MICROBIAL QUALITY OF FRESH FRUIT JUICES PREPARED IN JUICE HOUSES OF DANGILA TOWN, AWI ZONE, AMHARA REGIONAL STATE, ETHIOPIA

M.Sc. Thesis

Biresaw Alemayehu

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MICROBIAL QUALITY OF FRESH FRUIT JUICES PREPARED IN JUICE HOUSES OF DANGILA TOWN, AWI ZONE, AMHARA REGIONAL STATE, ETHIOPIA

A Thesis Submitted to the College of Natural and Computational Science School of Biological Sciences and Biotechnology, School of Post Graduate Program Directorate HARAMAYA UNIVERSITY

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Biresaw Alemayehu

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APPROVAL SHEET
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As thesis research advisors, we here-by certify that we have read and evaluated this thesis prepared under our guidance, by Biresaw Alemayehu entitled; **"Microbial Quality of Fresh Fruit Juices Prepared in Juice Houses of Dangila Town ,Awi Zone, Amhara Regional State ,Ethiopia"** were commend that it be submitted as fulfilling the thesis requirement.

Ameha Kebede (PhD)	_____	_____
Name of Major Advisor	Signature	Date
Sissay Menkir (PhD)	_____	_____
Name of Co-advisor	Signature	Date

As member of the Board of Examiners of the M.Sc. Thesis open defense Examination, we certify that we have read, evaluated the thesis prepared by Biresaw Alemayehu and examined the candidate. We recommended that the Thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Biology.

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DEDICATION

This piece of work is dedicated to my parents, sisters and brothers and Adane Mihiret who made my way bright and helped me from the beginning to the end. It is also dedicated to my lovely Beza Zeleke

STATEMENT OF THE AUTHOR

First of all, I declare that this thesis is my own work and all sources of materials used for it have been accordingly acknowledged. The thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at the Haramaya University and is deposited at the University's Library to be made it available to borrowers under rules of the library. I gravely declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree or diploma.

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Name: Biresaw Alemayehu

Place: Haramaya University

Date of submission: November 2018

BIOGRAPHICAL SKETCH

The author was born on 19 May 1985 in Awi, Amhara Regional State, Ethiopia. He completed his elementary education at Lideta Primary School and Kossober Junior Secondary Schools from1993-2000, and attended his Secondary and Preparatory School education from 2001 to 2004 in Injibara Comprehensive Higher Education Preparatory School. In 2005, he joined Addis Ababa University and graduated with B.Ed.degree in Biology in August 2007. After graduation, he worked for six years in Haddya Zone, Hossana town. For two years, he worked at West Gojjam, Damot, and then he joined the summer program of Haramaya University in July 2015 to pursue his study in biology for his Master of Science degree in biology.

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LIST OF ACRONYMS AND ABBREVIATIONS

ASFBC	Aerobic Spore Forming Bacterial Count
BGLBB	Brilliant Green Lactose Bile Broth
BHI	Brain Heart Influision
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
EE	Enterobacteriaceae Enrichment
EMB	Eosin Methyle Blue Agar
FCC	Faecal Coliform Count
FDA	Food and Drug Administration
FMHE	Federal Ministry of Health Ethiopia
HACCP	Hazard Analysis and Critical Control Point
LB	Lactose Broth
MHA	Muller Hinton Agar
MPN	Most Probable Number
MSA	Manitol Salt Agar
NA	Nutrient Agar
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
SCA	Simmons Cirate Agar
SC	Staphylococcus Count
SSA	Salmonella Shigella Agar
TAVBC	Total Aerobic Viable Bacterial Count
TCC	Total Coliform Count
TSA	Triptic Soya Agar
XLD	Xylose Lysine deoxycholate Agar
YMC	Yeast and mould count

TABLES OF CONTENTS

TABLES OF CONTENTS (Continued)

TABLES OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES IN THE APPENDIX

MICROBIAL QUALITY OF FRESH FRUIT JUICES PREPARED IN JUICE HOUSES OF DANGILA TOWN, AWI ZONE, AMHARA REGIONAL STATE, ETHIOPIA

ABSTRACT

Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits or their juices. Improperly prepared fresh fruit and vegetable juices are recognized as one of the major causes of food borne illnesses. Therefore, this study was aimed at assessing the microbial quality of fresh fruit juices (avocado and mango) prepared in juice houses of Dangila Town and their hygienic conditions of preparation. Twenty four fresh fruit juice samples were collected from four different sites of Dangila town and analyzed for their microbial quality. Microbial quality was determined by quantifying the total aerobic viable bacterial count (TAVBC), Aerobic spore forming *bacterial* count (ASFBC), *Staphylococcal count (SC), Yeast and Mould count (YMC), using spread plate count method with appropriate media and Entrobactriaceae count, Total coliform count (TCC) and Faecal coliform count (FCC), were determined by using the most probable number (MPN) method. Structured questionnaires were distributed for 30 participants working in juice houses to obtain preliminary information on hygienic and safety practices of fruit juice makers and handlers. Results show that the mean of TAVBC, ASFBC, SC, YMC, were 1.702 x 10^5cfu/ml, 2.75 x 10^4cfu/ml, 2.00 x 10^4cfu/ml, 1.52 x 10^4cfu/ml, 1.35 x 10^4cfu/ml, respectively for mango juices and 2.77 x 10^5, 1.32 x 10^4, 9.31 x 10^4, 1.97 x 10^4, 1.13 x 10^4 cfu/ml, respectively for avocado juices. Mean of total entrobaceriaceae counts were 261*$\pm$*40*MPN/ml in mango juices and 587±136MPN/ml in avocado juices. Mean of total coliform counts were 367±110MPN/ml in mango juice and 822±124MPN/ml in avocado juices. Mean faecal coliform counts were 28±2MPN/ml in mango juices and 58±21MPN/ml in avocado juices. Most venders obtained fruits from the open market and most juice makers lacked special training in food hygiene and safety. According to the current study, the results may be attributed to contamination during processing and handling of fresh fruit juices. Therefore, regular surveillance and training on food hygiene, handling of fruit juices and hygiene of venders can improve the quality of fresh fruit juices. Vendors should use refrigerator to store fruits to reduce microbial contamination. Pathogenic bacteria such as E.coli, Staphylococcus aureus, Shigella spp. and Salmonella spp. were isolated following standard methods. The bacterial isolates were tested for their sensitivity to common antibiotics using the disc diffusion method on MuellerHinton Agar. Most isolates were susceptible to gentamicin, ciprofloxacin and chloramphenicol. Almost all isolates were resistance to erythromycin, and tetracycline.*

Keywords : *Antibiotic, Avocado, Dangila, Juice, Mango, Microbial, Quality, Susceptibility,*

1. INTRODUCTION

Fruit juices are well recognized for their nutritive value, mineral and vitamin content and are common in many tropical countries. Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits or their juices (Shakir *et al.,* 2009). Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (Kalia and Gupta, 2006).

Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust, water and by handling at harvest or during postharvest processing. Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ofor *et al*., 2009).

In developing countries, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations (Amoah *et al.,* 2009). Thus despite their nutritional and health benefits, the report of current outbreak of cholera in Addis Ababa city related to raw contaminated foods including fruits and vegetable products and water can be considered as evidence (FMHE, 2016)

Improperly prepared fresh fruits and vegetable juices are recognized as an emerging cause of food borne illnesses (Sandeep *et al.,* 2004). Even in developed counties microbial food-borne diseases affect an estimated one-third of the population each year (Andargie *et al.*, 2008). There are reports of food borne illnesses associated with the consumption of fruit juices worldwide (Sandeep *et al*., 2001). According to the study conducted in Jimma town, most of the fruit juices being served in Jimma had higher microbial loads than the specification set for fruit juices in some parts of the world and these products were thought be the cause of health problems (Tsige *et al.*, 2008).

In response to the increasing number of food borne illnesses, governments all over the world are intensifying their efforts to improve food safety (Sudershan *et al.*, 2009). Besides, in Ethiopia, especially in large cities no continuous survey/assessment of food safety has been implemented in fruit juice houses where fresh fruit juices are sold (Fekadu.2017) and no published information exists on the microbial quality of the most popular juices, i.e. avocado and mango juices, prepared in Dangila town in particular. Therefore, this study was aimed at determining the microbial quality of fresh fruit juices prepared in juice houses of Dangila town with the following objectives.

General objective: -

- To assess the degree of fresh fruit juice (Avocado and Mango) contamination by microbes and asses level of awareness about food safety and pathogenic microorganisms as well as the hygienic conditions of the fruit juice processing from all juice houses of Dangila Town.

Specific objectives were

- To determine the levels (loads) of indicator microbial groups in fresh fruit juices consumed in all juice houses of Dangila Town
- To identify some bacterial pathogens present in fresh fruit juices collected from juice houses of Dangila Town
- To investigate the antimicrobial susceptibility of the bacterial pathogens isolated from fresh juices to some of the commonly prescribed antibiotics in the study area

2. LITRATURE REVIEW

2.1. Fruit Juice

Fruit juice are defined in the most general sense as the extractable fluid contents or tissues of the fruit or aqueous liquid squeezed or extracted usually from one or more fruits (Bello *et al*., 2014). It is the aqueous liquid expressed or extracted from one or more fruits or vegetables, purees of the edible portions of one or more fruits or vegetables, or any concentrates of such liquid (FDA, 2002).). Fruit juices are important sources of nutrients and contain several important therapeutic properties that may reduce the risk of various diseases. They contain large amounts of antioxidants, vitamins C and E, and possess pleasant taste and aroma (Abbo *et. al.*, 2006). Fruit juices are nutritious drinks with great taste and health benefits (Suaads and Hamed, 2008). Fruit juices are mainly used for their nutritional value, refreshing nature also for their medicinal importance. In detoxification of human body and in improvement of blood lipid profile in patients of hypercholesterolemia, fruit and vegetable juices play great roles.

2.2. Composition of Fruit Juice

The major component of the fruit juice is water. The other most common constituent is carbohydrates which comprise sucrose, fructose and glucose. Also, limited amount of protein and minerals are found in fruit juices. Fruit juices are known as considerable sources of ascorbic acid (vitamin C). Especially citrus fruits and juices are good sources of ascorbic acid, folic acid, vitamin B1, thiamine and potassium. It was noted that a cup of citrus juice (240 ml) provides vitamin C in the quantity of more than daily requirement (Bates *et al,* 2001). Mango juices are perfect to replenish salts, vitamins and energy after physical exercise. In gall bladder cancer a protective effect of mangoes consumes has been proven. Mango juice also contains a lot of tryptophan, the precursors of serotonin (Bates *et al,* 2001).

