MICROBIOLOGICAL PROFILE OF LOCALLY PREPARED FRESH FRUIT JUICES IN ADAMA TOWN, EAST SHOWA, ETHIOPIA

MSc THESIS

WUBIT FEKADU

MAY 2017
HARAMAYA UNIVERSITY, HARAMAYA

Microbiological Profile of Locally Prepared Fresh Fruit Juices in Adama Town, East Showa, Ethiopia

A Thesis Submitted to the Postgraduate Program Directorate
College of Natural and Computational Sciences, Department of Biology

HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN BIOLOGY

Wubit Fekadu

May 2017
HARAMAYA UNIVERSITY, HARAMAYA

APPROVAL SHEET
HARAMAYA UNIVERSITY
Postgraduate Program Directorate

We here by certify that we have read and evaluated this thesis titled, ***'Microbiological Profile of Locally Prepared Fresh Fruit Juices in Adama Town, East Showa, Ethiopia'*** prepared under our guidance by Wubit Fekadu. We recommend that it be submitted as fulfilling all the thesis requirements.

Ameha Kebede (PhD)	__________________	__________________
Name of Major Advisor	Signature	Date
Misrak Kebede (PhD)	__________________	__________________
Name of Co. Advisor	Signature	Date

As member of the board of the examiners of the MSc Thesis open defenses of the examination, we certify that we have read and evaluated the thesis properly by **Wubit Fekadu.** We recommend that the thesis can be accepted as fulfilling the Thesis requirement for the degree of *Master of Science in Biology*.

__________________	__________________	__________________
Name of Chair person	Signature	Date
__________________	__________________	__________________
Name of Internal Examiner	Signature	Date
__________________	__________________	__________________
Name of External Examiner	Signature	Date

DEDICATION

I dedicate this thesis manuscript to my parents for their love, affection, unrestricted encouragement during this research work and every success in my life. It is also dedicated to my daughter yididiya samuel and all the researchers who have shown their ingenuity to change our world.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my original work I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

This thesis has submitted in partial fulfillment of the requirements for the degree of Master of Science in biology at the Haramaya University. The thesis is deposited in Haramaya University Library and made available for borrower under the rule of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Brief quotations from this thesis may be made without special permission provided that accurate and complete acknowledgment of the source is made. Request for permission for extended quotation from or reproduction of this thesis in a whole or in part may be granted by the Head the school or Department when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Wubit Fekadu

Place: Haramaya University, Haramaya

Date of Submission: June, 2017

Signature**: __________________**

BIOGRAPHICAL SKETCH

The author was born in September 1968 E.C in Nekemete Town, Oromia Regional State. She attended her elementary education at Harar Senga Ber Primary School in Harar town, her secondary school education at Wolaita Sodo Compressive School in Wolaita Sodo town. In 1985, she joined Adama TTI and graduated with certificate in the same year. After graduation the author was employed by Oromia Regional State Education Bureau and has been working as a teacher at Boset Secondary School in Boset *Woreda*. In 1992, she joined the Diploma program in Adama Teachers College and graduated with diploma in Biology in 1994. In 1996 she joined Mekele University and graduated with BEd degree in Biology in 2000. After graduation, she has been working as a Biology teacher at Goro Preparatory School in Adama town until she joined the post-graduate program directorate of Haramaya University in 2004 to pursue M.Sc. study in Biology at the Department of Biology, College of Natural and Computational Sciences.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Ameha Kebede (PhD) and Misrak Kebede (PhD) for their valuable help starting from topic selection, proposal development, and the actual project work and final write up of the final thesis.

My great gratitude also goes to Miss Asnakech Getahun who helped me in coordinating, organizing, and providing valuable information and materials throughout the research period.

I am also grateful to Mr Ebisa Firdisa for being with me during the laboratory work and providing me with the most basic laboratory equipment and basic culture media required. My thanks also go to Mr. Adinew Zewdu for his help during data entry and analysis.I would like to express my heartfelt thanks and deep appreciation to Mr. Wake Abebe for his all support during laboratory work.

I would like to thank Oromia Public Health Research Laboratory for providing with full bench setup required for performing my laboratory tests.

I am thankful to Haramaya University Post graduate Program Directorate for the financial support that enabled me conducts this project. I also extend my gratitude and respect to all staff of the Department of Biology for providing me with the basic theoretical background which had led me to develop my future career.

My heart-felt appreciation goes as well to my mother Desta Tolsa for her sacrifice in providing continuous encouraegement and special treatment morally and psychologically.

I also express my deepest gratitude to all my sisters Welela Fekadu, Enanu Fekadu and Kasech Fekadu who helped me throughout my research work.

My heartfelt gratitude also gose to Solomon Gezajegn and Asnake for their amazing cooperation and sacrifice of their precious time during data analysis

LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Description
ANVOA	Analysis of Variance
CHF	Congestive Heart Failure
DHSS	Department of Health and Social Security
FAD	Food and Drug Administration
FCC	Fecal Coliform Count
MPN	Most Probable Number
PDA	Potato Dextrose Agar
SFBC	Spore Forming Bacterial Count
TAMBC	Total Aerobic Mesophilic Bacterial Count
TCC	Total Coliform Count
TSC	Total Staphylococcal Count
VRBA	Violet Red Bile Agar
X LD	Xylose Lysine Deoxycholate Agar
YMC	Yeast and Mold Count
OPHRCBQAL	Oromia Public Health Research Capacity Building Quality Assurance Laboratory

TABLE OF CONTENTS

LIST OF TABLES

Table **page**

LIST OF FIGURES

LIST OF APPENDIX TABLE

Appendix **page**

Microbiological Profile of Locally Prepared Fresh Fruit Juices in Adama Town, East Showa, Ethiopia

ABSTRACT

Fresh fruits are essential components of the human diet and there is a considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits. However, during processing, contamination from raw materials, equipment or food handlers could be easily transferred to the final product of fruit juices resulting in food-borne illnesses. Thus, this study was conducted with the aim of determining the microbiological quality of fresh fruit juices prepared and marketed in Adama town. Questionnaire, observation and laboratory based cross-sectional survey study was employed to assess the source of fruits, the hygienic conditions of fruit juice processing and the personnel involved in the production of fresh juices as well as the bacteriological quality of fresh papaya, mango and avocado juices. Accordingly a total of 15 respondents and 81 fruit juice samples were used for the questionnaire survey and microbial an alysis in the laboratory, respectively. The results indicated that 25.9% of the yeast and mold count were marginally accepted and 69.1% of total viable bacteria, 88.9% of the total coliforms, 81.5% of fecal coliforms and 45.7% of staphylococci were found in the fresh juices with greater than the permitted maximum microbial load. The mean counts of yeast and mold, viable bacteria, coliforms, fecal coliforms, and staphylococci were $9.1x10^{2}$*cfu/ml,* $9.1x10^{4}$*cfu/ml,* $2.4x10^{4}$*cfu/ml,* $6.9x10^{3}$*cfu/ml, and* $8.6x10^{3}$*cfu/ml, respectively. The results also showed that the mean counts of yeast and mold, viable bacterial, total coliforms, total fecal coliform and total staphylococcus counts showed statistically significant difference among avocado, mango and papaya juice(*$P<0.05$*) samples except total spore forming bacterial counts. In this study pathogenic bacteria were detected from avocado, mango and papaya juice samples. Generally E. coli, Salmonella* spp. *and S. aureus had an overall prevalence of 67.9%, 14.8%, and 61.7%, respectively. Out of the* 13.6% *Cryptococcus species detected from all fruit juice samples*, 12.3% *were identified from avocado alone. Most venders obtained fruit from the open market and all juice makers lacked special training in food hygiene and safety. Thus, on the basis of the above findings, regular monitoring of the quality of fruit juices and health education on food hygiene and safety is recommended to be introduced in the town to avoid any occurrence of bacterial disease outbreak in the future.*

KeyWords*: Avocado, Food-borne pathogens, Fresh juice, Mango, Papaya, Risk factors.*

1. INTRODUCTION

Fruit juices are popular drinks as they contain antioxidants, vitamins, and minerals that are essential for human beings and play important roles in the prevention of heart diseases, cancer, and diabetes. They contain essential nutrients which support the growth of acid tolerant bacteria, yeasts, and moulds (Kamal *et al.*,, 2014).

Juices are fat-free, rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health. For example, orange juice is rich in vitamin C, an excellent source of bio-available antioxidant photochemical (Franke *et al.*, 2005). It also significantly improves blood lipid profile in people affected by hypercholesterolemia (Kamal *et al.*,, 2014).

Fruit juices are available in essentially the same form almost anywhere in the world. From polar regions to the tropics and from the largest developed countries to the smallest developing countries, fruit juices are available in bottles, cans, laminated paper packs, pouches, cups and almost every other form of known packaging. In recent years, these juices have been included significantly in the diet of most people irrespective of age groups as they are known to promote detoxification in the body of humans (Franke *et al.*, 2005).

Unpasteurized juices are preferred by consumers because of the fresh flavor and the absence of preservatives. Often consumers have preference for fresher fruit juices over the bottled juices as they are considered to be more nutritious foods that also happen to meet the needs of busier lifestyles. Fresh fruit juices have no artificial color and sweetness, and that is why these are preferred over the bottled ones (Addo *et al.*,, 2008; Melbourne, 2005). Additionally, fresh fruit juices processed under hygienic conditions could play important roles in enhancing consumers' health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Ketema *et al.*, 2008). Freshly squeezed juices are simply prepared by extracting the liquid and pulp of mature fruit usually by mechanical means or blenders. Thus the final product is an unfermented, unclarified, untreated juice, ready for consumption (Melbourne, 2005)

However, in the absence of good fruit preparation and juice making practice, the nutritional richness of freshly squeezed fruit juices makes the product good medium for bacterial growth, vehicle of food-borne pathogens and associated complications (Al-Jedah, 2002). Source of fruits, quick methods of cleaning fruits and utensils, handling practices of fruits, mechanical methods of squeezing juices, and unhygienic conditions of juice houses also contribute a lot to bacterial contaminations of juices (Al-Jedah *et al.*,, 2002). For example, the outside surface of fruits may not be washed properly before it is placed into a juice machine for extracting the juice. **(**Mahale, 2008).

Fruit juices contain a micro flora which is normally present on the surface of fruits during harvest and postharvest processing which include transport, storage, and processing (*V.* Tourna***s*** *et.al.*200).

Many microorganisms such as acid tolerant bacteria and fungi (moulds, yeasts) use them as a substrate for their growth. Yeasts form the main flora of fruits before processing because of acidic pH. The major genera include *Candida*, ,*Dekkera, Hanseniaspora*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces .Penicillium*, *Byssochlamys,* ,*Aspergillus*, *Paecilomyces*, *Mucor*,*Cladosporium*, *Fusarium*, *Botrytis*, *Talaromyces*,and *Neosartorya* are filamentous fungi most frequently isolated from fresh fruits and juices. Among bacteria, lactic acid bacteria and acetic acid bacteria have been isolated from fruit juices (ICMSF, 2005).

Furthermore, high pH and storage temperature of fruits and juices may also favor growth of pathogenic bacteria (Tambekar, 2009) like *Salmonella, Staphylococcus aureus* and *Escherichia coli* (Doyle, 2001). As a result, contaminated juices are unacceptable for human consumption and create significant health problems for young children, the elderly and people with weakened immune system (Ahmed, 2009).

The quality of fruit juices is strictly maintained in developed countries under several laws and regulations. For instance, the U.S. Food and Drug Administration introduced regulations in response to several outbreaks of illnesses in the 1990s associated with raw juices processed at commercial facilities. Accordingly, it decided to introduce a regulation where by 100% of fruit/vegetable juices sold at wholesale must be produced under a Hazard Analysis and Critical Control Point plan (FDA, 2001).

Here the regulation applies to the entire domestic as well as 100% of imported juice products and as a result it had implications on juice producers in countries that export juice to the United States. Thus, currently any plan for the extraction of fruit juices must be supported by good manufacturing practices and sanitary standard operating procedures. In addition, the regulation requires juice processors to apply a treatment resulting in at least a 5-log reduction of the "pertinent microorganism" which is considered as the most resistant microorganism of public health significance that is likely to occur in the juice (FDA, 2001).

It is known that in Ethiopia, particularly in large urban areas, fruit juices are available in supermarkets in canned or bottled forms as well as in fresh fruit juice vending houses, which are produced from different types of fruits and being served in fresh forms. Nowadays, the rapid increasing number of such juice venders has extended the consumption of juice products across the country specifically in Adama town (Abadias *et al.*,, 2008).

Generally, food-borne or waterborne microbial pathogens are leading causes of illnesses in developing countries, killing an estimated 1.9 million people annually at the global level. Even in developed countries, an estimated one-third of the population is affected by microbiological food borne diseases each year (Andargie *et al.*, 2008). There are also reports of food-borne illnesses associated with the consumption of fruit juices in several places of India and elsewhere in the world (Sandeep *et al.*,, 2001). In the same way most of the fruit juices being served in Jimma had high microbial loads; and as a consequence, these products were found to be the cause of health problems and potential vehicle of food borne outbreaks (Ketema *et al.*, 2008).

Fruit juices have their pH values in the acidic range (<4.5) which serve as important barrier for microbial growth. However, food-borne pathogens such as *E. coli* and *Salmonella* spp. survive in the acidic environment of fruit juices due to acid stress response is through immediate pumping of hydrogen ions using ATP. Moreover, it has been reported that contamination of fruit juices sold in restaurants, cafes and even road side stalls could be sometimes unacceptable for human consumption and could create significant health problems (Lewis *et al.*,, 2006). This is what motivated the researcher to conduct the study in order to evaluate the microbiological quality of some selected fruit juices being served in Adama town, Eastern Ethiopia.

