



**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
DEPARTEMENT OF BIOTECHNOLOGY**

**TITLE: MICROBIOLOGICAL QUALITY AND SAFETY ASSESSMENT OF FRESH
FRUIT JUICES SOLD IN JUICE HOUSES IN GUBRYE SUBCITY, WOLKITE TOWN**

**A SENIOR RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT
OF BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN
BIOTECHNOLOGY**

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**MAY, 2024
WOLKITE, ETHIOPIA**

Title Page

Microbiological Quality and Safety Assessment of Fresh Fruit Juices Sold in Juice Houses in Gubrye Subcity, Wolkite Town

**A senior research project submitted to The Department of Biotechnology In
Partial Fulfillment Of The Requirements For The Degree of Bachelor Of
Science In Biotechnology**

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APPROVAL SHEET

The Senior Research Project entitled “**Microbiological Quality and Safety Assessment of Fresh Fruit Juices Sold in Juice Houses in Gubrye Subcity, Wolkite Town**,” Submitted to Department of Biotechnology, College of Natural and Computational Science In Partial Fulfillment Of The Requirements for The Degree Of Bachelor of Science In Biotechnology. Therefore, I recommended that the students has fulfilled the requirements and hence here can submit to the department.

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ACKNOWLEDGMENTS

Our deepest and wonderful appreciation goes to our advisor Mr. Aklilu Banjaw who advised us strongly starting from the time of topic selection, development of proposal, research work, and the final write-up and shaping of the Senior Research Project. We would like to extend our thanks to the Mr. Ayansa Kebenessa laboratory technicians of molecular laboratory of wolkite university who helped us to done our laboratory experiment with laboratory room and full materials .Our genuine appreciation is extended to Natural and computational science of and Department of Biotechnology for their give us this opportunity ,follow up and help us in need And we would like to thanks Biology Department for they help us by giving us chemicals.

LIST OF ABBREVIATION

Cm	Centimeter
Ph	Power of Hydrogen
ml	Milliliter
°C	Degree Centigrade
CFU	Colony Forming Unit
FDA	Food and Drug Administration
Km	Kilometer
FAO	Food and Agricultural Organization
Hr	Hour
SNNPR	Southern Nations, Nationalities and Peoples Region

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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. However, during processing contamination from raw materials, equipment or food handlers could be easily transferred to the final product of fruit juices resulting food-borne illnesses. Therefore, the aim of this research was to evaluate the microbiological safety of avocado and papaya fruit juices and to examine the association between the risk factors and the microbial loads of the juice. A cross sectional study was conducted from May 07 /2024 to May 17/2024 in Gubrye subcity .Total *plate count*, *yeast and mold count*, *total coliform*, and the presence of pathogenic microorganisms such as *Staphylococcus aureus*, and *Salmonella* spp. were analyzed following standard methods. The *total coliform* count of avocado and papaya ranged from 1.1×10^6 cfu/ml - TNTC (to numerous to count) and 1.3×10^5 - 2.2×10^5 cfu/ml respectively. The total *Staphylococcus* count obtained from avocado and papaya ranged between 3.5×10^5 - 7×10^5 cfu/ml and 1.5×10^5 - 3.2×10^5 cfu/ml, respectively. The total *E.coli* count obtained from avocado and papaya ranged between 2.6×10^6 - 1.48×10^7 cfu/ml and 3.6×10^5 - 1.3×10^6 cfu/ml, respectively. The total *salmonella and shigella* counts count obtained from avocado and papaya ranged between 3.8×10^5 - 2×10^7 cfu/ml and 6.5×10^5 - 1.7×10^7 cfu/ml, respectively. The total *yeast and mold* counts obtained from avocado and papaya ranged between 6.9×10^5 - 6.1×10^7 cfu/ml and 3.1×10^6 - 7.7×10^6 cfu/ml, respectively. Such results might have been attributed to contamination during processing and subsequent handling of the fresh fruit juices. Therefore, regular supervision and training on safe processing and handling practices of fruit juices as well as monitoring the hygiene of venders are suggested to be implemented to improve the quality of fresh fruit juices in the study area.

Keyword: Avocado, papaya, gubrye subcity, fresh fruit juice

CHAPTER 1:INTRODUCTION

1.1. Background of The Study

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 fruit or more fruits (Bello *et al.*, 2014). Fruit juices are well recognized for their ingredient value, such as mineral and vitamin content and are common in much tropical and desert area of many countries. Most 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). The constituent of juice predominantly is water and also contains carbohydrate, sucrose, fructose, glucose, sorbet and small amount of protein (Pao *et al.*, 2001).

Most people may use fresh fruit juice. The microorganisms may be the potential sources of fruit spoilage which are known to shorten shelf life of fruits (Chen *et al.*, 2010). Moreover, the use of unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notable *E. coli* 0157:H7, species of *Salmonella*, *Shigella* and *Staphylococcus aureus* (Joy *et al.*, 2006). However, the organisms that have become internalized within a fruit can be able to survive during processes to the final product until they reach the consumer (FDA, 2008).