2.3. Microbial Quality of Fruit Juice

The International Organization for Standardization defines quality as the totality of features or characteristics of a product that bear on its ability to satisfy the stated or implied needs. The

quality of vegetables and fruit can only be maintained after harvest; thus it is absolutely imperative to harvest promptly especially at the peak quality period (Bello *et al.,* 2016b). This is because overripe or immature fruit may have short shelf life in storage compared with those picked at appropriate maturity levels (Eni *et al.,* 2010).

According to recent study conducted in Bahir Dar, Ethiopia, reported that mean total viable count was 7.49 *log* cfu/ml (Mekonen and Tadele, 2016). The study conducted in Bair Dar, Ethiopia, reported 4.76*log*cfu/ml mean total viable bacterial count in fresh Mango juices (Asmamaw and Mulugeta, 2012). According to the study conducted in Jima town (Tsige *et al.,* 2008), the mean aerobic mesophilic bacteria counts (cfu/ml) of avocado, papaya and pine-apples were 8.0 $x10^6$, $3.1x10^7$, and $7.9x10^6$, respectively. The counts of yeasts were relatively higher in avocado ($4.5x10^5$cfu/ml) and pine-apple (5.0 x 10^6cfu/ml) as compared to that of papaya ($6.2x10^3$cfu/ml). Unpublished study conducted in Debre-Markos,North-Western Ethiopia, reported mean Total aerobic viable count (TAVBC), Aerobic spore forming bacterial count (ASFBC) ,Staphylococcal count (SC), Yeast count (YC) and Mould count (MC), were 2.2 x 10^6, 1.3 x 10^4 ,4 x 10^2, 1.1 x 10^6, 1.5 x 10^4 (cfu/ml) respectively in fresh mango juices and 3.6 x 10^6, 8 x 10^3, 2.7 x 10^4, 1.2 x 10^6, 2 x 10^3(cfu/ml) in fresh avocado juices respectively (Kindu, 2015).

According to the study conducted on the microbiological quality of freshly squeezed or freshly prepared fruit juices sold by local market vendors in Dhaka city, the total fungal counts were in the range of 1.0×10^1 to 8 $.05\times10^4$ cfu/ml **(**Shakir *et al*., 2009). Rashed *et al.* (2012) investigated to resolve the microbiological attributes of the fruit juices collected from different areas around Dhaka city. To check the total bacterial load, coliforms and staphylococci 26 vendor fruit juices and 15 packed juices were examined. Samples were found to harbor viable bacteria within the range between 10^2 -10^7cfu/ ml. According to this research, thirty samples exhibited the presence of staphylococci. Total coliforms were detected in 31 samples within the range of 10^2 - 10^6cfu/ ml which were further detected as *Escherichia coli* and *Klebsiella* spp. Fecal coliforms were found in 4 vendor fruit juice samples (10^2cfu/ ml), while in the industrially packed samples, they were completely absent. Overall, the study demonstrates that

the quality of both packed and fresh juices was unsatisfactory and hence the products need to be microbiologically controlled in order to ensure the overall health safety.

Another study by Tasmina *et al.,* (2010) conducted their study to assess the microbial quality of fresh and commercially packed available juices collected from different locations of Dhaka city. A total of six fresh juice and nine commercially packed juice samples were collected. Standard culture techniques were followed to assess total viable count (TVC), total Staphylococcal count (TSC), total *Bacillus* count (TBC) and total fungal count (TFC) on different culture media. The TVC varied from the range from 10^2 to 10^5 cfu/ ml with the higher of 2.4 x10^5cfu/ml. A large number of Staphylococci and *Bacillus* was also found from several samples. Total coliform and fecal coliform was found in six and five (out of fifteen) samples, respectively. Among total coliforms, *Klebsiella* spp., *Enterobacter* spp. along with *E. coli* were detected. From all the assessment it was determined that the microbial quality of commercially packed juice was fairer than that of fresh juice collected from local market.

The study which was conducted in Jessore city in Khulna, Bangladesh by Munjur *et al*., (2014) investigated to resolve the microbiological attributes of the fruit juices collected from different areas. Ten fresh fruit juices and ten commercially packed fruit juices were collected. Standard plate count techniques were followed to assess total viable count (TVC), total coliform count (TCC) and total Staphylococcal count (TSC) on different culture media. Samples were found to harbor viable bacteria within the range between 10^3-10^8 cfu/ml. Nineteen samples exhibited the presence of Staphylococci. Total coliforms were detected in 17 samples within the range of 10^3-10^6 cfu/ ml which were further detected as *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. From all the assessment, the study demonstrates that the quality of both packed and fresh juices was unsatisfactory and hence the products need to be microbiologically controlled in order to ensure the overall health safety.

The research which was conducted in South India by (Joy *et al.,* 2006) aimed at examining the quality and safety of freshly squeezed fruit juices, in a metropolitan city (Visakhapatnam) , based on standard techniques (e.g. culturing on selective media), showed that in most localities the street vended fruit juices remained hygienically poor since bacterial loads (Total viable counts and Total coliforms) on the whole are abnormally high (HVC 0.88-33.6×10^4 cfu/ 100

ml; TC 0.8-22.2×10^4 cfus/ 100 ml). Based on the presence of fecal coliforms (0.4-11.0 cfu/ 100 ml) and fecal Streptococci (0.0-6.6 cfu/ 100 ml), it is concluded that fruit juices in certain areas inside the city are highly impacted and unfit for human consumption.

Overall, it is contended that contamination is mainly due to poor quality of water used for dilution, prevailing unhygienic conditions related to washing of utensils, maintenance of the premises, and location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and overcrowding. The occurrence of pathogenic *E. coli*, *Streptococcus faecalis*, *Salmonella typhi* and *Salmonella typhimurium* is alarming enough for an immediate action by the suitable agency. It is suggested that regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks.

Another study was aimed and done to assess the microbial quality of fruit juices sold for immediate consumption in the markets of Kashmir valley. Twelve fruit juice samples (three from each apple, orange, pineapple and mango juices) were procured from different markets and tested for their microbiological quality. Microbial quality was determined by enumerating the total viable count. About 25% of the samples (orange juice) did not comply with the standards of microbial quality as per the guidelines (Appendix II) for microbiological quality of ready to eat foods while as apple, mango and pineapple juices complied with the standards. The microbial load in orange juice was comparatively higher than that in the apple, pineapple and mango juice which had the microbial load within acceptable limits (Gulzar *et al.,* 2013).

2.4. Water Supply

Water used in processing establishments must be clean unless it is used solely for fire protection or auxiliary services and there must be no connection between the system for that water and the system for potable water. The other serious problem associated with food borne illness is unhygienic water supply that may be used for dilution of fruit juices. According to the research conducted in Hawassa Town, Ethiopia, a total of sixty (60) water samples (30 from the containers and 30 directly from taps) from 10 venders were analyzed for bacteriological quality of water which was used for preparation of unpasteurized fruit juices

and washing of glasses and other equipments (Mesfin. 2011). Among these, the most probable number of water from the tap of each vender was zero (0 MPN/ml) whereas the most probable numbers (MPN/100ml) of water samples from the container of each vender were in range from one (1MPN/100ml) to nine (9 MPN100ml) .

2.5. Pathogenic Microorganisms Found in Fruit Juice

Microbial growth is unlikely at low pH, it is well documented that pathogenic microorganisms may survive in fruit juices, become adapted to the acid environment, and cause outbreaks of food borne illnesses (Parish, 2009). Orange juice (pH 3.1) did not allow the survival or growth of the test organisms at proper temperatures (Yigeremu *et al.,* 2001). Contamination of fruit juices sold in restaurants, cafes and even roadside stalls are sometimes unacceptable for human consumption and create significant health problems **(**Shakir *et al*., 2009).

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 (Neha and Tumane, 2011). Fruit juices and minimally processed fruits and vegetables have also been involved in food borne disease outbreaks. It appears that the acidic property of some juices does not always prevent the survival of organisms like *E. coli, Salmonella,* viruses and *Cryptosporidium* (SCF, 2002).

2.6. 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 and 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).

TAVB (Total Aerobic Viable Bacteria)

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 $3.00x10^2$ to $9.60x10^8$. *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.

Coliform bacteria

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. According to research conducted in Visakhapatnam City, India, all street vended fresh fruit juices in many parts of the city showed contamination with faecal coliforms and faecal Streptococci (Lewis *et al.*, 2006). This study conducted in Vishakhapatnam city reported that in pineapple juice the total viable count was 18.8×10^4 cfu/ml and it had total coliforms ranging from 11.4 to 22.4×10^4 cfu/ml, which indicates a very high contamination by coliforms. The presence of *E. coli* and other coliform bacteria could be due to inadequate hand washing by food workers and the absence of good manufacturing practices (Tambekar *et al.*, 2007).

Fecal coliform bacteria

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 coliform group is indicative of organisms originating in the intestinal tract of humans and some animals.

Spore formers

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.

Staphylococci

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℃ and have the ability to grow at low aw (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 micro flora present on/in several parts of the human body (Nester, 2001).

Yeasts and moulds

Most fruit juices are acidic enough and have sufficient sugar to favor the growth of yeasts. 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).

According to the study conducted on the microbiological quality of freshly squeezed or freshly prepared fruit juices sold by local market vendors in Dhaka city, the total fungal counts were in the range of 1.0×10 to 8.05×10^4 cfu/ml (Shakir *et al*.,2009). 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 (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).

Some of these properties include the low pH of fruit juices, the positive oxidation-reduction potential of the fruit juices, and the rich nutrient composition of the juice (Obire *et al*., 2008; Okigbo and Obire, 2009). In developing nations like Nigeria, it has not been possible to have

control over the processing of hawked foods, because most of the vendors lack the adequate knowledge of food processing and handling practices (Essien *et al*., 2011).