General objective

The major objective of this study was to assess the microbiological quality of locally prepared fresh fruit Juices (mango, avocado and papaya) marketed in Adama town.

Specific objectives

- To determine the bacteriological quality of locally prepared fresh fruit juices in terms Of the loads of indicator microbial groups.
- To evaluate the hygienic conditions of facilities used during processing and the personnel involved in the production of fresh juices through observation and questionnaire administration.
- To identify bacterial pathogens from the fresh fruit (avocado papaya and mango) juices of Adama town.

2. LITERATURE REVIEW

2.1. Fruit juice

Juices are the aqueous liquids expressed or otherwise extracted usually from one or more fruits or vegetables, purees of the edible portion of one or more fruits or vegetables, or any concentrates of such liquids or purees (Fraternale, 2011). Generally, fruit juices avilaable in the market are divided into unpasteurized juice and pasteurized juice.
Unpasteurized juice/cider does not undergo treatment. Often it can be purchased as freshly pressed from local orchards, roadside stands, farmers' markets, country fairs and juice bars. Unpasteurized juice/cider may also be found on ice or in refrigerated display cases and in produce sections at grocery stores (Health Canada, 2006).

Juice/cider that is pasteurized has been treated to kill harmful bacteria and to extend shelf-life (Health Canada, 2006). Not only the locally prepared fruit juices but also juices imported are another important problem in resulting food borne illness. A study conducted in Kumasi, Ghana, on microbiological analysis shows that some imported fruit juices indicate significant increase bacterial load in the apple and mango fruit juices as they stayed for long period in shelves (Abadias *et al.*,, 2008)

2.2. Factors that Affect the Safety of Fruit Juices

Food safety is defined as the assurance that the food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. On the other hand quality is defined by the International Organization for Standardization (ISO) as "the totality of features and characteristics of a product that bear on its ability to satisfy stated or implied needs." In other words, good quality exists when the product complies with the requirements specified by the client (van Reeuwijk, 1998). This means quality is a term defined by the consumer, buyer, grader, or any other client based on a number of subjective and objective measurements of the food product. These may include measures of purity, flavor, color, maturity, safety, wholesomeness, nutrition, or any other attribute or characteristic of the product (Jaeger *et al.*,, 2009).

2.2.1. Fruit Storage Practices

Ideally, fruit should be pressed as soon as possible after picking to avoid increases of pH that would favor growth of pathogens during storage. The lower the pH, the worse the conditions will be for the growth and survival of pathogens. However, if fruit needs to be stored, rapid cooling to as close to 0°C as possible (0 to 4°C) and achievement of adequate storage conditions will maintain fruit condition. Storage facilities must be clean, secure from rodents and insects and suitable for storing food (Canada food agency, 2001). A research conducted in Ethiopia stated that Papaya and avocado juices had initial pH values of >5.7 and allowed all test strains to reach numbers >10^7cfu/ml at ambient temperature. At refrigeration temperatures, at least no elimination was observed. In pineapple juice (pH 3.8), the *E. coli* test strains were eliminated at both holding temperatures within 16 h whereas slight increase in counts of *Salmonella* test strains was observed at ambient temperature holding (Yigeremu *et al.*,, 2001).

2.2.2. Handling and processing

Poor handling and processing of fresh fruit juices are some of the main cause of food associated illness to the community who live in developing countries. In most case a number of pathogenic organisms are isolated and identified from locally prepared fruit juices. According to study conducted in Dhaka, Bangladesh, the total viable count of samples ranged from 3.00×10^2 to 9.60×10^8. Out of 114 freshly prepared fruit juices samples collected, 113 samples (99%) showed the presence of coliform and *E. coli*. The other bacteria like *B. cereus, Staphylococcus aureus, Salmonella*, *Streptococcus* were found in 64.91%, 6.14%, 7.89% and 5.26% of the tested samples, respectively.

The number and type of microorganisms recovered from the freshly squeezed fruit made them unsafe for drinking. It was concluded that due to unhygienic fruit handling in the un sanitary environmental conditions under which the vendors operate the juices become contaminated with harmful bacteria. The consumption of unhygienic quality of popular types of market vended freshly squeezed fruit juices may cause risk to the consumers (Shakir *et al.*,, 2009).

Since Ethiopia is among the developing counties, food-borne illness in the country is common. For this health problem, poor handling and processing of locally prepared juices take their part. According to the study conducted in Jimma, in Ethiopia, most of the fruit juices being served in many areas had higher microbial loads than the specification set for fruit juices standards. As these products could be the cause of health problems and potential vehicle of food borne outbreaks, high level of workers hygiene should be enforced and the use of disinfectant better practiced to improve the microbial quality, safety, and shelf-life of the final product (Ketema *et al.* 2008).

2.2.3. Contamination

Several factors can act as source of contamination such as use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust. Such juices have been shown to harbor bacterial pathogens notably *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* (Buchmann *et al.*,, 1999; Sandeep *et al.*,, 2004; Barro *et al.*,, 2006).

The most likely cause of the contamination is fruit coming in contact with animal feces, or water, workers, containers or processing equipment contaminated with animal faces. Cattle, deer and sheep, are the most common reservoirs for the pathogen, but usually do not show symptoms themselves. Birds, rodents, insects and poor hygiene may also contribute to the contamination. One contaminated piece of fruit could affect an entire batch of juice or cider (FDA, 1999; Canada food agency, 2001). A pathogen that has become internalized within a fruit or vegetable must be able to survive in the product until it reaches the consumer in order to become a public health hazard.

Most fruit juice is sufficiently acidic to inhibit the growth of pathogenic organisms. Studies conducted on the survival or growths of microorganisms in juices have showed a number of pathogenic Organisms can be present and survive in a wide range of fruit and vegetables (FDA, 2008).

The study conducted in Nigeria on food safety and hygienic practices of street food vendors stated that are several health hazards associated with them. The study found that women made up 66.67% of the vendors while males made up 33.33%. 42.86% did not use aprons; 47.62% handled food with bare hands and 52.38% wore no hair covering while 61.90% handled money while serving food. 19.05% wore jewelry while serving food and 28.57% blew air into polythene bag before use. 9.52% of the vendors stored food for serving openly in the stalls while 23.81% stored them in the wheelbarrows. 42.86% had leftovers for serving the next day with poor storage facilities. 47.62% of the vendors washed their utensils with dirty water which is recycled and used severally in 28.57% despite the fact that only 9.52% of them complained of water shortages. The study recommended that there is need for health education of these vendors in order to ensure food safety for the consumers (Chukuezi *et al.* 2010).

2.2.4. Water Supply

Water used for juice preparation can be a major source of microbial contaminants including coliforms, faecal coliforms, faecal streptococci, etc (Tasnim *et al.*, 2010). Problem associated with food borne illness is unhygienic water supply that may be used for dilution of fruit juices. According to research conducted in Visakhapatnam City, India, overall the results of the study indicate that all street vended fresh fruit juices in many parts of the city showed contamination with *faecal coliforms* and *faecal streptococci.* It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils and maintenance of the premises (Lewis *et al.,*, 2006).

The location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and overcrowding seem to add to the contamination. Such locations should be avoided for establishing a street vender juice shop. Lack of sanitary conditions in street vended juice shops and the occurrence of pathogenic *E. coli*

O157:H7, *Shigella* and *S. typhimurium* is alarming enough for an immediate action by the suitable agency. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks (Lewis *et al.*,, 2006).

A survey on the bacteriological quality of both drinking water and flavored drinks from coin-operated vending machines explains that forty-four per cent of 25 drinking water samples examined contained coliforms and 84% had viable counts of greater than 1000 organisms per ml at 30^0C. Thirty-one flavored drinks were examined; 6% contained coliforms and 39% had total counts greater than 1000 organisms per ml. It is suggested that the D.H.S.S. code of practice on coin-operated vending machines is not being followed. It is also suggested that drinking water alone should not be dispensed from such machines (Hunter *et al.*,, 1986).

2.2.5. Equipment

Equipment should be made of stainless steel as it is easier to clean, sanitize and maintain than equipment made from other materials. All lubricants and surfaces coming into contact with foods should be made of food grade materials. Galvanized buckets, pipes or sheeting should not be used. Equipment that comes into contact with fruit juice/cider should not be made of a material that could lead to undesirable or unacceptable migration or leaching of chemicals into juice/cider, for example, brass equipment should not be used since the acidity of the juice/cider could leach the copper out of the brass (Canada food agency, 2001).

2.2.6. Personnel

All workers must be free from communicable diseases. They should be trained not only for their task, but also to keep the venders clean and to practice personal hygiene. Written requirements for personal hygiene should be available. Workers must have ready access to clean washrooms and proper hand washing (hot water and soap) facilities with disposable towels and closed trash containers. All persons must wash their hands upon entering food handling areas, before starting work, after handling contaminated materials, after breaks, and after using toilet facilities. Where necessary to minimize microbiological contamination, employees should use disinfectant hand dips. Washrooms must be segregated from production and storage areas. Employees having open cuts or wounds must not handle food or food contact surfaces unless the injury is completely protected by a secure waterproof covering (eg. rubber gloves) (Canada food agency, 2001).

All of the individuals entering food handling areas should remove jewelry and other objects which may fall into or otherwise contaminate food. Protective clothing, hair covering, footwear and/or gloves, appropriate to the operation in which the employee is engaged should be worn and maintained in a sanitary manner (Canada food agency, 2001).

Without personal hygiene of food handlers, safe processing of fruit juices alone has no value to improve the community health. Therefore, all rounded safety precautions should be applied by food handlers as well as during processing. According to study conducted in Gondar, Ethiopia food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms (Andargie*et al.*, 2008).

2.2. Risk of Consumption of Unpasteurized Products

While most people can safely consume unpasteurized fruit juice and cider, food safety experts don't recommend that children, pregnant women, older adults and people with a weakened immune system consume unpasteurized juice and cider. Facilities that serve juice such as schools, child and adult daycares, and hospitals, should make sure it is pasteurized. Children on field trips to farms or farm markets should not drink unpasteurized juice (Health Canada, 2006).

2.3. Prevalence of food-borne bacterial pathogens in fruit juice

Even in developed countries, an estimated one-third of the population is affected by microbiological food borne diseases each year. According to the survey conducted across Victoria, Australia collected 291 juice samples between March 2004 and May 2004 from retail businesses. All samples submitted were analyzed for *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes* and coagulase positive staphylococci; sample pH was also determined. The results showed that *Salmonella* was not detected in any of the juice samples collected (Melbourne, 2005). However, *E. coli* was detected in seven juice samples, two of which had levels greater than 100cfu/ml. *Listeria* spp. were detected in nine juice samples; *L. monocytogenes* was detected in one of these at a level of 25000cfu/ml and was assessed as being potentially hazardous. All juice samples analyzed for coagulase positive staphylococci contained less than 100cfu/ml and were assessed as satisfactory. Overall, the microbiological quality of the juice samples submitted in this survey was good despite the one sample that was assessed as being potentially hazardous (Melbourne, 2005).

A study conducted in Pakistan stated that microbiological quality of all the products was well outside of the Gulf Standards for fruit juices, and coliform counts usually exceeded 1,000 cfu/ ml. In one sample of mixed fruit juice, the coliform count was above 1.0×10^6. Routine examination of foods for a range of pathogenic microorganisms is impractical. In order to assess the microbiological safety from food borne pathogens, widespread use of groups or species which are easily enumerated and whose presence in foods indicates exposure to conditions that might introduce hazardous organisms and/or allow their growth, are used. These groups are referred to as indicator organisms (Department of health directorate, South Africa, 1997).

2.4. Indicator microorganisms

Indicator organisms are organisms that provide insight to the history of a sample or to potential associations with other organisms or conditions (e.g. they can indicate the potential presence of pathogens or spoilage organisms). Coliform bacteria have been used as indicators of unsanitary conditions in water and foods for over a century. This concept originated in the late 1800’s after *E. coli* was found to be ubiquitous in feces, and its detection in water was used to “indicate” an increased likelihood that pathogens such as *Salmonella typhi* (causative agent of typhoid fever) were in the water as well (i.e., an indicator of unsanitary conditions). Indicators have been applied to both food and water safety and quality. The indicator organisms should meet the following criteria: - easily distinguishable from other microorganisms common to a sample; easily detected &

enumerated in a relatively short period of time (e.g., rapid tests); show direct or indirect association with reduced safety or loss of quality; and be able to survive as well as the associated organism(s) in the water/food being tested (Jay *et al.*,, 2005).

2.2.7. Total Viable Bacteria (TVB)

Different freshly prepared fruit juices contain significant amount of microorganisms. In a previous study, Shakir *et al.*, (2003) demonstrated that the mean total viable count (microbial load) showed the presence of bacteria in all the freshly prepared fruit juices in the range of 3.00×10^2 to 9.60×10^8. *Staphylococcus aureus* was detected in almost all the samples of fruit juices as well as of cold drinks. *Escherichia coli* were obtained in all fruit juices but not in cold drinks (Neha and Tumane, 2011). The presence of total aerobic viable bacteria in food can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing and dilution, cross contamination from rotten fruits and vegetables, or the use of dirty processing utensils like knife and trays (Khalil,1994)

2.2.8. Total Coliform count (TCC)

Coliform are a heterogeneous group of Enterobacteriaceae (e.g. *E.coli*, *Entrobacter*, lactose positive biotypes of Citrobacter, Serratia and Hafnia). They are facultative anaerobes, gram negative, non-spore-forming rods that ferment lactose with the production of acid and gas within 48 hours at 35°C (32-37°C). They are indicator organisms, which are closely associated with the presence of pathogens but not necessarily pathogenic themselves (Banwart, 1989).