Improperly prepared fresh fruits and vegetable juices are recognized as an emerging cause of food borne illness (Sandeep *et al.*, 2004). The impact of foodborne diseases caused by enteropathogenic bacteria, including *Salmonella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Staphylococcus aureus*, is indeed a global concern with significant public health implications. Here's a citation highlighting the common occurrence and impact of foodborne disease outbreaks associated with these pathogens:(Majumder T *et al.*, (2020)).Therefore, the consequence of in appropriate manipulation and storage conditions, both pathogenic and/or deteriorative microorganisms may be contaminated product, thus increasing the risk of microbial diseases and spoilage (Diaz Cinco *et al.*, 2005). In fact, the number of outbreaks and cases of illness caused

by consumption of fresh cut fruits and unpasteurized juices has increased in the last years (Harris *et al.*, 2003).

In Ethiopia, particularly in large urban areas, fruit juices are available in supermarkets in canned or bottled forms. In addition, fruit juice vending houses, which have been serving different types of fruit juices in fresh forms, are proliferating. However, information on the safety of the fruit juices prepared and consumed in Ethiopia is scanty in general). 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 (Tsige *et al.*, 2008). Therefore, in many parts of Ethiopia continuous assessment of food safety have not yet been conducted on fruit juices that have been prepared in cafes. The same like it in the campus and the available of mass population their use of fresh fruit juice are contaminated. Therefore, the aim of this study could be tray to determine the bacteriological assess (analyze) and quality of fruit juices preparing in cafes. And no published information exists on the microbiological safety of the most popular juices, i.e. Avocado and Papaya juices, consumed in Gubrye Subcity in particular. It could investigate the results generated in the present study would be useful for both the health of consumers and to juice manufacturers to improve microbial safety and hygiene quality.

1.2. Statement of the problem

Microbial have significant effect for quality of squeezed fruit juices which are sold in Fruit juice house. Also, it is difficult to get enough and qualified juices from any Fruit juice house without any problem for consumption. Because, due to any no care for the preparation of juice and lack of fruit storage practice, in almost all juice houses , the fruit juices and other fruit products was contaminated by pathogenic microbes especially *bacteria* and *fungi* that makes fruit juices unacceptable for human's consumption. Keeping the problem in mind, taking care for preparation of juice as well as fruit storing on temporal cleaned area and at optimum temperature is very important for consumers.

1.3. Objective of the study

1.3.1. General objective

- The General objective of this study was microbiological quality and safety assessment of fresh fruit juices sold in juice houses in Gubrye Subcity, Wolkite Town, Southwestern Ethiopia.

1.3.2. Specific objectives

- To isolate and count diverse microorganisms those contaminate fruit juices
- To assess the dominant microorganisms in the fresh juices sold juice houses
- To determine whether fruit juices are free from different microbial contamination
- To determine whether the fruit juices are safe for human consumption or not

1.3. Significance of the study

The significance of this study would be information sources for fruit juice producers and users peoples in order to get some awareness about importance, the sources of contamination which influence the quality of locally prepared fruit juices as well as the way that generate the favorite conditions for the growth of pathogenic bacteria in fruit juices. The study also help to create awareness who to solve the problem have occurred in the gubrye. It also significant for the managers of juice houses to make the juices in right way and to know the amount of prepared juices must be coordinated with his/her buyer in order to minimize the expiration rate. It also help to provide information for further study and to know which type of juice can easily contaminated by food born disease.

1.4. Scope of the study

This study was conducted in Wolkite University, College of Natural And Computational Science, and Department of biotechnology which was mainly focus on microbiological quality and safety assessment of fresh fruit juices sold in fruit juice houses in Gubrye Subcity, Wolkite Town. This Senior Research project was conducted by taking two fresh of fruit juice wick are avocado and papaya from two different juice houses.

CHAPTER 2:LITERATURE REVIEW

2.1. Fruit

Fruit, in strict botanical terms, is the fleshy or dry ripened ovary of a plant, which encloses the seed or seeds. Fruits, either fresh or processed, form an important part of our daily diet, and demand is increasing in all affluent countries of the world (Health Canada, 2006). Recent advances in agricultural technology have contributed significantly to the improved production of fruits throughout the world (FDA, 2002). In addition, the critical advances in fruit processing technologies, refrigeration, transportation, storage, and distribution have made it possible for consumers to enjoy these products year-round. Fruits are very perishable in nature because these are living and respiring tissues (Health Canada 2006).Fruits are important in human diets due to their contributions of vital nutrients, most especially vitamin C, they are very low in fats and proteins but high in sugar as they contain large amount of glucose, fructose, and sucrose. In addition, most fruits are often consumed fresh due to their cherished flavor/palatability and they contribute immensely to nutrients intake since no nutrients intake loss are recorded as a result of cooking as in other cooked staple foods (Abalaka *et al.*, 2013).

Papaya (*Carica papaya* L.) stands foremost among the fruits which have attained a great popularity in recent years. This is mainly due to its easy cultivation, quick returns, adaptability to diverse soil and climatic conditions and above all, it is attractive, delicious and wholesome fruits having multifarious use Papaya are now being cultivated commercially in almost all the tropical and subtropical countries of the world.Wide array of papaya-based products, including canned papaya pulp, juice, nectar, squash, beverages, jams, chutney, pickles, papaya leather, and raw papaya powder and the processing, nutritional attributes, sensory characteristics, and potential market applications of these diversified papaya products(Garcia E, *et al.* (2019)). Minimally processed avocado products meet the consumer demand of having fresh-like products; however, the shelf life of these products is relatively short, from one to several weeks (Oluseyi, 2003). The microbial contamination that is present in the fruit peel, due to lousy agricultural practices (particularly in developing countries). This contamination is hard to eliminate because of the

rough texture of the peel, especially in the Hass variety. Usually, the preservation factors are a combination of additives and refrigeration (FAO, 2004).