2.7. Microbial Spoilage Related to Fruit Juices

Food spoilage is defined as a change in the appearance, smell or taste of a food that makes it unacceptable to the consumer. Spoilage of fruit and vegetable juices is primarily due to the proliferation of their natural acid tolerant and osmophillic microflora. Fresh vegetables and fruits become contaminated with microorganisms during production, harvest, packing, and distribution (Bartz and Wei, 2003). Spoilage microorganisms also can enter plant tissues during fruit development, either through the calyx (flower end) or along the stem, or through various specialized water and gas exchange structures of leafy matter. Successful establishment, however, requires the spoilage microbe to overcome multiple natural protective barriers. Fruits and vegetables possess an outer protective epidermis, typically covered by a natural waxy cuticle layer containing the polymer cutin (Lequeu.*et al*., 2003). A diverse community of epiphytic microorganisms that present a further competitive barrier to the spoilage organism also typically colonizes the outermost fruit

Many fruits and vegetables present nearly ideal conditions for the survival and growth of many types of microorganisms. The internal tissues are nutrient rich and many, especially vegetables, have a pH near neutrality. Their structure is comprised mainly of the polysaccharides cellulose, hemicellulose, and pectin. The principal storage polymer is starch. Spoilage microorganisms exploit the host using extra cellular lyric enzymes that degrade these polymers to release water and the plant's other intracellular constituents for use as nutrients for their growth. Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage (Miedes and Lorences, 2004). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas, 2005b).

The causative agents of microbiological spoilage in fruits and fruit juices can be bacteria, as well as yeasts and molds. The main spoilage agents can be considered as due to the low pH of most fruits. Some bacteria such as *Campylobacter* spp., *E. coli O157:H7*, *Salmonella* spp.,

Listeria monocytogenes, Staphylococcus aureus, *Shigella* spp, *Erwinia* spp*., Enterobacter* spp., *Alicyclobacillus* spp., *Propionibacterium cyclohexanicum*, *Pseudomonas* spp., and lactic acid bacteria can cause spoilage in fruit and fruit juices (Walker and Phillips, 2008).

2.8. Food borne disease outbreaks

Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetable borne outbreak, occurred in Japan in 1996 in which 11,000 people affected and about 6,000 cultures were confirmed. The outbreak involved the death of three children and was carried by *Escherichia coli.* The Centers for Disease Control and Prevention (CDC) reported the occurrence of 6,647 food borne disease outbreaks between 1998 and 2002 (CDC, 2006), 55% of them being associated with bacterial pathogens. Among these, almost 5% were associated with vegetables, fruits, nuts, and related products revealing a low contribution of these products to the total number of outbreaks. However, the importance of juices as vehicles of food borne pathogens has increased in recent

In the USA, in 2013, the Centers for Disease Control and Prevention (CDC) reported 818 food borne disease outbreaks, resulting in 13,360 illnesses, 1,062 hospitalizations and 16 deaths. In the developing world epidemiological data on food borne diseases remain scarce. Even the most visible food borne outbreaks often go unrecognized, uninvestigated or unreported and may only be visible if connected to major public health or economic impacts.

Similarly coliforms were observed, in fresh fruit and vegetable juices sold by the street vendors of Nagpur city (Titarmare *et al*., 2009). However, outbreaks occurrence, mainly since the 1980s, resulted in more attention being given to acidic fruit juices, which were further implicated in food borne disease outbreaks. In more recent times, the rapid dissemination and search for exotic fruits or juices from high pH fruit, such as melon and watermelon, has brought a new challenge to the fruit juice industry. The challenge is related to the fact that these juices provide good conditions not only for the survival, but also for the growth of food borne pathogens. The occurrence of these cases displays the need for research concerning deferent aspects of these microorganisms and their control (Bevilacqua, 2008a, b).

Information obtained from investigations on food borne disease outbreaks and spoilage episodes are very useful in order to further develop the practices adopted during fruit juice production. Classical outbreaks such as those involving *E. coli O157:H7* in apple cider (FDA, 1996) and the increased number of outbreaks associated with fruit and vegetables (Sivapalasingam *et al.,* 2004) resulted in significant changes in fruit/fruit juice production regulations/guidelines.

For example, it resulted in the adoption of regulations that demand the use of :*1)* label warning consumers of potentially harmful bacteria in juices or beverages containing juices that have not been pasteurized and/or submitted to any cumulative processing steps in order to prevent, reduce or eliminate the pertinent pathogen by achieving a 5-log reduction (FDA ,1998, 2002), 2*)*The Hazard Analysis and Critical Control Point (HACCP) rule (FDA, 2001), and 3) the guide line to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (FDA 1998), that highlights the importance of achieving temperature differentials (either by heating the water or by air cooling the fruit or vegetable before immersion in cold water) in order to avoid pathogens present on fruit surfaces or in the water being internalized.

2.9. Antibiotic Resistance

The antimicrobial resistance of bacteria isolated from food and other sources has increased from time to time (Vicas and Singh, 2010). Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst food-borne pathogens (Farzana *et al.*, 2009). The incidence of resistant bacteria in foodstuff is a worldwide phenomenon. The study which was conducted in different areas around Dhaka city (Rashed *et al.,* 2012) to resolve the microbiological attributes of the fruit juices, drug resistance among the isolates was found against ampicillin, ciprofloxacin, amoxicillin, erythromycin, chloramphenicol, ceftriaxone, piperaciline, trimethoprime-sulfomethoxazole, nalidixic acid and vancomycin.

The study which was conducted in Ethiopia, MetamaYohhanes (Muammed, 2016), the results revealed that the susceptibility of *E.coli* was higher in ciprofloxacin followed by norfloxacin and ceftraxone with 86%, 82% and 76% respectively. The lowest percentage of susceptibility

was manifested against tetracycline (10.7%) and ampicillin (14.2%). *E. coli* isolate showed moderate resistance to cotrimoxazole and amoxicillin. As in case of *Staphylococcus aureus* the results revealed that the susceptibility was 100% for ciprofloxacin followed by norfloxacin and amoxicilin with 93%, 81% sensitivity respectively. The lowest percentage of sensitivity was manifested against ampicilin (12%). *S.aureus* isolates showed moderate sensitivity to Cotrimoxazole. *Salmonella* and *Shigella* have been reported to be resistant to antibiotics such as tetracycline and chloramphenicol by Beyene *et al*. (2011). As in case of *Shigella* spp. the results revealed that the susceptibility was higher in almost all antimicrobial drugs used in this study except for cefoxitin and tetracycline with 100% intermediate and 100% resistance respectively. *Shigella* spp. in this study seem to be highly susceptible to gentamicin, this is unlike studies in other places Asrat (2008).

Another study conducted in Ethiopia, Axum Town (Haftom, *et al.* 2017), most isolates were susceptible to ampicillin, gentamicin, ciprofloxacin, and chloramphenicol. All isolates were resistance to erythromycin, and most isolates were resistance to ciprofloxacin, amox-clavul acid, ceftriaxone, and tetracycline. According to the finding, erythromycin was not active against all bacterial isolates. All isolates of *S. aureus* were resistance to erythromycin and amox-clavul acid. 17.6% and 58.8 of isolates were resistance to tetracycline and ciprofloxacin, respectively, 41.1% and 64.7% to gentamicin and chloramphenicol, respectively. All isolates were sensitive to penicillin and cotrimoxazole. High rates of drug resistance were observed for *Staphylococcus* spp. against ampicillin (93%) and amoxicillin (92%). Some *E. coli* isolates were resistant to amox-clavul acid, cotrimoxazole, ampicillin, and gentamicin. 50% were resistant to chloramphenicol and 83.3% were resistance to. The other study which was conducted in Addis Ababa, (Fekadu,2017), all *E. coli* isolates were completely resistance (100%)to vancomycin and most isolates were moderately resistance to penicillin (78%),ampicillin(67%),sulphonamides(52%),nitrofurantoin(63%),sulphonamides(70%),ciprof loxacin,oxytetracycline(96%),chloramphenicol(96%),and trimethoprim,(85%)were moderately susceptible to *E. coli*. Regarding *Salmonella* isolates all of them were completely resistance (100%) penicillin, ampicillin and vancomycin. However, they were complete susceptible (100%) to ciprofloxacin, oxytetracycline, chloramphenicol, and trimethoprim.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Dangila town from February 2018 to May 2018. Dangila town is located in Western Ethiopia, in the Awi zone of Amhara Region. It is also located at 78 km from Bahir Dar, capital city of Amhara Regional State, and it is located at 485 km from Addis Ababa, capital city of Ethiopia. This town has an altitude and longitude of 11º 16' N, 36º 50' E, respectively, with an elevation of 2137meteres above sea level. In the town, there were four juice houses that prepare and sell unpasteurized mango and avocado fruit juices that can be consumed by the people of the town and visitors.

3.2. Study Design

The design of the study was a cross– sectional survey involving questionnaire-administration to determine the factors related to microbiological quality of fruit juices and laboratory based investigation of the prevalence of selected bacterial pathogens, the levels (loads) of indicator microbial groups, the antibiotic susceptibility of the bacterial pathogens isolated from fresh fruit juice samples collected from Dangila town.

3.3. Sample Size

A total of 24 fruit juice samples of two types (Mango and Avocado) from four sites, i.e.12 samples of each juice type, were collected. This was achieved by collecting three samples for each type of fruit juice in three rounds.

3.4. Sample Collection

Twenty four samples of Avocado and Mango of locally prepared unpasteurized fruit juices were collected from Dangila town in three rounds i.e. two juice samples (one Avocado and one Mango juice) from each juice house in different days. All the samples were collected aseptically on a voluntary basis from participating juice houses in sterile beakers (250 ml), labeled, and immediately transported to Bahir Dar University Microbiology Laboratory in an icebox where they were processed immediately.

3.5. Fruit Juice Sample Processing

For analysis, 25ml of fruit juice was measured using measuring cylinder and transferred to 225ml of sterile peptone water and homogenized by shaking in an aseptic environment, which was achieved by cleaning and disinfecting using alcohol as well as by using Bunsen burner flame. Serial dilutions (10^{-1},10^{-2}, 10^{-3}, 10^{-4} , 10^{-5}) were prepared by taking 1ml from a homogenized sample and adding to sterile test tubes containing 9ml of sterile peptone water and mixing properly (Roberts and Greenwood ,2003).

3.6. Data Collection Methods

Two basic data collection methods were used in this study: Questionnaire-survey and laboratory investigation.

3.6.1. Questionnaire administration

A structured questionnaire was distributed to 30 respondents working in juice houses. The questionnaire was used to obtain information on the demographic characteristics of the respondents, sources of fruit, storage conditions, water source for juice preparation as well as for cleaning purpose, the practice of washing the fruits before making juices, , whether or not the juice makers have had training in food hygiene and safety, awareness about microbial contamination, awareness of symptoms of disease as result of eating contaminated food and individual history of illness in relation with food born disease.