2.2.9. Total fecal coliform cont (TFCC)

Some strains of coliform bacteria can be further classified as "fecal coliforms," which are defined as Gram-negative facultative rods that ferment lactose at 44.5°C and produce acid and gas from lactose within 48 hrs. The fecal coliforms consist primarily of *E. coli*, but a few Enterobacter and Klebsiella strains can produce gas in lactose broth at 44.5°C (Duncan and Razzell 1972). The fecal coliforms are relatively specific for fecal material of warm-blooded animals. Fecal coliforms can be found in animals, plants, and foods derived from them, as well as in contaminated soil and water (Banwart, 1989). The fecal coliform group is indicative of organisms originating in the intestinal tract of humans and some animals (Thomann and Mueller, 1987).

2.2.10. Total Spore formers (TSFB)

Spore forming bacteria that are present in foods are important because the formation of the spores by the bacterium allows it to be resistant to heat, freezing, chemicals, and other adverse environmental changes that our food undergoes during processing and preparation. Although the vegetative cell is killed by these conditions, the spores can survive and need harsher conditions to be inactivated. Some of the bacteria that are important belong to the genus Bacillus, which are aerobic to facultative anaerobic rod-shaped microbes.

These microbes can either grow under mesophilic temperatures (by definition, they grow at 35ºC but not at 55ºC) or some grow under thermophilic temperatures (grow at 55ºC but not at 35ºC). These Bacillus species can cause food spoilage or some cause food-borne illnesses. The other important groups of spore forming bacteria belong to the genus Clostridium. These are anaerobic bacteria that can grow at temperatures that are both mesophilic and thermophilic, depending on the species involved. They are of interest in foods because they also cause food spoilage and some species cause food-borne diseases. The most well-known food-borne disease caused by a Clostridium species is botulism. Spores are found in soil, water, and intestinal contents of humans and animals; hence, they can find their way into foods (Cousin, 1989).

2.2.11. Total Staphylococci count (TSC)

Staphylococci are spherical bacteria (cocci) which on microscopic examination appear singly, in pairs or bunch of grape-like clusters. They are Gram-positive, facultative anaerobes, but grow rapidly under aerobic conditions. They are mesophiles with a growth temperature range of 7 to 48^0C and have the ability to grow at low a_W (0.86), low pH (4.8), and high salt and sugar concentrations of 15% and in the presence of NO_2. *S. aureus* are naturally present in the nose, throat, skin, and hair of healthy humans, animals and birds (Neeraj and Sharma, 2007). *S. aureus* is considered one of the main food-borne pathogens worldwide, as they produce coagulase, heat stable nuclease or enterotoxins (Jay *et al.,* 2005). The presence of these bacteria might be entered into the street foods during handling, processing or vending. It also due to the fact that it forms the normal microflora present on/in several parts of the human body (Nester, 2001).

2.2. 12. Total Yeasts and mold count (TYMC)

Most fruit juices are acidic enough and have sufficient sugar to favor the growth of yeasts (Hooves, 1997). Moulds are generally considered to be the least important group of microorganisms causing spoilage in fruit juice because of their limitation, inability to grow in the absence of air (Parish, 1991), with the exception of few moulds such as Penicillium and Aspergillus (Parish and Higgin, 1989). Fungal fruit infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer (Al-Hindi *et al.*, 2011). Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal growth which in turn may result in their decay (Singh and Sharma, 2007; Al-Hindi *et al.*,, 2011). Yeasts (*Saccharomyces spp.*,*Candida spp., Hanseniaspora spp*) and moulds (*Cladosporium spp., Penicillium spp., Aspergillus spp., Botrytis spp*.) are more favored as spoilage agents of fruit juices compared to bacteria because of the physical and chemical properties of the fruit juices (Obire *et al.*,, 2008; Okigbo and Obire, 2009).

2.5. Outbreaks Associated with Unpasteurized Fruit Juices

A number of outbreaks of human infections were documented in association with the consumption of raw fruits, vegetables, and unpasteurized fruit juices. According to the Centers for Disease Control and Prevention, in the U.S. the number of reported produce-related outbreaks per year doubled between the period 1973-1987 and 1988-1992.During both time periods, the etiologic agent was unknown in more than 50% of outbreaks. Outbreaks with identified etiology were predominantly of bacterial origin, primarily *Salmonella*.

According to a study by Buck *et al.,* (2003), salmonellosis has been linked to tomatoes, seed sprouts, cantaloupe, mamey, apple juice, and orange juice. *Escherichia coli* O157:H7 infection has been associated with lettuce, sprouts, and apple juice, and enterotoxigenic *E. coli* has been linked to carrots. Documented associations of shigellosis with lettuce, scallions, and parsley; cholera with strawberries; parasitic diseases with raspberries, basil, and apple cider; hepatitis A virus with lettuce, raspberries, and frozen strawberries; and Norwalk/Norwalk-like virus with melon, salad, and celery have been made. Among the greatest concerns with human pathogens on fresh fruits and vegetables are enteric pathogens (e.g., *E. coli* O157:H7 and *Salmonella*) that have the potential for growth prior to consumption or have a low infectious dose.

2.6. Health Benefits of Fruit Juices

In recent years, juices have been included significantly as the diet of many communities, irrespective of age. They are nutritious drinks and can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits (FAO, 2006).

The high potassium and low sodium characteristic of most juices help maintain a healthy blood pressure, furthermore the lack or near absence of fat in fruit juices is beneficial for the cardiovascular system. The fortification of juices with calcium and phytosterol provide some supplemental bone and cardiovascular benefits (Delichatsios and Welty, 2005).

Vitamins have a special role since they are essential for life and most are not produced by the body. Vitamin C (ascorbic acid), naturally presents to most juices, is necessary for the body to form collagen, cartilage, muscle, and blood vessels, and aids in the absorption of iron. More recently, the influence of vitamin C in gene modulation and biochemical pathways modifications has been shown, particularly in blood vessel endothelium (Wu *et al.*,, 2007) and atherosclerosis (FrikkeSchmidt and Lykkesfeldt, 2009).

Among the vitamins found in fruit juices, folate from citrus and pineapple is essential for the prevention of spina bifida (Bell and Oakley, 2009) and premature birth (Bukowsky *et al.*,, 2009). It also helps in maintaining a low level of the amino acid homocysteine, a marker of inflammation that has been associated with a higher risk for heart disease, stroke, and heart failure (Sánchez-Moreno *et al.*, 2009). The health benefits of minerals, vitamins, and micronutrients have been well characterized but many beneficial properties of juices have been shown to come from phytochemicals, mainly polyphenols, carotenoids and limonoids (Holst and Williamson, 2008).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

Adama is one of the central cities of Ethiopia and surrounded by East Showa Zone, which distance is located about 99 km southeast of Addis Ababa at 8°54'N and 39°27' E latitude and longitude, respectively, and at elevations ranging from 1600-1712 m.a.s.l. The city sits between the base of an escarpment to the west, and the Great Rift Valley to the east and is situated in a basin-like flat land surrounded by sloping hills on all slides except the one facing the south. Climatically, Adama falls within the low land zone and its average annual temperature is about 20^{o}C with the maximum temperature slightly exceeding 30-32^{o}C during the month of May. Summer temperatures are low giving mild and pleasant weather condition. Annual rainfall, which comes during the month of June, July and August ranges between 700 and 900mm.Freshly squeezed fruit juices are available in restaurants, cafes and juice houses for human consumption.

In the town, there are 90 restaurants and cafeterias prepare and sell unpasteurized fruit juices to visitors and the people of the town. Restaurants and cafeteria use tap water for preparation of juice, none of them use antiseptic for cleaning facilities and washing utensils, they store fruit on the shelf in the way exposed to light and dust this may contribute to a rapid growth of contaminant microbes in fruits. Mango, papaya and avocado fruit juice are the most popular consumed by ones.

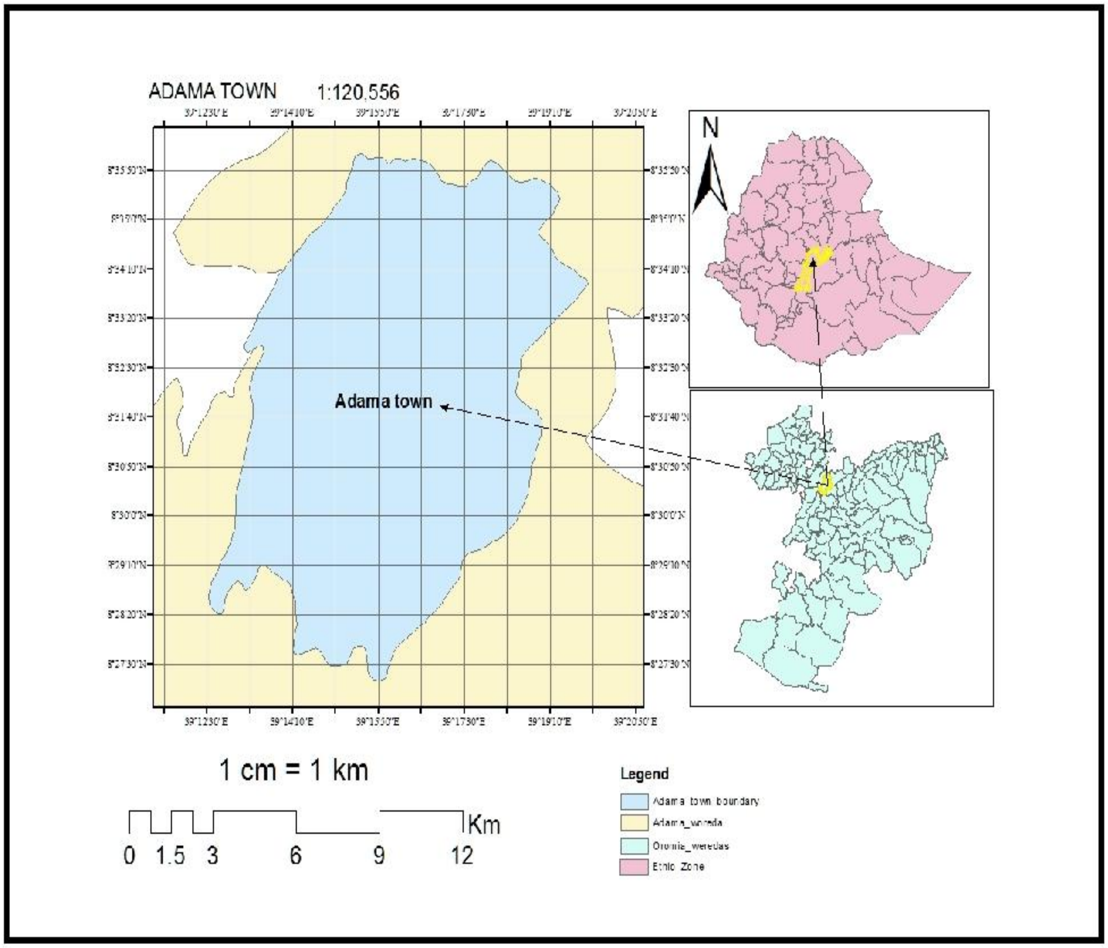

Figure 1: Map of the study area

3.2. Source of Samples

The sources of samples in this study were restaurants and cafeteria that prepare fresh fruit juices in Adama town.

3.3. Method of Data Collection

In this study questionnaire survey was used to obtain preliminary information on the demographic characteristics of fruit juice makers, servers, and cares being taken during processing 0f the fruit juices as well as the utensils used for preparation of juice.In addtion of this observation and laboratory examination were used as amethod of data collection.

3.4. Study Design

A cross-sectional survey design was employed to determine the microbiological profiles of locally prepared fresh fruit juices in Adama town. Study participants were administered with questionnaires to obtain preliminary information on the demographic characteristics of the fruit juice makers, servers, and cares being taken during processing of the fruit juices as well as the utensils used for preparation of the juice, handling and other information related to juice processing. All the personnel involved in the processing and/or serving of the fruit juices in the selected cafés/restaurants were included in the study. Information about the personal hygiene and of fruit juice vendors were collected through observation using checklists.

Microbial analyses were done in the laboratory using randomly collected juice samples from cafes/restaurants of the study area. Three fruit juice samples (papaya, avocado, and mango) were collected from cafes and restaurants and serially diluted. Aliquots of the appropriate dilution were then spread plated on appropriate media. After incubation, total coliform count, total staphylococci, total fecal coliform, total spore forming bacteria, total aerobic mesophilic bacteria and yeast and mold counts were determined. The isolation, identification, and determination of microbial loads were done in Oromia Public Health Research Capacity Building Quality Assurance Laboratory (OPHRCBQAL), Adama. The results of all bacteriological analyses were compared with microbiological criteria for food stuffs provided by the Gulf Standards (2000).

3.5. Study Samples and Sampling Houses

Three types of fruit juices, namely avocado, mango and papaya were chosen for the study on basis of the consumers ‘preference for the juice. The study population constituted 90 fresh juice producers in Adama town.

3.6. Sample Size Determination

Even though there are many juice vending houses in the city including cafes and restaurants, according to information obtained from Adama Town Trade and Industry Office, 90 juice vending cafeteria and restaurants were found in the town. A total of 81 juice samples, 3 samples from each of the 3 types of juices, i.e. avocado, papaya and mango, in 9 juice vending houses were collected using purposive sampling technique out of the 90 cafés/restaurants found in Adama town. As some of the fruit juices vending cafés were serving either one, two or three types of the fruit juices, only those serving the maximum number of fruit juices including, avocado, mango and papaya were considered in this study.

