MICROBIAL QUALITY AND HYGIENIC PRACTICES OF SELECTED FRESH FRUIT JUICES IN HARAR CITY, EASTERN ETHIOPIA

M.Sc. THESIS

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As thesis Research advisors, we hereby certify that we have read and evaluated this Thesis, prepared, under our guidance by Dejene Gurme, entitled **Microbial Quality and Hygienic Practices of Selected Fresh Fruit Juices in Harar City, Eastern Ethiopia.** We recommend that it be submitted as fulfilling the thesis requirement.

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DEDICATION

I dedicate this Manuscript to my mother;Jifare Tulu,my beloved wife Desta Ejersa my brothers and my sisters to achieve at the Master Degree level and success of my life.

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ACRONYMS AND ABBREVIATIONS

AMB	Aerobic Mesophic Bacteria
APHA	American Public Health Association
BGBB	Brilliant Green Bile Broth
BHI	Brain Heart Infusion
BPW	Buffered Peptone Water
CDC	Center for Disease Control
CFU	Colony Forming Unit
DF	Dilution Factor
ECB	Enrichment Culture Borth
EEB	Enterobacteriaceae Enrichment Borth
EMB	Eosin-Methylene Blue Borth
FDA	Food and Drug Agency
HACC	Hazard Analysis and Critical Control
MSA	Manitol Salt Agar
NA	Nutrient Agar
PCA	Plate Count Agar
PDA	Potato DextroseAgar
PH	Power of Hydrogen
SDA	SabouraudusDextros Agar
SD	Standard Deviation

SPSS	Statistical Package for Social Science
SSA	Salmonella Shigella Agar
TC	Total Coliforms
TCC	Total Coliform Count
VRBA	Violet Red Bile Agar
XLDA	Xylose Lysine Deoxycholate Agar
USA	United States of America
WHO	World Health Organization

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ABSTRACT

Freshly prepared fruit juices are widely consumed in urban centers of Ethiopia due to their nutritional value, affordability, and accessibility. However, their microbiological safety remains a growing public health concern, particularly when juices are prepared under poor hygienic conditions. This study assessed the microbial quality and hygienic practices associated with mango (Mangifera indica) and avocado (Perseaamerican)juices vended in Harar city. A total of 20 juice samples (10 Mango and 10 Avocado) were collected from local juice houses such as Addot, Selam, Silase, TeshitaJuice, and Berger house between April and May 2022. Standard culture-based methods were employed to enumerate indicator organisms (total coliforms, fecal coliforms, Escherichia coli) and spoilage microbes (yeasts, molds, Staphylococcus species), and to detect selected pathogens (E. coli, Staphylococcus aureus, Salmonella, and Shigella). Additionally, a structured questionnaire was administered to 30 vendors and consumers to assess socio-demographic characteristics, awareness, and hygienic practices. Data was analyzed using descriptive statistics and chi-square tests. Results showed that both mango and avocado juices were contaminated with coliforms, E. colicounts ranging from 2.42 ± 0.05 to2.77 ± 0.08 log_{10} cfu/mL. Staphylococcal counts were significantly higher in avocado juices (4.97 ± 0.15 log_{10} cfu/mL) than in mango (4.30 ± 0.12 log_{10} cfu/mL, $p \leq 0.05$). Yeasts and molds exceeded Codex limits (≤3 log_{10} cfu/mL) in all samples. Pathogen prevalence included E. coli (30% of samples), S. aureus (40–50%), Salmonella (10–20%), and Shigella (20% in avocado only). All juice samples failed to meet Codex microbiological standards, indicating potential health risks to consumers. Survey data revealed that most respondents were young (67% aged 21–23 years), lacked formal training in food hygiene (83%), and relied exclusively on untreated tap water during preparation.Hence, freshly prepared mango and avocado juices sold in Harar are unsafe for consumption due to high microbial loads and the presence of pathogens. So, it needs urgent interventions on food hygiene training for vendors, stricter regulatory monitoring, improved water quality, and consumer awareness campaigns to safeguard public health.

Keywords: Avocado, Hygienic practices, Mango, Pathogens

1. INTRODUCTION

Fruits are vital components of a healthy diet because of their nutritional and health-promoting properties. They are rich sources of essential vitamins (especially vitamin C), minerals, dietary fiber, and bioactive phytochemicals that act as antioxidants and help protect the body against oxidative stress (Slavin & Lloyd, 2012). Consumption of fruits and their products has been linked to numerous health benefits, including improvement of blood lipid profiles, detoxification, prevention of vitamin deficiencies, reduction of cholesterol levels, regulation of blood pressure, and lowering the risk of non-communicable diseases such as cardiovascular disorders and certain types of cancer (Hung et al., 2004; Minich & Bland, 2007). For these reasons, regular consumption of fruits or their juices is widely recommended as an integral part of a balanced diet (Bhat et al., 2011).

In urban centers, particularly in developing countries, freshly prepared fruit juices have become increasingly popular. They are consumed for their refreshing qualities, relatively low cost, and convenience compared to whole fruits. Among the most widely consumed fruit juices in Ethiopia are mango (*Mangifera indica*) and Avocado (*Persea americana*), both of which are valued not only for their taste and nutrient density but also for their availability across different seasons. These juices are sold in small-scale juice houses, restaurants, and street-side vendors in towns such as Harar, where they are consumed by all income and age groups.

Despite their nutritional advantages, fruits and fruit juices are not inherently sterile. During harvesting, transport, storage, and processing, fruits are exposed to contamination from soil, water, dust, and handling (Ogofure et al., 2017). While whole fruits possess natural protective barriers such as the epidermal peel, cutting, peeling, and juicing remove this protection, leaving the inner tissues susceptible to microbial invasion (Barro et al., 2006). Vendors often slice and prepare fruits in open environments where they are exposed to vectors such as flies, cockroaches, and rodents, as well as environmental contaminants like dust and heat.

The microorganisms associated with fruit juices include natural non-pathogenic microflora, spoilage organisms such as yeasts and molds, and foodborne pathogens of public health significance. Coliform bacteria, particularly *Escherichia coli*, are frequently used as indicators of fecal contamination and hygiene during processing. Other pathogens commonly reported in fruit juices include *Salmonella*, *Shigella*, *Staphylococcus aureus*, and *Campylobacter*species (Mensah et al., 2002; Muinde & Kuria, 2005). The presence of these organisms indicates poor hygienic practices and the potential risk of foodborne outbreaks.

