Microbial quality, Pathogens

1. INTRODUCTION

1.1 Background of the Study

Fruit juices are an excellent source of health-promoting phytochemicals, such as vitamin C, polyphenols, beta-carotene, lycopene, and other antioxidants that are reactive to free radicals and exhibit antioxidant activity, which may help ensure microbiological safety by preventing the growth of pathogens (Evrendilek and Özkan, 2024; Dippong *et al.*, 2025). Freshly squeezed juices are consumed worldwide due to their nutritional benefits, but the microbial quality and safety of juices are often poorly monitored and affect developing countries (WHO, 2026; Pius, 2023). Fruit juices are rich in nutrients that serve as an ideal medium for the growth of pathogenic microorganisms. Most freshly made fruit juice samples lack safety and quality, and are contaminated with pathogenic bacterial species that exceed the maximum permissible levels. This is associated with poor personal hygiene, contact with contaminated surfaces, contaminated water, and improper storage, which lead to high microbial load and rapid deterioration of taste, nutrients, and colour changes, which create risk for consumers (Kaddumukasa *et al*., 2019; Mengistu *et al.*, 2022).

Among fruit types, watermelon (*Citrullus lanatus*) is commonly consumed by people in tropical countries (Marie-Michel *et al*., 2020). It contains up to 92% water and excellent source of various health-promoting bioactive compounds such as lycopene, phenols, flavonoids, tannins, carotenoids, vitamin C, citrulline, phytofluene, phytoene, lutein, and neurosporene (Kalu and Uchenna, 2022; Marie-Michel *et al*., 2020). These antioxidants are beneficial for reducing cancer, cardiovascular disorders, diabetes, high blood pressure, and obesity (Lum *et al*., 2019). Despite its nutritional and health-promoting benefits, watermelon's shelf life is limited. The physiological and biochemical activities that occur during fresh-cut watermelon processing of fruit lead to changes in sensory, nutritional, and microbiological quality (Quandoh and Albornoz, 2025).

The sugarcane (*Saccharum officinarum*) juice has a pleasant flavour, is simple to process, and is well-accepted by the population, but the microbiological quality is not always assured, which can result in risks for consumption (Ed-Dra *et al*., 2025). A study in Brazil found a total coliforms in all sugarcane juice samples; of these, 57.1% had counts above 1600 MPN/mL, and thermo-tolerant coliforms were present in every

sample, with 33.3% exceeding the acceptable limit. Additionally, 19.1% tested positive for *Escherichia coli*. Among the 21 samples analysed, one contained *Salmonella typhimurium*. These results showed that the number and variety of microorganisms in sugarcane juice sold in Goiania require the need for better hygiene and sanitary practices (Cristine *et al.*, 2024). In Pakistan, municipal areas, bus stations, and crowded marketplaces sell mechanically extracted and prepared sugarcane juice that is ready for consumption, but has not been pasteurized and has a high microbial load on its surface due to unhygienic transportation from the field to the food processing place. Sugarcane is not properly cleaned before extraction, which causes foodborne microorganisms to enter the extract and cause contamination (Shah *et al*., 2020).

Pineapple juice has 81.2- 86.2% moisture content and 13-19% total solids such as glucose, sucrose, and fructose, and 0.4% fibre (Agbaje *et al*., 2020). It possesses antioxidant activity and contains polyphenolic compounds. It is an important source of vitamin C, which is useful for collagen synthesis in the human body (Lu *et al*., 2014).

Regular consumption of balanced foods, such as fruits are important to prevent deficiencies of different vitamins, such as vitamin A and vitamin C, and also reduce the risk of diseases, such as stroke, breast cancer, heart failure, Alzheimer disease, cataracts, urinary tract infection, and also improve blood lipid profiles in people affected by hypercholesterolemia (Rahaman *et al.*, 2023; Fan *et al.*, 2023). Similarly, the growing consumption of unpasteurized juices has increased disease outbreaks related to *Staphylococcus*, *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* (Pius *et al*., 2023). In the study of 100 fresh or blended and self-service unpasteurized juices, foodborne pathogens such as *Salmonella* spp, *Escherichia coli*, and *Listeria monocytogenes* were detected, and the microbiological quality, such as total aerobic mesophilic bacteria, total coliform, moulds, and yeasts, and indicates the contamination of the juices (Neggazi *et al*., 2024).

In other cases, physicochemical properties of juice such as pH, titratable acidity, and total soluble solids are usually the permissible standard levels, which allows the growth of acid-tolerant microorganisms and affects the juice shelf life (Wang *et al*., 2022). Freshly made unpasteurized sugarcane juice usually shows variable acidity, which are associated with higher bacterial load and reduced product stability and shelf life (Issa-Zacharia and Rwabunywenge, 2024a).

Foodborne diseases are a significant public health issue and affect health care centres, causing diarrhoea, the highest burden on children under five years old and those living in low-and middle-income countries (WHO, 2026). In addition to environmental contamination, pollution in water, soil, air, food processing areas, and unsafe food storage are factors causing foodborne diseases (Mehlhorn, 2016). Food safety challenges are mainly faced by vulnerable population groups, including immunocompromised individuals (Njoagwuani *et al*., 2023). Despite the wide consumption by millions of people in developing countries, the prevalence of foodborne illness linked to the intake of freshly squeezed juices sold by street vendors is increasing (Issa-Zacharia and Rwabunywenge, 2024a).

A study conducted in Arba Minch Town, Ethiopia, found that fresh unpasteurized fruit juice samples were positive for *E. coli* 48(50%), *Salmonella spp*. 40(41.67%), and *Staphylococcus aureus* 65 (67.71%), which may harm the health of consumers (Bikila and Kadire, 2019). The other study also revealed that freshly made unpasteurized fruit juices are increasing in urban areas, but they fail to meet microbiological quality and safety standards. For example, total coliforms, fecal coliforms, and higher viable counts, along with the presence of *Salmonella*, *Staphylococcus*, and *Escherichia coli*, were frequently detected due to contamination during handling and juice preparation (Mengistu *et al*., 2021; Kechero *et al*., 2019; Demisie and Melese, 2024).

According to the Dessie City Administration Trade and Industry Office report, the number of juice vending houses is increasing due to population growth and rising consumer demand. A previous local study assessed only the microbiological safety of mango, avocado, and papaya juice (Kibret *et al*., 2025). However, the study did not include commonly consumed sugarcane juice, pineapple juice, and ready-to-eat chopped watermelon. These juices and ready-to-eat chopped watermelon are widely consumed in Dessie Town, but they are mainly prepared under poor hygienic conditions. Most studies in Ethiopian lacked information on the association of handling practices, pH, TA with microbial contamination together with isolating antimicrobial resistance pathogens from freshly prepared juices. Hence, there is a limited information on their safety; the actual health risk remains a concern in the area. Therefore, this study aimed to evaluate the microbiological quality and physicochemical characteristics of freshly prepared sugarcane juices, pineapple juices, and ready-to-eat chopped watermelon in Dessie Town.

1.2 Statement of the Problem

Fresh-squeezed juices are consumed worldwide due to their nutritional benefits (Beckett *et al*., 2025), but the microbial quality and safety of juice are often poorly monitored and a source of risk for developing countries (WHO, 2026; Pius, 2023). For example, the sugarcane (*Saccharum officinarum*) juice has good sweetness with nutritional benefits, but studies reported a high coliform count above the microbiological safety limit and the presence of *Salmonella* and *E.coli* (Cristine *et al.*, 2024; Ed-Dra *et al*., 2025). Currently, the WHO has announced the global burden of foodborne diseases from 2000 – 2021, more than 866 million cases of foodborne illnesses and 1.52 million deaths occurred in 2021. The burden of foodborne diseases falls disproportionately on children under 5 years of age, with the highest burden in low-and middle- income countries (WHO, 2026).

The presence of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Shigella*, and *Staphylococcus aureus in* fresh fruit juices is due to poor hygienic practices, contaminated water, inadequate sanitation of equipment, improper waste management, and long-term storage at room temperature (Neggazi *et al*., 2024; Balali *et al.*, 2020; Shiferaw and Kibret, 2018). In other cases, physicochemical properties of juices, such as pH and titratable acidity, influence the growth of microorganisms, stability of juice, or shelf life (Vi *et al*., 2025). Both pH and titratable acidity influence microbial growth in fresh juices and fresh-cut fruits, for example, sugarcane and watermelon juices, with low acidity, support the growth of *Salmonella* spp., *E. coli*, and *Staphylococcus aureus*, while pineapple has higher acidity, in which acid-tolerant microorganisms survive, however, the lack of integrated information on these parameters may increase gastrointestinal infections (Ed-Dra *et al*., 2025).

In Ethiopia, freshly prepared fruit juices and ready-to-eat fruit are increasingly consumed in urban areas due to nutritional and health benefits. However, studies conducted in different urban areas of Ethiopia revealed that freshly prepared unpasteurized fruit juice and ready-to-eat fresh-cut fruit fail to meet microbiological safety standards, and *Salmonella*, *E.coli*, *Staphylococcus aureus*, and coliforms have been frequently detected, indicating microbial contamination during handling, storage, and preparation (Kechero *et al*., 2019; Mengistu *et al.*, 2022).

In Dessie Town, a study by Kibret *et al*., (2025) assessed only the microbiological quality and safety of mango, papaya, and avocado juices, such as total aerobic mesophilic bacterial count, coliforms, yeast and mold count, and pathogens, such as *Salmonella*, *E.coli,* and *Staphylococcus aureus.* But the study did not incorporate other widely consumed popular juices and ready-to-eat fresh-cut fruit available in juice houses, such as sugarcane juice, pineapple juice, and ready-to-eat chopped watermelon, along with their association of physicochemical properties with microbial contamination, such as pH and titratable acidity. Poor hygiene, contaminated utensils, and poor sanitation practices may contribute to microbiological contamination and transmission of antimicrobial-resistant bacteria to consumers and reduce the effective treatment of infections. Due to the increase in juice vending houses and population growth in Dessie Town, the demand for consuming sugarcane juice, pineapple juice, and ready-to-eat chopped watermelon has increased, but there has been limited attention to their microbiological safety, and the current public health risks of these commonly consumed juices and ready-to-eat chopped watermelon remain uncertain. Furthermore, the relationship between handling practices, pH, and titratable acidity and the microbial load has not been determined in the area. Hence, generating integrated evidence on the microbiological and physicochemical quality of juices and fresh-cut watermelon, along with isolating AMR bacterial isolates, was necessary to strengthen hygiene and promote safe handling practices among vendors. Therefore, this study aimed to assess the microbiological quality and physicochemical properties of locally prepared sugarcane juices, pineapple juices, and ready-to-eat chopped watermelon sold in Dessie Town.

1.3 Objective of the Study

1.3.1 General Objective

- To assess the microbial quality, physicochemical characteristics, and handling practices of locally prepared juices and ready-to-eat chopped watermelon in Dessie Town.

1.3.2 Specific Objectives

- To determine the microbiological quality of pineapple juice, sugarcane juice, and ready-to- eat chopped watermelon.
- To isolate *Escherichia coli* and *Salmonella* from pineapple juice, sugarcane juice, and ready-to-eat chopped watermelon.
- To determine the antimicrobial susceptibility pattern of the bacterial isolates (*Escherichia coli* and *Salmonella*).
- To evaluate the relationships between pH and titratable acidity and the microbial load of the samples.
- To assess the association between the handling practices of vendors and microbial load.

1.4 Significance of the Study

To promote food safety practices, assessing the physicochemical properties and microbiological quality of pineapple juice, sugarcane juice, and ready-to-eat sliced watermelon is essential. This study provides comprehensive information on microbiological safety, antimicrobial susceptibility patterns of bacterial isolates, and physicochemical characteristics of these products, contributing to a better understanding of local food safety practices. The findings will serve as baseline evidence for governmental, non-governmental, and private organizations to strengthen hygiene and sanitation measures for freshly prepared juices and ready-to-eat fresh-cut fruit. Moreover, the study will help vendors to improve processing and storage practices. Furthermore, the findings can be published and used as baseline scientific information for the research community, supporting future studies on the safety, quality, and public health implications of locally prepared pineapple juice, sugarcane juice, and ready-to-eat sliced watermelon in the study area.

2. LITERATURE REVIEW

2.1 Microbiological Quality of Fresh Juices and Foodborne Diseases

Juices made from fresh fruits that were improperly prepared are one of the main causes of foodborne illnesses. A study reported that out of 200 fresh fruit juice samples, such as mango, orange, pineapple, pomegranate, and sugarcane, Bacillus, *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus*, *Acetobacter*, *Salmonella*, and *Shigella* were investigated, and also *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* spp., were the predominant moulds found in fruit juices such as sugarcane and orange juices (Kumari *et al.*, 2023). Yeast such as *Saccharomyces*, *Candida*, and *Rhodotorula* were also reported in freshly prepared fruit juices. It has been determined that the microbial load in fresh fruit juices is significantly greater than the normal permissible limits, suggesting that it may play an important role in food spoilage and food-borne illnesses (Kumari *et al.*, 2023; Areum *et al.*, 2021).

Food-borne diseases are a global public health issue, with 1 in 10 people falling ill after eating contaminated food every year (Romero-Calle *et al.*, 2023). In addition, according to CDC data, over 9,000 foodborne illness outbreaks were reported between 2011 and 2022 across all 50 states. Foodborne illness has widespread economic consequences, costing Americans an estimated 75 billion dollars annually in 2023, annually in medical care, lost productivity, and premature deaths, and according to a United States Department of Agriculture report, people are developing secondary chronic illnesses after being exposed to foodborne risks (Matters, 2025). Each year, an estimated 800 million people fall ill, and 1.5 million people die from unsafe food, resulting in the loss of 57.1 million healthy or disability-adjusted life years. Children under 5 years of age are at particularly high risk, accounting for 29% of the total disability-adjusted life years (WHO, 2026).

2.1.1 Fruit juice microbial contamination and foodborne diseases in Africa

A meta-analysis of 20 studies conducted in African countries reported that out of 1531 fruit juice samples, the prevalence of bacterial contamination was recorded as 86.96%, at 95% CI (81.25 - 90.93). The potential bacterial pathogens such as *Staphylococcus*, *Escherichia coli*, *Bacillus*, and *Salmonella*, are observed across studies. It indicated a

higher prevalence of bacterial contamination with both commensals and pathogenic bacteria, indicating juices and potential sources of foodborne illness (Pius *et al*., 2023).

A previous study analysed the microbiological quality of sugarcane juice and found the TVBC, TCC, FCC, Staphylococci, yeas,t and mold to be 5.79 ± 0.36, 3.78 ± 0.33, 1.67 ± 0.34, 2.81 ± 0.23, and 3.73 ± 0.23 log CFU/mL, respectively. Additionally, *Escherichia coli* was detected in 77% of samples, and *Staphylococcus aureus* was found in 7% of samples, while *Salmonella* and *Listeria monocytogenes* were not detected (Ed-Dra *et al*., 2025).

2.1.2 Fruit juice microbial contamination and foodborne diseases in Ethiopia

Many studies conducted in Ethiopia have continuously reported poor microbiological quality of locally prepared fruit juices and ready-to-eat sliced fruits like watermelon, causing serious public health concerns. A study conducted by Mengistu *et al.*, (2022), found that out of 78 analysed juice samples, 85.9% of the samples had total aerobic mesophilic bacterial count, 64.1% had total coliform count, 60.3% had fecal coliform, and 33.3% of the samples had *Escherichia coli* higher than the maximum permitted level of Gulf Standard 2000. The high contamination levels were attributed to poor personal hygiene, unclean utensils, and unsafe water sources. The other similar study also confirmed the presence of *Escherichia coli, Shigella, Salmonella,* and *Staphylococcus* in locally prepared fruit juices (Mengistu *et al*., 2021).

2.1.3 Antibiotic- resistant bacterial pathogens in freshly prepared juices

Microbial contamination of fruit juices has caused major outbreaks of food-borne diseases, leading to significant mortality and morbidity in developing countries. The hygiene and safety practices of juice vendors are the primary risk factors for the microbial contamination of juices. For example, 42.86% of *S. aureus* isolates, and 40.26% of *E. coli* isolates from street-vended sugarcane juice were resistant to ampicillin, penicillin, tetracycline, and streptomycin (Ed-Dra *et al.,* 2025). Antibiotic resistance in street-vended foods and beverages is frequently reported; for example, Enterobacteriaceae and *Staphylococcus* species are resistant to β-lactams, tetracycline, and aminoglycosides, where antibiotic resistance genes have also been detected. In addition, these pathogens exhibit substantial resistance to tetracycline, ceftriaxone, and ampicillin exhibited substantial levels of resistance. The prevalence of antibiotic resistance bacteria in street food ranged from 5.2% to 70.8%. Environmental variables

that contribute to the spread of antibiotic resistance and multidrug-resistant bacteria, such as *Salmonella, Staphylococcus, Escherichia coli,* and *Klebsiella,* to many classes of antibiotic drugs (Fusaro *et al.*, 2024).

In Ethiopia, Staphylococc*i* and Enterobacteriaceae are significantly resistant to many antibiotics. Out of the 525 food samples, 279 (53%) had bacterial contamination, and among these, 95 (54.3%) of the 175 water samples had *Escherichia coli* contamination. A total of eleven bacterial species were isolated from two samples. The most common isolated bacterial species, *Staphylococcus aureus, Salmonella, Shigella,* and Klebsiella, have statistically significant relationships with food type, and they are resistant to streptomycin (17%), amoxicillin(14%), and erythromycin (54%) (Moges *et al.*,2024).