3.7. Sampling Procedures

Three types of fruit juices that were being consumed highly by the consumers were selected for the study (Mango, Avocado and papaya). A total 81 juice samples were collected using purposive sampling technique. Those juice houses that were not producing the three types of juices were removed out of the sampling frame. Then from the list of selected juice houses, ten percent of the total juice houses (9) were selected by purposive sampling system. From each of the selected fruit juice houses, samples of 25 ml of each of the three types of juices were collected using sterile flasks in three rounds. Samples collected in each round were transported immediately to OPHRCBQAL and kept in refrigerator and analyzed within 3-4 hours of their collection.

3.8. Isolation and enumeration of microorganisms from juice samples

Isolation and enumeration of microorganisms were done by using juice samples diluted from 10^{-1}up to 10^{-7} with sterile normal saline solution. 1ml of each dilution was evenly spread on the nutrient agar medium and incubated at 37^{o}C for 24 hours. After incubation colonies were counted and the actual numbers of bacteria were estimated as colony forming units per ml (cfu/ml) using the formula: CFU/ml = Number of colonies counted X Dilution factor / volume of sample plated.

Thus, Total viable bacteria Count (TVBC), Total coliform count (TCC), total fecal coliform count (TFCC) and Total staphylococcal count (TSC), Total Spore Forming Bacterial Count (TSFBC), total Yeast and Mold Count (TYMC) were determined using the above formula on appropriate culture media. Isolates were then identified according to the Bergey's manual of determinative bacteriology (Buchanan and Gibbon, 1984) and manual for the identification of medical bacteria (Cowan, 1975**).** Plate Count Agar (PCA) and Manitol Salt Agar (MSA) were used as a culture media for total aerobic mesophilic count (TAMC) and *Staphylococcus aureus*, respectively.

One milliliter of sample from each of the three serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}**)** was taken aseptically and pour plated on to three triplicate plates of agar. The plates were then incubated at 37^{o}C for 24-48 hours. Plates with colonies ranging from 30-300 and 20-200 were used for estimating the total colony forming units for TAMC and Total Staphylococcal Count (TSC), respectively (Ahmed *et al.*,, 2009). Catalase test was done on presumptive golden yellow colonies of *Staphylococcus aureus* by adding few drops of 3% H_2O_2 on plates of an over-night culture of the pure isolates (Ahmed *et al.*,, 2009)

3.8.1. Total Yeast and Mold Count (TYMC)

Serial dilutions were prepared for each of the juice samples. The stock culture was prepared by taking 50 ml of juice samples and mixed with 450 ml of sterile peptone water. Each sample was serially diluted (10 fold dilution) in sterile peptone water. An aliquot of 0.1ml of each dilution was plated out on potato dextrose agar (PDA) and incubated at ambient temperature (26-28^{0}C) for 48-72 hours. The mean number of colonies was used to calculate the YMC as colony forming units (cfu)/ml as described in section 3.7. The discrete isolated colonies (pure cultures) were also picked out and purified by re-streaking on PDA plates and maintained on slants of the same medium at 5^0C in the refrigerator. The pure isolated fungi were identified using cultural and morphological features according to the most documented keys developed for fungal identification (Samson and Varga, 2007).

3.8.2. Total Viable Bacterial Count (TVBC)

Tenfold serial dilutions of the juice samples were made up to 10^{-7} with sterile normal saline solution. 0.1ml of each dilution was then evenly spread on Plate Count Agar (PCA) and incubated at 30ºC for 24 hours. After incubation, the plates were examined for the presence of discrete colonies which were used for estimating the actual number of bacteria. Colonies were counted from replicate plates and the average was taken to estimate the TVBC. The mean numbers of colonies were used to calculate colony forming units (cfu)/ ml using the formula shown in section 3.7 (Cowan, 1975; Titarmare *et al*.,, 2009).

3.8.3. Total coliform count (TCC)

From each serial dilution, one ml was transferred into sterile Petri dishes. Following this, 15 ml of violet red bile agar (VRBA) medium (Oxoid company) which has been previously sterilized and kept in water bath at 45ºCwas pour plated and incubated at 37ºC for 24-48 hours. Coliform colonies lower the pH of the medium, subsequently causing their colonies to look red and to precipitate the bile salts. Thus, typical purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of precipitates were considered as belonging to coliforms (Tasnim *et al.,* 2010).

3.8.4 .Total Fecal Coliform Count (TFCC)

Tenfold serial dilution was prepared up to 10^{-7} with sterile normal saline solution. 0.1ml of each dilution was evenly spread on MacConkey agar medium and incubated at 37ºC for 24 hours. After incubation, the plates were observed for the presence of discrete colonies and the actual numbers of bacteria were estimated as colony forming units per ml (cfu/ml) following the method used by Tasnim *et al.*, (2010).

3.8.5. Total Spore Forming Bacterial Count (TSFBC

For spore-forming bacterial count, 1:10 dilution of the juice sample in distilled water was made in a test tube and heated to 80ºC for 10 minutes. The sample tube was immediately cooled and 1ml of the heat shocked sample was transferred to dextrose tryptone agar plate containing bromcresol purple. The plate was subsequently incubated at a temperature of 55ºC for 48 hrs. Yellow zone formation around the colonies resulting from acid production indicated spore-forming bacteria and these were counted to determine the total spore-forming bacterial count (ASFM, 1982).

3.8.6. Detection of some pathogens

Selected pathogens (*Salmonella* spp., *E. coli* and *Cryptospococcus* spp.) were detected following the procedures outlined by Food and Drug Administration (FAD, 2001).

3.8.7. Total Staphylococcal Count (TSC)

From each sample of the serial dilution, one ml was transferred into sterile Petri dishes and 15 ml of Mannitol Salt Agar (MSA) medium (Oxoid Company) previously sterilized and kept in water bath at 45ºC was pour plated and incubated at 37ºCfor 24-48 hours. Yellow and orange colonies surrounded by yellow zones due to mannitol fermentation were enumerated and further tested by coagulase test after overnight sub-culturing in nutrient agar plates (Tasnim *et al.*,, 2010).

3.9. Data Analysis

Data were entered into the computer and analysis was done using SPSS version16.0. ANOVA was used to compare means of microbial counts among juice types. In the analysis, P-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

4.1. Socio-demographic Characteristics, Handling Practices, and Personal Hygiene of Fruit Juice processors

A total of fifteen juice makers participated in the study to obtain data on socio-demographic characteristics, fruit juice processing, source of fruits and storage of fruits from randomly selected juice houses in Adama Town. Among the respondents, ten (66.7%) were females and five (33.3%) were males. Nine (60%) of the respondents had attended secondary school and three (20%) had attended elementary school and the other three (20%) had attended preparatory school(Table1).The data clearly indicate that the majority of workers in juice houses were educated up to secondary school level (Table 1). Females with the least educational attainments of high school level and less than the age of 40 practice safer food preparation and handling (Klontz *et al.*,, 1995). In this study, 12 (80%) of the respondents were aged between 18-25 and two (13.33%) were with ages greater then 25, and the remaining 1(6.7%) was below 18 yrs. Five (33.33%) of the respondents were waiters while 10(66.7%) were juice makers (**Error! Reference source not found.**).

Table 1. Socio-demographic characteristics of the participants of the study (n=15).

Factors	Categories	Number	Percentage
Sex	Male	5	33.33%
	Female	10	66.7%
Age	<18yrs	1	6.7%
	18-25yrs	12	80%
	>25yrs	2	13.33%
Education	Primary	3	20%
	Secondary	9	60%
	Preparatory	3	20%
Occupation	Waiter	5	33.33%
	Juice maker	10	66.7%

Concerning the source of fruits, the majority (77.8%) of the respondents responded that the fruits were obtained from open markets. In addition, most (77.8%) of the vendors were using shelves for temporary storage of fruits (**Error! Not a valid bookmark self-reference.**).

Table 2. Sources of fruits and their temporary storage places of the juice makers prior to juicing

Parameter	Type of fruit source and storage	frequency	Percentage
Source of fruit	Open market	7	77.8%
	Directly from producers	2	22.2%
	Others	0	0%
Storage site	Shelf	7	77.8%
	Basket	1	11.1%
	Refrigerator	1	11.1%

Majority of respondents reported that fruits were purchased from open market retailers and temporarily stored on shelves. But, during observation of juice maker houses, it was revealed that the majority of juice vending houses kept fruits outside in a condition that is exposed to sunlight (high temperature) and dust. This may contribute to a rapid growth of contaminant microbes in fruits. If the washing practice of these fruits is poor, microbes may get entry during juice making process (Tambekar *et al.*, 2009*).*

Most of the fruit juice makers had no training related to safe handling and processing of fruit juice. Moreover, none of fruit juice makers had the experience of using antiseptics prior to preparation of fruit juices. All of the vendors were using tap water for dilution of fruit juices (**Error! Reference source not found.**). As can be seen from Table 3, most of the juice makers (93.7%) do not wear apron while 6.7% wear apron, respondents that wear apron were further questioned on frequency of changing their aprons. Among these, 6.7% of the respondents change their apron once per week(Table3). Wearing apron during fruit preparation, juicing, and serving may protect bacterial contamination of juices (Mekonnen *et al.* 2012*).* Food may be contaminated from hair and unclean hands (Kekkonen *al.*, 2012).

Table 3. Juice handling practices and personal hygiene of the juice makers (n=15)

Variables	Categories	Frequency	Percentage
Training	Yes	1	6.7%
	No	14	93.3%
Wear apron	Yes	1	6.7%
	No	14	93.7%
How often do you change your apron	Once per day	0	0%
	Twice per day	0	0%
	Once per week	1	6.7%
	twice per week	0	0%
	As per the condition	14	93.7%
Wash hands before preparation	Yes	15	100%
	No	0	0%
Washing hands by	Water only	12	80%
	Water and soap	3	20%
Source of water	Tap	15	100%
	Well	0	0%
Washing agents	Water	12	80%
	Water+ soap	3	20%
	Water+soap+antiseptic	0	0%

All the respondents indicated that they wash their hands before preparation. The majority (80%) indicated that they wash their hands using water only and 20% of the respondents wash their hands with water and soap(Table3). Hand washing practice of food handlers is in agreement with a study reported by (Mulugeta *et al.*, 2012*)*. Thus proper training on food handling is important to reduce food borne illnesse.

From the observation of the fruit juice preparation most of the checklist points seem to be good, that is all juice makers wash hands and utensils before preparing juices, have neat clothes (77.8%) and work places (66.7%), had short finger nails (66.7%), and do not wear jewelries (73.3%). However, 93.3% of the venders wipe their hands with their dresses and all the juice maker prepare the juice with their bare hand and the type of the juice container they use for provision costumer are non-disposable (Table 4). All this indicate, there is high possibility of contamination during juice preparation.

Table 4: Observed handling practices and hygienic conditions of the juice make (n=15)

Parameter		Variables	Frequency	Percentage
Juice makers	Hand washing while preparing	Yes	15	100%
		No	0	0%
	Vendor's cloth	Neat	13	77.8%
		Untidy	2	22.22%
	Handle juice with bare hand	Yes	15	100%
		No	0	0%
	Long finger nail	Yes	3	33.33%
		No	12	66.7%
	Wear hand jewelries	Yes	4	26.7%
		No	11	73.3%
	Wipe hand with their owns dresses	Yes	14	93.3%
		No	1	6.7%
Juice house	Washing utensils with water	Yes	9	100%
		No	0	0%
	Types of glasses used	Disposable	0	0%
		Non disposable	9	100%
	Work place	Neat	6	66.7%
		Untidy	3	33.33%

4.2. The Microbiological Quality of Fruit Juices

According to Gulf Standards (2000), any sample with a count above the maximum count permitted shall be designated as "defective" while any sample with a count above Maximum count anticipated shall be designated as "marginally acceptable" (Table 5). Generally, from 81 juice samples, 56(69.1%), 72(88.9%), 66(81.5%), 37(45.7%), and

6(7.4%) were above the gulf standards for TVBC, TCC, TFCC, TSC, and TSFBC respectively (Table6).

Table 5: The recommended microbial standards for a fruit juice (n=81)

Parameter	Total viable (CFU/ml	Total Coliforms (CFU/ml)	Total Fecal coliforms (CFU/ml)	Total Staphylococci count (CFU/ml)	TSFBC (CFU/ml	Yeast & mold (CFU/ml)
Maximum count(load) anticipated	5.0×10^{3}	10	0	100	0	100
Maximum count(load) permitted	5.0×10^{4}	100	0	1.0×10^{3}	0	1.0×10^{3}

Source: Gulf Standards (2000). Key- TSFBC=

4.2.1. Yeast and mold count (YMC)

Fresh squeezed or pressed juices made from fruits and vegetables have a very high consumer preference both in terms of taste and health effects throughout the world. Total yeast and mold counts of freshly prepared fruit juice samples of avocado, mango and papaya collected from three different areas, of Adama city (Mebrat Hail, Postabet, and Menahiria) were investigated. Out of a total of 81 juice samples, 21(25.9%) of the juice had marginally acceptable yeast and mold count by the Gulf standard. Furthermore, of the 21 marginally accepted juice samples 11 were identified belong to mango juices (Table 6).

The mean yeast and mold count in all fruit juice was $9.1\text{x}10^{2}$cfu/ml. The mean yeast and mold counts among fruit juice samples were found to be statistically significantly different (p=0.004) (Table7). The mean yeast and mold count in avocado, mango, and papaya were $4.15\text{x}10^{2}$ cfu/ml, $1.5\text{x}10^{3}$cfu/ml and $8\text{x}10^{2}$ cfu/ml, respectively. The highest count identified in mango than avocado and papaya but study showed (Ketema *et al.*, 2008) in Jimma town in 2006, the counts of yeasts were relatively higher in avocado ($4.5\text{x}10^{5}$CFU/ml) as compared to that of papaya ($6.2\text{x}10^{3}$CFU/ml). This difference among fruit juice might be due to the ripeness of the fruit or the nature of the fruit. There were also a statistical significance difference (P=0.000) .in each juice of avocado, mango and papaya were observed, which also indicate that there is a variation of ripeness of fruit.