Although fruit juices generally have acidic pH values (2.0–4.5) that limit microbial growth, acid-tolerant pathogens have been shown to survive and remain infectious. Notably, *E. coli* O157:H7 and *Salmonella*spp. have been implicated in outbreaks of foodborne illness linked to unpasteurized juices in several countries (Raybaudi-Massilia et al., 2009). Thus, fruit juices are now recognized as emerging vehicles for foodborne diseases (Dewanti-Hariyadi, 2013).

Microbial spoilage of juices can occur through yeasts, molds, and acid-tolerant bacteria that withstand pasteurization or inadequate cleaning. Spoilage manifests as cloud loss, off-flavor development, gas production, discoloration, and textural changes (Lawlor et al., 2009; Sospedra et al., 2012). Reported spoilage bacteria include *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Gluconobacter*, *Lactobacillus*, and*Zymomonas* species. Yeasts such as *Saccharomyces*, *Candida*, *Pichia*, and *Rhodotorula*, and molds such as *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria*have also been detected in fruit juices (Bevilacqua et al., 2011; ICMSF, 2005). Beyond spoilage, some strains, particularly *Staphylococcus aureus*, can produce heat-stable toxins that remain harmful even after bacterial cells are killed.

The risk associated with fruit juices is compounded by the informal nature of juice vending in developing countries. Vendors often lack formal education on food safety and hygiene, and preparation typically takes place without adequate storage, refrigeration, or protective measures (Muinde & Kuria, 2005). Fruits are frequently washed with untreated tap water or left unwashed, while utensils and containers may be inadequately cleaned (Oliveira et al., 2006). Such practices facilitate cross-contamination and enhance the risk of pathogen transmission to consumers.

In addition to microbial assessments, it is important to evaluate the hygienic practices and awareness levels of vendors and consumers. Several studies have shown that limited knowledge of microbial contamination and foodborne illness symptoms among handlers contributes significantly to unsafe juice preparation (Chumber et al., 2007; Tasnim et al., 2010). Therefore, socio-demographic characteristics, food safety training, and past experiences with foodborne illness are important factors in understanding contamination risks.

Globally,several outbreaks of foodborne diseases have been linked to consumption of unpasteurized fruit juices (Parish, 1997; Ghenghesh et al., 2005). In sub-Saharan Africa, outbreaks associated with contaminated fruit juices are rarely documented due to limited surveillance, but available evidence suggests high microbial loads in street-vended juices (Mosupye& Holy, 2000; Nwachukwu et al., 2008). In Ethiopia, fruit juice consumption is increasing rapidly in urban areas, yet little is known about the microbiological safety of these products. Harar city, a cultural and commercial hub in eastern Ethiopia, has numerous small-scale juice houses where mango and avocado juices are widely consumed. However, microbial quality assessments in this region are scarce, and regulatory oversight remains weak.

Given the rising consumption of fresh juices and the absence of adequate safety monitoring in Ethiopia, it is critical to investigate the microbial quality of juices sold in Harar city. Such evidence is essential for identifying contamination risks, informing public health authorities, and designing interventions to improve food safety. The present study therefore focused on microbial load assessment (coliforms, *E. coli*, *Staphylococcus aureus*, yeasts, molds, *Salmonella*, and *Shigella*) in Mango and Avocado juices. In addition, the study assessed hygienic practices and awareness levels among vendors and consumers, to get a holistic understanding of juice safety in the study area.

General Objective:

- To assess the microbiological quality, and hygienic practices associated withfreshly prepared fruit juices in Mango and Avocado sold in Harar city.

Specific Objectives:

- To determine the levels of indicator microorganisms (total coliforms, fecal coliforms, and *E. coli*) in freshly prepared fruit juices.
- To identify selected bacterial pathogens (*E. coli*, *Staphylococcus aureus*, *Salmonella*, and*Shigella*) present in the juices.
- To assess the hygienic practices and level of awareness regarding food safety among juice vendors and consumers in Harar city.

2. LITERATURE REVIEW

2.1. Spoilage of Fruit Juice

Due to their low PH and sugar content, spoilage of fruit juices is generally caused by yeast and to lesser extent, lactic, acetic and propionic acid bacteria. Various sugar fermenting yeasts (*Debaryomyces, Dekkera, Hanseniaspora Pichia, Zygo saccharomyces*) can be found, and most notably preservatives resistant yeast *Zygo saccharomycesbailii.* This yeast is acid tolerant, xerophillic, extremely resistant to weak acid preservatives and produces carbon dioxide that may lead to distortion or leakage of can or bottles (ICMSF, 2005).To a lesser extent, *Gluconobacter, Acetobacter* and *Lactobacillus*, *Leuconostoc*arealso found to cause fruit juice spoilage. Meanwhile, *Propionate bacterium cyclohexanicum*has been isolated from deteriorated orange juice. These spoilage microorganisms are readily inactivated by pasteurization, thus they are more problematic in unpasteurized fruit juices. Pasteurized or heat treated juices, however, may become spoiled by a thermo acidophilic Bacteria.*Allycloclobacillusacidoterresis.* The outgrowth of *Allycloclobacillus* is influenced by oxygen; type of product and residual spore level afterheat treatment Contamination of this strictly Aerobic spore forming bacterium in fruit juice produces off-flavor and visible growth ((FDA, 2001; Dewanti-Hariyadi, 2013).