A comparative study between fruit juices and vegetable salads revealed that higher bacterial counts were found, and 58 (74.4%) of the fruit juice samples had *Staphylococcus* counts, 19(24.4%) had *Salmonella,* and 12 (15.4%) had *Shigella,* higher than the maximum permitted limit of the Gulf standard, 2000 set for any type of fruit juice. This study assessed poor environmental sanitation and vendor handling practices as major contributing contamination sources (Kechero *et al.*, 2019). Beyond fruit juice, the bacteriological quality of street-vended sliced watermelon in Adama City was found to have extremely high aerobic mesophilic counts (2.59×10^{6} to 2.86×10^{7} CFU/g) and the presence of *E. coli, Shigella,* and *Salmonella* spp., showing that ready-to-eat fruits are equally at risk (Tamire and Chala, 2024).

2.2 Source of Microbial Contamination of Fresh Fruit Juice

Despite the beneficial effects of fruit juices to consumers, improper handling, poor hygiene practices, and the lack of good environmental sanitation affect the quality and safety of fruit juices (Pius *et al.*, 2021). In the absence of good production practice, the nutritional richness of fruits and fruit juices makes the product a good medium for microbial growth, a vehicle of foodborne pathogens, and associated complications. The microorganisms present in fruit juices frequently originate from the raw materials used for the preparation and introduced during the processing. Several factors encourage, prevent, or limit the growth of microorganisms in juices; Such as ones are water activity (aw), pH, hygienic practice, and storage temperature and concentration of preservatives. The storage of products at refrigerator temperature is not always best for the maintenance of the desirable quality of some fruits (Mihiretie and Desta, 2015).

Water used for juice preparation can be a major source of microbial contaminants such as total coliforms, faecal coliforms, faecal streptococci, etc. Environmental fomites may also make the fruits unsafe and these may have a role in the spread of *Salmonella, m Shigella, Vibrio,* and *Escherichia coli* (Elamparithi *et al*., 2024).

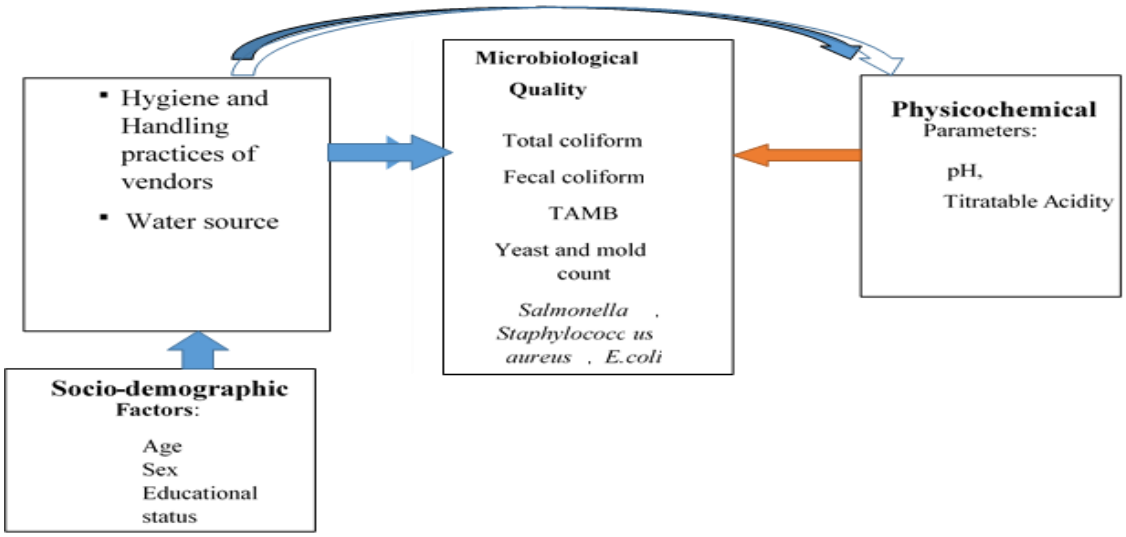


Figure2.1: Conceptual framework that shows the variables' effect on microbial contamination

2.3 The Common Microorganisms in Fruit Juices

2.3.1 Aerobic mesophilic bacteria

Aerobic mesophilic bacteria are a group of microorganisms that grow under aerobic conditions at moderate temperatures from 20-45°C. They are important indicators of general handling, hygiene, and microbial quality in food samples. Higher AMB count reflects poor sanitation, contaminated raw materials, and inadequate storage conditions. While many mesophilic bacteria are not pathogenic, they facilitate spoilage and change the juice flavor, odor, and turbidity (Alegre, 2024). According to Codex Alimentarius and UKHSA guidelines, low aerobic mesophilic counts ensure microbiological safety and shelf-life stability of juice.

2.3.2 Coliforms

Coliform bacteria are facultative anaerobic and rod-shaped Gram-negative, motile or non-motile, and non-spore -forming bacteria able to ferment lactose, which produce acid and gas when incubated at 35 to 37°C within 48 hours. Fecal coliforms are a group of coliform bacteria that ferment lactose at 44.5°C(Bacteriological Analytical Manual,

2020). They are mainly found in the human intestine, some are found in plants and soil, and are used as indicators of the sanitary quality of food and water (Çiftçi and Öncül, 2021).

2.3.3 Yeast and mold

Spoilage fungi cause significant financial losses in the food and beverage industry each year. These fungi thrive in adverse environmental conditions with low acidity, low water activity, and high sugar content, which are often found in fruit fillings used in pastry products. As a result, fruit fillings are very vulnerable to fungal spoilage. Fungal growth can lead to changes in sensory quality in foods, such as odour, appearance, flavour, or texture. It can also cause health risks due to the production of mycotoxins by some mold species (Akter *et al.*, 2024). Fruit juices contain water, protein, lipids, carbohydrates, acids, phenolic compounds, dietary fibre, vitamins, and essential minerals. These are necessary to maintain a healthy life. However, due to their short shelf life, these juices can be contaminated by mould and yeast and can become a source of health hazards (Alam *et al*., 2019).

2.3.4 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium, typically arranged in clusters that can colonize humans, animals, and the environment (Heaton *et al.*, 2020). Bacterial pathogens, including *Staphylococcus aureus,* are often involved in foodborne disease and pose a serious threat to human health. Staphylococcal enteric disease, specifically staphylococcal food poisoning (SFP), accounts for many gastrointestinal illnesses through the contamination of food with its enterotoxins, and its major impact on human health leads to a heavy economic burden in society (Phan *et al.*, 2025).

Food poisoning that involves multiple etiologies is reported from developing as well as developed nations. *Staphylococcus aureus* is a major pathogen of food poisoning that can occur in sporadic and epidemic form. It may cause life-threatening infections in children, the elderly, and immunocompromised persons. Staphylococcal food poisoning is responsible for 241,000 cases in the United States annually (Pal, 2022).

2.3.5 *Escherichia coli* O157:H7

The pathogenic strain of *Escherichia coli*, namely *E. coli* O157:H7, is a relevant pathogen related to MPF safety since it has been implicated in some outbreaks and sporadic cases. *Escherichia coli* strains are members of the gastrointestinal microbiota

of humans and mammals. Fecal matter can contaminate food and water, including irrigation and recreational waters. Human infections by pathogenic *Escherichia coli* strains occur after the consumption of contaminated food such as undercooked meat, contaminated fresh vegetables and fruit, or through contact with contaminated animals (Yoo *et al*., 2015).

2.3.6 *Salmonella*

Bacteria of the genus *Salmonella* are Gram-negative and move by means of flagella. The genus *Salmonella* is rod-shaped, belonging to the family Enterobacteriaceae. They have several antigens: O, H, and Vi, and are facultative anaerobes, chemo-organotrophs, carrying out aerobic metabolism and fermentation processes. The optimum temperature for their growth is 37 °C, but they grow over a wide temperature range from 2°C to 54°C (Eng *et al*., 2015).

Salmonella causes far more illnesses than you might suspect. For every case of *Salmonella* illness confirmed by laboratory tests, almost 30 other cases are not reported. That's because most people with symptoms of foodborne illness do not go to the doctor or submit a sample to a laboratory, so we never learn what germ made them sick. CDC estimates that *Salmonella* causes more than 1 million foodborne illnesses in the United States every year. Certain people are more likely to get a serious *Salmonella* infection. These people include children who are younger than 5, adults who are 65 and older, and people whose immune systems are weakened from certain medical conditions, such as diabetes, liver or kidney disease, and cancer. Symptoms usually start at 6 hours to 6 days after infection. They include diarrhoea that can be bloody, fever, and stomach cramps. Most people recover within 4 to 7 days without antibiotic treatment. But some people with severe diarrhoea may need to be hospitalized or take antibiotics (CDC, 2022).

2.4 Physicochemical Properties and their Effects on Microbial Contamination

A study conducted in Pakistan confirmed that out of 50 juice samples, 31 exceeded acceptable or permissible microbial limits, and *Escherichia coli* was observed, while physicochemical conditions such as pH and total solids are favoured for microbial growth. Overall, the authors conclude that many fresh juices sold in the area have both microbial hazards (pathogenic *E. coli* risk) and chemical (toxic metal) hazards to consumers and recommend that monitoring and better hygiene and quality-control

measures should be applied to prevent outbreaks and chronic exposure of the people (Zahoor *et al*., 2021).

The study in Imus, Cavite, Philippines on pineapple and watermelon reported that the total plate count of fresh-cut watermelon did not exceed 10^7 CFU/mL, but all supermarket watermelon samples and three out of six from open market samples exceeded the 10^7 limit for total plate count; four out of six supermarket samples and three out of six open market samples exceeded the limit for pineapple. The whole watermelon samples were within the acceptable limit for yeast and mold count, whereas five out of six samples from open markets and supermarkets had exceeded the limit. The total coliform in all watermelon samples, and pineapples from all supermarkets, three out of six open markets were exceeded the 10^2 limit. *E. coli* was not detected in all samples; However *Salmonella* was reported in one supermarket and three open market pineapple samples. In addition, two watermelon samples from both open and supermarket markets were positive for *Salmonella*. For watermelon, 2 samples each from supermarkets and wet markets were positive for *Salmonella,* while for pineapple, 1 sample from the supermarket and 3 from wet markets turned out positive (Piano and Castillo-Israel, 2019).

2.4.1 pH and Titratable acidity

In unpasteurized fruit juices, pH is a key factor that limits microbial growth. Many spoilages and harmful bacteria cannot multiply below pH 4. However, yeasts, moulds, and acid-tolerant bacteria can still survive and cause spoilages in these acidic conditions. Some strains of *Escherichia coli* and *Enterobacter aerogenes* can grow at pH levels lower than 4.5. Additionally, certain *Salmonella* strains can tolerate conditions as low as pH 3.9. Yeasts show a wide range of pH tolerance, thriving in a pH range of about 1.5 to 9 and growing at temperatures between 12°C and 50°C. Similarly, moulds can develop over a broad pH range of approximately 1.5 to 11 and can grow at temperatures from slightly below freezing up to about 55°C. These traits allow yeasts and moulds to significantly contribute to spoilage in acidic, unpasteurized fruit juices, despite their low pH environment (Dietetici and Spa, 1989).

The total free acid, often expressed as a percentage of citric or malic acid, is a primary trait that defines juice taste. It interacts inversely with pH and strongly affects both sensory quality and microbial stability of fruit juices (Al-farsi *et al.*, 2024). Total acidity

is an important, measurable indicator of flavour and plays a role in the juice's protective chemistry. However, effective microbial control, especially in unpasteurized or lightly processed juices, needs to combine total acidity and pH data with targeted microbial testing and appropriate processing or preservation measures. pH regulates the hydrogen-ion activity that controls the ranges of microbial growth, while total acidity reflects the buffering capacity and total acid content, which contribute to flavour and can influence microbial stress during processing and storage (Casolari, 2016).

2.5 International Guidelines for Examination of Fruit Juice

The United Kingdom Health Security Agency (UKHSA) provides guidelines for assessing the microbiological quality and safety of ready-to-eat foods placed on the market. It provides a practical and risk-based framework for interpreting microbiological test results of ready-to-eat foods. It gives context-dependent interpretation for presence or absence and uses numerical cut-off points for each microorganism as satisfactory, borderline, and unsatisfactory (UKHSA, 2024). The Codex Alimentarius Commission, general standard for fruit juices and nectars (CXS 247-2005) specifies that fruit juices and nectars must fulfil the minimum requirement of soluble solids (Brix) values for each fruit. The CXS 247-2005 ensures composition, quality, and labelling standards, but it does not specify quantitative permissible limits for pathogens in juices or other fruit products (Standard *et al*., 2005).

According to the United State Food and Drug Administration juice Hazard Analysis Critical Control Point (HACCP) regulation all industrial processed juice producers are required to follow a preventive science-based system that helps to identifies and control biological, chemical, and physical hazards in juice production. It is recommended that less than 5 log reduction of pathogens such as *E.coli* 0157:H7 and *Salmonella* via pasteurization with serious monitoring and sanitation. The HACCP principles, such as hazard analysis, hygiene, and controlling microbial contamination, are highly useful to improve microbial safety and handling practices of vendors that are involved in making unpasteurized freshly prepared fruit juice and fresh-cut fruit (NAFDAC, 2019). The European Commission Regulation No 2073/2005 establishes microbiological criteria for foodstuffs to determine the quantitative permissible limits for *Salmonella* (absence in 25 g) and *E.coli* (less than 100 CFU/ml). It complements Hazard Analysis and Critical Control Point principles by monitoring microbial load and taking corrective action.

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Dessie City administration which is found in the Eastern part of the Amhara Regional State. It is located about 401 km from Addis Ababa, which is the capital city of Ethiopia. Geographically, it is situated between 560000–578000 m E and 1222000–1246000 m N (Fig 3.1). Dessie is surrounded by Tossa Mountain, with an elevation ranging from 1915 to 3041 m above sea level. According to the World Population Review 2026 report, the total population of Dessie Town is estimated as 335,739.

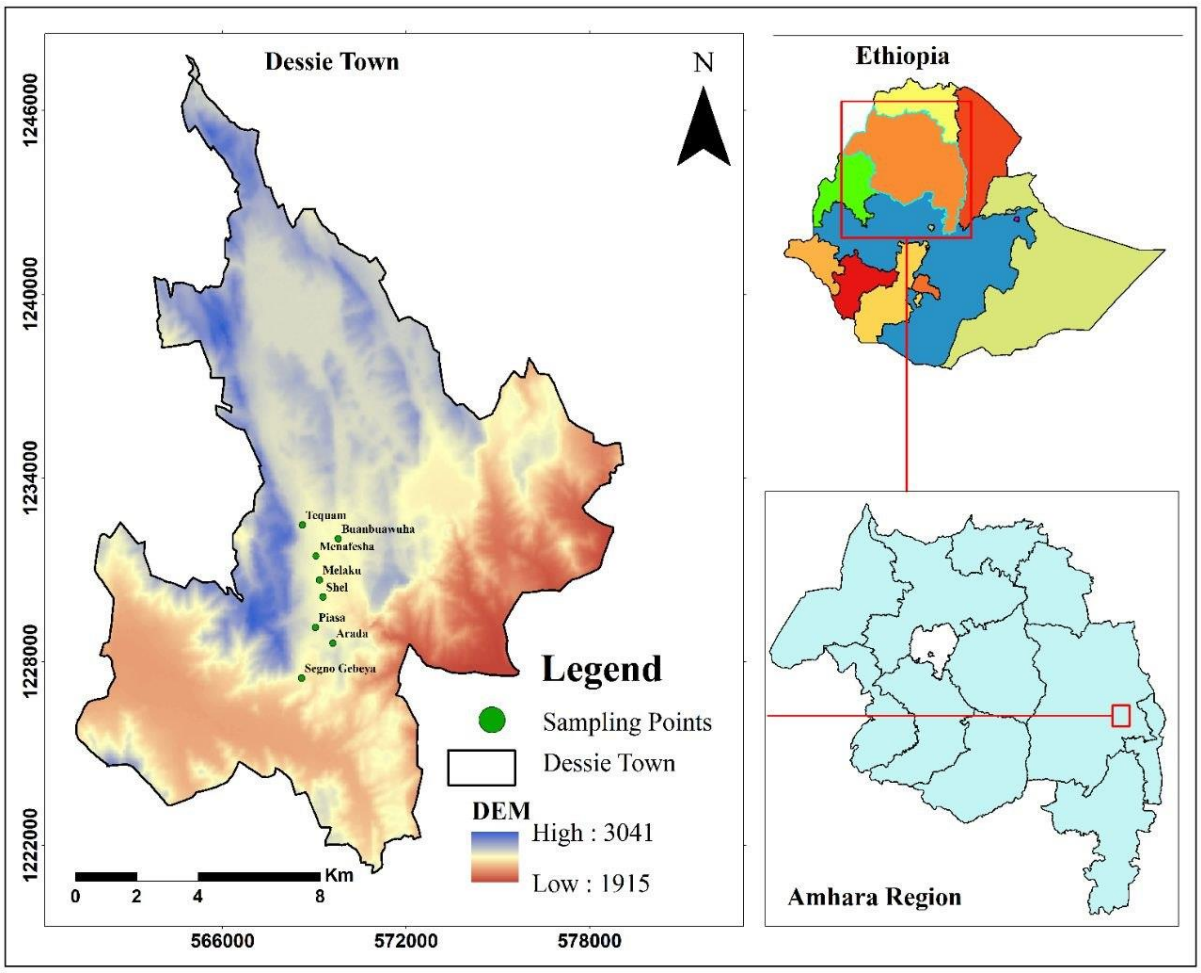


Figure 3.1: Map of the study area

3.2 Study Design

A laboratory-based cross-sectional prospective study design was used from January 2026 to August 2026 to determine the microbiological and physicochemical quality of freshly prepared pineapple juices, sugarcane juices, and ready-to-eat chopped watermelon.