Table 6. Number and Percentage of fruit juice samples with colony counts above the gulf region standards

Type of juice sample	No of juice samples	Microbial load of juice samples (permissible gulf standard)					
		TYMC (10^3cfu/ml)	TVBC (10^4cfu/ml)	TCC (10^2cfu/ml)	TFCC (0 cfu/ml)	TSC (10^3cfu/ml)	TSFBC
Avocado	27	4(5%)	21(25.9%)	23(28.4%)	23(28.4%)	17(21%)	5(6.2%)
Mango	27	11(13.6%)	13(16.1%)	24(29.6%)	21(25.9%)	6(7.4%)	1(1.2%)
Papaya	27	6(7.4%)	22(27.2%)	25(30.9%)	22(27.2%)	14(17.3%)	0%
Total fruit juice samples	81	21(25.9%)	56(69.1%)	72(88.9%)	66(81.5%)	37(45.7%)	6(7.4%)

Key: **TVBC**: total viable bacterial count **TCC**: total coliform count **TFCC**: total fecal coliform count, **TSC**: total staphylococcus count **TSFBC**: total spore forming bacterial count, **TYMC**: total yeast and mold bacterial count

Normally at room temperature, alcoholic fermentation and oxidation of alcohol and fruit acid take place by yeast and mold (Iqbal *et al.*,, 2016) As fruits usually carry a rich micro flora of yeasts and molds, it is not surprising, perhaps, that the counts in the juices were often very high, usually well above the Gulf Standard. However, it is relevant that juices of this type are consumed immediately after preparation and that few yeasts are pathogenic to humans, so that the actual health risk from this group should be minimal. So based on this study the fruit qua lity and the fresh ness of the fruit juice in Adam town is better than that of the other area.

7. Mean microbial counts for fresh fruit juice samples purchased from retail outlets in Adama.

Type of juice samples	No of juice samples	TYMC cfu/ml	TVBC cfu/ml	TCC cfu/ml	TFCC cfu/ml	TSC cfu/ml	TSFB cfu/ml
Avocado	27	$4.15x10^2$	$1.5x10^5$	$3.4x10^4$	$1.2x10^4$	$1.26x10^4$	$1.85x10^2$
Mango	27	$1.5x10^3$	$1.4x10^4$	$5.4x10^3$	$1.6x10^3$	$7.1x10^2$	1.2
Papaya	27	$8x10^2$	$1.1x10^5$	$3.3x10^4$	$7.5x10^3$	$1.2x10^4$	0.0
Total	81						
Mean		$9.1x10^2$	$9.1x10^4$	$2.4x10^4$	$6.9x10^3$	$8.6x10^3$	63
p-value		0.000	0.000	0.000	0.010	0.002	0.088

Key: **TVBC:** total viable bacterial count **TCC**: total coliform count **TFCC**: total fecal coliform count, **TSC**: total staphylococcus count **TSFBC**: total spore forming bacterial count, **TYMC**: total yeast and mold bacterial count

4.2.2. Total viable bacterial count (TVBC)

From 81 juice samples of different kinds (avocado, mango and papaya) 56(69.1%) of the juice sample had total viable bacterial count which was above the maximum bacterial load permitted (Table6). The finding is almost similar to Lahore city in Pakistan, 66.66% of samples had TVBC more than 4 log10 CFU/ml (Iqbal *et al.*,, 2015) this indicates that there is highly contaminated fruit juices are retailing in the city. The highest frequency of juice its TVBC above the maximum bacterial load permitted were observed in 21(25.9%) avocado and 22(27.2%) papaya than 13(16.1%) mango (Table 6).

The mean viable bacterial count in all fruit juice were $9.1x10^4$ cfu/ml, it was statistical significance difference (p=0.000) among all fruit juice. Which is similar study as that (Geta 2015) of Debre markos, it show statistically significant difference between avocado and mango fruit juices (P<0.05) (Table 7).

The mean count of TVBC of avocado, mango and papaya were $1.5x10^5$ cfu/ml, $1.4x10^4$ cfu/ml and $1.1x10^5$ cfu/ml, respectively. According to one study made in Jimma (Ketema *et al.,* 2008), the mean total viable bacterial counts of avocado, and papaya were 8.0 $x10^6$ and $3.1x10^7$, respectively. Other study at Debre markos (Geta 2015), the mean total viable bacterial count of avocado juice ($3.6\pm0.6x10^6$cfu/ml) was higher than that of mango juice ($2.2\pm0.48x10^6$ cfu/ml). In both studies the total viable bacterial count are higher than our study. Even though the count of TVBC in our study lowers than Jimma and Debre Markos, the mean bacterial count difference among fruit juice is similar to the two studies.

In another study in microbiological of vendor and packed fruit juices of Dhaka city, in Bangladesh, juice smples were found to harbor viable bacteria within the range between 10^2-10^7 cfu/ml (Rashed *et al.,*2013) which is somewhat similar to our study in the lower margin of the colony count which is $2x10^2$-$1.5x10^6$ cfu/ml range. All this, variability might be due to environmental factor, personal factor who prepare the juice and the type of the water they use for juice preparation.

Fruits would be containing large numbers of vegetative bacteria and also have been further contaminated at the vending sites during cutting, chopping, and mixing. Usually vendors cut these materials holding them in their bare hands. In addition, raw materials were left uncovered on the tables during the preparation which allowed for dust to settle leading to further contamination.

Dirty knives were also used to cut this fruits. The ice and water added during preparation were likely to provide possible sources of additional bacterial contamination (Bryan *et al.,*, 1998, Bryan *et al.,*, 1992, ICMSF, 1998). Furthermore, these fruit juices were left in ambient temperature which may have led to the proliferation of contaminating bacteria resulting in increased bacterial counts (Ahmed *et al.,* 2009). Improper processing methods and unhygienic conditions are indicated by higher counts of microbes in fruit juices. So there should be few microbes in the frozen juice concentrates (Iqbal *et al.,* 2016). All this information indicated that, our study finding show there is a poor preparation and storage of fruit juice before consumption

4.2.3. Total coliform count (TCC)

A total of eighty one (81) fresh fruit juices were cultured in order to determine total coliform count, of these 88.9 %(72) showed TCC, which was greater than the maximum total coliform count permitted (Table 6). In study of Pakista Lahore city, the total coliform count had observed in 46.66% of the juice sample which was more than 10^2 cfu/ml of the Gulf standard (Iqbal *et al.,* 2015). In another study (Rashed *et al.,* 2013) in Dhaka city, Bangladesh, the total coliforms were detected in 75 % (31) of juice samples of 41 which was within the range of 10^2-10^6 cfu/ml. So the total coliform count in our study (Table 6) was higher than in both studies which indicate there is high contamination than the other two studies. So the presence of coliforms in high numbers (CC >10^2 CFU/ml) is health hazard which caused spoilage of fruit juices and food borne diseases (Iqbal *et al.,* 2015).

In papaya juice, 25(30.9%), avocado 23(28.4%) and mango 24 (29.6%) of the sample were above the standard and its mean count were $2.4x10^4$ cfu/ml. The mean count of avocado mango and papaya were $3.4x10^4$cfu/ml, $5.4x10^3$cfu/ml, and $3.3x10^4$ cfu/ml, respectively (Table7). The maximum count observed in avocado and papaya over mango (Table6).

The mean count of TCC of avocado, mango and papaya juice are statistically significant difference (P=0.000) (Table7). So based on this, the highest contamination observed in avocado and papaya than mango. This contamination of mean count difference of avocado and papaya over mango might be due to the quality of the fruit or the preparation techniques among juice sample even though they prepared at the same condition.

The data reveals that all fruit juices samples collected from all houses were contaminated with coliform. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to improper washing of fruits, and utensils, inadequate storage of these at ambient temperatures in unhygienic places, maintenance of premises and personal hygiene of vendors. A number of genera within the coliform group are widely found on vegetable tissues and pose no hazard to humans, but it is the possible to observe pathogenic microorganism like *E. coli* and *Salmonella* spp. (Al-Jedah & Robinson, 2002). In general, most of the fruit juices being served in Adama city had higher microbial load than the specification set for fruit juices in some parts of the other study. So these fruit juice could be the cause of health problems and potential vehicle of foodborne outbreaks in the town.

4.2.4. Total fecal coliform count (TFCC)

From 81 fresh fruit juices samples, 81.5 %(66) (Table6).Juice sample were showed a growth for total fecal coliform count. This result is almost similar to Ahmed *et al.,* (2009) study where they identified *E.coli* in 99% of freshly squeezed fruit juices in Dhaka City, Bangladesh. But in a study (Rashed *et al.*, 2013) at Dhaka city, Bangladesh, fecal coliforms were found in 4 (9.7%) fruit juice samples of 41 (10^2cfu/ml) which is completely disagree with our study. Total fecal coliform counts were 28.4 % (23) in avocado, 25.9% (21) in mango and 27.2% (22) in papaya juice (Table 6).

The mean fecal coliform count in avocado, mango and papaya were $1.2x10^4$ cfu/ml, $1.6x10^3$ cfu/ml and $7.5x10^3$ cfu/ml, respectively (Table7). The mean coliform count of individual juice type in avocado, mango and papaya juice were statistically significant difference (p=0.010) (Table7). The highest mean coliform count was observed in avocado. This study was disagree with (Geta 2015) study done at Debre markos , its Fecal coliform counts were$0.2\pm0.1x10^4$ cfu /ml for avocado juice and0.06 ± 0.04 $x10^4$ cfu/ml for mango juice. The variation of these microbes in different study can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing and dilution, cross contamination from rotten fruits and vegetables, or the use of dirty processing utensils like knife, flies and trays.

The reason of higher fecal coliform count in our study related to a number of factors, one ,intermittent water supply and electric power, second personnel hygienic problem of the juice maker, third weather condition of the Adama town favor the growth of microorganism as compared to Jimma and Debremarkos. All this condition made our study finding of total fecal coliform count higher than the other study.

According to Demisse (2016) study on bacteriological quality of drinking water of Adama town, 43.75% coliform and 39.35% fecal coliform were observed, which showed that the drinking water used in the study area was contaminated by fecal coliforms. Thus, the water used for making juice was one of the major reasons for high TFC in the study area. So supplies of potable water, electricity, waste disposal services, good drainage system are crucial and the Government should take the provision of these services seriously in order to avoid contamination.

4.2.5. Total spore forming bacterial count (TSFBC)

The mean TSFBC ($1.85x10^2$) was detected only in 6 (7.4%) of total samples. (Table6). Even though the spore forming bacterial count observed only 6(7.4%) from 81 fruit juice sample which far less than (64.91%) study of Ahmed *et al.,* (2009) in Dhaka city Bangldish.

The highest count identified in avocado juice 5(6.2%) (Table6). Than the other fruit juice sample and the mean difference of total spore forming bacterial count is not statistical significant in all juice sample (p=0.088) (Table7). The mean count of spore forming bacteria in all fruit juice sample was 63 cfu/ml. However, this spore forming bacterial count mostly applied in frozen fruit juice samples to indicate there were contaminations for spore forming bacteria such as *Clostridium botulinum* (Iqbal *et al.,* 2016). So the finding of low spore forming bacterial count in our study might be due to test quality procedure or some other reason.

4.2.6. Total Staphylococcal count (TSC)

Out of eighty one specimens of fresh fruit juices 37 (45.7%) (Table6). Showed colonial growth on mannitol salt agar (MSA) for S*taphylococcus species* which is greater than the maximum bacterial load permitted. This finding is close to study in (Iqbal *et al.,* 2015), 51.66% of samples had TSC more than 10^3 CFU/ml and different from (Rashed *et al.,*

2013) study in Dhaka city, Bangladesh, vendor squeezed fresh fruit juice and packed fruit juice sample, with 30(73.2%) samples of 41 sample exhibited the presence of staphylococci. Of thes juice sample, the highest mean bacterial load were identified in avocado ($1.26x10^4$) and papaya ($1.2x10^4$) than mango ($7.1x10^2$). (Table7).

The frequency of juice sample which have greater than the maximum bacterial load permitted in avocado mango and papaya were 17(21%), 6(7.4%), and 14(17.3%) (Table.6) respectively. The mean count of all juice sample were $8.6x10^3$cfu/ml. which is statistical significant difference among fruit juice (P=0.002) (Table7). This result is higher than Rahman *et al.*, (2011) study in Dhaka City; mean staphylococcal count was 7.2 $x10^3$ cfu/ml. In another study (Iqbal *et al.*, 2015) Lahore city in Pakistan, Mean TSC in all fruit juice samples were (5.45±1.06 log10 CFU/ml) which is lower than our study. Higher number of TSC indicates the presence of contamination of juice during preparation due to poor hand hygiene and use of bare hand.

4.2.7. Detection of pathogens from avocado, mango and papaya juice sample

In this study, some pathogenic and potentially pathogenic bacterial species were detected in the squeezed fresh fruit juice samples retailed in vending juice houses of Adama city. The overall results showed that 52.7% (128) samples of fresh juice were contaminated with pathogenic microorganisms (Table 8).