2.2. Potential Hazards Associated with Fruit Products

In addition to quality issue growth of mold may pose health risk. *Bissochlamys*, *Penicillium* and *Aspergillus* are of concern since they can produce mycotoxin pineapple juice. Meanwhile, *A. niger*and *A, Carbonarius*arefound to produce other mycotoxin, i. e. ochratoxins, in grape juice. *P. expansum*, for example, is the most commonly found mold that produces patulin in apple juice. This mycotoxin is suspected to have carcinogenic properties although has low toxicity to human. The limit for patulin in apple juice has been set at 50 μg/kg (50 ppb) by various regulatory agencies such as Codex Alimentations Commission (1995), US Food and Drug Administration (2001) and the Indonesian National Agency for Drug and Food Control (NADFC, 2009). Ochratoxin, consisted of ochratoxin A and B, are commonly found in cereals but could also be present in coffee, wine, and grape juice. Ochratoxin A has-been shown to be nephrotoxic (toxic to kidney), hepatotoxic (toxic to liver), teratogenic (causing malformations of an embryo or fetus) and immune toxic (toxic to immune system) to several species of animals, also carcinogenic in mice and rats and causing tumors of the kidney and liver. The European Union as well as the Indonesian NADFC (2009) has set a limit of ochratoxin grape juice.

Unpasteurized fruit juices that do not receive heat treatment may be contaminated by pathogens such as vegetative bacteria, parasites or viruses from human, water and equipment. *Salmonella*, *E. coli* O157:H7, Norvirus and *Cryptosporidium* are the most commonly encountered. Pasteurizations commonly used to reduce the microbial load and inactivate pathogens in fruit juices. Depending on the initial microbial load, various temperature-time combination can be applied to achieve a 5-6log cycle reduction of the microorganisms while it still maintain the nutrient content in the fruit juices (FDA, 2001).

2.3. 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).

2.3.1. Aerobic viable bacterial

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.

2.3.2. 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 within48hours 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 18.8×10^4cfu/ml and it had total coliforms ranging from 11.4 to 22.4×10^4cfu/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).

2.3.3. Fecal coliform bacteria

Some strains of coliform bacteria can be further classified as“fecalcoliforms,”which are defined as Gram-negative facultative rods that ferment lactose at 44.5°C and produce acid and gasfromlactosewithin48hrs.Thefecalcoliformsconsistprimarilyof*E.coli*,butafew*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.

2.3.4. 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.

2.3.5. 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 of7 to 48°C and have the ability to grow low pH(4.8),and high salt and sugar concentrations of 15% and in the presence of NO2. *S. aureus* are naturally present in the nose, throat, skin, and hair of healthy humans, animals and birds(NeerajandSharma,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).

2.4. 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 wereintherangeof1.0×10to8.05×10^4cfu/ml(Shakir*etal*,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; OkigboandObire,2009).In developing nations, it has not been possible to havecontrol 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).

2.5. 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 extracellular 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 mostfruits.Somebacteriasuchas*Campylobacter*spp,*E.coliO157: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).

2.6. Food Borne Disease Outbreaks

Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetable borne outbreak, occurred in Japan in1996 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. 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 streevendorsofNagpurcity(Titarmare*etal*,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, 2008).

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 demandtheuseof:*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),The Hazard Analysis and Critical Control Point (HACCP) rule (FDA, 2001), and 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.

2.7. Control of Microbiological Quality and Safety Control of Fruit Juices

Although food borne outbreaks related to the consumption of unpasteurized fruit juice are often reported, it is possible to produce safe fruit juices. The control has to be placed along the production line starting from the receiving of fruit as the major raw materials. Only fruits produced with Good Agricultural Practices, appropriately mature and sound can be used. Sorting must be done to remove damaged and spoiled part of fruits. Trimming out of rotten apple for making apple juice, for example, has-been reported to reduce 90% of Pauline. Refrigeration is a must and length of storage should be limited. Several guidelines for good hygienic practices in the production of unpasteurized juices are available elsewhere (Dewanti-Hariyadi, 2013).

Properly pasteurized fruit juices are generally considered as safe and very rarely associated with food borne disease outbreaks. To reduce the use of heat, weak acid preservatives (citric acid, benzoic acid, sulfur dioxide or their combination) are generally added. In addition, refrigeration at 5^{o}C or lower has to be applied to control spoilage microorganisms that survive pasteurization. New technologies like high pressure processing has been demonstrated to inactivate microorganisms in certain fruit juices equals to pasteurization while maintaining their flavor since no heat is applied (Bull *et al.*, 2004).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Harar city (Fig 1), Eastern Ethiopia, from April to May 2022. Harar is located at 9°19′N latitude and 42°07′E longitude, with an elevation of 1,885 meters above sea level. The area experiences a mean annual rainfall of 596 mm and an average annual temperature of 24.0°C. Harar is an important commercial and cultural center in eastern Ethiopia, hosting a wide range of food establishments including juice houses, cafés, restaurants, hotels, and markets where fresh fruit juices such as mango and avocado are commonly sold for human consumption. The choice of Harar as a study area was based on the high consumption of fresh fruit juices and the diversity of juice-selling outlets.