2.2. Fruit juices

Juice is defined in the most general sense as the extractable fluid content of cells or tissues (Bates and Crandall, 2001). 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 or puree (FDA, 2002).

Fruit juice may have been concentrated and later reconstituted with water suitable for maintaining the essential composition and quality factor of the juice. The finished product from manufacturers is expected to have undergone proper production processes as well as good packaging that would guarantee consistent good quality of the product throughout its shelf life (Oluseyi, 2003).

Fruit juices are nutritious drinks with great taste and health benefits (Suaad and Eman, 2008). They are common beverages in many countries of the world. In hot climate areas, cafes, restaurants and roadside stalls have local facilities to extract the juice from fresh fruits and then serving the juice liberally dozed with ice, to the thirsty customers (Al-jedah, 2001).

The consumption of fruit juices could have both positive and negative effect on the part of consumers. Fruit juices processed under hygienic condition could play important role in enhancing consumer 's health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Tsige *et al.*, 2008). In absence of good manufacturing practice, however, the nutritional richness of fruit juices makes the product good medium for microbial growth, vehicle of food borne pathogens and associated complications (Al-jedah, 2001).

2.3. Composition of fruit juice

The major component of the fruit juice is water. The other most common constituent is carbohydrates which comprise sucrose, fructose, glucose and sorption. Also, limited amount of

protein and minerals are found in fruit. 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).

2.4. 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.*, 2016). 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).

2.5. 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. 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).

2.6. Coliform bacteria

Coli form is a heterogeneous group of *Enterobacteriaceae* (e.g., *E. coli*, *Enterobacter*, lactose positive biotypes of *Citrobacter*, *Serratia* and *Hafnia*). They are facultative anaerobes, Gram-negative, nonperforming rods that ferment lactose with the production of acid and gas within 48 hours at 35°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 *fecal coli forms and fecal Streptococci* (Lewis *et al.*, 2006).

2.7. Yeasts and moulds

Most fruit juices are acidic enough and have sufficient sugar to favor the growth of *yeasts*. *Molds* 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, and relatively low resistance to acidic and heat treatments"(Bevilacqua *et al* 2018)

2.8. Unpasteurized juice

Unpasteurized juice/cider does not undergo treatment. Often it can be purchased as freshly pressed from local orchards, roadside stands, farmers markets, country fairs and juice bars. Unpasteurized juice/cider may also be found on ice or in refrigerated display cases and in produce sections at grocery stores (Health Canada, 2006). However, consumption of unpasteurized fruit juices causes approximately 16,000 to 48,000 cases of illnesses in a year (Foley *et al.*, 2002). Previously it was believed that fruit juices are safe due to their low pH values. However,"recent outbreaks of foodborne illness associated with consumption of fresh produce, including unpasteurized juices, have heightened concern about the potential for acidic foods to carry pathogenic microorganisms(Sapers Gm,2008)

2.9. Risks of consuming unpasteurized products

While most people can safely consume unpasteurized fruit juice and cider, food safety experts don't recommend that children, pregnant women, older adults and people with a weakened immune system consume unpasteurized juice and cider (Health Canada, 2006).

2.10. Pasteurized juice

Pasteurization describes a mild heat treatment which is applied at temperatures below 100°C. The thermal pasteurization criteria for white grape juice are 90- 95 °C for 15-30 seconds (Cemeroglu, 2004). The first pasteurization, in order to deactivate enzymes, kills most microorganisms, leaving the juice or concentrate commercially sterile. For NFC juice (Not from Concentrate) there is a risk that microorganisms enter the juice during transport or bulk storage and recontamination the juice. If the juice or nectar is made from concentrate the recontamination may occur during storage and transport of the concentrate or during reconstitution with water. The water used for reconstitution should always be of high quality (Silva and Gibbs, 2004).

2.11. Handling and processing

Poor handling and processing of fresh fruit juices are some of the main cause of food associated illness to the community who live in developing countries. The number and type of microorganisms recovered from the freshly prepared fruit juices made them unsafe for drinking. It was due to unhygienic fruit handling in the unsanitary environmental conditions under which the vendors operate the juices become contaminated with harmful bacteria. The results of this study demonstrate the unhygienic quality of popular types of market vended freshly squeezed fruit juices and their risk to the consumers (Shakir *et al.*, 2009).

2.12. Safety and quality of fruit juices

Relatively large number of reports of food-borne illness associated with fruit juices indicates that unpasteurized juices pose a high public health risk. Several authors have reported that pathogens such as *Salmonella*, *Shigella*, *E. coli* O157:H7 can survive for long periods in refrigerated juices and acidified culture media. The ability of different pathogens to survive in low pH environments has been documented at length (Eribo and Ashenafi, 2003).