3.3 Source of Sample

Freshly prepared juices, such as, avocado, mango, papaya, strawberry, orange, pineapple, banana, and ready-to-eat chopped watermelon, were sold in juice houses, cafeterias, restaurants, and hotels. Among these, sugarcane juice, pineapple juice, and ready-to-eat watermelon were mostly sold and consumed in the study area at juice houses. In this study, the number of juice houses available in Dessie town was considered the study population. Therefore, sugarcane juice, pineapple juice, and ready-to- eat chopped watermelon were obtained from juice houses for microbiological and physicochemical laboratory analysis.

3.4 Sample Size Determination

The sample size was determined according to FDA Bacteriological Analytical Manual (2020) sampling unit guidance. According to the Dessie City Administration Trade and Industry Office report, 35 juice houses were available, except for cafeterias, restaurants, and hotels. Out of these, 90 samples (30 pineapple, 30 sugarcane, and 30 ready-to-eat watermelon) were taken based on the allocated sites available in the town.

3.5 Sampling Technique

A multistage sampling technique combining both purposive and simple random sampling techniques were used. A purposive sampling technique was used to select eight sites in Dessie town, such as Buanbuawuha, Melaku, Menafesha, Shel, Piasa, Tequam, Segnogebeya, and Arada. These sites are found around the main roads of the town where people were heavily populated for different day-to-day activities. Geographically, the sites are found consecutively; they represented all juice house locations available in the town. It did not mean excluding juice houses present in unpopulated areas of the town. According to Neyman proportional allocation method used in other recent studies, thirty juice houses were selected proportionally from eight allocated sites using the formula. $ni = Ni/N \times n$, *Where*, *ni*= Number of juice houses selected from site(i), *Ni* = total number of registered juice houses in sites (i), N= total

number of registered juice houses in all sites(35), n= total number of sample size(90)(Berihu *et al*., 2024:Mengistu *et al*., 2021:Kipuru *et al.,* 2024). Within each allocated site, juice houses were randomly selected using a lottery method to take samples of pineapple, sugarcane juice, and ready-to-eat chopped watermelon. From each selected juice house at eight sites, one vendor was randomly chosen for questionnaire administration. Finally, the hygiene and handling practices were assessed using structured questionnaires and an observation checklist among 30 randomly selected vendors.

3.6 Study Variables

3.6.1 Dependent variables

Total aerobic mesophilic bacterial count, *Staphylococcus aureus* count, total coliform count, fecal coliform count, yeast and mold count, *E. coli*, *Salmonella*, and antimicrobial susceptibility.

3.6.2 Independent variables

Handling practices, pH, and titratable acidity.

3.7 Sample Collection and Preparation

200 mL of each locally prepared fresh pineapple and sugarcane juice was collected using sterile sampling bottles, and 200g of chopped watermelon samples were collected using an aluminium-foil-capped beaker; each sample was transported using an Ice Box to the Wollo University Environmental Health Department, Microbiology Laboratory within 2-4 hours. A 25 mL or g of pineapple juice, sugarcane juice, and sliced watermelon samples were aseptically homogenised with 225 mL of sterile Buffered Peptone Water to get the initial dilution (10^{-1}). Then 1mL of diluted sample from the 10^{-1} dilution tube was transferred to the next dilution tube containing 9 mL sterile Buffered Peptone Water (10^{-2} to 10^{-6}). Dilutions were prepared until the dilution expected to give the desired colony count range of 30–300 CFU/mL per plate was obtained (FDA Bacteriological Analytical Manual, 2020).

3.8 Data Quality Management

Negative and positive controls were used to assure the validity and reliability of the microbiological testing. Reference strains of *Escherichia coli* and *Salmonella* were taken from the Amhara Public Health Institution laboratory (APHI), Dessie branch. The

structured questionnaire and observation checklist were pretested to ensure clarity and non-confusability. All equipment and media were sterilized via autoclave at 121˚C and at 15 pascal for 15 minutes. All sample analysis was conducted in triplicate with its control and done inside a biosafety cabinet. To prevent cross-contamination, 70% ethanol and flame were used to disinfect and sterilize materials.

3.9 Microbiological Analysis

3.9.1 Aerobic Mesophilic Bacterial Count

The aerobic mesophilic bacterial count was determined using plate count agar (PCA) (HiMedia, Nashik, India) (Neggazi *et al.*, 2024). One mL from each serially diluted sample (10^{-3}, $10^{-4,}$ and 10^{-5}) was transferred into three sterilized and labelled plates, followed by pouring sterile Plate Count Agar (PCA) (HiMedia, Nashik, India) on the sample-containing plates. The plates were incubated at 30±2°C for 24 hours under aerobic conditions. After incubation, plates with colonies between 30 and 300 were counted using a Digital Colony Counter (Stuart Scientific, UK), and the result was calculated as CFU/mL(UKHSA, 2024).

CFU/mL=(Number of colonies× Dilution factor)/(Volume plated(mL))

3.9.2 Enumeration of coliform

On Violet Red Bile Agar (VRBA)(HiMedia, Nashik, India), 0.1 mL aliquots from appropriate decimal dilutions were plated in triplicate and incubated for 24 hours at 32˚C (for total coliform) and 44.5˚C (for faecal coliform). Typical pink- red colonies on plates surrounded by a zone of bile acid were counted using a Digital Colony Counter (Stuart Scientific, UK) (ISO-4832, 2006).

3.9.3 *Staphylococcus aureus* count

Staphylococcus aureus was isolated and enumerated using Mannitol Salt Agar (MSA) (Alpha Chemica, Mumbai, India). A 0.1 mL aliquot from the dilution was dispersed on MSA and incubated for 24 hrs at 35±2 ˚C, after incubation, golden yellow colonies ranged from 20 to 200 were counted using the colony counter and recorded as *Staphylococcus* spp., in CFU/mL(Amare *et al.*, 2019; ISO 6888-3:2017, 2023).

3.9.4 Yeast and Mold Count

Sabour Dextrose Agar (SDA) (Microxpress®, Tulip Diagnostics(P)Ltd., Verna, Goa, India) supplemented with chlortetracycline was used to enumerate yeast and mold. One

mL from each dilution (10^{-3} to 10^{-5}) was inoculated into a sterile Petri plate, and 15 mL of SDA was poured and mixed gently. The plates were solidified for a minute and incubated in an inverted position at 25 ± 1 ˚C for five days. Finally, colonies were counted and determined as CFU/mL (Asghar *et al*., 2018).

3.9.5 Detection of *Escherichia coli*

Preliminary identification of *Escherichia coli* was carried out using MacConkey Agar (Oxoid Ltd, Basingstoke, United Kingdom), where colonies exhibiting pink to red coloration were selected for further confirmation. These colonies were subsequently streaked onto Eosin Methylene Blue (EMB) agar (Microxpress®, Tulip Diagnostics(P)Ltd., Verna, Goa, India) and incubated at 35±2°C for 18–24 h. Colonies that developed a green metallic sheen were presumptively identified as *E. coli* (Asghar *et al.,* 2018). Gram stain was performed following standard procedures for confirming *E.coli* as a Gram- negative bacterium.

A biochemical characterization of *E.coli* was performed following Bergey's Manual of Systematic Bacteriology and standard methods. The presumptive *E. coli* isolates were subjected to biochemical assays, including citrate utilization, triple sugar iron agar test, indole, and urease tests, following standard procedures.

In the citrate utilization test, colonies with pink-red or green metallic sheen from EMB agar were inoculated into Simmons Citrate Agar (HiMedia, Nashik, India) and incubated at 35±2 °C for 24–48 h. The slants remained green, indicating negative citrate utilization, which is consistent with *E. coli*. Triple Sugar Iron Agar (TSIA) (HiMedia, Nashik, India) test was performed by incubating cultures at 44.5 °C for 24 hours, where gas production, medium cracking, and bubble formation without hydrogen sulphide (H_2S) production confirmed the presence of *E. coli*. For the indole test, fresh cultures were inoculated into sterile test tubes containing 4 mL of Tryptophan broth (Oxoid Ltd, Basingstoke, United Kingdom) and incubated at 35±2 °C for 24-48 hours. After incubation, 0.5 mL of Kovac's reagent was added, and the formation of a red ring indicated indole production, confirming *E. coli*. Finally, in the urease test, cultures inoculated onto Urea Agar (Oxoid Ltd, Basingstoke, United Kingdom) and incubated at 35±2°C for 24 h showed no colour change, with the medium remaining yellow. This negative urease reaction was consistent with *E. coli* identification (ISO 16649-2:2001(E); FDA Bacteriological Analytical Manual, 2020).

3.9.6 Detection of *Salmonella*

Isolation of *Salmonella* was performed according to ISO 6579-1:2017/Amd 1:2020(E); and the FDA Bacteriological Analytical Manual (2020). A 25 mL or g of pineapple juice, sugarcane juice, and sliced watermelon samples were homogenized in 225 mL of sterile Buffered Peptone Water. The homogenate was incubated at 35±2˚C for 24 hrs for pre-enrichment of *Salmonella*. One mL from each dilution tube was dispersed on XLD and Salmonella-Shigella (SS) Agar (Microxpress®, Tulip Diagnostics (P) Ltd., Verna, Goa, India) and incubated at 35±2˚C for 24 hours. Gram stain was performed following standard methods for confirming *Salmonella* as a Gram-negative bacterium.

Furthermore, presumptive *Salmonella colonies* were identified and confirmed by using the Citrate test, Triple Sugar Iron agar test, Indole and Urease tests. Citrate utilization test was performed by inoculating the culture on Simmons citrate agar and observing the color change after 24 hours of incubation at 35±2˚C. The Triple Sugar Iron Agar (HiMedia, Nashik, India) test was used to assess sugar fermentation ability and production of Hydrogen sulphide (H2S) with black color formation. Indole production test was performed by adding Kovacs reagent to tryptophan broth and observing a red ring after 24 hrs. The urease test was done by inoculating culture on urea agar and incubating at 35±2˚C.

3.9.7 Antimicrobial susceptibility test for isolated *E.coli* and *Salmonella*

According to the Clinical and Laboratory Standards Institute Antimicrobial Susceptibility Testing guidelines (2026), the AMS patterns of *Salmonella* and *Escherichia coli* were determined through the Kirby-Bauer Disk Diffusion method by using Mueller-Hinton Agar (HiMedia, Nashik, India), The fresh bacterial colonies were transferred into sterile normal saline solution (0.9%) and adjusted to 0.5 McFarland turbidity standard (≈1× 10^8 CFU/mL). The inoculum of bacterial isolates was evenly spread on Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 10-15 minutes. Then, selected and standardized antibiotic disks (Ampicillin (10 µg), Amoxicillin (10 µg), Nalidixic Acid (30 µg), Tetracycline (30 µg), Cefradine (30 µg), Norfloxacin (30 µg)) were placed on each agar surface plate and incubated at 35±2˚C for 18-24 hours. After incubation, the diameters of the inhibition zones surrounding each antibiotic disc were measured in millimetres (mm) by using a ruler. Finally, the antimicrobial susceptibility of each isolate was recorded and interpreted as susceptible

(S), intermediate (I), and resistant (R) (CLSI, 2026). The standard reference strains of *Escherichia coli* (ATCC 25922) and Salmonella enterica serovar Typhimurium ATCC 14028 were used as a control for this study.

3.10 Physicochemical Analysis

The physicochemical parameters, such as pH and Titratable acidity, were measured using a calibrated pH meter (Eutech, Instrument (P) Pte Ltd, Singapore) and the titration method, respectively. Determination of titratable acidity was performed through the titration method, where 1 mL of centrifuged juice or aliquot was taken and diluted to 15 mL with carbonate-free double-distilled water. Two to five drops of phenolphthalein indicator were added, and titrate with standardized 0.1N NaOH solution, and the volume of NaOH used was noted until the pink colour appeared for at least 15 seconds or the pH reached 8.1. Then, the titratable acidity (%) was calculated as follows (Paul *et al*., 2010).

$$\text{TA}(\%)\ \text{g of dom. acid}/100\text{mL} = \frac{\text{V NaOH in mL} \times \text{N of NaOH} \times \text{Eq.W of Dominant organic acid} \times 100}{\text{Volume of titrated juice(mL)}}$$

3.11 Socio-Demographic and Handling practices of the study participants

Information on the associated risk factors of sanitary conditions and handling practices of pineapple juice, sugarcane juice, and sliced watermelon was collected using a structured questionnaires and an observation checklist from randomly selected 30 vendors in eight sites.

3.12 Data Analysis

Microbial counts were log-transformed before analysis. Statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Descriptive statistics (frequency, percentage, mean, and standard deviation) were calculated. Differences among sample types were assessed using One-Way ANOVA, while Spearman correlation was applied to evaluate the relationship between pH, titratable acidity, and microbial load. Principal Component analysis (PCA) was used to reduce multiple variables of handling practices to small representative and non-correlated variables. Following this, a multiple linear regression model was used to determine the association of predictors (handling practices, pH, and TA) with the overall bacterial load.

3.13 Microbiological Risk Assessment

The risk assessment was determined by linking the pH, titratable acidity, and sources of contamination (hygienic practices, water source, source of fruits, storage, sanitation of utensils, etc.) with the microbial load. The microbial load and detection of these pathogens were compared with the Gulf Standard guideline as satisfactory (low risk), borderline (moderate risk), and unsatisfactory (high risk) (G. Standard *et al*., 2002).

3.14 Ethical Consideration

The study proposal was approved by the Department of Biology and Bahir Dar University College of Science Ethical Review Committee. Then, concerned government officials at their hierarchical level were informed through formal letters written from the Department of Biology. Written consent was obtained from the Dessie Health Office after a detailed explanation of the purpose, confidentiality, benefits, risks, and procedures during data collection. Juice house owners were informed about the study purpose and confidentiality to make their participation voluntary. Written verbal consent forms were used for juice house owners before collecting any juice samples and fresh-cut watermelon.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Aerobic Mesophilic Bacterial Count

In the present study, the mean total aerobic mesophilic bacterial counts (TAMBC) of chopped watermelon, pineapple, and sugarcane juice were found to be 6.61 log CFU/g, 5.89 log CFU/mL, and 5.75 log CFU/mL, respectively. Out of 90 samples compared to the Gulf standard, 81(90%) were classified as borderline, while 9(10%) were classified as unsatisfactory. A One-Way ANOVA confirmed that there were significant differences in the mean TAMBC among sample juices and chopped watermelons ($p<0.001$). Post-hoc multiple comparisons of ANOVA showed chopped watermelon had higher TAMBC than pineapple juice and sugarcane juice with the mean difference of 0.72 log CFU/mL ($p<0.001$) and 0.86 log CFU/mL ($p<0.001$), respectively, while the difference among pineapple and sugarcane juice was not significant with the mean difference of 0.14 log CFU/mL ($p=0.674$) (Table 1).

Table 1: The overall mean comparison of microbial count of freshly prepared pineapple, sugarcane juice, and ready-to-eat chopped watermelon (n=90

Microbiological parameters	Mean and Ranges	Chopped watermelon(n=30	Pineapple (n=30	Sugarcane (n=30	P-value
TAMBC	Mean ±SD	6.61±0.56	5.89±0.53	5.75±0.76	0.000
	Range	5.23-7.86	4.35-6.63	4.50-7.65	
TCC	Mean ±SD	3.87 ±2.81	3.59±2.61	3.89±1.85	.868
	Range	0-6.65	0-5.79	0-5.54	
FCC	Mean ±SD	1.74±2.73	1.88±2.53	0.73±1.66	0.123
	Range	0-6.64	0-5.53	0-4.61	
SAC	Mean ±SD	4.94±1.79	2.83±2.72	3.63±2.84	0.06
	Range	0-6.94	0-6.41	0-6.71	
YMD	Mean ±SD	5.85±0.75	4.13±2.42	5.98±0.75	0.000
	Range	4.30-7.56	0-6.73	4.61-7.78	

TAMBC: Total Aerobic Mesophilic Bacterial Count, TC: Total Coliform Counts, FC: Fecal Coliform Count, SA: *Staphylococcus aureus* Count, YMD: Yeast and Mold Count.

As shown in Table 2, a multiple linear regression analysis model was statistically significant ($p<0.001$), with a multiple correlation coefficient of ($R=0.826$) and described 68.3% of variations in TAMBC ($R^2= 0.683$), with adjusted $R^2=0.638$, showing a better explanatory effect while adjusting for the number of predicting factors. Among other predictors, environmental sanitation and waste management practices of juice vendors had strong predictor association with TAMBC ($B = -0.614$, $\beta = -0.418$, $p<0.001$), followed by personal hygiene of respondents ($B = -0.297$, $\beta = -0.205$, $p = 0.019$), working materials or utensils sanitation ($B = -0.270$, $\beta = -0.182$, $p = 0.032$), and storage and processing safety ($B = -0.285$, $\beta = -0.190$, $p = 0.01$) were a negative significant predictors of a bacterial load. TA was a negative predictor of TAMBC ($B= -0.976$, $\beta= -0.221$, $p=0.045$), whereas pH was not a significant predictor ($p>0.05$). In contrast, water source, fruit source, sex, age, educational status, and pH of sample juices were not significant predictors of the microbiological quality of pineapple juices, sugarcane juices, and ready-to-eat chopped watermelon.