3.6.2. Laboratory based experiment

The laboratory based experiment involved avocado and mango juice sample collection, sample processing, determination of the level (load) of indicator microbial groups , identification of some bacterial pathogens present from the juice sample and testing antibiotic susceptibility of bacterial pathogens .

3.6.3. Microbiological analysis of fruit juice

Microbiological analysis was done using appropriate media designed for enumeration and identification of different microbial groups following standard procedures (Buchanan and

Gibbons, 1974). The total colony count was done by spread plate method using plate count agar for bacteria and potato dextrose agar for fungi (Lateef *et al*., 2005).

3.6.4. Enumeration of microorganisms

In this study different types of microbial loads were observed and microbial counts such as, total aerobic viable bacterial count (TAVBC), aerobic spore-forming bacterial count (ASFBC), staphylococcal count (SC), yeast count (YC), and mould count (MC) were made using the spread plate count method. Entrobacteriaceae count, Total coliform and Faecal coliform counts were determined by using the most probable number (MPN) method (Thomas, 1942). Calculation of MPN was done from the completed test results using the formula employed by Thomas (1942).

$$\text{MPN} = \frac{P}{\sqrt{TN}}$$

Where: P= the number of positive tubes

T= Total quantity of sample in all tubes in ml

N= Total quantity of sample in negative tubes in ml

Total microbial counts were determined as colony forming units per milliliter (cfu/ml), using the following formula

$$N = A \times D$$

Where: N=number of colonies/ml

A=is average colony count per volume plated

D=dilution factor

3.6.4.1. Total aerobic viable bacterial count (TAVBC)

The total colony count of bacteria was performed on plate count agar (Oxoid) in three triplicates using the spread plate method. Plate count agar was prepared based on the manufacturer's instruction. 0.1ml from each of appropriate serial dilutions (10^{-3}, 10^{-4} and 10^{-5}) was used to inoculate the plates of three triplicates. Inoculated plates were incubated at 30-32℃ for 24-48 h and the total colony counts were determined. Total microbial counts were determined as colony forming units per milliliter (cfu/ml).

3.6.4.2 Aerobic spore-forming bacterial count (ASFBC)

For enumeration of spore-forming bacteria, homogenized samples were heat treated at 80℃ for 10 minutes to destroy vegetative cells. A volume of 0.1 ml of appropriate dilution was transferred on to three triplicates of PCA by spread plating technique. Colonies were counted after incubation at 30℃ for 2 days **(**Roberts and Greenwood, 2003).

3.6.4.3. Staphylococcal count (SC)

Enumeration of Staphylococci was done using Manitol Salt Agar (MSA) in three triplicates following standard methods and procedures. From each sample of prepared triplicate serial dilutions (10^{-3}, $10^{-4}$and 10^{-5}), 0.1ml was transferred in to three triplicates of MSA and were spread plated. Three triplicates were incubated at 30ºC for 24-36 hours .Then, each plate was observed after 24 to 30 hours of growth and presumptive colonies were counted (Mahle *et al.,* 2008). For confirmation of *Staphylococcus aureus***,** coagulase test was performed. To do this, inoculums from each presumptive colony of MSA plate was transferred to a separate tube of Brain Heart Infusion (BHI) broth and incubated at 35℃ for 18-24 h under aerobic condition. Then, 0.2 ml of BHI broth culture was transferred into sterile tubes containing 0.5 ml certified coagulase plasma and mixed thoroughly. The mixture was incubated at 35℃ and examined after 1h and 4h. A firm clot, which did not move when the tube is tipped on its side (coagulase reaction), was considered a positive test for *Staphylococcus aureus***.** If no clot was observed, it was considered as a negative test for *Staphylococcus*. Gram staining was also done for confirmation of *Staphylococcus aureus* by preparing smears from the deep yellow opaque colonies.

3.6.4.4 .Yeast count (YC)

From appropriate dilutions (10^{-3}, 10^{-4} and 10^{-5}), 0.1ml of juice sample was plated by the spread plate technique in three triplicates using potato dextrose agar supplemented with 0.1g streptomycin. Colonies were counted after incubating the plate at 25℃ for 5 days. Smooth (non-hairy) colonies without extension at periphery were counted as yeasts.

3.6.4.5 .Mould count (MC)

From appropriate dilutions (10^{-3}, 10^{-4} and 10^{-5}), 0.1ml of juice sample was plated by the spread plate technique in three triplicates using potato dextrose agar supplemented with 0.1g streptomycin. Colonies were counted after incubating the plate at 25℃ for 5 days. Hairy colonies with extension at periphery were counted as molds

3.6.4.6. Enterobacteriaceae count

Enterobacteriaceae were obtained by MPN technique, 1ml of each of the 10^{-1}, 10^{-2} and 10^{-3} dilutions were inoculated into three test tubes of buffered peptone water with Durham's tube and incubated at 37℃ for 18±2 hours. Each presumptive positive tube of buffered peptone water gently swirled and 1ml of each positive culture was transferred to tubes of Entrobacteriaceae Enrichment (EE) broth. Entrobacteriaceae Enrichment (EE) broth tubes were inoculated and incubated for 24 ± 2 hours at 37°C.Tubes of EE broth which were turbid and yellow-green, were considered as positive test for Enterobacteriaceae. Only tubes, which were positive in the medium within 24 hours, were used in the calculation of Enterobacteriaceae.

3.6.4.7. Total coliform count (TCC)

The three-tube procedure using lactose broth (Fawole et al., 2002; Bakare *et al.,* 2003) in three replicates were used to detect the coliform and determine the most probable number (MPN) of coliform .One ml of each of the 10^{-1}, $10^{-2}$and 10^{-3}dilutions were inoculated into three test tubes of LB each containing Durham's tube. It was incubated for 24 hours, the number of tubes in each set of three tubes that shown positive for acid and gas production were recorded. Negative test tubes were re incubated for additional 24 hrs. Each presumptive positive tube of lactose broth (LB) was gently swirled and a loopful of each positive culture was transferred to tubes of brilliant green lactose bile 2% broth(BGLBB) using a sterile inoculating loop. Inoculated BGLBB tubes were incubated for 48 ± 3 hours at 35 ± 0.5°C and examined for gas formation. Formation of gas in any amount in the inverted via at any time within 48 ± 3 hours was recorded as a positive confirmed test. Those tubes which formed gas as a result of incubation process were evaluated according to the MPN table and results of a test were reported as MPN per ml of sample (FDA, 2001).

3.6.4.8. Faecal coliform count (FCC)

One ml of each of the 10^{-1} 10^{-2} and 10^{-3} dilutions were inoculated into three test tubes of LB with Durham's tube and incubated at 37℃ for 48 hours. Each presumptive positive tube of lactose broth was gently swirled and a loopful of each positive culture was transferred to tubes of Escherichia coli (EC) broth using a sterile inoculating loop. Inoculated EC broth tubes were incubated for 48 ± 3 hours at 45 ± 0.5°C. Gas production in an EC broth culture was considered as positive fecal coliform reaction. Only tubes, which were positive in the medium within 24 hours, were used in the calculation of fecal coliforms. Results of a test were reported as MPN per ml of sample (FDA, 2001).

3.6.5. Detection of major bacterial pathogens found in fruit juices

Detection of the pathogens was done using appropriate selective media and procedures outlined by Food and Drug Administration (FDA, 2001).One ml of juice sample was mixed with 9 ml of normal saline solution and incubated at 37℃ for 24 hrs. After overnight incubation, one loopful from the mixture was aseptically transferred and streaked on the prepared different culture media.

3.6.5.1. Detection of *Salmonella* spp**.**

All homogenized samples were incubated at 37℃ for about 24 h. Subsequently, 1 ml of the pre-enrichment culture was added to 10 ml of Selenite Cystine broth and incubated for 24 h at 37℃. A loopful of culture from the selective-enrichment broth was sub-cultured onto Xylose Lysine Deoxycholate (XLD) agar and then incubated under aerobic atmosphere at 37 ℃ for 24 hours. Colorless and red colonies with/without black center on XLD agar were picked and were streaked onto Nutrient agar for purification purpose. After 24 hours of incubation at 37 ℃ under aerobic atmosphere, a single colony of bacteria was taken from the nutrient agar and inoculated into Tryptic Soy agar slant. The slant was incubated at 37^{o}c under aerobic atmosphere for 24 hours. Finally, after 24 hours of incubation the slant was preserved in 5^{o}C for the purpose of biochemical test.

3.6.5.2. Detection of *Shigella* spp.

All homogenized samples were incubated at 37°c for about 24 h. Subsequently, 1 ml of the pre-enrichment culture was added to 10 ml of Selenite Cystine broth and incubated for 24 h at 37^{o}C. A loopful of incubated Selenite cystine broth was streaked on *Salmonella Shigella* agar (SS agar) and incubated at 37^{o}c for 24 hours. Colorless less than 2mm colonies were assumed as *Shigella* spp. The suspected colonies were cultured in sterile dextrose broth. One loopful organism was sub cultured in to the biochemical test.

3.6.5.3. Detection of *Staphylococcus aureus*

One ml of homogenized juice sample was transferred into sterile Petri dishes and 15 ml of sterile molten Mannitol Salt Agar (MSA), was poured, swirled and allowed to solidify. Finally the plates were incubated at 37ºC for 24-48 hours and growths of yellow and orange colonies surrounded by yellow zones were observed. Microscopic investigation for Gram reaction and morphological features of suspected colonies were determined using standard method of Gram staining. *Staphylococus aureus* is a Gram positive spherical bacterium (coccus) which on microscopic examination appears as clusters or bounded grape like aggregates of cells.

3.6.5.4. Detection of *E.coli*

One ml of juice sample was added in to 9ml of lactose broth (LB) and incubated at 37℃ for 24hrs.Then positive tubes of LB was gently swirled and a loopful culture was transferred to tubes of 10 ml EC broth using a sterile inoculating loop. Inoculated EC broth tubes were incubated at 45℃ for 24hrs in order to identify thermo tolerant *E.coli* via Gram staining and biochemical test. Then a loopful positive EC broth tubes were streaked on EMB agar medium and incubated at 35℃ for 24hrs and examined for typical nucleated dark centered colonies with or without sheen. Typical colonies were present and picked from EMB plates by touching needle to the center of colony and transferred to a PCA slant for biochemical tests.