2.13. Contamination

The most likely cause of the contamination is fruit coming in contact with animal faces, or water, workers, containers or processing equipment contaminated with animal faces. Cattle, deer and sheep, are the most common reservoirs for the pathogen, but usually do not show symptoms themselves. Birds, rodents, insects and poor hygiene may also contribute to the contamination. One contaminated piece of fruit could affect an entire batch of juice or cider (FDA, 1999; Canada food agency, 2001).

The contaminated juice products contained harmful bacteria such as *Salmonella* and *E. coli*, *kelbesila*, *proucidenia*. Although fruits that are used to make juice do not naturally contain harmful bacteria, or parasites, they can become contaminated in the farm environment, through handling, processing or transportation. Contaminated unpasteurized juice and cider can potentially pose a health risk to consumers (Health Canada, 2006). Rural producers, vendors and urban consumers of and use various methods to improve keeping quality. Wrapping the products with fresh inset leaves and burying them in pits are the most frequently used method by rural producers. They can store the products from two to three months using this method. Urban consumers could store the products only for 2-3 weeks (Ashenafi *et al.*, 1996).

2.14. Sources of fruit juices contamination

Fruit juices contain water, sugars, organic acids, vitamins, and trace elements thus providing an ideal environment for spoilage by microorganisms. On the other hand, they generally have a lower pH thus the common feature of their potential spoilage agents is that they must be acid-loving microorganisms. The most commonly encountered microbial genera are *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Clostridium*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Saccharobacter*, *Zymomonas*, and *Zymobacter* (Keller and Miller, 2006).

2.15. 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. Potable water, hot and cold under pressure, should be provided (Canada food agency, 2001). The other serious problem associated with food borne illness is unhygienic water supply that may be used for dilution of fruit juices. According to research conducted in Visakhapatnam City, India, over all the results of the study indicate that all street vended fresh fruit juices in many parts of the city showed contamination with *fecal coliform* and *fecal streptococci*. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic condition related to washing of utensils maintenance of the premises. The location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and overcrowding seem to add to the contamination. Such locations should be avoided for establishing a street vender juice shop. Lack of sanitary conditions in street vended juice shops and the occurrence of pathogenic *E. coli* O157:H7, *Shigella* and *S. typhimurium* is alarming enough for an immediate action by the suitable agency. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks (Lewis *et al.*, 2006).

2.16. Pathogenic microorganisms found in fruit juice

Contamination of fruit juices sold in restaurants, cafes and even road side stalls are sometimes unacceptable for human consumption and create significant health problems (Shakir *et al.*, 2009). Water used for juice preparation can be a major source of microbial contaminants including coli forms, fecal coli forms, and fecal streptococci. Thus, the transmissions of certain human diseases through juice and other drinks have become a serious problem (Tasnim *et al.*, 2010).

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). The prominent pathogens involved in un-pasteurized juice-borne disease outbreaks have been identified as *E. coli* O157:H7, *Salmonella* sp. and *Cryptosporidium* (Burnett, 2001). 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* and *Cryptosporidium* (SCF,

2002). Contaminated juices have shown to be potential sources of bacterial pathogens notably *E. coli* O157:H7, species of *Salmonella*, *Shigella* and *S. aureus* (Sandeep *et al.*, 2001).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts, and splits that occur during growing or harvesting. Contamination from raw materials and equipment, additional processing conditions, improper handling, prevalence of unhygienic conditions contributes substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables.

Practice good personal hygiene, avoid cross contamination, keep foods at safe temperatures, avoid foods and water from unsafe sources are basic rules for prevent food borne diseases (VGDHS, 2005; Oliveira *et al.*, 2006; Nicolas *et al.*, 2007).

CHAPTER 3: MATERIALS AND METHODS

3.1. Study area

The study was conducted in Wolkite University, College of Natural and Computational Science, Department of Biotechnology, Molecular Biology laboratory, Southwestern Ethiopia. Gubrye is SNNPR, Guraghe Zone, 189 km South West of Addis Ababa on the way to Jimma Latitude 8.2° or 8° 12' north Longitude 37.8° or 37° 48' east with an Elevation 1,918 metres (6,293 feet), The mean annual temperature is 27°C.

3.2. Sample Collection

Samples of fresh fruit juices of Avocado and papaya, was collected from two different Juice houses using sterilized Fulcon tube by putting in plastic bag and transported to the Molecular Biology laboratory, Department of Biotechnology, College of Natural and Computational Science, University of Wolkite. All samples was kept at 4°C for further analysis.

3.3. Equipments and chemicals

In order to conduct the research, the equipments and chemicals that was used is the following: laminar flow, electronic balance, flasks, graduated cylinder, autoclave, test tubes, laminar air flow, incubator, Bunsen burner, micropipette, cotton, Petri dish, conical flasks, plastic bag, refrigerator, sterilized Fulcon tube, magnetic stirrer, spatula, colony counter, inoculating loop, flasks and water bath, distilled water, manitol salt agar, salmonella and shigella agar, eosin methylene blue agar, MacConkey agar, malt extracted agar, avocado, papaya sample.