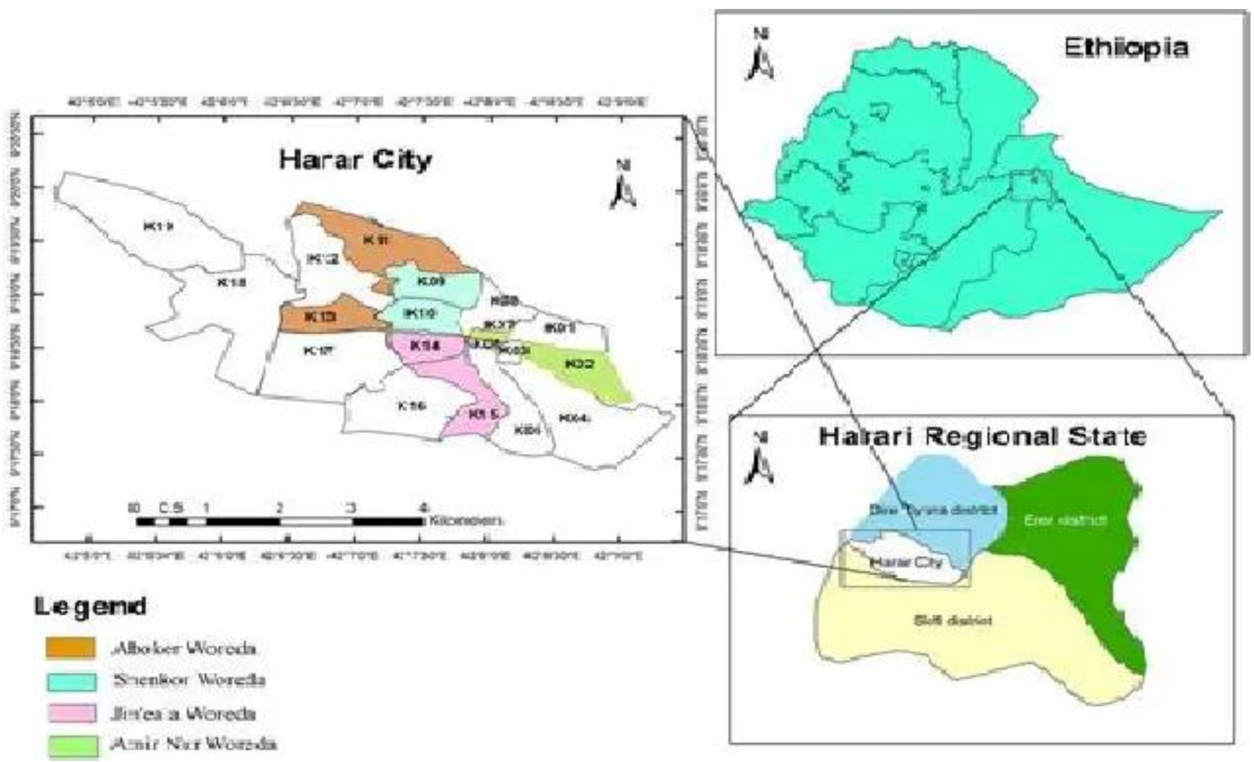

Figure 1. Map of Harar City.

3.2. Study Design

A cross-sectional study design was employed. The study had two major components: Laboratory-based microbiological investigation to determine the presence, prevalence, and load of indicator microorganisms (total coliforms, fecal coliforms, *E. coli*, *Staphylococcus aureus*, yeasts, and molds) and selected foodborne pathogens (*Salmonella*, *Shigella*, and *E. coli*) in freshly prepared mango and avocado juices. Questionairesbased survey of juice vendors to assess socio-demographic characteristics, hygienic practices, awareness of microbial contamination, and history of foodborne illness. This design enabled a comprehensive assessment of both microbial quality of fruit juices and the human factors influencing contamination, providing evidence for potential food safety interventions.

3.3. Sample Collection and Preparation

A total of 20 fresh fruit juice samples, comprising 10 mango and 10 avocado juices, were collectedpurposively from four juice houses in Harar city:Addot Juice House, Silase Juice House, Selam Juiceand Teshite Juice and Berger House.These juice houses were selected based on accessibility willingness to participate in the study and prepaireboth fresh fruite and fresh fruit juice. Each juice sample (25 mL) was collected aseptically in sterile screw-capped flasks and immediately transported to the Microbiology Laboratory at Haramaya University in an icebox maintained at 4°C. Samples were processed within four hours of collection to prevent microbial proliferation.

Prior to microbial analysis, the pH of each juice sample was measured using a digital pH meter. For this, 10 mL of each juice was homogenized in 90 mL of distilled water, and the measurement was conducted in triplicate. Serial tenfold dilutions of each juice were prepared using sterile Butterfield’s phosphate buffer to facilitate microbial enumeration. Appropriate dilutions, ranging from 10^{-1} to 10^{-5}, were used for plating in duplicate.

3.4. Study Population, Sampling Method, and Sample Size

The study population included juice vendors and consumers in Harar city. Purposive sampling was applied to select vendors based on their service quality and willingness to participate in the study. Consumers were randomly selected during the sampling period to provide additional information regarding juice consumption and handling practices.

A total of 30 individuals (vendors and consumers) participated in the questionnaire survey. The sample size was determined according to Kish (1965), who suggested that 30–200 respondents are adequate when the attribute of interest occurs 20–80% of the time in the population and the distribution approximates normality.

3.5. Microbiological Analysis

Microbiological analysis focused on total coliforms, fecal coliforms, E. coli, Staphylococcus aureus, yeasts, and molds. All analyses were performed in duplicates or triplicates as appropriate.

3.5.1. Total coliform count (TCC)

Total coliforms were enumerated on Violet Red Bile Agar (VRBA). Colonies appearing purplish-red with a precipitated bile zone were counted. Microbial counts were calculated using the formula:

$$\text{TCC (cfu/mL)} = \frac{\text{number of colonies x dilution factor}}{\text{Volume of sample plated (mL)}}$$

Where: Number of colonies = colonies counted on the agar plate. Dilution factor = inverse of the dilution used (e.g., 10^3 for 10^{-3} dilution). Volume plated = volume of diluted sample plated (mL). Counts were expressed in $\log_{10}$ cfu/mL**.**

3.5.2. Fecal coliform count (FCC)

One mL of diluted juice (10^{-1}, 10^{-2}, 10^{-3}) was inoculated into lactose broth with Durham tubes and incubated at 37°C for 48 h. Tubes showing gas formation were transferred to EC broth and incubated at 45°C for 24 h. Gas production in EC broth indicated fecal coliforms.

$$\text{MPN/mL} = \frac{\text{Number of positive tubes x dilution factor}}{\text{Volume of sample inoculated (mL)}}$$

Where MPN tables were used to confirm results according to FDA (2001).

3.5.3. E. coli Enumeration

Presumptive coliform-positive cultures were transferred to Enterobacteriaceae Enrichment (EE) broth and incubated at 37°C for 24 h. Turbid and yellow-green tubes were considered positive for *E. coli*. Positive cultures were streaked on EMB agar and confirmed using Gram staining and biochemical tests. Counts were calculated using the standard formula for colony-forming units:

$$\text{E. coli (cfu/mL)} = \frac{\text{number of confirmed colonies x dilution factor}}{\text{Volume plated (mL)}}$$

3.5.4. Staphylococcus aureus count

Serial dilutions (10^{-3}, 10^{-4}, 10^{-5}) were plated on Mannitol Salt Agar (MSA) in triplicate and incubated at 30°C for 24–36 h. Yellow colonies were presumptively identified as *S. aureus*. Confirmation involved Gram staining and coagulase testing in Brain Heart Infusion (BHI) broth. Counts were calculated and expressed as:

$$\text{SC (log10 cfu/mL)} = \text{log10}\left(\frac{\text{number of colonies x dilution factor}}{\text{volume of plated (mL)}}\right)$$

3.5.5. Yeasts and molds

Diluted samples (10^{-3}, 10^{-4}, 10^{-5}) were plated on Potato Dextrose Agar (PDA) supplemented with 0.1 g streptomycin. Plates were incubated at 25°C for 5 days. Smooth colonies were counted as yeasts, while hairy colonies with peripheral extensions were counted as molds. The microbial load was calculated as:

$$\text{Yeast/Mold count (cfu/mL)} = \frac{\text{number of colonies x dilution factor}}{\text{Volume plated (mL)}}$$

All counts were expressed as $\log_{10}$ cfu/mL.