3.6.6. Prevalence of bacterial pathogens.

The frequency of occurrence of bacterial pathogens such as *Salmonella* spp. thermo tolerant *E.coli*, *Shigella* spp. and *S.aureus* isolates from avocado and mango juices were carried out by using percentage of positive samples.

3.6.7. Biochemical test

Microorganisms were collected from different culture media according to their growth pattern, morphology, appearance and compared with the morphology of suspected microorganisms. After comparing with suspected organisms the isolates were then sub-cultured and some specific biochemical tests were done for identification. Before biochemical confirmation was done, the presumptive colonies were streaked to nutrient agar aseptically for purification purpose and incubated at 37℃ for 24 hours. The pure cultures were then subjected to biochemical tests as described by (Ganguli *et al.* 2004). Pure colonies were also transferred aseptically from Nutrient Agar (NA) to Triptic Soya Agar (TSA) slants as stock cultures and stored in refrigerator at 4℃. Then organisms were sub cultured in to different biochemical tests such as Triple sugar iron agar, Simmons citrate, Urea broth, Motility test medium and Indol test.

3.6.7.1. Biochemical tests for identification of *Salmonella* and *Shigella* spp.

To identify *Salmonella* spp. and *Shigella* spp*,* the suspected colonies performed previously were sub cultured into sterile dextrose broth and incubated at 37℃ for 24 hrs until the broth being cloudy. Different biochemical tests such as Urea agar, Simmons citrate and Triple sugar iron agar were used for identification of *Salmonella* spp. and *Shigella* spp.

3.6.7.2. Biochemical test for identification of coagulase positive *S.aureus*

Coagulase test was performed by a tube coagulase test. The selected *Staphylococcus* was sub cultured into brain heart infusion broth (BHI) and incubated at 37 ℃ for 24hrs. Then, 0.5 ml of broth culture and 0.5 ml of sterile rabbit plasma were put into a narrow sterile tube and placed in an incubator with a control tube containing a mixture of 0.5 ml of sterile brain heart infusion broth. The tubes were incubated at 37℃ and examined after 4 and 24hrs of incubation

for clot formation. Any sign of coagulation of plasma when compared to the control was regarded as positive for the test.

3.6.7.3. Biochemical test for identification of *E.coli*

Isolates of *E. coli* were subjected to standard biochemical test and Gram staining. The series of biochemical tests were carried out involving Indole test, Simmon Citrate test, Urease test, H_2S gas production test, and glucose fermentation test. A loopful of bacterial culture from pure colonies on eosin methyl blue (EMB) agar was picked and stabbed into Plate count agar (PCA) slant and incubated at 35℃ for 24 hours. For indole test, 5 to 6 drops of Kovac's reagent was used to see the color change from pink to red ring on top for a positive *E. coli* test.

3.7. Antimicrobial Susceptibility Test

From twenty isolates of pathogenic bacteria, total fifteen isolates were tested for their sensitivity to the antibiotics by means of the disc diffusion method using Mueller-Hinton Agar (Difco,Detroit,MI), described previously by Bauer *et al*. (1966)) using *E. coli* ATCC 25922 and *S.aureus* ATCC 25923 as standard cultures obtained from Microbiology Laboratory of Bahir Dar University. Cefoxitin (Cx 30µg), Ciprotoxacin (Cip 5µg), Chloramphenicol (C 30µg), Cotrimoxazole (Cot 25µg), Gentamicin (Gen 10µg), Tetracycline (Te 30µg), Erythromycin (E 15µg) and Vancomycin (Va 30µg) were used for susceptibility test.

The test was done by picking five colonies of each isolate and introducing them into 5 ml of Nutrient broth. The inoculated broth was incubated for 24 hours; this was used as source of standard inoculums. Sterile cotton swab was dipped into the suspension and spread evenly over the entire surface of Mueller-Hinton Agar. Then antibiotic discs were placed onto the surface of the inoculated plates and incubated at 37^{o}C for 16-18hrs. The zone of inhibition of growth around each disk was then measured in millimeter and zone of diameters were interpreted in accordance with standards as susceptible, intermediate and resistant (CLSI, 2017). If clear zone diameter was larger than resistant diameter scale and less than susceptible diameter, this was intermediate, which means the specific bacteria is neither resistant nor susceptible to the specific antibiotic. On the other hand if no clear zones or very small clear

zones were observed around to the antibiotics, they were interpreted as resistant; and if clear zones were larger than the intermediate, they were interpreted as susceptible to the antibiotics.

3.8. Data Analysis

After the completion of data collection, each measurement of the different variables were systematically organized into tables and subsequently subjected to statistical analysis. Data analysis was done using the SPSS computer software version 21. ANOVA was used to compare mean values of different parameters .Statistically significant differences were indicated by P values ≤ 0.05.

4. RESULTS AND DISCUSSION

4.1. Demographic Characteristics of the Respondents

The socio-demographic profile of respondents was presented in Table1. Among 30 respondents, 11(36.7%) were males and 19 (63.3%) were females. About 20(66.7%) of the respondents were aged between 21 - 23 years. About 24 (80%) respondents had completed High School grade and 2 were attending in Elementary School. Four respondents were graduated College diploma. There was no University joined respondents among the study participants. Reasons for proliferation of microorganisms in fruit juices could also be attributed to the fact that the most juice producers lacked special training in food hygiene and safety, as it was established in this study that 25(83.3%) of participants did not have such training, 5(16.7%) of the participants had training related to safe handling and processing of fruit juices. Of course it does not mean only trained personnel prevent contamination of fruits without constant surveillance and good manufacturing processes. Regarding previous history of illness, 23(76.7%) study participants had history of sickness in relation with food borne disease.

Demographic characteristics of respondents were disagree with the work of Tsige *et al.* (2008) who reported that all the ninety fruit juice makers interviewed were females and 54 (60.0%) of them were younger than 30 years. Although 45 (50%) of them had completed or were attending high school education, none of the fruit juice makers had any exposure to professional training related to their current career.

Table1: Demographic profile of respondents in juice houses of Dangila Town (N=30**)**

Variables		A		B		C		D			
		N	%	N	%	N	%	N	%	Tot	%
Sex	M	2	18.2	7	63.3	1	9.1	1	9.1	11	36.7
	F	0	0	7	36.8	7	36.8	5	26.3	19	63.3
Age	15_17	0	0	0	0	0	0	0	0	0	0
	18_20	0	0	3	37.5	5	62.5	0	0	8	26.7
	21_23	1	5	10	50	3	15	6	30	20	66.7
	Above 23	1	50	1	50	0	0	0	0	2	6.7
Educational status	Illiterate	0	0	0	0	0	0	0	0	0	0
	1_8	0	0	1	50	0	0	1	50	2	6.7
	9_10	1	5	7	35	8	40	4	20	20	66.7
	11-12	1	25	2	50	0	0	1	25	4	13.3
	Diploma	0	0	4	0	0	0	0	0	4	13.3
	Degree	0	0	0	0	0	0	0	0	0	0
Training in food hygiene & safety	Yes	1	20	4	80	0	0	0	0	5	16.7
	No	1	4	10	40	8	32	6	24	25	83.3
History of illness in relation with food born disease	Yes	2	8.7	11	47.8	4	17.4	6	26.1	23	76.7
	No	0	0	3	42.9	4	57.1	0	0	7	23.3

Key: A, B, C and, D represent juice houses, N=number of respondents, Tot=total

In four sites of juice houses, the participants reported the source of fruits used for the processing of juices was primarily from the open market. According to the temporary storage of fruits, only one juice house was using baskets as storage which was reported by 8(26.7%) respondents and another one was using refrigerators as reported by juice producers and handlers 14(46.7%) , but the rest two were storing on shelves reported by 8(26.7%) as shown from Table 2. But, observation reveals that two of juice vending houses stored fruits outside in a condition that is exposed to temperature abuse and dust. This may contribute to rapid growth of contaminant microbes in fruits. If the washing practice of these fruits is poor, microbes may get entry during juice making process. The percentage of respondents was in line with the work of Bello *et al.,* (2014) who reported that source of fruits used for the processing of juices

were majorly from the open market; the temporary storage sites of fruits were shelves (33.33%), baskets (41.67%) and refrigerators (10%).

Table 2: Sources of fruits and storage places in juice houses of Dangila Town (N=30)

		Percentage of respondents from juice houses									
Variables		A		B		C		D			
		N	%	N	%	N	%	N	%	Tot	%
Sources of fruits											
	Open market	2	6.7	14	46.7	8	26.7	6	20	30	100
	From producers	0	0	0	0	0	0	0	0	0	0
Storage place	Basket	0	0	0	0	8	26.7	0	0	8	26.7
	Refrigerator	0	0	14	46.7	0	0	0	0	14	46.7
	Shelf	2	25	0	0	0	0	6	75	8	26.7

Key: A, B, C and D stand juice houses, Tot=total, N=number of respondents

4.2. Respondents' level of awareness towards: Microbial Contaminations, Food Safety as well as Hygienic Conditions of the Fruit Juice Processing

As can be seen from Table 3, a lot of participants (66.7%) had the knowledge that microorganisms can contaminate fruits and fruit juices while (33.3%) respondents did not have awareness. Sixteen respondents (53.3%) had the knowledge of symptoms of disease as a result of eating contaminated foods while 14(46.7%) did not know symptoms of disease resulting from eating contaminated food. Almost all of respondents reported that fruits were cleaned during processing and tap water was used for cleaning of fruits and for juice making purposes. Observation reveals that lack of potable water for various activities, venders re used the water for cleaning utensils and used materials. But, none of them reported that well water was used for fruit juice preparation in any of the juice houses. None of the fruit juice makers was practicing using antiseptics to wash fruits required in preparation of fruit juices.