Table 2: Multiple linear regression model predicting bacterial load (n=90)

				95% Confidence Interval		
Variables	B	SE	β	LB	UB	P-value
Sex	-.003	.117	-.002	-.237	.230	.977
Age	-.007	.065	-.009	-.136	.121	.909
Educational status	.012	.082	.012	-.151	.176	.880
pH	.205	.123	.180	-.039	.450	.098
TA	-.976	.479	-.221	-1.929	-.022	.045
Environmental safety and waste management practices	-.614	.138	-.418	-.888	-.340	0.000
Personal hygiene of vendors	-.297	.123	-.205	-.543	-.051	.019
Processing and storage safety	-.285	.108	-.190	-.500	-.069	.010
Juice machine and utensils sanitation	-.270	.124	-.182	-.516	-.024	.032
Fruit handling practices	-.013	.109	-.009	-.231	.205	.907
Water Safety and handling	-.039	.109	-.027	-.257	.178	.720

$R=0.826$, $R^2=0.683$, adjusted $R^2=0.638$, TA (%) = Titratable acidity, LB: Lower bound, UB: Upper bound.

4.1.2 Coliform Count

In this study, the mean total coliform count was higher in sugarcane juice (3.89±1.85 log CFU/mL), followed by chopped watermelon (3.87±2.81 log CFU/g), and pineapple juice (3.59±2.61 log CFU/mL). Coliform counts in most samples of chopped watermelon, pineapple, and sugarcane juices exceeded the recommended Gulf Standard limit (<2 log CFU/mL) for ready-to-eat fruits and fresh juices (G. Standard *et al*., 2002). Out of the total 90 samples, 25(28%) were found to be satisfactory, while the other 65(72%) were classified as unsatisfactory. The One-Way ANOVA revealed there was no significant difference in all pairwise comparisons of microbial contamination between the sample juices and chopped watermelon (p=0.868) (Table 1).

4.1.3 Fecal coliform

In this study, the mean fecal coliform counts of chopped watermelon, pineapple, and sugarcane juices were 1.74 log CFU/g, 1.88 log CFU/mL, and 0.73 log CFU/mL, respectively. Pineapple juice showed higher contamination, followed by sliced watermelon and sugarcane juice. In accordance with the Gulf Standard, 72(80%) samples were categorized as satisfactory, whereas 18(20%) samples were found to be unsatisfactory. Fecal coliform was detected in 6(20%) of chopped watermelon, 7(23.3%) of pineapple, and 5(16.7%) of sugarcane juice. A One-Way ANOVA Comparison analysis showed that there was no significant difference in faecal contamination among the three sample types (p= 0.123) (Table 1).

4.1.4 *Staphylococcus aureus* Count

The mean *Staphylococcus aureus* counts in chopped watermelon (4.94 log CFU/g), sugarcane juice (3.63 log CFU/mL), and pineapple juice (2.83 log CFU/mL) samples were above the legal Gulf Standard limit (2 log CFU/mL. Out of the 90 samples, 27(30%) were classified as satisfactory, whereas 63(70%) were as unsatisfactory. A One-Way ANOVA showed no significant difference in the mean of *Staphylococcus aureus* count among the sample types (p =0.06). Post Hoc analysis found that chopped watermelon had higher *Staphylococcus aureus* count than pineapple juice with the mean difference of 2.11 log CFU/mL (p = 0.003). However, no statistical significant mean difference between chopped watermelon and sugarcane juices (p=0.092) or between pineapple juice and sugarcane juice (p =0.517) (Table 1).

4.1.5 Yeast and Mold Count

The mean yeast and mold count in sugarcane juice (5.98±0.75 log CFU/g) was higher than chopped watermelon (5.85±0.75 log CFU/g) and pineapple juice (4.13±2.42 log CFU/mL), which was beyond the recommended Gulf Standard guideline (>3.0 log CFU/mL). Out of the 90 samples, 83(92%) were categorized as unsatisfactory, while 7(8%) were satisfactory. A One-way ANOVA showed there were significant differences in yeast and mold between chopped watermelon, pineapple, and sugarcane juices ($p<0.001$). The yeast and mold counts were higher in chopped watermelon and sugarcane juice as compared to pineapple, with the mean difference of 1.73 log CFU/m ($p=0.002$) and 1.85 log CFU/mL ($p=0.001$), respectively. However, no significant difference was observed between sugarcane juice and chopped watermelon ($p=0.811$) (Table 1).

As shown in Table 3, the overall mean microbiological quality analysis showed majority of the 90 samples failed to meet the Gulf Standard. Among the samples, chopped watermelon had the highest microbial contamination as compared to pineapple juice and sugarcane juice. The mean total aerobic mesophilic bacterial counts among the samples were found to be above the Gulf Standard acceptable limit (4 log CFU/mL). In most samples, the total coliform counts exceeded the acceptable limit (2 log CFU/mL). In addition, the detection of fecal coliform in 20% and *E.coli* in 14.4% of the total samples also did not meet the acceptable limit of the Gulf Standard. The mean *Staphylococcus aureus* counts in chopped watermelon, followed by sugarcane juice and pineapple juice did not meet the acceptable limit (<1.3 log CFU/mL). *Salmonella* spp., was detected in two chopped watermelon and three pineapple juice samples, while it was not detected in the sugarcane juice samples (Table 3).

Table 3: Overall comparison of mean microbial load of sample juices with the Gulf Standard 2000 (n=90)

				Microbiological Criteria's limit (log CFU/mL)		
Variables	Chopped Watermelon	Pineapple	Sugarcane	Satisfactory	Borderline	Unsatisfactory
TAMBC	6.61	5.89	5.75	<4	4≤7	>7
TC	3.87	3.59	3.89	≤ 2		>2
FC	1.74	1.88	0.73	ND		Detected
E.coli	6	4	3	ND		Detected
Salmonella	2	3	ND	ND		Detected
SA	4.94	2.83	3.63	< 1.3	1.3 ≤ 4	>4
YMD	5.85	4.13	5.98	≤2	2- 3	>3

TAMBC: Total Aerobic Mesophilic Bacterial Count, TC: Total Coliform Count, FC: Fecal Coliform Count, SA: *Staphylococcus aureus* Count, YMD: Yeast and Mold Count

4.1.6 Isolation and characterization of *E. coli* and *Salmonella* from sample Juices and chopped watermelon

4.1.6.1 Morphological characteristics of bacterial pathogen isolates

The distinct colonies from selective and differential media were picked and characterized. Colonies with pink coloration on MacConkey Agar (Oxoid Ltd, Basingstoke, United Kingdom) and green metallic sheen on EMB (Microxpress®, Tulip Diagnostics(P)Ltd., Verna, Goa, India) agar were identified as *E.coli*, while colourless colonies with a black-centred on Salmonella-Shigella Agar (Microxpress®, Tulip Diagnostics (P)Ltd., Verna, Goa, India), were identified as S*almonella*. A Gram stain was confirmed for both isolates as a Gram-negative rods (Table 4).

Table 4: Morphological characteristics of isolated bacteria in selective and differential media

Sample types	Positive samples	Cell Shape	Colony colour	Arrangement	Gram stain	Bacterial Isolates
Chopped Watermelon	6	Rod	Pink-red, Green metallic shine	Single	-	*E. coli*
	2	Rod	Colourless with black centre	Single or in pairs	-	*Salmonella*
Pineapple	4	Rod	Pink-red, Green metallic shine	Single	-	*E. coli*
	3	Rod	Colourless with black centre	Single or in pairs	-	*Salmonella*
Sugarcane	3	Rod	Pink-red, Green metallic shine	Single	-	*E. coli*
						ND

ND: Not detected

4.1.6.2 Biochemical characteristics of bacterial pathogens

A combined biochemical identification of *E. coli* and *Salmonella* in sample juices and chopped watermelon is presented in Table 5. The citrate utilization test was negative for *E. coli* isolates, the medium remained green in color, while *Salmonella* was citrate positive and characterized by a change in the medium from green to blue. In Triple Sugar Iron Agar, the formation of gas, cracking of the medium, and gas bubbles in t medium, or forming a gap in the medium without H_2S production, is recorded as *E.coli* positive, whereas *Salmonella* produced hydrogen sulphide with the formation of a blackish color on the medium. *E. coli* showed the formation of a red ring in tryptophan broth after the addition of Kovac's reagent, while *Salmonella* didn't show a red ring color on the medium. Both isolates were negative for the urease test (Table 5).

Table 5: Biochemical characterization of bacterial isolates from samples (n=18)

Sample types	Isolates ID		Citrate		Urease		TSIA		Indole	
	E. coli	*Salmonella*	*E .coli*	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*	*E.coli*	*Salmonella*
Chopped watermelon	Mk9	Mk11	-	+	-	-	Y/Y, Gas	R/Y, H_2S	+	-
	pw17	Aw23	-	+	-	-	Y/Y, Gas	R/Y, H_2S	+	-
	pw19		-		-		Y/Y, Gas		+	
	Aw22		-		-		Y/Y, Gas		+	
	Aw26		-		-		Y/Y, Gas		+	
	Sgn27		-		-		Y/Y, Gas		+	
Pineapple	Bp2		-		-		Y/Y, Gas		+	
	Mp7	Mkp11	-	+	-	-	Y/Y, Gas	R/Y, H_2S	+	-
	Sp12	Pp19	-	+	-	-	Y/Y, Gas	R/Y, H_2S	+	-
	Ap23	Ap23	-	+	-	-	Y/Y, Gas	R/Y, H_2S	+	-
Sugarcane	Bsg4	ND	-				Y/Y, Gas		+	
	Ssg12		-				Y/Y, Gas		+	
	Asg24		-				Y/Y, Gas		+	

ND: Not detected

4.1.6.3 Detection of *Escherichia coli*

In this study, *Escherichia coli* was detected in 6(20%) samples of chopped watermelon, 4(13.3%) of pineapple juice, and 3(10%) of sugarcane juice (Fig 4.1). According to the Gulf Standard 2000 guideline, *E. coli* must not be detected, but 13 samples were unsatisfactory (Table 5).

4.1.6.4 Detection of *Salmonella*

In the present study, *Salmonella* was detected in 2(6.7%) samples of chopped watermelon and 3(10%) of pineapple juice samples, but it was not detected in sugarcane juice (Table 5). Gulf Standard recommended that *Salmonella* must be absent in 25 g of a sample. Therefore, all sugarcane juice samples fulfilled this limit, except two chopped watermelon and three pineapple samples (Fig 4.1).

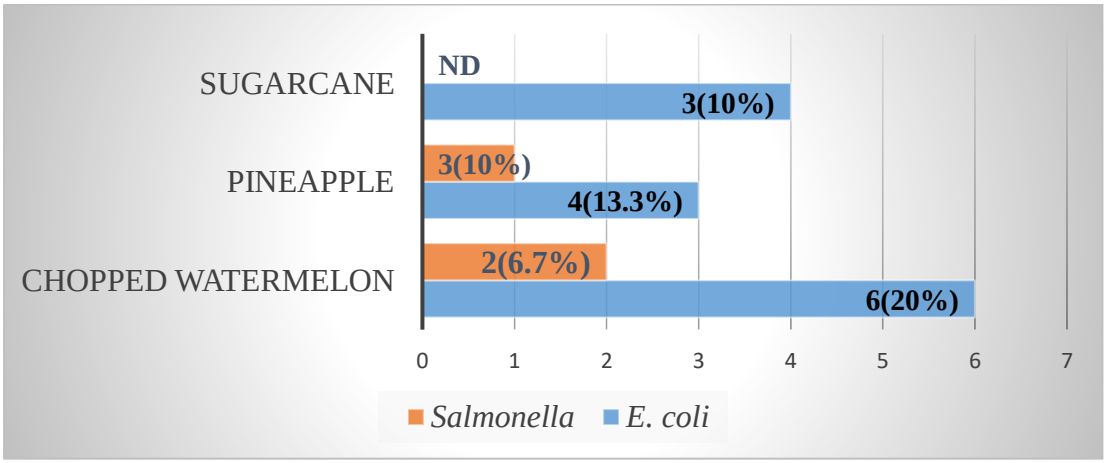


Figure 4.1: The number of bacterial isolates in sample juices (n=18)

4.1.7 Antimicrobial Susceptibility profile of bacterial isolates

4.1.7.1 Antimicrobial Susceptibility pattern of *Escherichia coli* isolates

In this study, all 13(100%) *E.coli* isolates were resistant to ampicillin, while 7(53.8%) were resistant and 6(46.2%) were intermediate resistance to amoxicillin. However, all isolates were susceptible to norfloxacin, and tetracycline. Among the 13 isolates, 1(7.7%) isolate was found resistant, 7(53.8) isolates were found intermediate susceptibility, and 5(38.5%) isolates were found susceptible to nalidixic acid. Generally, *E. coli* isolates were resistant to ampicillin, followed by amoxicillin, while all isolates were susceptible to norfloxacin and tetracycline (Table 6).

4.1.7.2 Antimicrobial Susceptibility profile of *Salmonella* isolates

In the present study, 4(80%) *Salmonella* spp., isolated from chopped watermelon and pineapple were resistant, while 1(20%) was susceptible to ampicillin. Three (60%) *Salmonella* isolates were resistant, 1(20%) intermediate resistant, and 1(20%) was susceptible to amoxicillin. All *Salmonella* isolates were susceptible to norfloxacin. Three (60%) of isolates were susceptible, and 2(40%) showed intermediate susceptibility to tetracycline (Table 6).

Table 6: Antimicrobial susceptibility patterns of bacterial isolates (n=18)

Isolates	Category (%)	AMP	A	T	NA	NOR
E. coli (n=13)	R	13(100%)	7(53.8%)	0(0.0%)	1(7.6%)	0(0.0%)
	I	0(0.0%)	6(46.2%)	0(0.0%)	7(53.8%)	0(0.0%)
	S	0(0.0%)	0(0.0%)	13(100%)	5(38.5%)	13(100%)
Salmonella (n=5)	R	4(80%)	3(60%)	0(0.0%)	NR	0(0.0%)
	I	0(0.0%)	1(20%)	2(40%)	NR	0(0.0%)
	S	1(20%)	1(20%)	3(60%)	NR	5(100%)

R: Resistant, I: Intermediate, S: Susceptible, AMP: Ampicillin, A: Amoxicillin, T: Tetracycline, NOR: Norfloxacin, NA: Nalidixic acid, NR: Not reported

Multiple antimicrobial resistance showed that 53.8% of *E. coli* isolates were resistant to two antibiotics, while 7.6% were resistant to three antibiotics (Table 7). The overall resistance rate was 31.1%.

Table 7: Multiple antimicrobial resistance patterns of bacterial isolates (n=18)

Resistance types	*E. coli* (n=13)	*Salmonella* (n=5)
Resistance to two antibiotics		
AMP, A	7(53.8%)	3(60%)
AMP, NA	2(15.4%)	
Resistance to three antibiotics		
AMP, A, T	1(7.6%)	0%
Resistance to four antibiotics	0%	0%
Resistance to five antibiotics	0%	0%

AMP = Ampicillin; A = Amoxicillin; T = Tetracycline; NA = Nalidixic acid

4.1.8 Determination of pH and Titratable Acidity

As shown in Fig 8, the present study measured the pH and TA of juice samples to examine their association with the microbial load. The mean pH values of chopped watermelon, pineapple, and sugarcane juices were 5.23±0.37, 3.83±0.22, and 4.79±0.24 respectively, while the corresponding TA values were 0.037%, 0.41%, and 0.26 %. A One-Way ANOVA reveals that there were a significant differences in pH and TA values among pineapple juices, sugarcane juices, and chopped watermelon ($p < 0.001$) (Table 8).

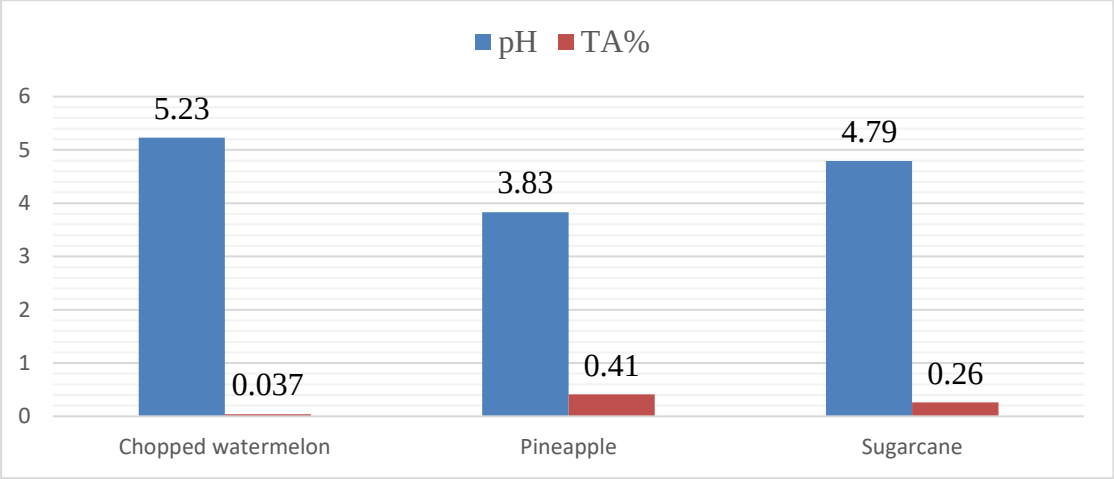


Figure 2.2: The mean of pH and Titratable acidity among chopped watermelon, pineapple, and sugarcane juices (n=90)

Table 8: The mean value of pH and Titratable acidity (n=90)

		N	Mean	Std. Deviation	Min	Max	P-value
pH	Chopped watermelon	30	5.23	.37	4.72	6.02	.000
	Pineapple	30	3.83	.22	3.48	4.26	
	Sugarcane	30	4.79	.24	4.27	5.25	
TA (%)	Chopped watermelon	30	.037	.02	.02	.10	.000
	Pineapple	30	.41	.07	.32	.51	
	Sugarcane	30	.26	.06	.11	.38	

In the present study, a Spearman correlation analysis showed that neither pH nor titratable acidity was significantly correlated with microbial load of chopped watermelon samples (p>0.05). In pineapple juice, pH showed a significant negative correlation with the TAMBC (r= - 0.397, p=0.031) and YMD (r = -0.51, p = 0.004), while TA showed a significant negative correlation with YMD (r = -0.584, p= 0.001). In sugarcane juice, pH was significant negative correlation with YMD (r = -0.473, p = 0.008), while TA showed no significant correlation with any microbial parameters. Generally, significant correlations were observed between pH or TA and yeast and mold count, specifically in pineapple and sugarcane juice (Table 9).