3.6. Detection of Major Bacterial Pathogens

Pathogen detection followed standard FDA protocols (2001).

- *Salmonella*: Juice samples were pre-enriched in normal saline, selectively enriched in Selenite Cystine broth, plated on Xylose Lysine Deoxycholate (XLD) agar, purified on nutrient agar, and confirmed biochemically.
- *Shigella*: Samples were pre-enriched, plated on Salmonella Shigella (SS) agar, cultured in sterile dextrose broth, and confirmed with standard biochemical tests.
- *E. coli*: Positive EC broth cultures were plated on EMB agar and confirmed by Gram staining and biochemical assays.
- *Staphylococcus aureus*: Confirmed via MSA growth, Gram-positive cocci morphology, and coagulase reaction.

3.7. Questionnaire

A structured questionnaire was administered to 30 respondents to collect socio-demographic information, history of foodborne illness, food hygiene training, fruit washing practices, water sources, and awareness of microbial contamination and symptoms. Responses were coded, entered, and summarized for statistical analysis.

3.8. Data Analysis

Data were recorded, organized, and analyzed using Microsoft Excel 2010. Microbial load comparisons between mango and avocado juices were performed using one-way ANOVA. Chi-square tests were used to evaluate associations between vendor hygiene practices, awareness, and microbial contamination levels. Statistical significance was set at $p \leq 0.05$. Descriptive statistics such as mean ± SD and prevalence percentages were used to summarize results.

4. RESULTS AND DISCUSSION

4.1. Bacteriological Analysis of Fruit Juices

The bacteriological analysis revealed that both mango and avocado juices contained coliform bacteria, though at varying levels. *E. coli* counts ranged from 2.42 ± 0.05 $\log_{10}$ cfu/mL in mango to 2.77 ± 0.08 $\log_{10}$ cfu/mL in avocado juice, while total coliforms averaged 2.56 ± 0.07 and 2.91 ± 0.09 $\log_{10}$ cfu/mL, respectively (Table 1). Fecal coliforms were comparatively lower, with 1.36 ± 0.05 $\log_{10}$ cfu/mL in mango and 1.76 ± 0.06 $\log_{10}$ cfu/mL in avocado juices.

Although avocado juices consistently exhibited slightly higher counts across all coliform parameters, these differences were not statistically significant ($p > 0.05$). Notably, all juice samples exceeded the Codex standard for *E. coli***,** which should be absent, but total and fecal coliform counts were mostly within or marginally above the recommended limits (≤100 and ≤10 cfu/mL, respectively). This suggests potential post-harvest contamination or inadequate hygienic handling during juice preparation, highlighting a microbiological safety concern, especially regarding fecal contamination.

Table 1. Mean log10 counts (cfu/mL) of coliforms in mango and avocado juices

Parameter	Mango Juice (Mean ± SD)	Avocado Juice (Mean ± SD)	CodexLimit (cfu/mL)
E. coli	2.42 ± 0.05	2.77 ± 0.08	Absent
Total Coliforms	2.56 ± 0.07	2.91 ± 0.09	≤100
Fecal Coliforms	1.36 ± 0.05	1.76 ± 0.06	≤10

4.2. Staphylococcal, Yeast, and Mold Counts

The analysis of spoilage microorganisms showed that both mango and avocado juices had high microbial loads. Staphylococcus counts were significantly higher in avocado juice (4.97 ± 0.15 $\log_{10}$ cfu/mL) than in mango juice (4.30 ± 0.12 $\log_{10}$ cfu/mL, $p \leq 0.05$) (Table 2). Yeasts and molds, however, did not differ significantly between the two juices, with yeast counts of 4.18 ±

0.14 and 4.29 ± 0.13 log_{10} cfu/mL, and mold counts of 4.13 ± 0.11 and 3.98 ± 0.10 log_{10} cfu/mL in mango and avocado juices, respectively.

All recorded counts for Staphylococcus, yeasts, and molds exceeded the Codex recommended limit of ≤3 log_{10} cfu/mL. This indicates a high risk of spoilage and potential toxin production, particularly by Staphylococcus aureus, which can produce heat-stable enterotoxins that pose health risks even if the bacteria are later inactivated.

Table 2. Mean log10 counts (cfu/mL) of Staphylococcus, yeasts, and molds in mango and avocado juices

Parameter	Mango Juice (Mean ± SD)	Avocado Juice (Mean ± SD)	CodexLimit (cfu/mL)
Staphylococcus	4.30 ± 0.12	4.97 ± 0.15	≤3
Yeasts	4.18 ± 0.14	4.29 ± 0.13	≤3
Molds	4.13 ± 0.11	3.98 ± 0.10	≤3

4.3. Prevalence of Bacterial Pathogens

The prevalence study (Table 3) demonstrated that *E. coli* was present in 30% of both mango and avocado samples, and *S. aureus* was more frequent in avocado (50%) than mango juice (40%). *Salmonella* was detected in 10% of mango and 20% of avocado samples, while Shigella was absent in mango but present in 20% of avocado juices.

Chi-square analysis ($\chi^2 = 1.71$, df = 3, $p = 0.63$) indicated no statistically significant difference in the distribution of pathogens between mango and avocado juices. The presence of these pathogens clearly exceeds the Codex limits (absent or ≤3 log_{10} cfu/mL), suggesting that the juices are unsafe for consumption and could pose a significant risk of foodborne illness. The slightly higher pathogen prevalence in avocado juice may be attributed to its higher nutrient content and consistency, which can favor microbial growth.