Table.3: Respondents' level of awareness towards microbial contaminants, awareness of symptoms of disease resulting from eating contaminated foods, hygienic conditions of fruits and source of water for fruit juice processing in juice houses of Dangila Town(N=30)

Variables		Percentage of respondents from juice houses									
		A		B		C		D			
		N	%	N	%	N	%	N	%	Tot	%
Water sources for fruit juice preparation	Tap water	2	6.7	14	46.7	8	26.7	6	20	30	100
	Well water	0	0	0	0	0	0	0	0	0	0
	Spring water	0	0	0	0	0	0	0	0	0	0
Habit of cleaning fruits during processing	Yes	2	6.7	14	46.7	8	26.7	6	20	30	100
	No	0	0	0	0	0	0	0	0	0	0
How fruits are cleaned?	With water only	2	6.7	14	46.7	8	26.7	6	20	30	100
	Water+soap	0	0	0	0	0	0	0	0	0	0
	Water+soap+antiseptic	0	0	0	0	0	0	0	0	0	0
Are you aware that Microorganisms contaminate fruits?	Yes	1	3.3	9	30	8	26.7	2	6.7	20	66.7
	No	1	3.3	5	16.7	0	0	4	3.3	10	33.3
Awareness of symptoms of disease resulting from eating contaminated food	Yes	1	3.3	10	33.3	4	13.3	1	3.3	16	53.3
	No	1	3.3	4	13.3	4	13.3	5	16.7	14	46.7

Key: A, B, C and D represent juice houses, N=number of respondents, Tot=total

4.3. Enumeration of Microorganisms

4.3.1. Total aerobic viable bacterial count (TAVBC)

As depicted in Table 4, a total of twenty four (24) prepared fresh fruit juices samples were cultured for total aerobic viable bacterial count (T AVBC). The mean of total viable count of avocado was 2.77 x 10^5cfu/ml where as the mean count in mango juice was 1.70 x 10^5 cfu/ml. With regard to sample sites, there was statistical difference among the mean TAVBC of mango juice (p=.019) and avocado juice (p=.013). The results of the present study showed that all of the fruit juice samples showed much higher viable bacterial counts than the permitted counts. The specifications for fruit juices served in the Gulf region recommend that the maximum count permitted for total aerobic bacterial count, coliforms, yeast and molds

should be 1×10^4,100, and 1.0×10^3cfu/ml, respectively (Gulf Standards, 2000). The mean total aerobic viable bacterial counts of both samples were found to be above Gulf standards which did not comply with standards. The probable reason for the variation in the mean total sample viable bacterial count may be source of fruit and vegetable salad, geographical variation, microclimate change, seasonal variation, pH and moisture variation, water used for washing and dilution, time of sample collection, hygiene, and incubation time (Yigeremu *et al*.**,** 2001). Also the location by the side of a busy road with heavy vehicular traffic (airborne particles) and overcrowding seem to add to the contamination.

The study conducted in Bangladesh (Shakir *et al*., 2009) demonstrated that the mean total viable count (microbial load) showed the presence of bacteria in all the freshly prepared fruit juices. Shakir *et al.* (2009) also reported that the total aerobic bacteria count of 8.00×10^3 - 8.05 x 10^8cfu/ml for mango juices and the mean total viable count (microbial load) showed the presence of bacteria in all the freshly prepared fruit juices in the range from 3 x 10^2 to 9.6 x 10^8cfu/ml. Mean bacteria count of both juice sample of this study was lower than the finding of the author. Mean total viable bacterial count of both fruits of present study showed microbial load ranging from 2.0 x 10^3 – 4.96 x 10^5cfu/ml.

According to the study conducted in Visakhapatnam City, India, total viable bacterial counts were 0.8-33.6 $\times$ 10^4cfu/100 ml) in all the samples (Lewis *et al*., 2006). Another Study conducted in Vishakhapatnam city by Lewis *et al*. (2006) reported that in pineapple juice the total viable count was 1.88×10^5cfu/ml. In Jimma town Tsige *et al*. (2008) also reported that the mean aerobic mesophilic bacteria counts of avocado, papaya and pine-apples were 8.0 x 10^6, 3.1x 10^7, and 7.9 x 10^6cfu/ml, respectively.

4.3.2. Aerobic spore-forming bacterial count (ASFBC)

The mean ASFBC of mango and avocado was 2.75 x 10^4cfu/ml and 1.32 x 10^5cfu/ml respectively as can be seen from the Table 4. All samples collected from juice houses were contaminated with aerobic spore-forming bacteria. With regard to sample sites, there was statistical difference among the mean ASFBC of mango juice (p=.025) and mean ASFBC of avocado juice (p=.00). The presence of ASFB in almost all the fruit juice may be attributed to

its ability to form spores which are heat resistance. The presence of ASFB in this study might also be attributed to poor handling of fruit juices.

4.3.3. Staphylococcal Count (SC)

In the present study, almost all juice samples were found to be contaminated with *Staphylococcus* spp. This was in agreement with an earlier work done in Nagpur city, India by Bagde *et al. (*2011*).* The presence of *Staphylococcus* spp. in almost all the juice samples can be attributed to contamination via handling. This may be due to poor personal and domestic hygiene indicating lack of knowledge of hygienic practices and safety of food products (Tambekar *et al.*, 2009). The results of the present study showed that the highest Staphylococcal count (3.05 x 10^5cfu/ml) for avocado juice was found in juices samples collected from juice house C, but mean Staphylococcal count of avocado was 9.31 x 10^4cfu/ml and mean SC of mango was 2.00 x 10^4cfu/ml. According to study conducted in Nigeria, the highest number of *Staphylococcus* species (3.5 x 10^4cfu/ml) was observed in avocado juices (Bello *et al.,* 2014). Even though the type of juices to show high number of *Staphylococcus* species was similar in both study, but *Staphylococcal count* was relatively high in this study.

The difference in colonial count between the studies may attribute to different factors such as geographical variation, seasonal variation, hygiene, incubation time, sample transportation time, handling, processing and storage. With regard to sample sites, there was statistical significant difference among the mean SCs (2x10^4cfu/ml) of mango juice ($p = 0.00$) and the mean SCs (9.31 x 10^4cfu/ml) of avocado juice *(*$p = 0.00$)

4.3.4. Yeast and mould count (YMC)

The mean mould count was 1.35 x 10^4cfu/ml in mango juices and 1.13 x 10^4cfu/ml in avocado juices. The presence of yeast and molds in many of the juices suggest that handling of the fruits and extraction of the juices methods may fall short of acceptable standards (Al- Jadah and Robinson, 2002). The mean yeast count of avocado juice (1.97 x 10^4cfu/ml) recorded in this study was lower than yeast count (3 x 10^4cfu/ml) reported in the work of Bello *et al.,* (2014), and mold count (1.13 x 10^4cfu/ml) was also lower than the author's finding (4 x 10^4cfu/ml). According to the study conducted in Jimma town by Tsige *et al.* (2008) found that

of yeasts count of (4.5×10^5, 5.0×10^6 and 6.2×10^3) cfu/ml in avocado pine-apple and papaya respectively.

Shakir *et al.* (2009) showed that the presence of fungi in all the freshly prepared fruit juices in the range from 1.00×10^2 to 8.05×10^4 and 1.05×10^2- 8.05×10^4 for mango juices. While yeast and mold count of the current study is lower than the authors report. According to the research conducted on microbiological safety of fruit juices consumed in cafes and restaurants of Debre-Markos Town, the mean yeast count was 1.1×10^6cfu/ml in fresh mango juices and 1.2×10^6cfu/ml in fresh avocado juices. The mean mould count was 1.5×10^4cfu/ml in fresh mango and 2×10^3cfu/ml in fresh avocado juices (Kindu, 2015) which exceeds the present study.

In present study, the mean count of yeast was relatively higher in avocado juices (1.97×10^4cfu/ml) as compared to mean count of mango juices(1.52×10^4cfu/ml) but mean mould count was lower in avocado(1.13×10^4cfu/ml) as compared to mean count of mango juices(1.35×10^4cfu/ml). Across each juice houses, the mean YC (1.52×10^4 cfu/ml) of mango juice ($p=0.071$) and the mean YC (1.97×10^4 cfu/ml) of avocado juice ($p=0.110$) were not statistically significant while mean MC of avocado juice was statistically significant ($p \leq 0.05$)

Table.4: Microbial counts of fresh fruit juices (Mango and Avocado) prepared in juice houses of Dangila Town

	TAVBC		ASFBC		SC		YC		MC	
Sample source	Mango	Avocado	Mango	Avocado	Mango	Avocado	Mango	Avocado	Mango	Avocado
A	0.365	3.039	0.221	0.477	0.047	0.062	0.118	0.181	0.222	0.200
B	0.270	0.387	0.217	0.232	0.043	0.216	0.052	0.082	0.043	0.038
C	3.037	4.127	0.342	3.608	0.415	3.058	0.276	0.335	0.149	0.055
D	3.136	3.529	0.322	0.961	0.294	0.390	0.165	0.193	0.127	0.160
Mean	**1.702**	**2.771**	**0.275**	**1.320**	**0.200**	**0.931**	**0.152**	**0.197**	**0.135**	**0.113**
P-value	**0.019**	**0.013**	**0.025**	**0.00**	**0.00**	**0.00**	**0.071**	**0.110**	**0.161**	**0.015**

Key: A, B, C and D stand for Juice houses where samples were collected. TAVBC =total aerobic viable bacterial count, ASFBC=aerobic spore forming bacterial count, SC=staphylococcal count, YC=yeast count and MC=mould count. Data represent mean microbial counts of 3 samples (three triplicates) per each juice house, mean and one way ANOVA of mean microbial count of 4 juice houses**.**

4.3.5. Total entrobacteriaceae, total coliform and faecal coliform count

Total entrobacteriaceae counts were in the range of 35-460MPN/ml in mango juices and 36->1100MPN/ml in avocado juices. Total coliform counts were in the range of 9.2 - > 1100 MPN/ml in mango juice and 20 - > 1100 MPN/ml in avocado juices. Fecal coliform counts were in the range of 11-28MPN/ml in mango juices and 3-210MPN/ml in avocado juices. Juices had total coliform counts >100 MPN/ml which is maximum permitted level for any juice sold in the Gulf Region (Gulf standards, 2000). Avocado juice was highly contaminated with total coliforms as compared with mango juices. Thus such high MPN/ml of coliforms of juices may probably came from contaminated water used for washing and juicing purpose (Lewis *et al*., 2006).

According to research conducted in Visakhapatnam City, India, all street vended fresh fruit juices in many parts of the city showed contamination with faecal coliforms and faecal Streptococci Lewis *et al.* (2006). The study conducted in Bahir Dar city reported that Total coliform counts were in the range of 9.2 to > 1100 MPN/ml in mango and from < 3 to > 1100 MPN/ml in pineapple juices (Asmamaw and Mulugeta, 2012).

Similarly coliforms were observed, in fresh fruit and vegetable juices sold by the street vendors of Nagpur city Titarmare *et al.* (2009). The presence of *E. coli* and other coliform bacteria could be due to inadequate hand washing and the absence of good manufacturing practices (Tambekar *et al.*, 2007)**.** Coliforms are indication of unsanitary conditions, unhygienic practices during or after production and poor quality of source of water used (Durgesh *et al.,* 2008).