3.4. Study design

Identification of the bacterial species affecting the quality and safety of juice was carried out in the laboratory using the collected juice samples from juice houses of the Gubrye subcity. Two types of fruit juice (i.e. papaya and avocado fruit juice) samples were collected and portions transferred to appropriate culture media. Indicator microbial groups were enumerated from cultures using standard methods. *Staphylococcus aureus*, *Total coliform*, *E. coli*, *Salmonella* spp and *fungi and yeast* were detected from samples (fruit juices). The bacteriological analysis was

done in Wolkite university molecular laboratory. The results of all bacteriological analyses were compared with microbiological criteria for food stuffs (Gulf Standards, 2000).

3.5. Experimental procedures

3.5.1. Sample preparations

Initially serial dilutions 10^{-1} to 10^{-7} of each sample was made with sterilized distilled water.

3.5.2. Microbiological load analysis

One milliliter of each liquid juice sample was taken and transferred into sterilized cotton plugged test tubes containing 9 ml of sterile distilled water using pipette, mixed thoroughly by shaking many times for 10-fold serial dilution aseptically under laminar flow. Appropriate dilutions of 1 ml and 0.1ml of juice sample was inoculated on surfaces of respective media for microbial count using spread plate technique .

3.5.2.1. Total plate count

Standard plate count agar (Oxide) was used to estimate total bacterial count/ml of fresh fruit juices. Total of 4 juice samples (one avocado and one papaya samples from one juice houses and the other also from another juice houses) from Gubrye subcity were cultured for total plate count. Each sample was diluted serially 10^{-2} up to 10^{-5} dilutions in distilled water and 1 ml of each dilution was transferred to a sterilized Petri dishes. Then 20 ml of sterilized molten (45°C) standard plate count agar was poured in the Petri plates. Medium was mixed well with sample by thoroughly rotating the plate. After that agar was allowed to settle down at room temperature and then inoculated and incubated at 35°C for 48 hr. After incubation, to numerous to count colonies was resulted from all samples.

The number (N) of cfu/g or ml of test sample was calculated as follows:

Formula **$\text{CFU}=\text{C}/\text{Vd}$**

Where CFU: Colony forming units

C: C is the sum of colonies on all plates counted

V: is the volume applied to each plate

d: is the dilution from which the first count was obtained

3.5.2.2. Total coliform

MacConkey agar plate was used to estimate *total coliform* count/ml of fresh fruit juice about 1ml of 10^{-2} to 10^{-5} serial dilution of each sample in distilled water transfer to the plates. These plates was incubated at 35°C for 48 hr . After incubation, colonies ranging 65 -220 were counted and the results were expressed as colony forming units (cfus)

3.5.2.3 Staphylococcus aureus count

From 10^{-2} to 10^{-5} dilution, 0.1 ml was plated onto manitol salt agar media and inocula were distributed evenly using sterile glass rod. The plates was then incubated at 35°C for 48 hrs. After incubation, colonies ranging 32 -150 were counted and the results were expressed as colony forming units (cfus)

3.5.2.4. E.coli count

From 10^{-4} and 10^{-5} dilution, 0.1 ml was plated onto eosin methylene blue agar media for selective isolation of *E.coli* and inocula was distributed evenly using sterile glass rod. The plates was then be incubated at 35°C for 48 hrs. After incubation, colonies ranging 15 -148 were counted and the results were expressed as colony forming units (cfus)

3.5.2.5. Salmonella and Shigella counts

From 10^{-4} and 10^{-5} dilutions, 0.1 ml was plated onto *salmonella and shigella* agar media and inocula will be distributed evenly using sterile glass rod. The plates will then be incubated at 35°C for 48 hrs. After incubation, colonies ranging 6 -170 were counted and the results were expressed as colony forming units (cfus)

3.5.2.6. Yeast and mold count

Malt Extract agar was added for *yeast and mould* count/ml. About 1 ml of each sample serially diluted 10^{-4} and 10^{-5} dilutions in distilled water was taken and 20 ml of malt extract agar will be added in each Petri plates. Each plate was mixed well and allowed to settle and incubate at 30°C for 5 days. One milliliter (1 ml) of the sample was serially diluted to nine fold sterile distilled water of which zero point one (0.1 ml) taken from each dilution (10^{-4} and 10^{-5}) of the serially diluted sample was spread on malt extract agar media prepared . The plates were incubated at 30

for 5 days. After incubation, colonies ranging 27 -179 were counted and the results were expressed as colony forming units (cfus) .

Table 1. Types of microbes with colour formation on the media

Microbes	Colour formation
Total plate count	Straw colored colonies
Total coliform	Pink/Fucshia
E.coli count	Slight sheen
Salmonella and Shigella	Slight pinkish colonies
Yeast and mold	Smooth creamy white colonies
Staphylococcus aureus	Golden to orange-yellow

3.6. Antimicrobial susceptibility testing

In vitro test was used to confirm susceptibility of isolates to chosen antimicrobial agents, or to detect resistance in the isolated human pathogens by means of a disc diffusion method on Mueller-Hinton Agar. Sterile cotton swabs was dipped into the suspensions and spread evenly over the entire agar surface. Two(2)commercially prepared fixed concentration paper antibiotic (cefepime and vancomycin) discs was used in the experiment for each isolate. Inoculates plates was incubated for 24 hrs at 34°C. The diameters of zone of inhibition will be measured and recorded in millimeter .

3.7. Data Analysis

After the proper collection of data, the data was analyzed through both quantitative and qualitative meaning that the expected result were expressed in numbers, tables and percentage .