Table 9: Correlation analysis between pH and TA with microbial load (n=90)

Sample Types	Mean ±SD		TAMBC	TTC	FCC	SAC	YMD
Chopped watermelon	pH	5.23±0.37	r=0.325	r = 0.200	r=0.218	r =0.172	r = -0.252
			p=0.080	p = 0.289	p=0.246	p=0.363	p = 0.179
	TA (%)	0.037±0.02	r=0.198	r = 0.178	r = 0.145	r = 0.175	r = -0.282
			p=0.295	p=0.346	p=0.445	p=0.356	p = 0.131
Pineapple juice	pH	3.83±0.22	r= -0.394	r = 0.321	r=0.342	r= 0.209	r = -0.510
			p=0.031	p = 0.084	p=0.064	p = 0.267	p = 0.004
	TA (%)	0.41±0.07	r = -0.274	r = -0.043	r=0.250	r = 0.125	r = -0.584
			p=0.143	p=0.823	p=0.183	p= 0.511	p = 0.001
Sugarcane juice	pH	4.79±0.24	r = -0.124	r= - 0.302	r = 0.105	r = -0.199	r = -0.473
			p = 0.513	p=0.105	p=0.581	p = 0.291	p =0.008
	TA (%)	0.26±0.06	r = -0.062	r= -0.031	r = -0.074	r = 0.076	r = 0.122
			p = 0.746	p=0.869	p= 0.697	p = 0.690	p = 0.522

TAMBC= Total Aerobic Mesophilic Bacterial Count, TCC =Total Coliform Counts, FCC = Fecal Coliform Count, SAC= *Staphylococcus aureus* Count, YMD = Yeast and Mold Count, TA= titratable acidity

4.1.9 Socio-demographic characteristics and Handling practices

4.1.9.1 Sociodemographic characteristics of the respondents

The socio-demographic characteristics of the respondents are shown in Table 10. The majority of the juice vendors were female 23(76.6%), while 7(23.3%) were male. In terms of age distribution, 13 respondents (43.3%) were between 18 to 26 years, 9(30%) were between 27 and 35 years, and 8(26.7%) were older than 35 years. All respondents had some level of formal education; 4(13.3%) had completed primary education, 15(50%) had completed secondary or preparatory education, and 11(36.7%) held a diploma or higher education (Table 10).

Table 10: Sociodemographic characteristics of the respondents (n=30)

Variables	**Category**	**Frequency**	**Percentage (%)**
Sex	Male	7	23.3
	Female	23	76.6
Age	18-26	13	43.3
	27-35	9	30
	>35	8	26.7
Educational status	Illiterate	0	0
	Primary education	4	13.3
	Secondary and preparatory education	15	50
	Above Diploma	11	36.7

4.1.9.2 Sources of fruits, spoilage assessment and addition of additives

Majorly, fruits for juice and fresh-cut fruit preparation were bought from wholesaler 17(56.7%), and 13(43.3%) from local or open market retailers (Table 11). Around 27(90%) juice vendors performed physical spoilage assessment of fruit before the juices and sliced fruit were prepared. A 26(86.7%) juice vendors used additives such as lemon and sugar, mainly for pineapple juice and lemon only for sugarcane juice, and 4(13.3%) used commercial citric acid. All juice vendors use tap water for washing and preparation of fresh juices (Table 11).

Table 11: Preliminary spoilage assessment, source fruits, and water (n=30)

Variables	**Category**	**Frequency**	**Percentage**
Fruit source	Local market	13	43.3
	Wholesaler	17	56.7
	From own farm	0	0
Water source	Stream water	0	0
	Tap water	30	100
Spoilage pre-assessment	Yes No	27 3	90 10
Addition of additives	Sugar and lemon	26	86.7
	Citric acid	4	13.3

4.1.9.3 Hygienic practices

The findings showed that 22(73.3%) of locally prepared juice vendors had not taken hygiene training, while only 8(26.7%) had been trained. Regarding hand hygiene, 11(36.7%) of vendors reported washing their hands with water and soap before preparing juice and fresh-cut fruit, whereas 19(63.3%) washed only with water. None of the vendors reported using hand sanitizers. In addition, 9(30%) of juice workers used mobile phones while preparing juice, while 21(70%) did not. Isolation of ill vendors was not implemented in 23(76.7%) of juice houses, while 7(23.3%) were isolated. 23(76.7%) of vendors wore appropriate aprons and hair covers while preparing juice and fresh-cut watermelon (Table 12).

Table 12: Hygienic practices of the respondents (n=30)

Variables	**Category**	**Frequency**	**Percentage**
Hygiene training	Yes	8	26.7
	No	22	73.3
Protective clothing	Apron	3	10
	Hair cover	4	13.3
	Both	23	76.7
Handwashing practices	With soap and water	11	36.7
	Only water	19	63.3
	Only Sanitizer	0	0
Mobile phone use	Yes	9	30
	No	21	70
Isolation of ill vendor	Yes	7	23.3
	No	23	76.7

4.1.9.4 Juice preparation practices

In this study, the juice preparation practices among juice houses are presented in Table 14. Among 30 juice houses, 19(63.3%) of juice vendors were storing fruit on shelves and 11(36.6%) in a refrigerator. All juice makers always peeled fruit and prepared the juice in the backroom. In 21(70%) juice houses, juice makers sold the juice immediately, while 9(30%) stored it for an hour (Table 13).

Table 13: Juice preparation practices of the respondents (n=30)

Variables	Category	Frequency	Percentage
Preparation room	Backroom	30	100
	Home	0	0
	In front of customer	0	0
Peeling	Always	30	100
	Sometimes	0	0
	Never	0	0
Selling of juice	Immediate sale	21	70
	Stored	9	30
Storage place	Refrigerator	11	36.6
	Shelve	19	63.3

4.1.9.5 Cleaning practices

In the present study, the frequency of washing utensils after one use or in between was applied in 4(13.3%) juice houses, while 26(86.7%) were washed once a day. Twelve (40%) of juice houses used uncleaned juice machines, whereas 18(60%) used clean machines. In this study, 22(73.3%) of the juice houses put waste near them in an open basket, while 7(23.3%) near juice houses had closed baskets, and 1(3.3%) away from juice house with open basket (Table 14).

Table 14: Cleaning practices of the respondents (n=30)

Variables	Category	Frequency	Percentage (%)
Frequency of cleaning utensils (knife, and chopping board)	After once use	4	13.3
	At evening	26	86.7
	Rarely	0	0
Re-using of uncleaned juice machine	Yes	12	40
	No	18	60
Waste disposal place	Near juice house with closed basket	7	23.3
	Near juice house with open basket	22	73.3
	Away from juice house with closed basket	0	0
	Away from juice house with open basket	1	3.3

4.1.9.6 Observation analysis

In the present study, observation analysis reveals that 24(80%) of the respondents wore clean aprons and hair covers, while 6(20%) of juice makers did not. In all juice houses, watermelon fruit is directly sliced or chopped without washing the external fruit surface, this may be a cause for the entrance of microorganisms from the external part of the fruit to the edible or juice part. In 23(76.7%) juice houses, there were not observed clean cutting boards, knives, and juice processing surface, whereas 23.3% were visibly cleaned. A 60% of juice makers in this study handle cash without washing their hands, this might be a cause for cross-contamination. The majority of juice processing houses put their sliced and whole fruits outside or near roads and canals, where they are exposed to dust particles, flies, and extended temperature. The butchery and liquid waste released from different café and hotels have been seen in canals and found mainly in front of juice houses, where flies have been observed in 73.3% of juice houses (Table 15).

Table 15:On-site observation analysis of handling practices in juice houses (n=30)

Variables	**Category**	**Frequency**	**Percentage (%)**
Vendor wears clean protective clothes	Yes	24	80
	No	6	20
Juice processing surface, cutting boards, and knifes visibly clean	Yes	7	23.3
	No	23	76.7
Flies or pests present near juicing area	Yes	22	73.3
	No	8	26.7
Butchery waste and liquid wastes present near juice houses	Yes	21	70
	No	9	30
Vendor handles cash without washing hands	Yes	18	60
	No	12	40
Fruits stored properly	Yes	11	36.7
	No	19	63.3

4.2 Discussion

4.2.1 Bacterial load

As shown in Table 1, according to One-Way ANOVA analysis chopped watermelon had a higher bacterial load than pineapple and sugarcane juices. It confirmed significant differences in mean TAMBC among chopped watermelon, pineapple juice, and sugarcane juices ($p<0.001$). The mean TAMBC values were 6.61 log CFU/g for chopped watermelon, 5.89 log CFU/mL for pineapple juice, and 5.75 log CFU/mL for sugarcane juice (Table 1). This finding indicated that all three sample juices exceeded the acceptable limits of Gulf Standard 2000 (>4 log CFU/mL). Similarly, this finding was comparable to a study conducted in Adama City, Ethiopia, which found that bacterial contamination of sliced watermelon ranged from 6.41 to 6.43 log CFU/mL(Tamire and Chala, 2024). In contrast, the total aerobic mesophilic bacterial count in chopped watermelon (6.61 log CFU/mL) was higher than that reported for minimally processed watermelon (3.80-5.79 log CFU/mL) sold in Port Harcourt (Obinna-Echem and Koanyie, 2020), indicating higher bacterial contamination in sliced samples. It also exceeded the bacterial load reported for street- vended watermelon reported in Ghana (4.76 log CFU/mL) (Shitu *et al*., 2024). The higher bacterial load of chopped watermelon may be attributed to contaminated slicing boards, knives, exposure of sliced watermelon to air, flies, and dust released from roads and canals. Moreover, its contamination might be associated with higher water activity (aw) and moisture content of the fruit (Lima and Pinto, 2026).

The mean total aerobic mesophilic bacterial count of pineapple juice in the present study was 5.89 log CFU/mL, which lies on the borderline of Gulf Standard Ranges between 4 and 7 log CFU/mL. This finding was consistent with the bacterial mean count of 4.94 log CFU/mL reported in Tudun, Wada Region (Shitu *et al.*, 2024). Likewise, it was comparable with the mean aerobic mesophilic bacterial count found as 5.18 log CFU/mL(Cherinet, 2025), which indicated a moderate level of contamination compared to this study. Similarly, another study found that four out of six supermarket pineapple samples and three out of six wet market pineapple samples were above the maximum tolerance level of Gulf Standard (>10^7 CFU/mL) (Piano and Castillo-Israel, 2019). This is likely due to poor personal hygienic practices and contamination during processing time.

The mean total aerobic mesophilic bacterial count of sugarcane juice in this study is 5.75 log CFU/mL, which was close to the 5.59 and 5.64 log CFU/mL reported in Darse Selam, Tanzania (Issa-Zacharia and Rwabunywenge, 2024a), and it was also comparable with the 5.79 ± 0.36 log CFU/mL found in Morocco (Ed-Dra *et al*., 2025). In contrast, the present study is compared with 6.41 log CFU/mL reported in Dhaka University, Bangladesh (Khan *et al*., 2015). The elevated counts may be explained by prolonged exposure of fresh-cut sugarcane to ambient temperatures, contamination from flies and dust, poor water quality, and inadequate hygienic practices of vendors.

As shown in Table 2, the multiple linear regression analysis model explained 68.3% of the variation in total aerobic mesophilic bacterial load (R^2=0.683), with an adjusted value of R^2 = 0.638, indicating that 31.7% of the variation might be attributable to other external factors. Among other predictors, environmental sanitation and waste management practices of juice vendors had strong predictor association with TAMBC (B = -0.614, β = -0.418, p<0.001), followed by personal hygiene of respondents (B = -0.297, β = -0.205, p = 0.019), working materials or utensils sanitation (B = -0.270, β = -0.182, p = 0.032), and storage and processing safety (B = -0.285, β = -0.190, p = 0.010), all of which were significant negative predictors of bacterial load. The titratable acidity was significantly negatively associated with TAMBC (B = -0.976, p = 0.045), where a one-unit increase in titratable acidity corresponded to a 0.976 decrease in TAMBC. On the other hand, the Standardized Coefficient (β = -0.221) showed that TA was the primary significant predictor, while pH was not significantly associated with TAMBC (p>0.05). In contrast, water source, fruit source, sex, age, educational status, and pH of sample juices were not significant predictors of the microbiological quality of pineapple juices, sugarcane juices, and ready-to-eat chopped watermelon. This finding aligns with Mengistu *et al*., (2022), who reported that pH was not statistically associated with total viable bacterial count. Similarly, the present study is consistent with Cherinet, (2025), who found that higher titratable acidity in pineapple juice was associated with reduced bacterial counts.

4.2.2 Coliform Count

In the present study, the mean total coliform count of chopped watermelon is 3.87±2.81 log CFU/mL, which is consistent with a study conducted in the Accra Region of Ghana, where mean total coliform counts ranged from 4.20-4.49 log CFU/mL (Table 1). The study reported no significant difference among vendors ($p>0.05$), and recommended that contamination was more likely due to poor handling practices than fruit source differences (Olu-Taiwo *et al*., 2021). Similarly, a study in Nigeria found the mean coliform loads in watermelon ranged from 3.74 -5.83 log CFU/mL(Obinna-Echem and Koanyie, 2020), which showed a comparable level of contamination. In another case, the mean coliform counts of sugarcane juices reported in Saudi Arabia were 3.78±0.33 (Ed-Dra *et al.*, 2025), which is comparable to the present study with the total mean coliform counts of sugarcane juice (3.89 ± 1.85 log CFU/mL). This could be due to the faecal contamination of water used for washing and preparation of fruit juices.

In contrast, the study in Adama City found that the mean coliform counts of sliced watermelon were higher, ranging from 6.21- 6.43 log CFU/mL, and suggested that poor hygienic practices in the area (Tamire and Chala, 2024). Likewise, in Bangladesh, the mean coliform counts of sugarcane juice ranged from 4.60 -5.92 log CFU/mL (Akter *et al*., 2024). Whereas the other study reported the total coliform counts of pineapple juice in Bahir Dar ranged from <3 to > 1100 MPN/mL (Leul and Kibret, 2012). In contrast, a prevalence study in the Philippines found that three out of six samples from open markets and all supermarket samples exceeded the recommended coliform count level (≤ 2 log CFU/mL)(Piano and Castillo-Israel, 2019). Despite this study, the total coliforms were reported in 100% of all sugarcane juice samples with a count of >1600MPN/mL in 57.1% (Cristine *et al*., 2024).

The mean fecal coliform counts of sugarcane juices observed in this finding were lower than 1.67±0.34 log CFU/mL (Ed-Dra *et al.*, 2025)**.** Likewise, the other study in Tanzania found 1.79 and 2.10 log CFU/mL fecal coliform counts for unpasteurized raw and iced sugarcane juice, respectively (Issa-Zacharia and Rwabunywenge, 2024a). Conversely, thermo-tolerant coliforms were found in 100% of samples, with 33.3% of samples above the acceptable limit (Cristine *et al*., 2024), but in the present study, 5(16.7%) of samples were positive for faecal coliform, with 4(13.3%)of samples positive for *Escherichia coli*. The higher coliform contamination of chopped

watermelon and sample juice samples may be associated with faecal contamination of water and cross-contamination.

4.2.3 *Staphylococcus aureus* Count

A One-way ANOVA analysis showed no significant difference in mean *Staphylococcus aureus* count among the sample types (p=0.06). The mean *Staphylococcus aureus* count in chopped watermelon (4.94 log CFU/mL), sugarcane juice (3.63 log CFU/mL), and pineapple juice (2.83 log CFU/mL) samples exceeded the legal Gulf Standard limit (2 log CFU/mL). The higher contamination of chopped watermelon, sugarcane juice, and pineapple juice, may be due to the use of an unwashed chopping board and knives at the time of slicing watermelon and extracting the juice. Comparably, the mean *Staphylococcus aureus* counts for sliced watermelon (4.94 log CFU/g) in this study were consistent with 4.26-5.00 log CFU/mL, as reported in Nigeria (Obinna-Echem and Koanyie, 2020). However, the mean value of sugarcane juice (3.63 log CFU/mL) was higher compared to 2.81 log CFU/mL reported in Morocco (Ed-Dra *et al*., 2025). A study in Bahir Dar Town found a mean *Staphylococcus aureus* count (3.74 log CFU/mL) in pineapple juice that was higher than the value of this study (2.83 log CFU/mL)(Leul and Kibret, 2012). This may be because a higher concentration of titratable acidity in pineapple juice inhibits the growth of acid-intolerant microorganisms.

4.2.4 Yeast and Mold Counts

The mean counts of yeast and mold in three sample juices were beyond the recommended safety guideline (>3.0 log CFU/mL). A One-way ANOVA showed there were significant differences in yeast and mold between chopped watermelon, pineapple, and sugarcane juice ($p<0.001$). Likewise, the mean yeast and mold count in sugarcane juice (5.98 log CFU/mL) was consistent with a study found in Bangladesh (5.5-5.8 log CFU/mL), and Morocco (3.73 log CFU/mL) (Akter *et al.*, 2024; Ed-Dra *et al*., 2025). Nevertheless, this value was higher than a study reported in Nigeria (3.89-4.45 log CFU/g (Obinna-Echem and Koanyie, 2020). In contrast, the mean counts of yeast and mold in pineapple juice were shown to be lower than 3.69 log CFU/mL and 4.51 log CFU/mL, respectively (Cherinet, 2025). In addition, the yeast and mold in the watermelon juice sample were reported as 1.37 log CFU/mL (Buraimoh and Orieruo, 2025), which was contradicted by the present study, which found 5.85 log CFU/mL.