Table.5: Ranges of Total Entrobacteriaceae, Total Coliform and Total Fecal Coliform Count of fresh fruit (Mango and Avocado) juices prepared in Dangila Town

Sample source	Entrobacteriaceae				TCC				FCC			
	Mango		Avocado		Mango		Avocado		Mango		Avocado	
	Range	**Mean±SEM**	**Range**	**Mean±SEM**	**Range**	**Mean±SEM**	**Range**	**Mean±SEM**	**Range**	**Mean ±SEM**	**Range**	**Mean± SEM**
A	35-460	235±123	36->1100	449±329	9.2-1100	375±362	440->1100	887±213	28-35	33±2	20-150	64±43
B	150-290	227±41	27-1100	446±331	29-460	260±125	20-460	200±133	11-16	14±2	3-15	11±4
C	150-460	300±90	460-1100	887±213	150-460	357±103	1100->1100	1100	20-28	25±3	3.6-210	83±64
D	150-460	283±92	150-1100	570±279	120->1100	477±312	1100->1100	1100	15-21	19±2	28-160	75±43
Mean±SEM		**261±40**		**587±136**		**367±110**		**822±124**		**28±2**		**58±21**
Range	**35-460**		**36->1100**		**9.2-1100**		**20->1100**		**11-28**		**3-210**	

Key: A, B, C and D represent juice houses where samples were collected, SEM=standard Error of Mean

4.4. Detection of Bacterial Pathogens from Fruit Juices

Four bacterial genera were detected from the fruit juices and these were characterized as *Sallmonella* spp*, Shigella* spp., *Staphylococcus aureus* and *E. coli***.** Among these isolates, the dominant organism was *Staphylococcus aureus* with 37.5% (9/24) followed by thermo tolerant *E.coli* with 25% (6/24) while the lowest were *Shigella* spp. and *Salmonella* spp. with 8.3 % (2/24) and 12.5% (3/24) respectively**.** This result was in line with the study of Bello *et al*. (2014) who reported that *Staphylococcus aureus* was isolated from avocado juice. Adesetan *et al*. (2013) also reported that *Staphylococcus aureus* and *E. coli*, in their study on street-vended pineapples, pawpaw, watermelons, and coconut.

According to the present study 37.5% of fresh fruit juices were positive for *S.aureus.* The occurrences of *S.aureus* in fruit juices might be attributed to contamination as result of improper handling, processing and storage of fruit juice. The presence of *E.coli* in 25% of fresh fruit juice samples indicate evidence of fecal contamination which may be attributed to poor hygiene practice of juice makers, cross-contamination of juice samples and may be correlated with vendors' awareness. The finding from the questioner about 30(100%) respondents used open market as sources of fruits support this.

Table.6: Bacterial pathogens detected from Avocado and Mango fruits juices collected from juice houses of Dangila Town (N=24)

Isolates	Juices	N	Positive samples	%	P-value
E.coli∗	M	12	3		
	A	12	3		
	T	24	6	25	1.00
S.aureus	M	12	4		
	A	12	5		
	T	24	9	37.5	.689
Salmonella spp.	M	12	1		
	A	12	2		
	T	24	3	12.5	.557
Shigella spp.	M	12	0		
	A	12	2		
	T	24	2	8.3	.152

E.coli∗=thermo tolerant *E.coli,* M=mango, A=avocado, N=number of samples, T=total

4.5. Prevalence of Bacterial Pathogens

In the current study, the incidence of *Salmonella* species and *Shigella* spp. were 15% and 10 % out of 20 fruit juice samples respectively. Similar study in Bangladesh showed that the overall finding of *Salmonella* species was 7.89% in unpasteurized fruit juices (Shakir *et al*, 2009). Another study conducted in India reported that 50% of fruit and vegetables juices were positive for *Salmonella* spp. (Titarmare *et al*, 2009).

According to the study conducted in Amravati city, India the incidence of bacterial pathogen recorded were *E.coli*, *Salmonella* spp. and *S. aureus* 40%, 16% and 6% in street vended fruit juices samples respectively (Tambekar *et al*, 2009). The present study was contrary to a study in Bangladesh which showed that *Staphylococcus* was 6.14%, of the tested samples and (99%) the tested samples showed the presence of *E. coli* (Shakir *et al*, 2009). Another study conducted in India documented that 27.7%, 16.6%, 38.8% of fruit juices were positive for *E. coli*, *Shigella,* and *Salmonella* spp. respectively (Lewis *et al*, 2006). The current study showed prevalence of *Staphylococcus aureus* was 45% out of 20 fruit juice samples and *E. coli* was 30% out of 20 fruit juice samples.

Table.7: Frequency of occurrence of bacterial isolates from Avocado and Mango fruit juice collected from Dangila town (n=20)

	Mango juice		Avocado juice	
Isolate	Frequency	%	Frequency	%
E.coli ∗	3	15	3	15
S.aureus	4	20	5	25
Salmonella spp.	1	5	2	10
Shigella spp.	0	0	2	10
Total	8	40	12	60

4.6. Antimicrobial Susceptibility Testing

From twenty bacterial isolates, a total fifteen isolates were selected for antibiotic susceptibility test and were checked for their antibiotic sensitivity pattern towards eight common antibiotics: Cefoxitin (Cx 30µg), Ciprofloxacin (Cip 5µg), Chloramphenicol (C 30µg), Cotrimoxazole (Cot 25µg), Erythromycin (E 15µg) , Gentamicin (Gen 10µg), Tetracycline (Te 30µg) and Vancomycin (Va 30µg), and the results of the antibiotic sensitivity test were interpreted as the resistant, intermediate and susceptible to the antibiotics based on zone of inhibition (Table 8).

Most isolates were susceptible to gentamicin, ciprofloxacin and chloramphenicol. Except *Shigella* spp., isolates of present study were resistance to erythromycin, and tetracycline. Susceptibility of *S.aureus* was higher in gentamicin followed by ciprofloxacin with 100%, 83% respectively and was sensitive to chloramphenicol and tetracyclin with 66%. Lateef (2004) reported that *S.aureus* were sensitive to erythromycin, gentamicin, and chloramphenicol was agree with this finding. The lowest percentage of *S.aureus* susceptibility was manifested against co-trimoxazole (17%). *S.aureus* isolate showed resistance to co-trimoxazole (66%) and intermediate resistance to erythromycin (50%). As in case of *E.coli* the results revealed that the susceptibility was higher in gentamicin, ciprofloxacin, co-trimoxazole followed by cefoxitin with 100%, 80%, 80%, and 60% sensitivity respectively. *E.coli* was highly resistant to tetracycline (80%) and chloramphenicol (60%). Another study by Rashed *et al*. (2012) found the *E. coli* isolates were highly resistant against ciprofloxacin (61%), was disagree with present study.

Salmonella spp. was highly susceptible to the ciprofloxacin, gentamicin, chloramphenicol cefoxitin and vancomycin with 100%. *Sallmonela* spp. was resistant to erythromicin and tetracycline with 50%. This finding was agree with finding in Ethiopia, *Salmonella* and *Shigella* have been reported to be resistant to antibiotics such as tetracycline and chloramphenicol by Beyene *et al*. (2011) but was disagree with finding of Brooks *et al.(*2006) who reported that *Salmonella* isolates in Kenya were resistant to gentamicin. As in case of *Shigella* spp. the results revealed that the susceptibility was higher in almost all antimicrobial drugs used in this study except for cefoxitin and tetracycline with 100%

intermediate and 100% resistance respectively. *Shigella* spp. in this study seem to be highly susceptible to gentamicin, this is unlike studies in other places Asrat (2008). Even though a reduced level of resistance was detected for tetracycline (70.6%), was reported by Asrat (2008) which was disagreed with present study. This may reflect underlying variations in strain patterns from place to place.

Table.8: Antimicrobial susceptibility patterns of pathogenic bacterial isolates from Mango and Avocado juice samples (%)

¤	Bacterial isolates											
	S.aureus (n=6)			*E.coli* (n=5)			*Salmonella* spp.(n=2)			*Shigella* spp.(n=2)		
Antibiotics	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
Cx	33	33	33	20	20	60	-	-	100	-	100	-
Cip	-	17	83	-	20	80	-	-	100	-	-	100
C	17	17	66	60	-	40	-	-	100	-	-	100
Cot	66	17	17	20	-	80	-	50	50	-	-	100
E	17	50	33	40	20	40	50	-	50	-	-	100
Gen	-	-	100	-	-	100	-	-	100	-	-	100
Te	17	17	66	80	20	-	50	50	-	100	-	-
Va	17	17	66	20	40	40	-	-	100	-	-	100

Key:Cx=Cefoxitin,Cip=Ciprofloxacin,C=Chloramphenicol,Cot=Cotrimoxazole,Gen=Gentamicin,E=Erythromycin,Te=Tetracycline,Va=Vancomycin,R=resistant,I=intermediate, S=susceptible.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits or their juices .Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases. This study was aimed at determining the microbial quality of fresh fruit juices prepared in juice houses and hygienic conditions of the fruit juice processing.

The most juice producers lacked special training in food hygiene and safety skills, as it was established in this study that 25(83.3%) of participants did not have such training; 5(16.7%) of the participants had training related to safe handling and processing of fruit juices. The results of the present study showed that all of the fruit juice samples showed much higher microbial counts than the permitted. The microorganisms present in fruits and vegetables are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce

Based on morphological and biochemical test four bacterial genera were isolated from the fruit juices and these were characterized as *Sallmonella* spp*., Shigella* spp., Staphylococcus *aureus* and thermo tolerant *E.coli.* According to current study, the incidence of *Salmonella* species and *Shigella* spp. was15% and 10 % out of 20 positive fruit juice samples respectively. The current study showed prevalence of *Staphylococcus aureus* was 45% and *E. coli* was 30% out of 20 positive fruit juice samples. Most isolates were susceptible to gentamicin, ciprofloxacin and chloramphenicol. Almost all isolates were resistance to erythromycin and tetracycline.

5.2. Conclusion

In the present study it can be concluded that, the most probable number analysis showed high levels of contamination in juice samples that were prepared in juice houses of Dangila town.

Of course, it doesn't mean that the presence of high number of total coliforms is always associated with presence of pathogens. This reflects the general hygienic conditions during juice processing or handling, be possible because of the poor quality of water may be used in juice preparation; moreover, water is one of the major sources of sewage contamination. Lack of training on food hygiene and safety; improper storage and processing of fruit juices may attribute to contamination of fruit during harvesting or poor processing and handling of fruit juices. 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.