Table 8. Bacterial pathogens detected from avocado, mango and papaya fruit juice collected from Adama town. Juice Samples (n=81)

Type of fruit juice	*E. coli*	*Salmonella* spp.	*S. aureus*	*Cryptococcus* spp.	Number of positive samples
Avocado	19(23.5%)	3(3.7%)	15(18.5%)	10(12.3%)	47(58%)
Mango	18(22.2%)	3(3.7%)	21(25.9%)	0	42(51.9%)
Papaya	18(22.2%)	6(7.4%)	14(17.3%)	1(1.2%)	39(48.2%)
Total	55(67.9%)	12(14.8%)	50(61.7%)	11(13.6%)	128 (52.7%)

As can be seen from the above table, 55 (67.9%) of the fruit juice samples were positive for *E.coli* and the proportion of positive sample among the fruit juice types were 19-18 for avocado, mango and papaya. The present study indicated that juice samples were less contaminated with human fecal as compared to those of Ahmed *et al.,* (2009) who reported that *E.coli* was isolated in 99% of freshly squeezed fruit juice samples of Dhaka City, Bangladesh.

The other detected pathogenic species was *Salmonella* spp. This species was isolated in 3(3.7%), 3(3.7%) and 5(7.6%) of avocado, mango and papaya juice samples, respectively. In papaya the prevalence of *Salmonella* isolates was somewhat higher than those of avocado and mango. The overall prevalence of Salmonella isolates from all fruit juice types was 14.8%, which was two times greater than that reported by Ahmed *et al.,* (2009), i.e. 7.89%, in Dhaka city, Bangladish.

S. aureus was more commonly isolated in mango juice than in avocado and papaya. From the total 81 samples, 61.7 % of the fruit juice samples were positive for this pathogen. Similar studies conducted at Dhaka city have reported the isolation of *S. aureus* in 6.14% of their juice samples, a prevalence which is so small compared to that of the present study. Another study conducted by Iqbal *et al.,* (2015) at Lahore city in Pakistan, showed that *Staphylococcus aureus* was isolated in 15.45% of fruit juice samples which is also far less than to the findings of the study. This high number of *Staphylococcus* isolation in our study indicates that there was a high degree of contamination of the fruit juices. The presence of high degree of contamination by *Staphylococcus aureus* suggests human contact as the most likely source of contamination, and clearly this level and type of contamination is totally unacceptable since *S. aureus* is a normal microflora of human skin and nose (Al-Jedah & Robinson 2002).

The other strange thing in our study was the presence of Cryptococcus species in the juice samples. Out of the total 81 fruit juice samples, 10(12.3%) and 1(1.2%) of the avocado and papaya juice samples, respectively, were positive for *Cryptococcus* species. *Cryptococcus* spp. was not detected in mango juice samples. This variation of the prevalence of *Cryptococcus* species in fruit juice samples could be due to differences in the pH of the fruit juices. Since mango juice has a lower pH value compared to others, it may not favor the growth of *Cryptococcus* species.

In general, most of the fruit juices being served in Adama town had higher microbial load than the requirement set for fruit juices standards. The principal pathogenic microbial groups isolated from the fruit juices were *Escherichia coli*, *Salmonella*, and *Staphylococcus aurous* and *Cryptococcus* species. This finding suggests that consumption of fruit juices at Adama juice vending houses could be potentially unsafe.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Fruit juices are an important part of the diet of all age groups due to the associated health benefits. Freshly squeezed juices are simply prepared by extracting the liquid and pulp of mature fruit usually by mechanical means or blenders. Improperly prepared fresh fruits and vegetable juices are recognized as an emerging cause of food borne illnesses Such juices have been found to be potential sources of bacterial pathogens. Notably *E. coli*, *Salmonella*, and *S. aureus.*

Contamination of fruit juices sold in restaurant and cafes are sometimes unacceptable for human consumption and create significant health problems. Fruit juice vending houses, which have been serving different types of fruit juices in fresh forms, are proliferating. Information on the safety of the fruit juices prepared and consumed in Ethiopia is no published information exists on the microbiological safety of the most popular juices, i.e. avocado, mango and papaya, consumed in Adama town in particular. Therefore the Present study was conducted with the aim of determining the microbiological quality of fresh fruit juices marketed in Adama town.

. In this study a total of 15 respondents were intended and 81 fruit juice samples were collected for the questionnaire survey and microbial analysis in the laboratory, respectively. Concerning the source of fruits, the majority (77.8%) of the respondents responded that the fruits were obtained from open markets and most (77.8%) of the vendors used shelves for temporary storage of fruits. In addition, most of the fruit juice makers had no professional training related to safe handling and processing of fruit juice.

So the microbial results showed that 66.7%, 96.3%, and 81.5%) and 45.7% of the fruit samples were found to show higher, total viable bacteria, total coliform count, total fecal coliform count and total staphylococcus counts, respectively, were above the specification set of Gulf region standard. The mean counts of viable bacteria, coliforms, fecal coliforms and staphylococci were $9.1x10^4$cfu/ml, $2.4x10^4$cfu/ml, $6.9x10^3$cfu/ml and $8.6x10^3$cfu/ml, respectively. Furthermore, high bacterial load was observed in Avocado than Papaya and Mango. Moreover, 67.9%, 16.1%, 61.7% and 13.6% of total of fruit samples were positive for *E. coli*, *Salmonella* species, *S. aureus* and *Cryptosporidium spp.*, respectively.

5.2. Conclusion

Generally, these studies clearly indicate the poor hygienic conditions of these juices and the consumers are at risk of contracting food borne infections. Based on these data avocado, mango and Papaya juices sold in Adama juice vending sites are contaminated with bacterial lode above specification set.

These juice preparations are also harboring pathogenic bacteria of fecal origin. Particularly juices prepared from papaya are highly contaminated with Salmonella species. Most of the fruit juices investigated in this study had higher microbial load than the specifications set for fruit juices in some parts of the world. Based on the gulf standards, it is clear that the colony counts of the microbial groups in these fruit juices exceeded the standard. These high counts, however, may pose hazard to the health of consumers especially if pathogenic species are present in the fruit juices to be consumed. They were also unaware of food regulations as well as lacking supportive services such as water supply of good and adequate quality, waste disposal systems that enhance their ability to provide safe food. The present study clearly demonstrates juice consumers at Adama town are at high risk of contracting food-borne diseases unless the concerned health authorities take regulatory measures in time.

5.3. Recommendations:

Based on the result of the study, the following points were recommended

- The juice vending houses should be regularly supervised.
- Vendors should use of antiseptics for cleaning equipment and refrigerator to store fruits to reduce microbial contamination
- Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future bacterial pathogen outbreak.
- There should, also, be regular training/retraining of handlers in all aspects of food hygiene and safety.
- Since current study was conducted on small sample size, it is also recommended that further study be made using large sample size a variety of juices made from different fruits.

6. REFERENCE

Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology* 123**:** 121–129.

Addo, M.G., Akanwariwiak, W.G., Addo-pordjour, P., Obiri–Danso, K. 2008. Microbiological and sensory analysis of imported fruit juices in Kumasi, Ghana, *Research Journal of Microbiology* **3:** 522-528.

Ahmed, M.S.U., Nasreen, T., Ferosa, B., Parveen S. 2009. Microbiological Quality of Local Market Vended Freshly Squeezed Fruit Juices in Dhaka City, Bangladesh. *Bangladesh Journal of Scientific and Industrial Research* .44**(**4**):** 421-424.

Al-Hindi RR, Al-Najada AR, Mohamed SA., 2011. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *Afr. J. Microbiolo. Res*. 5(4): 443-448.

Al-Jedah, J.H., Robinson, R.K. 2002. Nutritional Value and Microbiological Safety of fresh Fruit Juices sold through Retail Outlets in Qatar. *Pakistan Journal of Nutrition* **1:**79-81.

Andargie, G., Kassu, A., Moges, F., Tiruneh, M., and Huruy, K. 2008. Prevalence of Bacteria and Intestinal Parasites among Food-handlers in Gondar Town, Northwest Ethiopia,*J Health PopulNutr*.**26:**451-455**.**

Bacteriological Safety of Fruit and/or Vegetable Juices and Smoothies. 37 Gulf Standards (2000) Microbiological criteria for food stuffs-part 1. GCC, Riyadh, Saudi Arabia.

Bagde, N. I. and Tumane, P. M. 2011. Studies on microbial flora of fruit juices and cold drinks. *Asiatic Journal of Biotechnology Resources* **2** (4): 454-460.

Barro, N., Bello, A. R., Aly, S., Ouattara, C. A. T., Ilboudo, A. J. and Traoré, A. S. 2006. Hygienic status an assessment of dishwashing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). African Journal of Biotechnology 5 (11): 1107-1112.Bello, Olorunjuwon, O. *et al*.,, 2014. Microbiological quality of some locally-produced

Buchanan, R.and Gibbon, N. E. 1984. Bergey`s Manual of DetermminativeBacteriology, Baltimore. : William and Wilkins Co. New York.

Buck, J. W., Walcott, R. R., Beuchat, L. R. 2003. Recent trends in microbiological safety of fruits and vegetables. Online. *Plant Health Progress doi*:10.1094/PHP-2003-0121-01-RV.

Canadian Food Inspection Agency 2010. Code of Practice for the production and distribution of unpasteurized apple and other fruit juice/cidar in Canada. Retrieve from www.inspection.gc.ca/english/flavege/processed/codee.shtml. 36

Castillo, A, Villarruel-LO´ PEZ, A., Navarro-Hidalgo, V., Marti´Nez-Gonza, Lez,N.. E., andM., Torres-Vitela, R. 2006. *Salmonella* and *Shigella*in Freshly Squeezed Orange Juice, Fresh Oranges, and Wiping Cloths Collected from Public Markets and Street Booths in Guadalajara, Mexico: Incidence and Comparison of Analytical Routes. *Journal of Food Protection* 69: 2595–2599.

Cheesbrough, M. 2006. District Laboratory Practical in Tropical Countries, 2ed edition, part (2). Cambridge University press Cambridge.

Chukuezi, C.O. 2010. Food Safety and Hygienic Practices of Street Food Vendors in Owerri, *Nigeria Studies in Sociology of Science* 1: 50-57.

Demisse M. 2016. Parastological quality of drinking water and people's water handling practice in and around Adama Town, Oromia Region, Eastern Ethiopia Msc.

Department of health directorate, South Africa 1997. Food control guidelines for environmental health officers on the interpretation of microbiological analysis data of food.

Duncan, D. *W.*, and Razze! , W. E., 1972. *Klebsiella* biotypes among coli forms isolated from forest environments and farm produce. *Appl. Microbiol.* 24: 933-938.

Eze, V. C.*, Chimaeze, C.K., 2014. Microbiological and nutritional qualities of avocado fruit sold in Umuahia main market, Abia state, Nigeria. *American Journal of Food Science and Nutrition Research* 2014; 1(3), pp.17–22.Available at: http://www.openscienceonline.com/journal/fsnr

FDA (Food and Drug Administration), 2002. Bacteriological Analytical Manual, Enumeration of *Escherichia coli* and the Coliform Bacteria Online. Chapter 4: 16.

fruit juices in Ogun State, South western Nigeria. *E3 Journal of Microbiology Research*, 2(1), p.1–8.

FDA, 1999. Potential for Infiltration, Survival and Growth of Human Pathogens within Fruits,Vegetabl www] http://www.cfsan.fda.gov/~comm/juicback.html.

FDA, 2001, In Bacteriological Analytical Manual Online, USA. Chapter 12 p 1–6.

FDA, 2008. Internalization of Microorganisms into Fruits and Vegetables, . FDA. (2008) Literature Review of Common Food Safety [internet, www] http://www.cfsan.fda.gov.html.

Food safety, authority of Ireland, 2nd National Microbiological Survey. (2007). (07NS2)

Frikke-Schmidt H, Lykkesfeldt J. 2009. Role of marginal vitamin C deficiency in atherogenesis: invivo models and clinical studies. *Basic ClinPharmacolToxico*l**104**:419-433.

Geta, K., 2015. Microbiological Safety of Fruit Juices Consumed In Cafes and Restaurants of Debre-Markos Town, North Western Ethiopia, MSc Thesis, Haramaya University.

Ha, S.Y., Hwang ,Y.S., Yang, Y.J., Park, Y.M. 2007. Correlation between instrumental quality attributes and consumers' sensory evaluation in refrigerated-stored 'Campbell early' and 'Kyoho' grape. *Korean J HortSciTechnol.* 25:125-132.

Health Canada 2006. It is your health. Unpasteurized fruit juices/cidar. Retrieve from http:/www.hc-sc.gc.cawww/ha-vs/iyh-vsv/food-aliment/juice-jus-eng.php

Hunter, P. R., and Burge, S. H. 1986. Bacteriological quality of drinks from vending machines. *J. Hyg. Camb*. 97:497-500.

Iqbal, M.N. *et al.*,, 2015. Assessment of Microbial Load of Un-pasteurized Fruit Juices and in vitro Antibacterial Potential of Honey against Bacterial Isolates. *The Open Microbiology Journal, 9, pp.26–32*

Iqbal, M.N. *et al.*,, 2016. A Mini Review on Microbiological Quality of Commercial Fruit Juices in Pakistan. PSM Microbiology, 1(1), pp.26–32. Available at: www.psmpublishers.org Open

ICMSF, “Soft drinks, fruit juices, concentrates and food preserve in *Microorganisms in Foods 6:Microbial Ecology of Food Commodity*, Kluwer Academic, 2005.

James, CS (2004). Analytical Chemistry ofFood. Seale-Hayne Faculty of Agriculture, Food and Land use Department of Agriculture and Food Studies, University of Polymouth, UK1: 96–97.

Jaeger SR, Axten LG, Wohlers MW, Jay JM., M.J.Loessner, and D.A.Golden, 2005. *Modern Food Microbiology, 7th ed*. p 39. CBS Publishers and Distributors, New Delhi.

Sun-Waterhouse D. 2009. Polyphenol-rich beverages: insights from sensory and consumer science. *J, Sci, Food Agric*. **89**: 2356-2363.