The higher yeast and mold counts in sugarcane juice may be attributed to concentrated sugars, which help to promote growth. Furthermore, it may be due to poor personal hygiene, unsanitary equipment and utensils, entry of fungus from the outer stalk of sugarcane, flies, and dust present in the environment.

4.2.5 Detection of *Escherichia coli*

In this study, *Escherichia coli* was detected in 6(20%) samples of chopped watermelon, 4(13.3%) of pineapple juice samples, and 3(10%) of sugarcane juices. According to the Gulf Standard 2000 guideline, *E. coli* must not be detected, but 13 positive samples in this study were considered unsatisfactory (Table 5). Similarly, *E. coli* counts in watermelon samples ranged from 5.10 – 5.84 log CFU/g (Obinna-Echem and Koanyie, 2020), and another study reported its presence in street-vended sliced watermelon (Tamire and Chala, 2024). Additionally, a study in Bahir Dar found that *Escherichia coli* was detected in two pineapple juice samples (Leul and Kibret, 2012), which was lower than this study. Likewise, Cristine *et al*., (2024) reported *E.coli* in 19.1% of sugarcane juice samples, which was close to the present study. Iced and unpasteurized sugarcane juice were positive for *Escherichia coli* with 2.10 and 1.79 log CFU/mL, respectively (Issa-Zacharia and Rwabunywenge, 2024a). In contrast, the present study is lower than the study reported in Morocco, where *Escherichia coli* was detected in 77% of sugarcane juice samples (Ed-Dra *et al*., 2025). Nevertheless, the study by Piano and Castillo-Israel (2019) found that all fresh-cut watermelon and pineapple samples were negative for *E. coli*. The detection of *E. coli* in chopped watermelon and sample juices is possibly caused by faecal contamination of water and inadequate waste management practices.

4.2.6 Detection of *Salmonella*

In this study, *Salmonella* was detected in 2(6.7%) samples of chopped watermelon, and 3(10%) of pineapple juice samples, but it was not detected in sugarcane juice. Gulf Standard recommended that *Salmonella* must be absent in 25 g of a sample. Therefore, all sugarcane juice samples fulfilled the standard, except 2 chopped watermelon and 3 pineapple samples (Fig 4.1). Likewise, *Salmonella* was detected in two pineapple samples (Leul and Kibret, 2012) and three watermelon samples from the open market, and one sample from supermarkets was positive for *Salmonella* (Piano and Castillo-Israel, 2019). A study in Port Harcourt reported that *Salmonella* was ranged from 4.26

-5.00 log CFU/mL(Obinna-Echem and Koanyie, 2020). Comparable to this study, *Salmonella* spp., was not detected in any of the 60 sugarcane juice samples (Issa-Zacharia and Rwabunywenge, 2024a), and a study in Morocco found that *Salmonella* was not detected in sugarcane samples (Ed-Dra *et al*., 2025). However, out of 21 sugarcane samples, one sample was positive for Salmonella ser. Typhimurium (Cristine *et al*., 2024). The detection of *Salmonella* in chopped watermelon and pineapple juice may be attributed to exposure of sliced watermelon to the open environment, unsanitary equipment, poor personal hygiene, and cross-contamination during handling or processing.

4.2.7 Antimicrobial Susceptibility profile of bacterial isolates

4.2.7.1 Antimicrobial Susceptibility pattern of *Escherichia coli i*solates

As shown in Table 6, 13(100%) *E. coli* isolates were resistant to ampicillin, while 7(53.8%) were resistant and 6(46.2%) were intermediately resistant to amoxicillin. However, all isolates were susceptible to norfloxacin and tetracycline. Among the 13 isolates, 1 (7.7%) was resistant, 7 (53.8%) were intermediately susceptible, and 5 (38.5%) were susceptible to nalidixic acid. This study is consistent with a study conducted in Mekelle, which reported that 70% of the isolated *E.coli* were observed to be resistant to ampicillin, while 100% and 60% of isolates were found to be susceptible to norfloxacin and tetracycline, respectively (Tadesse *et al*., 2018). Similarly, a study reported that *E.coli* isolates were found to be 0% resistant to norfloxacin. This agrees with the present study, where 100% of *E.coli* isolates were found susceptible to norfloxacin. In contrast, Zeynudin et al. (2025) reported that 40% of isolates were resistant to tetracycline, whereas 100% of *E. coli* isolates in this study were susceptible to tetracycline. The possible reason for the higher resistance of *E. coli* to ampicillin and amoxicillin may be attributed to previous antimicrobial exposure and the production of β- lactamase enzyme, which deactivates and hydrolyses the β- lactam ring. However, the specific resistance gene through molecular methods was not investigated in this study.

4.2.7.2 Antimicrobial Susceptibility profile of *Salmonella* isolates

In the present study, 4(80%) *Salmonella* spp., isolated from chopped watermelon and pineapple were resistant, while 1(20%) was susceptible to ampicillin. 3(60%) *Salmonella* isolates were resistant, 1(20%) intermediate resistance, and 1(20%) was

susceptible to amoxicillin. All *Salmonella* isolates were susceptible to norfloxacin. 3(60%) of isolates were susceptible and 2(40%) showed intermediate susceptibility to tetracycline (Table 6). In agreement with this study, a study conducted in Addis Ababa reported that all *Salmonella* isolates were resistant to ampicillin (Asmelash *et al*., 2025). Likewise, the study found that 53.8% of their *Salmonella* isolates were resistant to ampicillin (Zeynudin *et al*., 2025). Even though the study was phenotypic, the knowledge gap on antimicrobial usage and the production of β- lactamase may be possible reasons for ampicillin-and amoxicillin- resistant *Salmonella* found in this study.

4.2.8 Association of physicochemical characteristics of juices with microbial load

The current study determined the pH and titratable acidity of chopped watermelon, pineapple, and sugarcane juice to associate with the microbial load. As shown in Fig 8, the mean pH and TA values of chopped watermelon, pineapple, and sugarcane juice were measured as 5.23±0.37, 3.83±0.22, 4.79±0.24, and 0.037%, 0.41%, 0.26 %, respectively. A One-Way ANOVA analysis revealed that there were significant differences in pH and TA values among sample juices and chopped watermelon ($p<0.001$)(Table 8). The mean pH value of sugarcane juice in the present study is consistent with the pH of raw sugarcane juice found to have a mean of 4.9±0.3 (Issa-Zacharia and Rwabunywenge, 2024a). Similarly, other findings reported that the mean pH value of fresh-cut watermelon and pineapple ranged from 5.19-5.80 and 3.67-4.05, respectively (Ann and Israel, 2021). A correlation analysis showed that both pH and titratable acidity were not significantly correlated with the microbial load of chopped watermelon samples ($p>0.05$). This indicates that acidity may not be associated with the microbial load in this sample. In pineapple juice, pH showed a significant negative correlation with the TAMBC ($r= - 0.397$, $p=0.031$) and YMD ($r = -0.51$, $p = 0.004$), while TA showed a significant negative correlation with YMD ($r = -0.584$, $p= 0.001$). In sugarcane juice, pH was significant negative correlation with YMD ($r = -0.473$, $p = 0.008$), while TA showed no significant correlation with any microbial parameters (Table 9). This study revealed that acidity may associate to microbial load variation in pineapple and sugarcane juices, but other factors such as, hygienic practices, handling practices of fruit and storage conditions may have a determinant predictors for the microbial quality of freshly prepared juices. In contrast to the present study, a higher

pH value in sugarcane juice can support the growth of microorganisms (Issa-Zacharia and Rwabunywenge, 2024b).

4.2.9 Personal hygiene and handling practices of the respondents

4.2.9.1 Hygienic practices of vendors

The findings showed that 22(73.3%) of locally prepared juice vendors had not taken hygiene training, while 8(26.7%) had been trained (Table 12). This finding is supported by another study, which reported that the growth of microorganisms in fruit juice might be due to a lack of hygiene and safety training (Cherinet, 2025)**.** Regarding hand hygiene, 11(36.7%) of vendors reported washing their hands using water and soap before making juice, whereas19 (63.3%) washed only with water. However, not all vendors used sanitizer for their hands. A 9(30%) of juice vendors used mobile phones while preparing juice, but 21(70%) didn't use. Isolation of ill vendors was not implemented in 23(76.7%) juice houses, while 7(23.3%) were isolated. In 23(76.7%) juice houses, vendors wore appropriate aprons and hair covers while preparing juice and fresh-cut watermelon, which disagreed with a previous report where 87.50% of study participants did not wear hair covers during juice preparation (Tilahun *et al*., 2025). In all juice houses, watermelon fruit is directly sliced or chopped without washing the external fruit surface, this may be a cause for the entrance of microorganisms from the external part of the fruit to the edible or juice part. In contrast, this finding is contradicted by the Codex Good Hygiene Principles (Alimentarius, 2026).

4.2.9.2 Fruit sources and processing practices

Majorly, fruits for juice and fresh-cut fruit preparation were bought from wholesalers (17(56.7%), and 13(43.3%) from local or open market retailers (Table 12). This study is opposed with other previous study findings, where the primary sources of fruits were from producers (68.75%) and 31.25% of juice makers bought from open market retailers (Tilahun *et al*., 2025). Around 27(90%) juice vendors performed physical spoilage assessment of fruit before the juice is prepared. In 26(86.7%) juice houses, juice vendors used additives such as lemon and sugar, mainly for pineapple juice and lemon only for sugarcane juice, and 4(13.3%) used commercial citric acid. All juice vendors use tap water for washing and preparation of fresh juices and chopped watermelon. Likewise, the study found that 93.75% of the juice houses primarily used

tap water for making juice (Tilahun *et al*., 2025). Among 30 juice houses, 19(63.3%) of juice makers were storing fruit on shelves and 11(36.6%) in a refrigerator (Table 14). This is consistent with a study reported by Mengistu *et al.*, (2022), where 15(57.2%) of juice houses were storing fruit on the shelves, but 11 (42.8%) used a refrigerator to keep the prepared juice from spoilage and contamination. All juice makers always peeled fruit and prepared the juice in the backroom. In 21(70%) of juice makers, the juice was sold immediately, while 9(30%) stored it for an hour.

4.2.9.3 Equipment handling practices

In the present study, the frequency of washing utensils (knife and cutting board) after one use or in between was poor in 4(13.3%) juice houses, while 26(86.7%) of juice makers washed once a day after they finished their daily activities. Similarly, all fruits were cut and blended using similar utensils without cleaning frequently or in between preparation time (Batool *et al*., 2013). Re-use of uncleaned juice machine in 12(40%) of juice vendors, while 18(60%) did not use unwashed machines. In 23(76.7%) juice houses, cutting boards, knives, and juice-processing surfaces were cleaned, whereas 7(23.3%) were visibly cleaned (Table 15). Similarly, a study found that cutting boards, juice machines, and knives were not frequently cleaned before juice and fresh-cut fruit preparation (Leul and Kibret, 2012). In this study, 22(73.3%) of the juice houses had put waste near them in an open basket, while 7(23.3%) had waste near the juice house in a closed basket, and 1(3.3%) away from the juice house in an open basket. In the present study, the observed handling of utensils was non- compliant with the Codex Hygiene Principles, such as cleaning of equipment, and adequate handling practices during processing (Alimentarius, 2026). These may cause cross-contamination and contribute to bacterial contamination of freshly prepared juices.

4.2.9.4 Environmental sanitation and waste management practices

In the current study, the majority of juice processing houses put their sliced and whole fruits outside or near roads and canals, where they are exposed to dust particles, flies, and extended temperatures. The butchery and liquid waste released from different cafés and hotels have been seen in canals, found mainly in front of juice houses, where flies have been observed in 73.3% of juice houses (Table 15). In line with this, a study reported that fruits stored outside are exposed to temperature abuse and dusts (Cherinet, 2025).

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study found that most of the chopped watermelon, followed by pineapple, and sugarcane juice samples were contaminated by microorganisms, which were above the Gulf Standard guideline and may cause health risks to consumers. Higher bacterial contamination was observed in chopped watermelon, while significant differences among the chopped watermelon, sugarcane, and pineapple juices were observed for total aerobic mesophilic count, and yeast and mold count; however, the *Staphylococcus aureus* and coliform counts did not show statistically significant differences. *E. coli* was detected in thirteen samples, while *Salmonella* was detected in five chopped watermelon and pineapple samples. Almost all isolates are phenotypically resistant to ampicillin and amoxicillin, while all isolates are susceptible to norfloxacin and tetracycline. Significant differences were observed in pH and titratable acidity of the sample juices and chopped watermelon, with lower pH and higher titratable acidity in pineapple juice. However, acidity alone may not reduce contamination when the handling and hygienic practices are insufficient. Generally, the major finding of the present study indicates low hygiene practices, exposure of fruits to the open environment (flies and dust), poor waste management, and cross-contamination among fruit juices and chopped watermelon due to repeatedly using unwashed chopping boards and knives as the primary associated factors for the microbial contamination of the chopped watermelon, pineapple, and sugarcane juices. Therefore, improving food safety practices and providing food safety training to juice vendors helps to control microbial hazards and the increase of antimicrobial-resistant bacteria in food.

5.2 Recommendations

Based on the main findings of the current study, the following recommendations are given:

- Creating awareness and food safety training programs should be provided for juice vendors to help them to follow good hygienic practices, such as, utilizing clean water for washing utensils and juice preparation, proper sanitation practices, and preventing cross-contamination while using a cutting board, knife, and juice grinding machine.
- Regular surveillance of the microbiological safety of fresh-cut fruits and freshly prepared juice should be applied by concerned governmental, and non-governmental organizations, along with improving liquid and butchery waste management.
- Advisable antimicrobial stewardship programs should be implemented to minimize misuse and overuse of antibiotics in humans.
- Develop innovative preservation methods and biodegradable edible coating for chopped watermelon.
- Finally, further studies should conduct seasonal investigations and source-tracking molecular studies that help to identify the major route of contamination and resistant genes in isolated bacteria.

6. REFERENCE

Akter, N., Murtaja, K., Linkon, R., Akter, M., and Hossain, A. (2024). Assessment of Physico-Chemical and Microbial Quality of Sugarcane Juice Sold by Street Vendors in Three Regions of Bangladesh. 263–276. https://doi.org/10.4236/fns.2024.154017

Al-farsi, M., Ullah, S., and Al-omairi, M. (2024). The Quality Characteristics of Different Fruit Juice Brands Consumed in Oman. Journal of Food Science and Nutrition, https://doi.org/10.24966/FSN-1076/100201

Alam, A., Shaheen, S., Ashfaq, M., Ali, M., Watto, J. I., Anjum, M. A., Khan, F., Maqsood, S., and Sajjad, M. (2019). Microbial examination of mould and yeast in fruit juices. Pakistan Journal of Agricultural Sciences, 56(3), 715–721. https://doi.org/10.21162/PAKJAS/19.6157

Alegre, I. (2024). Microbiological quality and safety of non-treated fresh and squeezed juices from supermarkets in Lleida , Spain. 4716–4722. https://doi.org/10.1111/ijfs.17198

Alimentarius, C. (2026). Codex Alimentarius: General Standard for fruit juices and nectars cxs 247-2005.

Amare, A., Worku, T., Ashagirie, B., Adugna, M., Getaneh, A., and Dagnew, M. (2019). Bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods and hygienic practice of vendors in Gondar town, Northwest Ethiopia: A cross sectional study. BMC Microbiology, 19(1), 1–9. https://doi.org/10.1186/s12866-019-1509-4

Ann, K., and Israel, C. (2021). Physico-chemical quality and microbial safety evaluation of ready-to-eat fresh-cut watermelon and pineapple sold in Imus, Cavite, Philippines. 3(January). https://doi.org/10.26656/fr.2017.3(6).139

Areum, A. J., Soyul, H., Lee, D. K. S., Jo, S., and Song, H. (2021). Evaluation of microbiological quality and safety of fresh-cut fruit products at retail levels in Korea. Food Science and Biotechnology, 30(10), 1393–1401. https://doi.org/10.1007/s10068-021-00974-0

Asghar, U., Nadeem, M., Nelofer, R., Mazhar, S., Syed, Q., and Irfan, M. (2018). Microbiological Assessment of Fresh Juices Vended in Different Areas of Lahore City.Electronic Journal of Biology, 14(4),106-110.

Bacteriological Analytical Manual Chapter 4 : Enumeration of Escherichia coli and the Coliform Bacteria October 2020 Edition. (2020). October.

Balali, G. I., Yar, D. D., Afua Dela, V. G., and Adjei-Kusi, P. (2020). Microbial Contamination, an Increasing Threat to the Consumption of Fresh Fruits and Vegetables in Today's World. International Journal of Microbiology, 2020. https://doi.org/10.1155/2020/3029295

Batool, S. A., Tahir, S. S., Rauf, N., and Kalsoom, R. (2013). Microbiological analysis of pasteurized and fresh fruit juice sold in Rawalpindi of Pakistan. Bangladish Journal of Scientific and Industrial research 48(3), 185–192. https://doi.org/10.3329/bjsir.v48i3.17329

Beckett, E. L., Fayet-Moore, F., Cassettari, T., Starck, C., Wright, J., and Blumfield, M. (2025). Health effects of drinking 100% juice: an umbrella review of systematic reviews with meta-analyses. Nutrition Reviews, 83(2), e722–e735. https://doi.org/10.1093/nutrit/nuae036

Berihu, T., Gebremariam, G., Weldu, Y., Kahsay, A., and Asmelash, T. (2024). Prevalence , antimicrobial susceptibility test and associated factors of Salmonella and Shigella in ready - to - eat fruit juices and salads in Mekelle , northern Ethiopia. BMC Infectious Diseases, 1–9. https://doi.org/10.1186/s12879-024-09066-w

Bikila, W., and Kadire, A. (2019). Assessment of Bacterial Load of Some Fresh and Packed Fruit Juices in. Journal of Nutrition and Food Sciences, 9(759), 1–7. https://doi.org/10.24105/2155-9600.1000759

Buraimoh, O., and Orieruo, A. (2025). Microbiological and Quality Assessment of Ready-to-serve Beverage Produced from Blends of Yoghurt and Watermelon Juice. October. https://doi.org/10.9734/ejnfs/2025/v17i101874

Casolari, A. (2016). Microbial Spoilage of Canned Fruit Juices. March. https://doi.org/10.1007/978-94-009-1113-0

Center for Disease Control and Prevention (CDC). (2022). *Salmonella* and food: what you need to know. Food Safety, 7–9. https://www.cdc.gov/foodsafety/communication/salmonella-food.html=CDCestimates that *Salmonella* causes,if it's not cooked thoroughly.