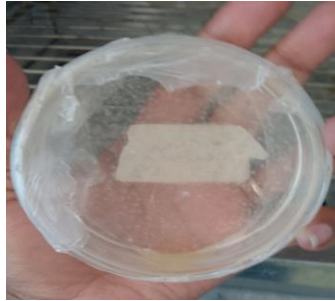
CHAPTER 4:RESULTS AND DISCUSSION

4.1.Microbial Enumeration in Fresh Fruit Juice Samples

Result of fresh juice type, numbers of samples and count ranges expressed in cfu/ml for , *total coliform*, *E.coli count*,*Staphylococcus Aureus*,*mold* and *yeast* and *Salmonella* and *Shigella* count was presented (Table 2).The total bacterial count of the fruit juice samples was calculated based on the bacterial count on each plate (10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} serial dilutions) . *Total coliform*,*Staphylococcus Aureus*,*mold* and *yeast* ,*Salmonella* and *Shigella* and *E. coli* were evaluated with Gulf Standard 2000.The recommended specifications for fruit juices such as Avocado, Mango, Pineapple and Papaya available for public consumption in Gulf region suggest that the maximum count for Total viable count, *Coliform* count,*Yeast-Mold* count, *E.coli* count,*Staphylococcus Aureus*, and *Salmonella* and *Shigella* are 1×10^4 , 1×10^2 and 1×10^3 cfu/mL, 1×10^1 , 1×10^1 and 1×10^1 respectively.

4.1.1.Total plate count

The total colony count of the bacteria was carried out using spread plate count method on plate count agar (PCA) .The *total plate* count was to many to count from all samples(from two juice houses of avocado and papaya).it was found that 100%(n=4) samples had total bacterial growth.almost all fruit juices samples showed much higher viable bacterial counts than the permitted count.



4.1.2. Total Coliform

The total coliform count of avocado and papaya ranged from 1.1×10^6 cfu/ml - TNTC (to numerous to count) and 1.3×10^5 - 2.2×10^5 cfu/ml respectively. From the TA (code of first juice house) juice house *total coliform* resulted from avocado as numerous to count from 3 dilutions from 10^{-2} - 10^{-4} . The Ketema, et al. reported in Jimma town that the range of microbial counts recorded in the avocado and papaya juice samples analyzed were from 6.2×10^3 - 3.1×10^7 cfu/ml and 4.2×10^3 - 3.1×10^5 cfu/ml. The study conducted in Hawassa town, Ethiopia reported that *total coliform* count in fruit juice samples of avocado was 2.54×10^5 cfu/ml. In another similar study conducted in Delhi, India the total coliform count was reported within juice samples of avocado was 3.1×10^2 - 4.9×10^5 cfu/ml. The variation among *coliform* count of the fruit juices of the two studies may be due to the different in ways of preparing and handling of the fruit juices.



4.1.3. Staphylococcus Aureus

The total *Staphylococcus* count obtained from avocado and papaya ranged between 3.5×10^5 - 7×10^5 cfu/ml and 1.5×10^5 - 3.2×10^5 cfu/ml, respectively. According to study conducted in Nigeria, the highest number *Staphylococcus* count of 3.5×10^4 cfu/ml was observed in avocado juices. Latef, et al, also reported that the total *Staphylococcus* count of 3.7×10^1 cfu/ml bacteriological loads in mango juices. According to study conducted in Adigrat town the highest number of *Staphylococcus* count was 3.78×10^1 cfu/ml observed in avocado juices.

Current study revealed that even though there was high total viable count it was low compared to the previous report. The difference may be linked to the factors like improper handling, use of contaminated water, use of unsafe processing utensils like knife and trays during juice preparation and contamination from rotten fruits that favor microbial growth and promotion as showed by few studies.



4.1.4. E.coli count

The total *E.coli* count obtained from avocado and papaya ranged between 2.6×10^6 - 1.48×10^7 cfu/ml and 3.6×10^5 - 1.3×10^6 cfu/ml, respectively.

Shakir, et al. in Dhaka Bangladesh also reported that 18% of the fruit juices studied were positive for *E. coli*. The reasons might be poor handling practices of fruits and fruit juices, improper storage sites and poor quality of water for dilution and washing equipment.

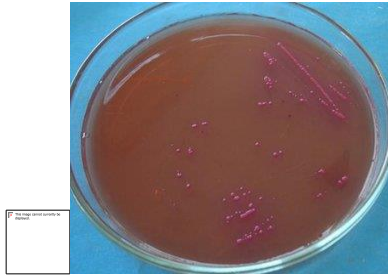


4.1.5. Salmonella and Shigella counts

The total *salmonella and shigella* counts count obtained from avocado and papaya ranged between 3.8×10^5 - 2×10^7 cfu/ml and 6.5×10^5 - 1.7×10^7 cfu/ml, respectively.

Poor handing practices in storage sites and poor quality of water for juices dilution can contribute for their prevalence. The prevalence of *Salmonella spp.* in the current study was lower than the study conducted in India which reported 50% of fruit and vegetables juices were positive for *Salmonella* species (*Titarmare et al., 2009*) and higher than the study conducted in

Bangladesh that showed an overall prevalence of 7.89% for *Salmonella* species in unpasteurized fruit juice samples (Shakir *et al.*, 2009). The probable reason for the difference may be attributed to fruit type, geographical variation, seasonal variation, sanitation habit and variation in diagnosis. Poor handling practices in storage sites and poor quality of water for juices dilution can contribute for their prevalence.