Kamal, R.A.,Romika, D.Neeraj, K. A.,Vikas, K. andManpreeet, K. 2014.*International Journal of Food Science* Article ID408085, 7 page Hindawi Publishing Corporation

Ketema, T., Gaddisa, T. & Bacha, K., 2008. Original article microbiological safety of fruit juices served in Jimma Town. *Ethiopian Journal of Health Sciences,* 18(3), p.98–100.

Khalil, K., G.B. Lindblom, K. Mazhar and B. Kaijser, 1994. Flies and water as reservoirs for bacterial enteropathogens in urban and rural areas in and around Lahore, *Pakistan. Epidemiol. Infect.* 113: 435-444.

Khan, M.M. *et al.*,, 2015. Assessment of microbiological quality of some drinks sold in the streets of Dhaka University Campus in Bangladesh. , p.0–4.

Klontz, K.C., Timbo B., Feins, S.,Vevy, A. 1995. Prevalence of selected food consumption and preparation behaviors associated with increased risks of food borne diseases. *Journal of Food Protection,* 58: 927-930.

Kurowska, E.M., Spence, J.D., Jordan, J., Wetmore, S., Freeman, D.J., Piche, L.A., Serratore, P., 2000. Cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. Am. J. Clin. Nutr., 72: 1095-100.

Lewis, J.E., Thompson, P., Rao, B., Kalavati, C., Rajanna, B. 2006. Human Bacteria in Street

Mahale D P, Khade RG, Vaidya VK.,(2008), Microbiological Analysis of Street Vended Fruit Juices from Mumbai City, India. Internet Journal of Food Safety, 10:31-34.

Mekonnen, H., Habtamu, T., Kelali, A. (2012). Source(s) of contamination of 'raw' and 'ready-to-eat' foods and their public health risks in Mekelle City, Ethiopia. *Journal of Food and Agriculture Science* 2(2): 20-29.

Melbourne, R.H. 2005. Microbiological survey of freshly squeezed juices from retail businesses across Victoria - web site at: www.health.vic.gov.au/foodsafety).

Mosupye, F.M., and A .von Holy. 1999. Microbiological Quality and Safety of Ready-To-Eat Street-Vended Foods in Johannesburg, South Africa. *Journal for Food Protection* 62(11):1278-1284.

Mulleta, D. and Ashenafi, M. 2001. *Salmonella*, *Shigella* and growth of other food-borne pathogens in Ethiopian street vended foods. *East African Medical Journal* **78:**576-580.

Mulugeta, K., Bayeh, A. 2012. The sanitary conditions of food service establishments and food safety knowledge and food handling practices of food handlers in Bahir Dar town. *Ethiopian Journal of Health Science* 22(1): 27-35.

Neeraj Dilbaghi and S. Sharma, 2007. *Food and Industrial Microbiology*; Food spoilage, food infections and intoxications caused by microorganisms and methods for their detection.

Neha I. Bagde and P.M., 2011. Studies on microbial flora of fruit juices and cold drinks Asiatic. *J. Biotechnolo. Resources* 2(04):454-460.Scale fruit juice processing. A Case Study of Visakhapatnam City, India: Internet *Journal of Food Safety* **8:**3538.

Nester, E.W., D.G.Anderson, C.E. Roberts, N.N. Pearsall and M.T. Nester, 2001. *Microbiology*: *Human Perspective*. 3rd Edn., McGaw-Hill,NewYork, ISBN: 0072318783, pp: 815-816

Obire, O., Ramesh .R. Putheti. Dick, A. A and Okigbo, R. N., 2008. Biotechnology Influence for the Production of ethyl Alcohol (Ethanol) from Waste Fruits. *E-Journal of Science and Technology* (e-JST). 3(3):17-32.

Parish ME, Higgins DP. , 1989. Yeast and Mould Isolated from Spoiling Citrus Products. *Journal Food Production* 52:261-263.

Rahman, T., Hasan, S. and Noor, R. 2011. An Assessment of Microbiological Quality of Some Commercially Packed and Fresh Fruit Juice Available in Dhaka City: A Comparative Study. *Stamford Journal of Microbiology* 1 **(1):** 13-1.

Rahman, T., Hasan, S. & Noor, R., 2011. An Assessment of Microbiological Quality of Some Commercially Packed and Fresh Fruit Juice Available in Dhaka City : A Comparative Study. *Stamford Journal of Microbiology*, 1(1):13–18.

Rashed, N., Aftab, U., Azizul, H., Saurab, K.M., Mrityunjoy, A. and Majibur, R. 2013. Microbiological study of vendor and packed fruit juices locally available in Dhaka city, *Bangladesh. International Food Research Journal* 20**(2):** 1011-1015.

Rashed, N. *et al.*,, 2013. Microbiological study of vendor and packed fruit juices locally available in Dhaka city, *Bangladesh. International Food Research Journal*, 20(2), pp.1011–1015.

Roberts, D. and Greenwood, M. 2003. *Practical Microbiology*, Third Edition, Blackwell Publishing Inc. USA.

Shakir, M., Ahmed, U., Nasreen, T., Feroza, B. and Parveen, S. 2009. Microbiological Quality of Local Market Vended Freshly Squeezed Fruit Juices in Dhaka City, Bangladesh. *Bangladesh J. Sci. Ind. Res*. **44:** 421-424.

Sudershan, R.V., Pratima, R. and Kalpagam, P. 2009. Food safety research in India: a review: *As. J. Food Ag-Ind.*

Sandeep, M., Diwakar. A. and Abhijit. G. 2004. Microbiological Analysis of Street Vende Fresh squeezed Carrot and Kinnow-Manderian Juices in Patiala City, India. Internet *Journal of Food safety***3:** 1-3.

Samson, R.A., Varga, J. 2007. Aspergillus systematics in the genomic era. CBS Fungal Biodiversity Centre, Utrecht. *J. Med. Microbial***25:** 73 – 74.

Subbannayya, K.G.K., Bhat, S., Shetty, J.V.G. 2007. How safe issugarcane juice? *IndianJ. Med. Microbia*l**25:** 73 – 74.

Tambekar, D.H., Jaiswal, V.J., Dhanorkar, D.V., Gulhane, P.B. and Dudhane, M.N. 2009. Microbial Quality and safety of street vended fruit juices: A case study of Amravati city Internet *Journal of Food Safety***10:** 72-76.

Tambekar, D.H., Murhekar, S.M., Dhanorkar, D.V., Gulhane, P.B., Dudhane, M.N. (2009). Quality and safety of street vended fruit juices: a case study of Amravati city, India. *Journal of Applied Bioscience,* 14, p.782 – 787,

Titarmare, A., Dabholkar, P., Godbole, S. 2009. Bacteriological Analysis of Street Vended Fresh Fruit and Vegetable Juices in Nagpur City, India. Internet *Journal of Food Safety***11:** 1-3.

Tasnim, F., Hossain, M. A., Nusrath, S., Hossain, M. K., Lopa, D. and Haque, K. M. F. 2010. Quality Assessment of Industrially Processed Fruit Juices Available in Dhaka City, Bangladesh. *Malaysian Journal of Nutrition* 16**(3):** 431-438.

Thormann, R: V and J, A, Mueller, 1987. Principles of surface water quality modeling and control. Harper and Row, New York.

Sandeep, M., Diwakar, A., Abhijit, G. 2004. Microbiological Analysis of Street Vended Fresh squeezed Carrot and Kinnow-Manderian Juices in Patiala City, India. Internet *J. Food safety***3:** 1-3.

V. H. Tournas, J. Heeres, and L. Burgess, “Moulds and yeasts infruit salads and fruit juices,” *Food Microbiology,* vol. 23, no. 7,pp. 684–688, 2006.

WHO (1996). Guidelines for drinking-water quality. Second edition, volume 3.World health organization, Geneva.

Yigeremu, B., Bogale, M., Ashenafi, M. 2001. Fate of *Salmonella* species and *E. coli* in Fresh-Prepared Orange, Avocado, Papaya and Pine Apple Juices. *Ethiop. Journal of Health Science***11:**89-94.

7. APPENDICES

Appendix Table 1 Bacterial load in vendor fruit juice samples

(n=81)

Sample Number	Sampling area	Type of juice fruit	TMBC(TVC)	TCC	FCC	TSC	TSFBC	TYMC
S1	Menahari ya	Avocado	**$3x10^4$**	**$2.3x10^4$**	**0**	**$6.9\ x10^3$**	**0**	**0**
S2		Avocado	**$1.6x10^4$**	**$1.2\ x10^3$**	**800**	**$2.1\ x10^3$**	**0**	**0**
S3		Avocado	**$3.5x10^5$**	**$1.3\ x10^5$**	**200**	**$6.9\ x10^3$**	**0**	**0**
S4		Avocado	**$7.7x10^4$**	**$1.2\ x10^4$**	**500**	**$7.2\ x10^3$**	**0**	**0**
S5		Avocado	**$1.6x10^5$**	**$8.6\ x10^4$**	**$7.4\ x10^3$**	**$9.5\ x10^4$**	**0**	**0**
S6		Avocado	**$4.7x10^4$**	**$1.1\ x10^4$**	**$1.6\ x10^3$**	**$1.1\ x10^3$**	**0**	**0**
S7		Avocado	**$9.9x10^5$**	**$9.7\ x10^4$**	**$9.6\ x10^3$**	**$2.1\ x10^3$**	**0**	**0**
S8	Hospital	Avocado	**$2.1\ x10^5$**	**$5\ x10^3$**	**$1.7\ x10^3$**	**0**	**0**	**600**
S9		Avocado	**$2.9\ x10^4$**	**$7\ x10^3$**	**0**	**$6.6\ x10^3$**	**0**	**0**
S10		Avocado	**$2.2\ x10^4$**	**$1.2\ x10^4$**	**$3.6\ x10^3$**	**$1.1\ x10^3$**	**200**	**$2\ x10^3$**
S11		Avocado	**$1.1\ x10^4$**	**$4.6\ x10^3$**	**$1.3\ x10^3$**	**$1.1\ x10^3$**	**$2.5\ x10^3$**	**0**
S12		Avocado	**$1\ x10^5$**	**$7.3\ x10^4$**	**0**	**$4.7\ x10^3$**	**0**	**500**
S13		Avocado	**$4\ x10^3$**	**$2.7\ x10^3$**	**$1.3\ x10^3$**	**0**	**0**	**0**
S14		Avocado	**$1.9\ x10^4$**	**$7\ x10^3$**	**700**	**$4.7\ x10^3$**	**0**	**0**
S15	Mebrat Haile	Avocado	**$1.5\ x10^6$**	**$3\ x10^5$**	**$1.6\ x10^5$**	**$7.6\ x10^4$**	**0**	**0**
S16		Avocado	**$3.2\ x10^4$**	**$1.3\ x10^4$**	**$8.8\ x10^3$**	**0**	**0**	**$1\ x10^3$**
S17		Avocado	**$1.6\ x10^3$**	**600**	**0**	**0**	**0**	**300**
S18		Avocado	**$1.4\ x10^4$**	**$8.3\ x10^3$**	**$1\ x10^3$**	**0**	**0**	**200**
S19		Avocado	**$1.5\ x10^4$**	**$2.\ x10^4$**	**$1.5\ x10^4$**	**960**	**200**	**200**
S20		Avocado	**$9\ x10^3$**	**600**	**200**	**100**	**$1.5\ x10^3$**	**0**
S21		Avocado	**$6.3\ x10^3$**	**$6.5\ x10^4$**	**$2.8\ x10^4$**	**$3.1\ x10^3$**	**0**	**$3.2\ x10^3$**
S22	Posta bet	Avocado	**$1.5\ x10^5$**	**$6.4\ x10^4$**	**$5.9\ x10^5$**	**$2.2\ x10^4$**	**0**	**0**
S23		Avocado	**$3\ x10^5$**	**$2.1\ x10^5$**	**$1.5\ x10^4$**	**$2.5\ x10^3$**	**0**	**0**
S24		Avocado	**$7.6\ x10^3$**	**$1.7\ x10^3$**	**600**	**100**	**0**	**0**
S25		Avocado	**$1.3\ x10^3$**	**$1.1\ x10^4$**	**700**	**300**	**0**	**0**
S26		Avocado	**$1.2\ x10^4$**	**$7.1\ x10^3$**	**300**	**600**	**600**	**900**
S27		Avocado	**$2.4\ x10^4$**	**$3.9\ x10^3$**	**500**	**$1.1\ x10^3$**	**0**	**$2.3\ x10^3$**
S28	Menahari a	Mango	**$4x10^4$**	**$1.7\ x10^4$**	**0**	**0**	**0**	**$3.2\ x10^3$**
S29		Mango	**$8.9\ x10^3$**	**$4.1\ x10^3$**	**$2.5\ x10^3$**	**$1.1\ x10^3$**	**0**	**0**
S30		Mango	**$1.7\ x10^4$**	**$4.4\ x10^3$**	**0**	**0**	**0**	**0**
S31		Mango	**$2.3\ x10^3$**	**$1.1\ x10^3$**	**0**	**0**	**0**	**0**
S32		Mango	**$1.8\ x10^4$**	**$5.4\ x10^3$**	**$2.6\ x10^3$**	**700**	**0**	**$1.2\ x10^3$**
S33		Mango	**$1.3\ x10^4$**	**$6.3\ x10^3$**	**$4.4\ x10^3$**	**$4.5\ x10^3$**	**0**	**0**
S34		Mango	**$1.7\ x10^4$**	**$8\ x10^3$**	**$4.8\ x10^3$**	**300**	**0**	**0**
S35	Hospital	Mango	**$8\ x10^4$**	**$4.3\ x10^3$**	**$1.7\ x10^3$**	**0**	**0**	**$6.1\ x10^3$**
S36		Mango	**$2.9\ x10^4$**	**$1.2\ x10^4$**	**$2.6\ x10^3$**	**200**	**0**	**$2.2\ x10^3$**
S37		Mango	**$2.2\ x10^4$**	**$1.6\ x10^3$**	**$3\ x10^3$**	**$1.9\ x10^3$**	**0**	**$2.5\ x10^3$**
S38		Mango	**$1.6\ x10^3$**	**$1.3\ x10^3$**	**400**	**0**	**0**	**0**