Varied degree of antimicrobial susceptibilities and resistances were observed on bacterial isolates.

5.3. Recommendations

Based on the findings of the present study the following recommendations are made

- Regular surveillance and training on food hygiene, handling of fruit juices and hygiene of venders can improve the quality of fresh fruit
- Vendors should use refrigerator to store fruits to reduce microbial contamination
- Handlers and vendors must have awareness of microbiological contamination can occur during the harvest, by workers, from the soil, from harvest equipment such as knives, from containers, or from transport vehicles.
- Damaged fruits must be avoided because they pose an increased risk for the growth of human pathogens.
- Health agencies must adopt measures to enforce adequate guidelines for juice preparations.
- Since current study was conducted on small sample size, it is also recommended that further study be made using large sample size of variety of juices made from different fruits
- Current study cannot address strains of isolated bacteria, it would have been better to identify up to strain level which would not be done due to absence of facility.

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7. APPENDICES

HARAMAYA UNIVERSITY
GRADUATE PROGRAM DIRECTORATE
SCHOOL OF BIOLOGICAL SIENCES AND BIOTECHNOLOGY

APPENDEX -I

QUESTIONNAIRE

Dear respondents,

My name is Biresaw Alemayehu. I was working as a principal investigator for a research conducted by Haramaya University, School of Biological Science and Biotechnology. My assistant was interview Dangila town juice makers and waiters from four juice houses to find information on the demographic characteristics of the respondents, sources of fruit, storage conditions, water source for juice preparation as well as for cleaning purpose, cleaning habit of the juice makers, the practice of washing the fruits before making juices, whether or not the juice makers have had training in food hygiene and safety, awareness about microbial contamination, awareness of symptoms as a result of eating contaminated foods and history of illness in relation with food born disease and its consequences for study purpose. Therefore, I kindly request you to be volunteer for participating in the interview.

Name of data collector: _____

Questionnaire format sheet to asses safety of fresh fruit juices to be filled by juice makers.

Mark by '√' in the box provided

1. Sex**: - 1.** Male ☐ 2. Female ☐

2. Age: 1. 15-17 ☐ 2. 18-20 ☐ 3. 21-23 ☐ 4.above 23 ☐

3. Educational status of juice maker/waiters:1. Illiterate ☐ 2. 1-8 ☐ 3. 9-10 ☐
4. 11-12 ☐ . 5. Diploma ☐ 6. Degree ☐

4. Source of fruit: - 1.Open market ☐ 2. Directly from producers ☐

5. Temporary storage site: - Basket 1. ☐ 2. Refrigerator ☐ 3. Shelf ☐

6. Water source for juice preparation: 1.Tap water ☐ 2. Well water ☐ 3. Spring water ☐

7. Do you have habit of cleaning fruits during processing? 1. Yes ☐ 2. No ☐

8. If yes, how do you do the cleaning? Mark by '√' in the box provided

1.With Water only ☐

2.With Water and soap ☐

3.With water, soap and antiseptic ☐

9. Do you have training in food hygiene and safety? 1. Yes ☐ 2. No ☐

10. Are you aware that microorganisms can contaminate fruits/fruit juices?

1. Yes ☐ 2. No ☐

11 .Are you aware of symptoms of disease as a result of eating contaminated foods?

1.Yes ☐ 2. No ☐

12. Do you have history of illness in relation with food born disease? 1. Yes ☐ 2. No ☐

Thank you!

April 2018

Dangila

የአማርኛ ቅጅ ቃለ መጠይቅ

ስሜ ቢረሳው አለማየሁ እባላለሁ፡፡ በሐረማያ ዩንቨርስቲ የድህረ ምረቃ ፕሮግራም ዳይሬክቶሬት የባዮሎጅካል ሳይንስና ባዮቴክኖሎጅ ት/ቤት የማስተርስ ዲግሪ ማሟያ ጥናትና ምርምር በዳንግላ ከተማ ውስጥ በሚገኙ ጭማቂ ቤቶች የሚዘጋጁ ፍሬሽ የማንጐና የአቦካዶ ጭማቂዎች ከጥቃቅን ተህዋሲያን ደህንነታቸውን በማጥናት ላይ እገኛለሁ፡፡ለዚህም ይረዳኝ ዘንድ የናንተ ቅን ተሳትፎ አስፈላጊ ስለሆነ ቀጥሎ የተዘረዘሩትን ቃለመጠይቆችን እንድትሞሉልኝ በአክብሮት እጠይቃለሁ፡፡ስለትብብራችሁ በቅድሚያ አመሰግናለሁ

ስም መግፍ አያስፈልግም

ለትክክለኛ ምርጫዎት የ"√" ምልክት በተዘጋጀው ሳጥን ውስጥ ይሙሉ

1. ፆታ ፡ ወንድ ☐ ሴት ☐
2. ዕድሜ ፡ ከ 15-17 ☐ ከ18-20 ☐ ከ21-23 ☐ ከ 23 በላይ ☐
3. የትምህርት ደረጃ ፡ ያልተማረ ከ 1-8 ☐ 9-10 ☐ 11-12 ☐ ዲፕሎማ ☐ ዲግሪ ☐
4. የፍራፍሬ ምንጭ ከየት ነው? ከገበያ ☐ ቀጥታ ከአምራቾች ☐
5. የፍራፍሬ ጊዜአዊ የማስቀመጫ ቦታ: በቅርጫት ☐ ማቀዝቀዣውስጥ ☐ በመደርደሪያ ☐
6. ጭማቂ ለማዘጋጀት የምትጠቀሙት ውሃ ፡ የቧንቧ ☐ የጉድጓድ ☐ የምንጭ ☐
7. ጭማቂ ሲዘጋጅ ፍራፍሬዎች ይታጠባሉ ወይ ?አዎን ☐ የለም ☐
8. ለተራ ቁጥር 7 አዎን ከሆነ እንዴት ነው የሚታጠበው? በውሃ ብቻ ☐ በዉሃና በሳሙና ☐ በውሃ ፣ በሳሙና እና በኬሚካል ☐
9. በምግብ ንጥህናና ደህንነት ስልጠና ወስዳችኂል? አዎን ☐ የለም ☐

10.ጥቃቅን ተህዋሲያን ጭማቂውን ሊበክሉ እንደሚችሉ ግንዛቤ አላችሁ ወይ ? አዎን ☐ የለም ☐

11.የተበከሉ ምግቦችን በመመገብ ለሚከሰቱ በሽታዎች ምልክቶችን ታውቃላችሁ ወይ? አዎን ☐ የለም ☐

12.ከምግብ ወለድ በሽታ ጋር በተያያዘ ከዚህ በፊት ታመው ያውቃሉ? አዎን ☐ የለም ☐

መረጃ ሰብሳቢ ስም ----------ፊርማ ----------ቀን ----------

አመሰግናለሁ

APPENDEX - II

Table i: Mean squares for source of variation of microbial counts in fruit juice

Mango juice					
Parameters	Juice houses mean square	Stan. error of mean	F	P	T-test Sig.
TAVBC	7676285735	50135.22	6.050	.019	.006
ASFBC	129635491	2094.27	5.458	.025	.00
SC	1030633400	5024.9	34.210	.00	.002
YC	267011220	3275.43	3.473	.071	.001
MC	164597920	3275.44	2.244	.161	.003

Avocado juice					
Parameters	Juice houses mean square	Sta.error of mean	F	P	T-test Sig.
TACVB	81680344503	50752.44	6.88	.013	.00
ASFBC	72566168540	42619.43	26.31	.00	.010
SC	60820885972	38784.36	30.23	.00	.035
YC	3257949559.8	3809.93	2.77	.110	.00
MC	188724825.2	2453.62	6.61	.015	.001

APPENDEX- III

Table ii: The Recommended Microbial Standards for Any Fruit Juices (Gulf Standard, 2000&ICMSF,2005)

Standard	Level	TVBC(cfu/ml	TCC(cfu/ml)	FCC(cfu/ml)
Gulf	MBLA	5×10^3	10	0
	MBLP	1.0×10^4	100	0
ICMSF	MBLA		10	0
	MBLB	4.9×10^6	100	10

Key: MBLA=Maximum Bacterial Load Anticipated, MBLA=Minimum Bacterial Load Permitted, ICMSF=International Commission on Microbial Specification for Food

APPENDEX IV

Table iii: Morphological and biochemical characteristics of bacteria isolates from mango and avocado fresh fruit juices prepared in Dangila town

Features	Isolate description			
Cultural shape	Rod	Rod	Cocci	Rod
Color	Red on XLD	Color less on SSagar	Yellow on MSA	Dark centers on EMB
Gram rxn	-	-	+	-
Motility test	Motile	Non motile	Motile	Motile
H_2S test	+	-	-	-
Indol test	-	-	-	+
Glucose fern	+	+	+	+
Citrate test	-	-	+	-
Catalase test	-	-	+	+
Coagulase test	-	-	+	-
Urea test	-	-	-	+
Most probable bacteria	*Salmonella spp.*	*Shigella spp.*	*S.aureus*	*E.coli*

Figure I. Gram staining

Figure II.Biochemical tests

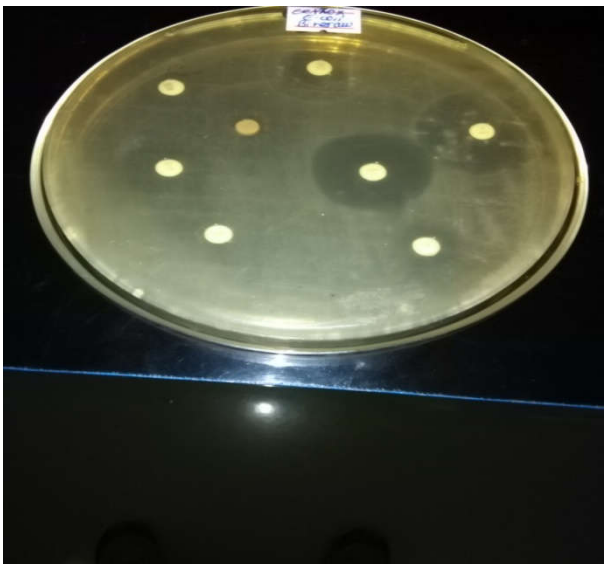

Figure III. Antimicrobial susceptibility test