4.1.6. Yeast and Mold count

The total *yeast and mold* counts obtained from avocado and papaya ranged between 6.9×10^5 - 6.1×10^7 cfu/ml and 3.1×10^6 - 7.7×10^6 cfu/ml, respectively. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed. *Yeasts and moulds* are more favored as spoilage agents of fruit compared to bacteria because of the physical and chemical properties of the fruit (Obire *et al.*, 2008). However the presence of fungi in many of the fruit may be due to the practice involved with the handling and processing of the fruit. Though the fungal isolates were not identified, their greater load showed the probability of the presence of strains that could pose spoilage of the fruit (Al-Hindi *et al.*, 2011 and Obire *et al.*, 2008).

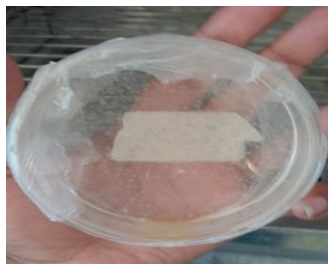


Table 2 : Microbes count from freshly collected fruit juices samples (cfu/ml)

Juices	Total Plate Count (CFU/ml)	Total Coliform (CFU/ml)	Total <i>Staphylococcus aureus</i> count (CFU/ml)	Total <i>E.coli</i> count(CFU/ml)	Total <i>Salmonella</i> and <i>shigella</i> counts (CFU/ml)	Total Yeast and mold count (CFU/ml)
TA Avocado	TNTC	TNTC	3.5×10^5	1.48×10^7	3.8×10^5	6.9×10^5
TA papaya	TNTC	2.2×10^5	1.5×10^5	1.3×10^6	6.5×10^5	7.7×10^6
NB Avocado	TNTC	1.1×10^6	7×10^5	2.6×10^6	2×10^7	6.1×10^7
NB Papaya	TNTC	1.03×10^5	3.2×10^5	3.6×10^5	1.7×10^7	3.1×10^6

Key:TA:stands for code for first juice house, NB: code stands for second juice houses, CFU: colony forming unit, TNTC: To numerous to count

4.2. Antimicrobial sustestbility test

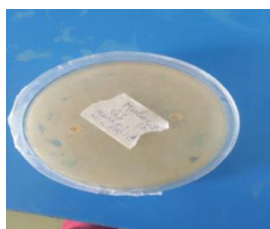
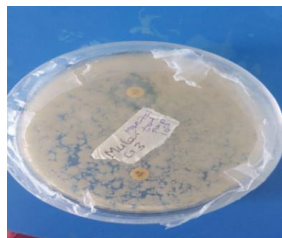
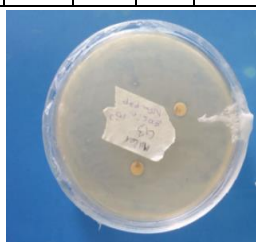
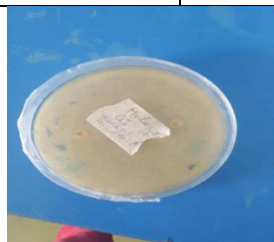
According to the finding of this study, isolates of *Total coliform* were resistance to cefepime 100% and vancomycin 100%. In this study, isolates of *S.aureus* were shown to have resistance cefepime 100% and Vancomycin 99%. Regarding the isolates of *E.coli* species they showed resistance to cefepime 99% and vancomycine 100%. *Salmonella* species were resistance to cefepime 98% and vancomycin 98%. Similar study indicates that *Salmonella* were resistant to multiple antibiotics. According to Nipa, *et al.* multiple drug resistance was observed in *Salmonella* 98.06%. Similar study reported that 85% of the resistant isolates were multiple drug resistant. The presence of *Salmonella* in fruit juices may be attributed to contaminated hands, poor food handing, poor hygienic practices and food safety. Yeast and mold species were resistance to cefepime 100% and and vancomycin 99%. Resistant means Small or no zone

diameter, indicating that the antibiotic is unlikely to be effective against that particular bacterial strain

Table 3: The antimicrobial susceptibility patterns of *Total coliform*, *S.aureus*, *E.coli*, *Salmonella* and *Shigella* and yeast and molds pathogenic bacteria isolate from avocado and papaya juice samples in Percent, %.

S = Sensitive, I = Intermediate, R = Resistant

Bacterial isolates																
Disk code	potency	<i>Total coliform</i>			<i>S.aureus</i>			<i>E. Coli</i>			<i>Salmonella and shigella</i>			<i>Yeast and mold</i>		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
cefepime	30µg	100	-	-	100	-	-	99	-	-	98	-	-	100	-	-
vancomycin	30µg	100	-	-	99	-	-	99	-	-	98	-	-	98	-	-



DISCUSSION

Laboratory examined samples of avocado and papaya juice were found to be contaminated with different groups of microorganism. The types of media that used under this studies are Standard plate count, MacConkey agar plate, Manitol salt agar, Eosin methylene blue, salmonella and shigella agar and Malt extract agar media was used for *total plate*, *Total coliform*, *S. aureus*, *E.coli*, *salmonella and shigella* and *yeast and mold* count respectively. From above media salmonella and shigella and distilled water was used from Biology departement. when the media was prepared the amount of media required measured from the media plate as direction was written, the direction was placed for 1000ml or for 1L but it's changed to as the media required amount of agar by the formula dilution ($C_1V_1=C_2V_2$). after colony formation was counted its was changed to colony forming unit (CFU) by the formula of :cfu equals to $\frac{n}{v}$ of colonies counted on the plate divides by volume of sample times by dilution factor .