S39		Mango	**3.4 x10^{3}**	**2.9 x10^{3}**	**0**	**100**	**0**	**0**
S40		Mango	**300**	**200**	**0**	**0**	**0**	**0**
S41		Mango	**7.6 x10^{3}**	**1.7 x10^{3}**	**600**	**100**	**0**	**1.2 x10^{3}**
S42	Mebrat haile	Mango	**8 x10^{3}**	**4.8 x10^{3}**	**1.7 x10^{3}**	**300**	**0**	**0**
S43		Mango	**1.7 x10^{4}**	**2.9 x10^{3}**	**400**	**0**	**0**	**3.5 x10^{3}**
S44		Mango	**2 x10^{3}**	**1.7 x10^{3}**	**1.2 x10^{3}**	**700**	**0**	**4 x10^{3}**
S45		Mango	**6.8 x10^{3}**	**1.1 x10^{3}**	**500**	**200**	**0**	**100**
S46		Mango	**7.4 x10^{3}**	**1.7 x10^{3}**	**200**	**0**	**0**	**3 x10^{3}**
S47		Mango	**7.5 x10^{4}**	**9.8 x10^{3}**	**8.6 x10^{3}**	**1.4 x10^{3}**	**0**	**7 x10^{3}**
S48		Mango	**3.2 x10^{4}**	**1.9 x10^{4}**	**1.2 x10^{3}**	**4.2 x10^{3}**	**0**	**0**
S49	Posta bet	Mango	**8 x10^{3}**	**4.8 x10^{3}**	**1.7 x10^{3}**	**300**	**0**	**0**
S50		Mango	**200**	**0**	**0**	**0**	**0**	**0**
S51		Mango	**1.2 x10^{4}**	**1.4 x10^{3}**	**1.2 x10^{3}**	**1.5 x10^{3}**	**0**	**0**
S52		Mango	**6.3 x10^{3}**	**3.2 x10^{3}**	**1.6 x10^{3}**	**800**	**0**	**0**
S53		Mango	**2.2 x10^{4}**	**9.6 x10^{3}**	**2.7 x10^{3}**	**900**	**0**	**6.1 x10^{3}**
S54		Mango	**1 x10^{3}**	**500**	**300**	**100**	**100**	**600**
S55	Menaharia	Papaya	**6.6x10^{4}**	**1.2 x10^{4}**	**1.2 x10^{3}**	**0**	**0**	**0**
S56		Papaya	**2.9 x10^{5}**	**1.9 x10^{5}**	**1.3 x10^{5}**	**1 x10^{5}**	**0**	**0**
S57		Papaya	**1.3 x10^{4}**	**4.9 x10^{3}**	**1.2 x10^{3}**	**6.6 x10^{3}**	**0**	**0**
S58		Papaya	**1.4 x10^{4}**	**1.6 x10^{3}**	**0**	**400**	**0**	**0**
S59		Papaya	**1.1 x10^{4}**	**6.9 x10^{3}**	**4 x10^{3}**	**1.1 x10^{3}**	**0**	**200**
S60		Papaya	**1 x10^{5}**	**1.4 x10^{4}**	**1 x10^{4}**	**1.3 x10^{4}**	**0**	**0**
S61		Papaya	**8.2 x10^{3}**	**9.6 x10^{3}**	**2.3 x10^{3}**	**0**	**0**	**0**
S62	Hospital	Papaya	**1.5 x10^{4}**	**6 x10^{3}**	**300**	**0**	**0**	**2.1 x10^{3}**
S63		Papaya	**1.1 x10^{4}**	**3.8 x10^{3}**	**2.5 x10^{3}**	**1.7 x10^{3}**	**0**	**200**
S64		Papaya	**7.5 x10^{5}**	**9.8 x10^{4}**	**2.7 x10^{3}**	**2.3 x10^{4}**	**0**	**500**
S65		Papaya	**1.1 x10^{3}**	**0**	**0**	**0**	**0**	**0**
S66		Papaya	**3 x10^{4}**	**9.4 x10^{3}**	**700**	**0**	**0**	**300**
S67		Papaya	**1 x10^{3}**	**100**	**0**	**100**	**0**	**500**
S68		Papaya	**1.3 x10^{5}**	**4 x10^{4}**	**0**	**6.8 x10^{3}**	**0**	**200**
S69	Mebrat Hail	Papaya	**1.9 x10^{5}**	**1.9 x10^{5}**	**1.1 x10^{4}**	**4.9 x10^{4}**	**0**	**7 x10^{3}**
S70		Papaya	**2.1 x10^{5}**	**1.9 x10^{4}**	**2 x10^{4}**	**0**	**0**	**1.6 x10^{3}**
S71		Papaya	**4.7 x10^{3}**	**2.6 x10^{3}**	**0**	**1 x10^{3}**	**0**	**1 x10^{3}**
S72		Papaya	**1 x10^{4}**	**1.4 x10^{3}**	**500**	**0**	**0**	**1.5 x10^{3}**
S73		Papaya	**9.6 x10^{4}**	**4.9 x10^{3}**	**1.3 x10^{3}**	**1.8 x10^{3}**	**0**	**700**
S74		Papaya	**2.4 x10^{4}**	**9.4 x10^{3}**	**3.6 x10^{3}**	**1.2 x10^{5}**	**0**	**700**
S75		Papaya	**1.1 x10^{5}**	**4.6 x10^{4}**	**1.3 x10^{3}**	**1.1 x10^{3}**	**0**	**0**
S76	Posta bet	Papaya	**8.2 x10^{4}**	**4.1 x10^{3}**	**200**	**0**	**0**	**0**
S77		Papaya	**4.2 x10^{3}**	**3.2 x10^{3}**	**1.1 x10^{3}**	**500**	**0**	**0**
S78		Papaya	**1 x10^{4}**	**7.9 x10^{3}**	**1.2 x10^{3}**	**0**	**0**	**0**
S79		Papaya	**3.9 x10^{4}**	**8.2 x10^{3}**	**1.8 x10^{3}**	**1.4 x10^{3}**	**0**	**0**
S80		Papaya	**4 x10^{4}**	**8.5 x10^{3}**	**200**	**0**	**0**	**3 x10^{3}**
S81		Papaya	**6.6 x10^{5}**	**1.1 x10^{4}**	**4.4 x10^{3}**	**0**	**0**	**2.2 x10^{3}**

Key

TVC: total Viable bacterial count **TCC**: total coliform count **TFC**: total fecal coliform count

TSC: total staphylococcus count   **TSFBC**: total spore forming bacterial count
TYMC: total yeast and mold bacterial count

Appendix Table 2 . Laboratory Juice processing form

Name of Juice house_______________ Type of Juice: _____________Lab.Serial No._______ Date of sample collection: __________

Type of test	Incubation date and Time	Dilution										Result reading date & time	CFU/ml
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}		
TVC(30oC,24hrs)													
TSFBC(55oC,48hrs)													
TYC(37oC)													
TMC(25oC)													
TSC(37oC,48hrs)													
TCC(37oC,48hrs)													
FCC(42oC,48hrs)													

Key

TVC: total Viable bacterial count **TCC**: total coliform count **TFC**: total fecal coliform count
TSC: total staphylococcus count **TSFBC**: total spore forming bacterial count
TYC: total yeast and mold bacterial count **TMC**: Total mold coun

Appendix Table 3. Observation Checklist

Parameter	Variables	Frequency	Percentage
Hand washing while preparing	Yes		
	No		
Vendor's cloth	Neat		
	Untidy		
Handle juice with bare hand	Yes		
	No		
Long finger nail	Yes		
	No		
Wear hand jewelries	Yes		
	No		
Wipe hand with their owns dresses	Yes		
	No		
Washing utensils with water	Yes		
	No		
Types of glasses used	Disposable		
	Non disposable		
Work place	Neat		
	Untidy		

Appendix 4 . Questionnaires

ሐረማያ ዩኒቨርስቲ

ድህረ ምረቃ ት/ቤት

ባዮሎጂ ዲፓርትመንት

አዲስ /ትኩስ/ ለሚዘጋጁ / ለሚጨመቁ ጁሶች ጥራትና ደህንነት አደጋ የሚሆኑ ነገሮችን ለማወቅ የተዘጋጁ የዳሰሳ ጥናት ጥያቄዎች፡፡

I. **ለሚከተሉትን ጥያቄዎች መልስዎን በተሰጠው ሳጥን ውስጥ የ √ ምልክት በማድረግ መልስ ስጡ፡፡**

የመልስ ሰጪዎች አጠቃላይ መረጃ

1. ፆታ- ወንድ ☐ ሴት ☐
2. ዕድሜ - ከ18 ዓመት በታች ☐ 18-25 ☐ ከ25 በላይ ☐
3. የጁስ አዘገጃጀት የት/ት ደረጃ
   አንደኛ ደረጃ - ☐ የመሰናዶ ት/ት ☐
   ሁለተኛ ደረጃ- ☐ ሌላ (እባክዎትን ይግለፁ) ____________________
4. የሥራ ሁኔታ
   አስተናጋጅ ☐ ጁስ አዘጋጅ ☐
5. የአገልግሎት ዘመን- ከአንድ አመት በታች ☐ 3-5 ዓመት ☐
   - 1-2 ዓመት ☐ ከ5 ኣመት በላይ ☐

II. **የፍራፍሬዎች አዘገጃጀት ሂደትና የመሳርያዎች ንፅህና አጠባበቅ**

6. በዚህ ጁስ ቤት ውስጥ የትኞቹ የጁስ አይነቶች በቦታው በትኩስ ይጨመቃሉ

የፍራፍሬ ጭማቂ ☐ ሌላ ካለ ይግለፁ___________________________

የአትክልት ጭማቂ ☐

7. የፍራፍሬዎች መገኛ ቦታ

ከገበያ ☐ ቀጥታ ከአምራቾች ☐

8. የፍራፍሬ ጊዜያዊ ማከማቻ ቦታ

መደርደሪያ ☐ ፍርጅ ማቀዝቀዣ ☐

ቅርጫት ☐ ሌላ ካለ ይግለፁ ___________________________

9. ለጁስ ዝግጅት የሚጠቀሙበት ውሃ

የቧንቧ ውሃ ☐ የጉድጓድ ውሃ ☐ የምንጭ ውሃ ☐

10. የሚጠቀሙበት ፍራፍሬ

ያልበሰለ ☐ የበሰለ ☐ ከመጥን በላይ የበሰለ ☐

11. ጁስ ከማዘጋጀትዎ በፊት ፍራፍሬውን ያጥባሉ

አዎ ☐ አይደለም ☐

12. ከላይ ላለው ጥያቄ 11 መልስዎ አዎ ከሆነ እንዴት

በውሃ ብቻ ☐ በውሃና በሳሙና ☐ ሌላ ካለ ይግለፁ____________________

13. ጁስ ከመጨመቁ በፊት ፍራፍሬውን እንዴት ያዘጋጁታል

በእጅ ይላጣል ☐ በቢላዋ በእጅ ይከተፋል ☐

ማሽን በመጠቀም ይቆራረጣል ☐ እንዳለ የመጭመቂያ ማሽን ውስጥ ይገባል ☐

14. ጁስ ከማዘጋጀትዎ በፊት የመጠቀሚያ ዕቃዎችን ያፀዳሉ

አዎ ☐ አይደለም ☐

15. ከላይ ካለው 14 ጥያቄ መልስዎ አዎ ከሆነ ፤ እንዴት ይተገብሩታል

በውሃ ብቻ ☐ በውሃ ፤ በሳሙናና በመድሃኒት ☐

በውሃና በሳሙና ☐ በምን ያህል ጊዜ ☐

16. ጁስ አዘጋጁ እራሱን ያፀዳል

ከእያንዳንዱ ጠቀሜታ በኋላ ☐

☐

በተለያዩ የጁስ አይነቶች መሃል

በቀን አንድ ጊዜ ☐

ሌላ ካለ ይግለፁ________________________________

III. <u>የጁስ አያያዝ ልማድና የግል ንፅህና</u>

17. የጁስ አያያዝን በተመለከተ ሥልጠና አሎት አዎ ☐ አይደለም ☐
18. የጁስ አገልግሎት ሲሰጡ ሽርጥ ይለብሳሉ አዎ ☐ አይደለም ☐
19. ለ 18 ጥያቄ መልስዎ አዎ ከሆነ በምን ያህል ጊዜ ሽርጥዎን ይቀይራሉ

በቀን አንዴ ☐ በቀን ሁለት ጊዜ ☐

በሳምንት አንዴ ☐ በሳምንት ሁለት ጊዜ ☐

ሌላ ካለ ይግለፁ ________________________________

20. ፍራፍሬና ጁስ ከማዘጋጀትዎ በፊት እጅዎን ይታጠባሉ

አዎ ☐ አይደለም ☐

21. ለ 20 ጥያቄ መልስዎ አዎ ከሆነ ምን ያህል ጊዜ ይታጠባሉ

ከእያንዳንዱ የጁስ ዝግጅት በፊት ☐

በተለያዩ የጁስ አይነቶች መሃል ☐

በቀን አንድ ጊዜ ☐

በቀን ከ2-3 ጊዜ ☐

22. በየትኛው ውሃ እጅዎን ይታተባሉ

☐

በቧንቧ ውሃ

በውሃና በሳሙና ☐

ሌላ ካለ ይግለፁ ________________________________

ስለ ትብብሮ አመሰግናለው።