Table 3. Prevalence of bacterial pathogens in mango and avocado juices (n=10 per juice type)

Pathogen	Mango Juice	Avocado Juice	CodexLimit (cfu/mL)
E. coli	3 (30%)	3 (30%)	Absent
S. aureus	4 (40%)	5 (50%)	≤3
Salmonella	1 (10%)	2 (20%)	Absent
Shigella	0 (0%)	2 (20%)	Absent

4.4. Socio-demographic Characteristics of Respondents

Among 30 respondents, 11 (36.7%) were male and 19 (63.3%) females. Most (20, 66.7%) were aged 21–23 years. Regarding education, 24 (80%) had completed high school, 4 (13.3%) had a college diploma, and 2 (6.7%) were attending elementary school. Most respondents (25, 83.3%) had no formal food hygiene training, while 5 (16.7%) had received training. A total of 23 (76.7%) reported a history of foodborne illness (Table 4).

Table 4. Socio-demographic profile of juice house respondents (N=30)

Characteristic	Number	Percentage (%)
Sex		
Male	11	36.7
Female	19	63.3
Age (years)		
21–23	20	66.7
>23	10	33.3
Education		
Elementary	2	6.7
High school	24	80.0
Diploma	4	13.3
University	0	0.0
Training in food hygiene		
Yes	5	16.7
No	25	83.3
History of foodborne illness		
Yes	23	76.7
No	7	23.3

4.5. Hygienic Practices and Awareness

All juice houses used tap water for washing fruits and preparing juices. Fruits were cleaned during processing, but no antiseptics were used. Awareness of microbial contamination was reported by 20 respondents (66.7%), while 16 respondents (53.3%) were aware of symptoms of foodborne illnesses. Chi-square analysis was performed for awareness variables (Table 5). Awareness of microbial contamination and awareness of symptoms were not significantly associated ($\chi^2 = 1.062$, df = 1, p = 0.303). Variables with zero counts in one category (water source, fruit cleaning, method) were not be analyzed.

Table 5**.** Hygienic practices and awareness among juice house respondents (N=30) with Chi-square analysis

Item	Category	Number	Percentage (%)	χ^2	df	p-value
Water source for juice preparation	Tap	30	100	NA	NA	NA
	Well/Spring	0	0			
Fruit cleaning habit	Yes	30	100	NA	NA	NA
	No	0	0			
Method of cleaning fruits	Water only	30	100	NA	NA	NA
	Water + soap/antiseptic	0	0			
Awareness: Microbial contamination	Yes	20	66.7	1.062	1	0.303
	No	10	33.3			
Awareness: Symptoms from contaminated food	Yes	16	53.3	1.062	1	0.303
	No	14	46.7			

4.6. Discussion

The present study revealed the presence of coliform bacteria, including *E.coli*, in both mango and avocado juices, with slightly higher counts in avocado juice, though differences were not statistically significant. The detection of E. coli in all juice types (2.42–2.77 $\log_{10}$ cfu/mL) is a serious concern because Codex standards require the absence of E. coli in ready-to-drink juices. Total and fecal coliform counts were mostly within or marginally above recommended limits, indicating potential post-harvest contamination or inadequate hygiene during juice preparation.

These findings are consistent with previous studies. For instance, Akinbode and Odumeru (2009) reported similar *E. coli* contamination levels in fresh fruit juices sold in Nigeria, while Gulumian et al. (2010) observed fecal coliform counts ranging from 1.0 to 2.0 $\log_{10}$ cfu/mL in fruit-based beverages in South Africa. The slightly higher counts in avocado juice may be attributed to its higher lipid and sugar content, which can support microbial growth (Badr et al., 2017). Collectively, these results underscore the persistent risk of fecal contamination in minimally processed fruit juices and highlight the need for improved hygiene and monitoring.

Staphylococcus aureus counts were significantly higher in avocado juice compared to mango, while yeast and mold loads were high in both juice types but not statistically different. All spoilage microorganism counts exceeded the Codex recommended limits of $\leq 3 \log_{10}$ cfu/mL. The elevated Staphylococcus counts are particularly concerning because S. aureus can produce heat-stable enterotoxins, which may cause foodborne illness even after pasteurization (Jay et al., 2005).

Comparable findings have been reported by Ahmed et al. (2012), who detected high Staphylococcus counts (4.0–5.0 $\log_{10}$ cfu/mL) in freshly squeezed fruit juices in Egypt, and by Kumar et al. (2015), who recorded yeast and mold counts exceeding acceptable limits in mango and mixed fruit juices in India. The slightly higher contamination in avocado juice may be linked to its viscous consistency and nutrient-rich matrix, which can protect microorganisms from environmental stresses and promote growth (Rahman et al., 2016). These results indicate that without proper sanitation and microbial control, fresh juices are highly susceptible to spoilage.

The study also confirmed the presence of foodborne pathogens, including *E. coli, S. aureus*, *Salmonella*, and *Shigella*. While chi-square analysis showed no statistically significant difference in pathogen distribution between mango and avocado juices, the prevalence rates (up to 50% for S. aureus in avocado) clearly exceeded safe limits. Similar studies have reported high prevalence of pathogens in fresh fruit juices sold in informal markets. For example, Al-Hindi et al. (2011) detected Salmonella in 15–20% of juice samples in Lebanon, while Ogbu et al. (2014) reported Shigella in 10–25% of street-vended fruit juices in Nigeria. The presence of multiple pathogens emphasizes the potential risk of foodborne outbreaks associated with unpasteurized fruit juices, particularly when served in settings lacking rigorous hygiene controls.

The demographic survey showed that most juice vendors were young (21–23 years) and had only completed high school, with the majority lacking formal food hygiene training. This aligns with findings by Abebe et al. (2018), who reported that street food handlers in Ethiopia generally have limited hygiene awareness, which contributes to microbial contamination of foods. Although two-thirds of respondents were aware of microbial contamination and half were aware of foodborne illness symptoms, this awareness did not translate into improved practices, as all respondents cleaned fruits using only tap water without antiseptics.