Cherinet, M. T. (2025). Physico-Chemical and Microbiological Safety of Fruit Juices Served

in Bahir Dar City, Northwest Ethiopia. September 2018.

Çiftçi, M., and Öncül, N. (2021). Turkish Journal of Agriculture - Food Science and Technology The Detection and Enumeration of Coliform Bacteria in Some Ready-to-Eat Unpackaged Food in Fethiye Region # Fethiye Bölgesinde Açıkta Satılan Tüketime Hazır Bazı Gı dalarda Koliform Bakteri Sayısının Belirlenmesi. 9, 2552–2559.

Cristine, A., Messias, C., Vinicius, E., and Paixão, R. (2024). original article microbiological quality of sugarcane juice sold in goiânia , goiás , and risks to. 53(4), 277–287. https://doi.org/10.5216/rpt.v53i4.80867

Clinical and Laboratory Standard Institue(CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 36th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2026

Demisie, K. N., and Melese, D. M. (2024). Assessment of bacterial and parasitic contamination of fruits gathered from specific local markets in Addis Ababa, Ethiopia. Frontiers in Sustainable Food Systems, 8. https://doi.org/10.3389/fsufs.2024.1402898

Dietetici, P., and Spa, A. (1989). MICROBIAL SPOILAGE OF CANNED. 65–75.

Dippong, T., Pop, F., Vo, Z., and Mihali, C. (2025). Quality of fruit juices in terms of antioxidant properties , evaluation of stability during storage. August, 1–17. https://doi.org/10.3389/frfst.2025.1656271

Ed-Dra, A., Alhudhaibi, A. M., Abdallah, E. M., and Nalbone, L. (2025). Quality and safety of fresh sugarcane juice sold by street vendors: a growing public health concern. Frontiers in Sustainable Food Systems, 9. https://doi.org/10.3389/fsufs.2025.1628211

Elamparithi, P., Hemapriya, A., Hemasruthi, G., Pooja, G., Priyadharshini, V., Sundarasan, K., Sakthi Pooja, S., Poovizhi, V., Prabhavadhani, K., and Ilakkiya, M. (2024). Studies on the Microbial Spoilage in Fruit Juice and Enhancing the Shelf Life using Bio-preservatives. International Journal of Current Microbiology and Applied Sciences, 13(10), 22–29. https://doi.org/10.20546/ijcmas.2024.1310.004

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G., and Lee, L. H. (2015). Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science, 8(3), 284–293. https://doi.org/10.1080/21553769.2015.1051243

Evrendilek, G. A., and Özkan, B. H. (2024). Pulsed electric field processing of fruit juices with inactivation of enzymes with new inactivation kinetic model and determination of changes in quality parameters. Innovative Food Sci. Emerg. Technol. 94, 103678. doi:10.1016/j.ifset.2024.103678

Fan, H., Han, X., Shang, X., Zhu, Z., He, M., Xu, G. Chen, Z., and Deng, R. (2023). Fruit and vegetable intake and the risk of cataract: insights from the UK Biobank study. Eye (Basingstoke), 37(15), 3234–3242. https://doi.org/10.1038/s41433-023-02498-9

Fusaro, C., Miranda-Madera, V., Serrano-Silva, N., Bernal, J. E., Ríos-Montes, K., González-Jiménez, F. E., Ojeda-Juárez, D., and Sarria-Guzmán, Y. (2024). Antibiotic-Resistant Bacteria Isolated from Street Foods: A Systematic Review. Antibiotics, 13(6), 1–20. https://doi.org/10.3390/antibiotics13060481

Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market Interpretation of test results generated by UKHSA Food Water and Environmental Microbiology Services Laboratories. (2024).

Heaton, C. J., Gerbig, G. R., Sensius, L. D., Patel, V., and Smith, T. C. (2020). Staphylococcus aureus epidemiology in wildlife: A systematic review. Antibiotics, 9(2). https://doi.org/10.3390/antibiotics9020089

International Organization for Standardization. (2020). Internaciona Standar Horizontal method for the detection , enumeration and serotyping of iteh standard preview amendment 1 : broader range of iteh standard preview. (The International Organization for Standardization), 2020, 9. https://cdn.standards.iteh.ai/samples/76671/O-6579-1-2017-Amd-1-2020.pdf

ISO-4832. (2006). International standard microbiology of food and animal feeding enumeration of coliforms colony-count iteh standard preview iteh standard preview. 2006.

ISO-6888-3:2017, I. (2023). resolution oiv-oeno 662-2023 horizontal method for the enumeration of coagulase- horizontal method for the enumeration of coagulase-Foreword. June, 1–7

Issa-Zacharia, A., and Rwabunywenge, S. P. (2024a). Evaluation of bacteriological quality and safety of sugarcane juice locally processed and vended in Dar es Salaam City, Tanzania. Journal of Food Safety and Hygiene, March.

https://doi.org/10.18502/jfsh.v9i4.15003

Issa-Zacharia, A., and Rwabunywenge, S. P. (2024b). Evaluation of bacteriological quality and safety of sugarcane juice locally processed and vended in Dar es Salaam City, Tanzania. Journal of Food Safety and Hygiene, February. https://doi.org/10.18502/jfsh.v9i4.15003

Kalu, C., and Uchenna, J. (2022). Physicochemical and Sensory Characteristics of Mixed Fruit Juices Prepared from Blend of Pineapple , Pawpaw and Watermelon Fruits Juices. 21(12), 28–35. https://doi.org/10.9734/AFSJ/2022/v21i12603

Kechero, F. K., Baye, K., Tefera, A. T., and Tessema, T. S. (2019). Bacteriological quality of commonly consumed fruit juices and vegetable salads sold in some fruit juice houses in Addis Ababa, Ethiopia. Journal of Food Safety, 39(1), 1–9. https://doi.org/10.1111/jfs.12563

Khan, M. M., Islam, T., Mehadi, M., Chowdhury, H., and Alim, S. R. (2015). Assessment of microbiological quality of some drinks sold in the streets of Dhaka University Campus in Bangladesh. 0–4. https://doi.org/10.1186/s40550-015-0010-6

Kibret, M., Siraj, W., and Mohammed, A. (2025). Microbiological quality of some selected fresh fruit juices served in Dessie town ' s charming cafes and restaurants , nestled in the heart of north - eastern Ethiopia. Discover Food. https://doi.org/10.1007/s44187-025-00479-3

Kipuru, M. M., Prof, A., Kibanja, G. M., and Matagi, L. (2024). East African Journal of Education Studies The Path to Persistence : How Conscientiousness and Social Integration Shape Academic Resilience in Higher Education. 8(1), 85–109. https://doi.org/10.37284/eajes.8.1.2557

Kumari, S., Aditya, P. K., and Mandal, R. K. (2023). Microbial Analysis of Fresh Fruit Juice Available in the Market. Environment and Ecology, 41(4), 2342–2347. https://doi.org/10.60151/envec/tcci5654

Leul, A., and Kibret, M. (n.d.). Bacteriological Safety of Freshly Squeezed Mango and Pineapple Juices Served in Juice Houses of Bahir Dar Town , Northwest Ethiopia. 24–35.

Lu, X., Sun, D., Wu, Q., Liu, S., and Sun, G. (2014). Physico-Chemical Properties, Antioxidant Activity and Mineral Contents of Pineapple Genotypes Grown in China.

8518–8532. https://doi.org/10.3390/molecules19068518

Lum, T., Connolly, M., Marx, A., Beidler, J., Hooshmand, S., and Kern, M. (2019). Acute Satiety Response and Cardiometabolic Risk Factors in Overweight and Obese Adults. https://doi.org/10.3390/nu11030595

Marie-michel, C. A., Koffi, N., Valery, P., Christine, E. A., Gnakri, K., and Salomée, A. (2020). Physicochemical , Microbiological and Sensory Characterization of Juice Produced from Watermelon (Citrullus lanatus) Consumed in Côte d ' Ivoire. 3214, 335–342. https://doi.org/10.36348/sjbr.2020.v05i11.009

Matters, W. T. (2025). Food Safety: Status of Foodborne Illness in the U.S. 1–23. https://www.gao.gov/products/gao-25-107606

Mehlhorn, H. (2016). Food-Borne Disease Burden Epidemiology Reference Group. Encyclopedia of Parasitology, 1068–1069. https://doi.org/10.1007/978-3-662-43978-4_3884

Mengistu, D. A., Baraki, N., and Gobena Tesema, T. (2021). Pathogenic Bacterial Species in Locally Prepared Fresh Fruit Juices Sold in Juice Houses of Eastern Ethiopia. Microbiology Insights, 14. https://doi.org/10.1177/11786361211060736

Mengistu, D. A., Mulugeta, Y., Mekbib, D., Baraki, N., and Gobena, T. (2022). Bacteriological Quality of Locally Prepared Fresh Fruit Juice Sold in Juice Houses of Eastern Ethiopia. Environmental Health Insights, 16. https://doi.org/10.1177/11786302211072949

Mihiretie, H., and Desta, K. (2015). Microbiological Criteria and Quality of Fruits and Fruit Juices in Ethiopia and International Experience. Journal of Medical Microbiology & Diagnosis, 04(04), 2–6. https://doi.org/10.4172/2161-0703.1000207

Moges, M., Rodland, E. K., Legesse, T., and Argaw, A. (2024). Antibiotic resistance patterns of Staphylococcus aureus and Enterobacteriaceae isolated from street foods in selected towns of Ethiopia. BMC Infectious Diseases, 24(1), 1–9. https://doi.org/10.1186/s12879-024-09266-4

National agency for food and drug administration and control (nafdac) fruit juice and nectar regulations 2019. (2019).

Neggazi, I., Colás-Medà, P., Viñas, I., and Alegre, I. (2024). Microbiological quality and safety of non-treated fresh and squeezed juices from supermarkets in Lleida, Spain.

International Journal of Food Science and Technology, 59(7), 4716–4722. https://doi.org/10.1111/ijfs.17198

Njoagwuani, E. I., Onyeaka, H., Mazi, I. M., Akegbe, H., Oladunjoye, I. O., Ochulor, C. E., Omotosho, A. D., Odeyemi, O. A., Nwaiwu, O., and Tamasiga, P. (2023). Food safety in vulnerable populations: A perspective on the challenges and solutions. FASEB Journal, 37(5), 1–8. https://doi.org/10.1096/fj.202201713R

Obinna-Echem, P. C., and Koanyie, L. (2020). Quality and Safety Characteristics of Minimally Processed Watermelon (Citrullus lanatus) Sold in Selected Locations in Port Harcourt. 2(3), 1–7.

Olu-taiwo, M., De-graft, B. M., and Forson, A. O. (2021). Microbial Quality of Sliced Pawpaw (Carica papaya) and Watermelon (Citrullus lanatus) Sold on Some Streets of Accra Metropolis , Ghana. 2021. https://doi.org/10.1155/2021/6695957

Pal, M. (2022). Staphylococcus Aureus: A Major Pathogen of Food Poisoning. Nutrition and Food Processing, 5(1), 01–03. https://doi.org/10.31579/2637-8914/074

Paul, V., Singh, A., and Pandey, R. (2010). Determination of Titrable Acidity (Ta). Research Gate, March, 23–25. https://doi.org/10.13140/RG.2.2.13335.50088

Phan, A., Mijar, S., Harvey, C., and Biswas, D. (2025). Staphylococcus aureus in Foodborne Diseases and Alternative Intervention Strategies to Overcome Antibiotic Resistance by Using Natural Antimicrobials. Microorganisms, 13(8), 1732. https://doi.org/10.3390/microorganisms13081732

Piano, A. M. P., & Castillo-Israel, K. A. T. (2019). Physico-chemical quality and microbial safety evaluation of ready-to-eat fresh-cut watermelon and pineapple sold in Imus, Cavite, Philippines. Food Research, 3(6), 684–692. https://doi.org/10.26656/fr.2017.3(6).139

Pius, T. . D. M. . O. E. I. . I. C. . O. U. B. . & O. A. S. (2023). A Systematic Review and Meta-analysis of the Microbiological Quality of Fruit Juices across Africa. *International Journal of Microbiology and Infectious Research*, 1(1). July. https://scholar.google.fr/scholar A+Systematic+Review+and+Meta-analysis of the Microbiological Quality of Fruit Juices

Pius, T., Mbina, S. A., and Tamale, A. (2021). Microbial Assessment and Sources for Contamination of Unpackaged Fruit Juice Served in Restaurants of Bushenyi-Ishaka

Municipality Western Uganda. OALib, 08(11), 1–13. https://doi.org/10.4236/oalib.1108115

Polytechnic, K., State, O., Sciences, H., and State, L. (2024). Qualitative Microbiological Assessment of Ready-to-Eat Fruits Sold in Selected Areas of Kaduna Metropolis. 9(3), 329–335.

Quality, B., Ababa, A., and Teklebirhan, G. (2025). college of health sciences.

Quandoh, E., and Albornoz, K. (2025). Fresh-cut watermelon : postharvest physiology , technology , and opportunities for quality improvement. February, 1–19. https://doi.org/10.3389/fgene.2025.1523240

R.B, A., T.A, I., and O.T, R. (2020). Physico-Chemical Properties and Sensory Qualities of Juices Extracted From Five Selected Fruits and Their Peels. International Journal of Engineering Applied Sciences and Technology, 04(11), 239–244. https://doi.org/10.33564/ijeast.2020.v04i11.041

Rahaman, M. M., Hossain, R., Herrera-Bravo, J., Islam, M. T., Atolani, O., Adeyemi, O. S., Owolodun, O. A., Kambizi, L., Daştan, S. D., Calina, D., & Sharifi-Rad, J. (2023). Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. Food Science and Nutrition, 11(4), 1657–1670. https://doi.org/10.1002/fsn3.3217

Romero-Calle, D. X., de Santana, V. P., Benevides, R. G., Aliaga, M. T. A., Billington, C., and Góes-Neto, A. (2023). Systematic review and meta-analysis: the efficiency of bacteriophages previously patented against pathogenic bacteria on food. Systematic Reviews, 12(1), 1–13. https://doi.org/10.1186/s13643-023-02352-9

Science, C., Science, C., and Canada, H. (2017). Bacteriological Assessment of Avocado Juice Vended in Cafeteria And Restaurants in Adigrat Town , Northern Ethiopia. 09(04), 593–596.

Shah, Y. A., Afzaal, M., Ahmad, M., and Mustafa, J. (2020). acta scientific microbiology (issn: 2581-3226) Microbiological Quality and Safety Assessment of Sugar Cane Juice and Ice Sold by Vendors in Faisalabad City, Pakistan. Acta Scientific Microbiology (Issn:, 3(7), 60–64.

Shiferaw, M., and Kibret, M. (2018). Microbial Quality of Avocado and Guava Fruits used for Preparation of Freshly Squeezed Juices from Juice Houses of Bahir Dar Town,

Northwest Ethiopia. International Journal of Scientific and Research Publications (IJSRP), 8(9). https://doi.org/10.29322/ijsrp.8.9.2018.p8115

Standard, G., Standardization, F. O. R., and Emirates, U. A. (2002). NO : 1016 / 2000 NO : 1016 / 2002 microbilogical criteria for food stuffs - PART 1. 1016.

Standard, I. (2001). international standard colony-count preview technique at 44 ° c using iteh standard preview. 2001.

Standard, T., Stan, C., S., Standard, G., Juices, F., Standards, I, Stan, C. Nectars, M. F. (2005). CODEX STAN 247 Page 1 of 19. 1–19.

Tadesse, H. A., Gidey, N. B., Workelule, K., Hailu, H., Gidey, S., Bsrat, A., and Taddele, H. (2018). Antimicrobial Resistance Profile of E. coli Isolated from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia. Veterinary Medicine International, 2018. https://doi.org/10.1155/2018/8903142

Tahsin, F., and Sciences, N. (2025). Public Health Risks of Fresh Fruit Juices : Bacterial Isolation , Fecal Contamination Assessment , and Antibiotic Resistance Patterns.

Tamire, S., and Chala, B. (2024). Bacteriological analysis of street-vended sliced watermelon (Citrullus lanatus) from selected sites in Adama City ,. 4(2), 444–452.

Testing, S. (2026). CLSI M100 ™ Performance Standards for Antimicrobial Susceptibility Testing 2026.