These findings suggest that educational interventions and formal training in food hygiene could significantly reduce microbial contamination. Similar studies (Mensah et al., 2002; WHO, 2006) have emphasized that training street food vendors in safe handling practices can reduce contamination by 30–50%.

5. SUMMARY, CONCLUSION, 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 the hygienic conditions of the fruit juice processing.

A cross-sectional study was conducted in Harar, Ethiopia (April–May 2022) to assess the microbial quality of freshly prepared mango and avocado juices. Twenty juice samples were analyzed using standard culture-based methods for indicator organisms and foodborne pathogens, while 30 vendors and consumers were surveyed on hygiene practices and awareness. Data were analyzed using ANOVA and chi-square tests, providing evidence of contamination risks and gaps in food safety practices.

The study revealed that freshly prepared mango and avocado juices sold in Harar city were microbiologically contaminated, raising significant food safety concerns. Both juice types contained coliform bacteria, with *E. coli* counts ranging from 2.42 ± 0.05 $\log_{10}$ cfu/mL in mango to 2.77 ± 0.08 $\log_{10}$ cfu/mL in avocado juices. Although total coliforms (2.56–2.91 $\log_{10}$ cfu/mL) and fecal coliforms (1.36–1.76 $\log_{10}$ cfu/mL) were within or slightly above Codex recommended limits ($\leq$100 and $\leq$10 cfu/mL, respectively), the detection of *E. coli* in all samples exceeded the Codex requirement of absence, indicating fecal contamination and poor hygienic handling during juice preparation. Avocado juices consistently showed higher microbial loads, though the differences were not statistically significant ($p > 0.05$).

Spoilage microorganisms were also abundant. *Staphylococcus aureus* counts were significantly higher in avocado juice (4.97 ± 0.15 $\log_{10}$ cfu/mL) than in mango juice (4.30 ± 0.12 $\log_{10}$ cfu/mL; $p \leq 0.05$), while yeast (4.18–4.29 $\log_{10}$ cfu/mL) and mold (3.98–4.13 $\log_{10}$ cfu/mL) counts did not differ significantly between juice types. All these values exceeded Codex limits of $\leq$3 $\log_{10}$ cfu/mL, suggesting a high risk of spoilage and potential toxin production, particularly by

S. aureus, which can produce heat-stable enterotoxins that remain active even after bacterial inactivation.

The prevalence of pathogens was alarming. *E. coli* was detected in 30% of both juice types, *S. aureus* in 40% of mango and 50% of avocado juices, *Salmonella* in 10% of mango and 20% of avocado juices, and *Shigella* in 20% of avocado but absent in mango juices. Chi-square analysis ($\chi^2 = 1.71$, df = 3, p = 0.63) indicated no statistically significant difference in pathogen distribution between mango and avocado juices, though the slightly higher prevalence in avocado could be attributed to its nutrient-rich and viscous matrix that favors microbial growth. Importantly, the presence of these pathogens violates Codex requirements (absence or $\leq 3 \log_{10}$ cfu/mL), making the juices unsafe for consumption and underscoring their potential role in foodborne disease transmission.

The socio-demographic survey of 30 respondents revealed that the majority were young (21–23 years, 66.7%) and female (63.3%), with most having completed only high school (80%) and lacking formal food hygiene training (83.3%). A striking 76.7% reported a history of foodborne illness. Although 66.7% of respondents were aware of microbial contamination and 53.3% recognized symptoms of foodborne illnesses, these awareness levels were not significantly associated with safer practices. All juice vendors used only tap water for fruit washing and juice preparation, without any use of antiseptics or disinfectants, and cleaned fruits using water alone, highlighting a critical gap between awareness and practice.

Collectively, these results demonstrate that the microbiological quality of fruit juices in Harar city falls below international safety standards. The simultaneous detection of indicator organisms, spoilage microbes, and pathogenic bacteria underscores systemic failures in hygiene and food handling practices at juice houses. The lack of formal training among vendors, coupled with reliance on minimal cleaning practices, further exacerbates the risk. These findings align with previous studies in Africa and elsewhere, which consistently report microbial contamination of unpasteurized fruit juices due to inadequate sanitation, poor infrastructure, and limited regulatory enforcement.

The implications of this study are significant: without urgent interventions—such as hygiene training for vendors, stricter enforcement of food safety regulations, consumer education, and possible consideration of pasteurization or alternative preservation methods—freshly prepared fruit juices in urban Ethiopian settings will continue to pose serious public health risks.

5.2. Conclusion

This study assessed the microbiological safety of freshly prepared mango and avocado juices sold in Harar city. The findings revealed widespread contamination with indicator organisms, spoilage microbes, and foodborne pathogens. *E. coli* was present in all samples at levels exceeding Codex standards, confirming fecal contamination and poor hygienic handling. *Staphylococcus aureus*, yeasts, and molds were detected in high numbers, suggesting inadequate sanitation during preparation and risks of spoilage and toxin production. Furthermore, pathogens including *Salmonella* and *Shigella* were isolated, underscoring the potential of these juices as vehicles for foodborne illnesses. The questionnaire survey highlighted major gaps in food hygiene knowledge and practices among vendors. Most were young, lacked formal training, and relied solely on untreated tap water for washing fruits and preparing juices. Although awareness of contamination was moderate, this did not translate into improved practices, reflecting systemic failures in food safety education and regulation. Overall, the study concludes that freshly prepared fruit juices sold in Harar city are not microbiologically safe for consumption. Immediate interventions are required to reduce public health risks associated with their consumption.

5.3. Recommendations

Based on the findings, the following recommendations were proposed:

- Local health authorities, in collaboration with universities and NGOs, should design and deliver regular training programs for juice vendors on hygienic practices, safe water use, and microbial contamination risks.
- Government agencies should strengthen monitoring and enforcement of food safety standards in juice houses. This includes routine microbiological testing and penalties for non-compliance.

- Vendors should be encouraged to use safe, treated, or boiled water for fruit washing and juice preparation. Subsidized access to disinfectants such as chlorine-based solutions could significantly reduce microbial loads.
- Municipalities should support juice houses in improving sanitary infrastructure, including clean working surfaces, storage facilities, and waste disposal systems.
- Public health campaigns should be conducted to raise consumer awareness of the risks of consuming unpasteurized juices and to promote demand for safer products.
- Future studies should evaluate the effectiveness of low-cost preservation methods (e.g., mild pasteurization, UV treatment, or natural antimicrobial extracts) that can extend shelf-life and reduce contamination without compromising nutritional quality.
- Food safety should be integrated into broader urban health and nutrition policies, ensuring that informal food sectors are not overlooked in public health planning.

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7. APPENDICE

Appendix I. Questionnaires for collecting information about hygienic practices and risk factors associated with fruit contaminations.

<u>Questionnaires to be filled by respondents from household family members</u>

Dear respondents you are expected to genuinely fill out the following questionnaires. The researcher would like to appreciate your cooperation in advance.

I. General information

1. Personal information

Name of respondent ______________________________

2. Sex: - 1) male 2) female

3) age_________

5. Residence area (village) _______________________________ Keble code___________

6. Marital status: a) single b) married c) divorced

7. Occupation (main job): a) waiter b) juice maker c) server) casher

8. Educational background: a. 1-4 b. 5-8 c. above 8 e. >8 & religious education

e) illiterate

II. Information about hygiene- sanitation practices of fruit vendors

1. Have you encountered any fruit juice born diseases? Yes ☐ NO ☐
2. If your answer is Yes, what kind of disease do you know from your experience

a. Typhoid fever

b. Diarrhea,

c. Parasites

d. pneumonia

3. What kind of sanitation practices do you practices in your shop for fresh fruit juices?
    a. The use of detergents for cleaning utensils
    b. Clean clothes/wears
    c. Clean rooms
4. Do you keep fresh juices in refrigerator? Yes/No
5. For how much time do you keep you fresh juices in refrigerators?

a. 1hr B. 4hr C. 8hrs D. 12hrs

6. Do you consider that refrigeration can overcome
   a. microbial contamination Yes/No
   b. prevent fruit spoilage, Yes/No
   c. loss of flavor and texture Yes/No
   d. has no effect
7. What kind of sanitation practices do you practice for fresh fruits surfaces before making juice
   a. Washing with water only
   b. Washing with detergents
   c. Don't wash
8. For how long do you wash your juice machine?
   a. half day
   b. per day
   c. per two days
9. Which cleaning technique do you think is recommended for washing your juice machine?
   a. use of detergent with cold water
   b. hot water with detergent
   c. cold water only

Appendix II. General information from customers

1. Personal information

Name of respondent ______________________________

2. Sex: - 1) male 2) female

3) age_________

5. Residence area (village) _______________________________ Keble code___________

6. Marital status: a) single b) married c) divorced

7. Occupation (main job): a) waiter b) juice maker c) server d) casher

8. Educational background: a. 1-4 b. 5-8 c. above 8 e. >8 & religious education

III. Information about hygiene- sanitation practices of fruit juice customers

1. Have you encountered any fruit juice born diseasees? Yes ☐ NO ☐
2. If your answer is Yes, what kind of disease do you know from your experience

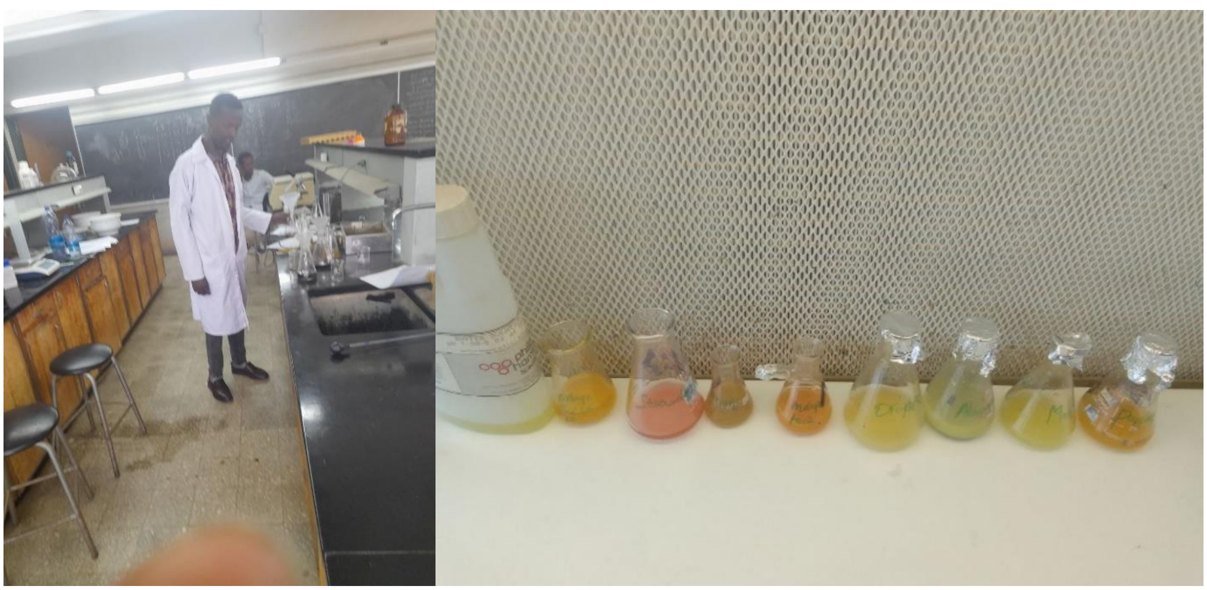
e. Typhoid fever
f. Diarrhea,
g. Parasites
h. pneumonia

3. Do you like to use lemon/ acheto (vinegar) during drinking of fruit juice?
4. What health benefits do you get from fruit?
   a. Vitamins/antioxidants
   b. Energy
   c. Proteins/body repair
   d. others
5. Do you take care of the sanitation practices of the vendors   Yes/No
6. Do you check expiry date of bottled juices Yes/No
7. Which fruit product do you prefer? Fresh fruits/ bottled juices
8. What do you recommend for fruit juice users
   A. preparation at their home
   B. use of good vendors
   C. use of bottled juices

Wish You All the Best!!!

Figure 2. List of Figures in Appendix

A. Media preparation B. Colon counting



C. Potato Deoxyterose Agar

D. Aerobic Mesophylic Bacterial Count

E. Yeast and Molds