Tilahun, T., Kumera, B., and Kibret, M. (2025). Microbial Quality and Hygienic Safety of Avocado Fruit Juice Sold in Merawi Town, Amhara, Ethiopia. Journal of Food Quality, 2025(1). https://doi.org/10.1155/jfq/8892198

Vi, I., Neggazi, I., Alegre, I., Bel, M., and Col, P. (2025). International Journal of Food Microbiology Impact of intrinsic factors and storage temperature on Escherichia coli O157 : H7 , Salmonella enterica subsp . enterica and Listeria monocytogenes survival infruit juices. 432(December 2024). https://doi.org/10.1016/j.ijfoodmicro.2025.111109

Wang, J., Wang, H., Lan, T., Liu, R., Gao, T., Yang, W., Zhou, Y., Ge, Q., Fang, Y., & Sun, X. (n.d.). Is overnight fresh juice drinkable? The shelf life prediction of non-industrial fresh watermelon juice based on the nutritional quality, microbial safety quality, and sensory quality. 1, 1–15.

World Health Organization (2026). WHO estimates of the global burden of foodborne

diseases 2000–2021: 2026 edition. https://www.who.int/teams/nutrition-and-food-safety/monitoring-nutritional-status-and-food-safety-and-events/foodborne-disease-estimates

Yoo, B. K., Liu, Y., Juneja, V., Huang, L., and Hwang, C. A. (2015). Growth characteristics of Shiga toxin-producing Escherichia coli (STEC) stressed by chlorine, sodium chloride, acid, and starvation on lettuce and cantaloupe. Food Control, 55, 97–102. https://doi.org/10.1016/j.foodcont.2015.02.040

Zahoor, S., Iqbal, S., Hashmi, I., Akbar, A., Ullah, H., Panezai, H., Khan, A., and Majid, S. (2021). Chemical and Biological Food Safety Threats Associated with Fresh Juices Consumed in Capital Territory Islamabad, Pakistan. Progress in Nutrition, 23(4). https://doi.org/10.23751/pn.v23i4.9012

Zeynudin, A., Degefa, T., Belay, T., Mumicha, J. B., Husen, A., Yasin, J., Abamecha, A., Wieser, A., and Abayneh, M. (2025). Detections of antimicrobial resistance phenotypes and extended-spectrum beta-lactamase (ESBL)- producing Salmonella spps and Escherichia coli O157:H7 in raw vegetables and fruits from open markets in Jimma town, Ethiopia and evaluation of hygiene and hand. One Health Outlook, 7(1). https://doi.org/10.1186/s42522-024-00125-5

7. APPENDIX

Date: ----------

❖ **Appendix 1:-** Structured questionnaire and observation checklist for microbiological and physicochemical quality of locally prepared unpasteurized fruit juice and ready-to-eat watermelon in Dessie town.

1. Socio-demographic variables

1.1Sex:-

1.2Age:-

1.3 Education status: ☐ illiterate ☐ Elementary ☐ High school ☐ Above Diploma

2. Source of ingredients and additives

2.1 Source of fruits: ☐ Local market, ☐Wholesaler ☐ From own farm ☐ others

2.2 Do you check whether the fruits are spoiled or not before use? ☐Yes ☐ No.

2.3 Where do you use the water source for washing and juice preparation?

☐ Stream water ☐tap water ☐ other:

2.4 Do you add ice to juice? ☐Yes ☐No

2.5 Do you use additives? ☐ Sugar ☐ Lemon ☐ Citric acid ☐other

3. Juice preparation

3.1 where is the juice prepared? ☐ Backroom ☐ At home and transport to the sale house ☐ In front of the customer

3.2 Fruit washing: ☐Always ☐ Sometimes ☐ Never

3.3 peeling fruit: ☐Always ☐ Sometimes ☐Never

3.4 Juice sold immediately or stored ☐ Immediately ☐ Stored

3.5 Storing condition of juice before sale ☐ in a shelf ☐ Refrigerator

4. Hygiene practices

4.1 have you taken food hygiene training? ☐Yes ☐ No

4.2 Do you have protective cloths? ☐No ☐yes. If yes, ☐Apron ☐Gloves ☐Hair cover

4.3 Do you use handwashing facilities?

☐ No ☐ Yes. If yes, which one? ☐ Soap and water ☐Sanitizer only ☐ water only

4.4 Do you use mobile phone during juice preparation? ☐ Yes ☐ No

4.5 Isolation of the vendor when he or she becomes ill and shows symptoms such as vomiting and diarrhoea. ☐Yes ☐ No

5. Cleaning and Sanitation of Utensils

5.1 Frequency of cleaning equipment: ☐ After use ☐ Daily ☐ Rarely

5.2 Re-usage of cleaned cup ☐ Yes ☐ No

5.3 Re-use of the uncleaned juice machine ☐ Yes ☐ No

5.4 Waste disposal: ☐ Near juice house with closed basket ☐ near juice house with open basket ☐ Away from juice house with closed basket ☐ Away from juice house with open basket

On-site Observation Checklist

No.	Observation Item	Yes☐	No ☐
1	Vendor wears clean clothes or apron		
2	Vendor wears gloves during juice preparation		
3	Handwashing material with soap or water		
4	Vendor washes hands before handling juice		
5	Cutting board and knife visibly clean		
6	Juice contact surfaces or working environment are clean		
7	Separate utensils for fruit and non-fruit items		
8	Clean water available for juice preparation		
9	Proper waste disposal observed		
10	Flies or pests present near juice area		
11	Raw animal products present near juice area		
12	Vendor handles cash without washing hands		
13	Juice containers covered during storage		
14	Fruits washed before juice preparation		
15	Fruits stored properly		
16	Equipment (blender or juicer) visibly clean		
17	Ice or water added to juice from clean source		
18	Serving cups or utensils clean and hygienic		
19	Juice preparation area free from garbage and dirt		
20	Vendor avoids touching face or hair while preparing juice		

Appendix 2: Gulf Standard 1016/2000 microbiological criteria for fruit juices

Microorganism	Satisfactory	Borderline	Unsatisfactory
Salmonella spp	Absent in 25 g or ml	---	Detected in 25 g
E.coli	Absent in 25 g or ml	$20 \leq 10^2$ CFU/ml	$>10^2$ /ml
S. aureus	< 20 CFU/g	$20 \leq 10^4$CFU/g	$>10^4$ CFU/g
TAMB	< 10000 CFU/g	$10^4 \leq 10^7$ CFU/g	$>10^7$ CFU/g
Coliforms	$\leq$100 CFU/ml	----	-----
Fecal coliform	Zero CFU/ml	------	------
Yeast and mold count	<100 CFU/ml	10^2- 10^3 CFU/ml	$>10^3$ CFU/ml

Appendix 3: CLSI 2026 Kirby-disk diffusion method Break point for antimicrobial susceptibility testing of *Escherichia coli* and *Salmonella* spp.

Antibiotics	Disk concentration	Pathogens	S	I	R
Ampicillin	10 µg	*E. coli*	≥17	14–16	≤13
		Salmonella spp.	≥17	14–16	≤13
Amoxicillin	10 µg	*E. coli*	≥17	14–16	≤13
		Salmonella spp.	≥17	14–16	≤13
Tetracycline	30 µg	*E. coli*	≥15	12–14	≤11
		Salmonella spp.	≥15	12–14	≤11
Norfloxacin	30	*E. coli*	≥17	13–16	≤12
		Salmonella spp.	≥17	13–16	≤12
Nalidixic acid	30 µg	*E. coli*	≥19	14–18	≤13
		Salmonella spp.	-	-	-
Cefradine	30 µg	*E. coli*	-	-	-
		Salmonella spp.	-	-	-

Appendix 4:- Physicochemical and Microbial result data recording table for freshly prepared juices and ready-to-eat chopped watermelon.

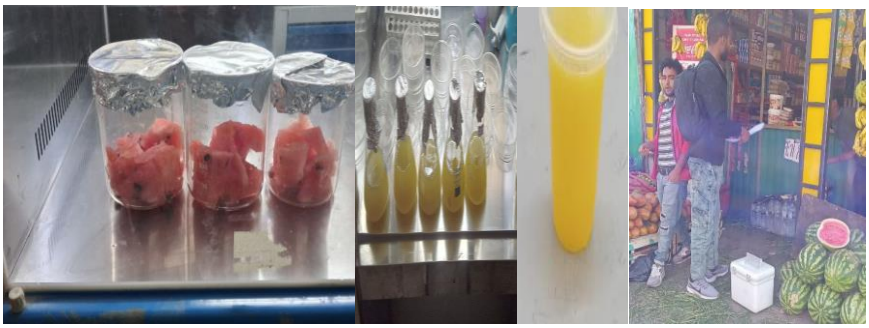
Number	Sample ID	pH	TA (%)	Mean of TAMB (CFU/mL	Mean of TC (CFU/mL	Mean of FC (CFU/mL	Mean of *S. aureus* (CFU/mL	*Salmonella*(CFU/mL	*E.coli* (CFU/mL	Mean of Yeast and Mold (CFU/mL	Remark
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											

26											
27											
28											
29											
30											

Appendix 5:- Sterilized equipment and culture media at 121˚C for 15 minutes.



Appendix 6: Samples collected from juice houses.





Appendix 7:- Serial dilution and inoculation inside biosafety cabinet.

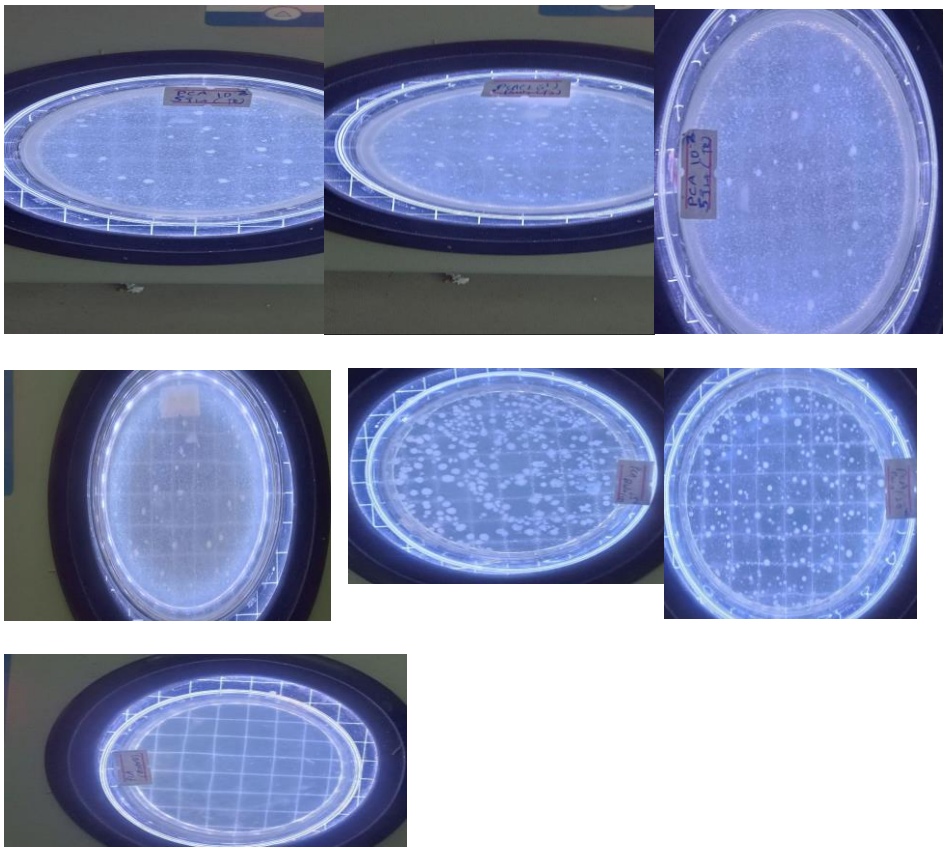


Appendix 8:- Measured value of pH and TA of samples

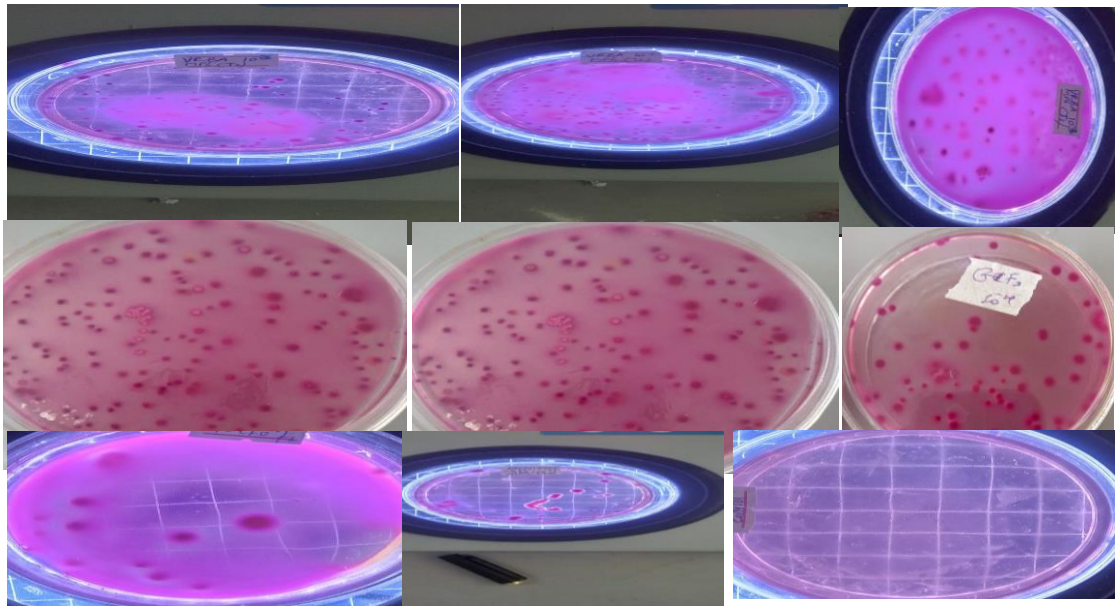




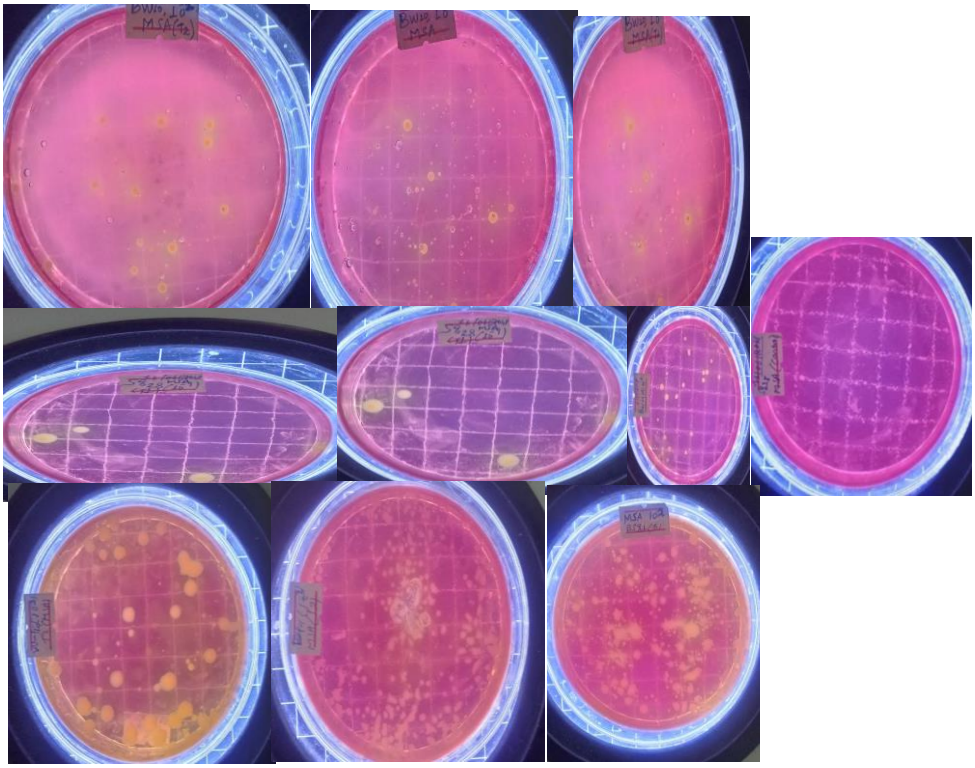
Appendix 9:- Total aerobic mesophilic bacterial count



Appendix 10:- Total and fecal coliforms



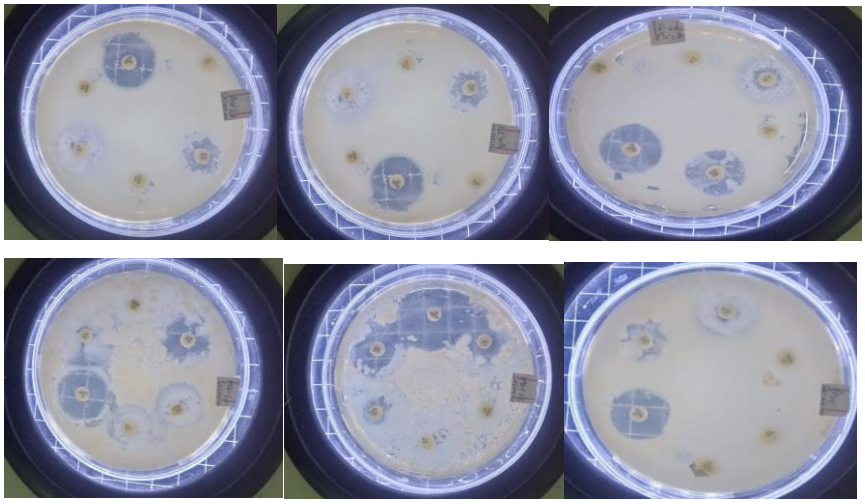
Appendix 11:- *Staphylococcus aureus* count



Appendix 12:- Biochemical identification of *E.coli* and *Salmonella* isolates.



Appendix 13: Antimicrobial susceptibility test through Kirby-Bauer Disk Diffusion Method.



Appendix 14:- Yeast and mold count