All samples of Avocado and Papaya of fruit juices were found to have microbial count in the range of 1.3×10^5 to 6.1×10^7 cfu/mL. *Ketema et al.* (2008) reported in Jimma town that the range of microbial counts recorded in the fruit juice analyzed were from 3.1×10^7 to 6.2×10^3 cfu/mL. The total viable bacterial count of all collected fruit juice in this investigation varies from 1.3×10^5 to 2.9×10^5 cfu/mL; this bacterial count was relatively lower than that of *Singh et al.* (2015) who reported that all samples of street vended fruit juices were found to have bacterial count in the range of 10^5 to 10^8 cfu/ mL. In other studies conducted by *Ankur et al.* (2009), the quantitative analysis of samples for total viable count (TVC) revealed that the range for TVC were between 1.0×10^4 to 4.0×10^6 cfu/mL. According to study conducted in Kolkata city India,

juice samples collected from most populated market places of and summarized account of the results obtained for the microbiological analysis of the juices; total viable counts were high ranging from $265-700 \times 10^4$ cfu/1000 mL (Mahuya *et al.*, 2011). In addition, lack of appreciation of basic safety issues by vendors augments the microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies, and airborne dust. Laboratory examined samples of avocado and papaya juice were found to be contaminated with different groups of microorganism. All samples of Avocado and Papaya of fruit juices were found to have microbial count in the range of 1.3×10^5 to 6.1×10^7 . Ketema *et al.* (2008) reported in Jimma town that the range of microbial counts recorded in the fruit juice analyzed were from 3.1×10^7 to 6.2×10^3 cfu/mL. According to the specification of Gulf standards, the colony counts of all microbial groups (total coliform count, Staphylococci count, total viable count and Yeast-mold count) in this study exceeded the standard by considerable margin.

However, the recommended specifications for fruit juices such as Avocado, Mango, Pineapple and Papaya available for public consumption in Gulf region suggest that the maximum count for *Total viable count, Coliform count and Yeast-Mold count* are 1×10^4 , 1×10^2 and 1×10^3 cfu/mL, respectively. According to the specification of Gulf standards, the colony counts of all microbial groups (*total coliform count, Staphylococci count, total viable count and Yeast-mold count*) in this study exceeded the standard by considerable margin. The finding of the current result revealed that the Staphylococci count was between 1.5×10^5 and 3.5×10^5 cfu/mL for all fruit juice. The presence of *Staphylococci* in high numbers (10^3 cfu/mL) is a health hazard as they cause spoilage of fruit juices and food borne diseases (Gulf standards, 2000). The existence of *S. aureus* in fruit juice can contribute to contamination through handling and processing conditions. The *total yeast and mould* counts for all fruit juice analyzed in the current investigation were in the range of 1.4×10^5 to 2.7×10^5 cfu/mL. 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 8.05×10^4 cfu/mL (Shakir *et al.*, 2009). Fungal fruit contamination may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing

conditions (*Al-Hindi et al.*, 2011). Fruits contain high levels of sugars and nutrient elements and their low pH values make them particularly desirable to fungal growth which in turn may result in their decay (Singh and Sharma, 2007), But the result of this study is more than the standard set by Gulf Region Mould and yeast count (1×10^3 cfu/mL).

This study also tried to address antimicrobial susceptibility testing by means of a disc diffusion method on Mueller-Hinton Agar. only two types of antibiotics was used because there is only two types of antibiotics was there in the our molecular laboratory. The results of the antibiotic sensitivity test were interpreted and are presented as the resistant of bacterial isolates to the antibiotics Pathogens isolated from fresh juices in this study showed resistance patterns to the tested antibiotics. Resistant explained as Small or no zone diameter, indicating that the antibiotic is unlikely to be effective against that particular bacterial strain. *Total coliform* resisted both tested antibiotics cefepime and vancomycin . Relatively tested microbes found resistant to cefepime and vancomycin.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

CONCLUSION

From the present finding, the microbiological quality of most of the vendor fruit juices was found not to be satisfactory for consumption as compared to Gulf region standard. *The total bacterial count, yeast and mould count, coliform count and Staphylococci count* of investigated fruit juice was also alarming. All samples of Avocado and Papaya of fruit juices were found to have microbial count in the range of 1.3×10^5 to 6.1×10^7 cfu/mL . Therefore, training about safe processing and handling is very crucial for juice vendors to improve microbiological quality of fruit juice. The fruit juices investigated in this study had higher microbial load than the specifications set for fruit juices in some parts of the world. It is clear that the colony counts of the microbial groups in our fruit juices exceeded the standard by considerable margin. Generally, this study indicated that samples of avocado and papaya juices examined were contaminated with different bacteria species.

Based on the antibiotic resistance total coliform had 100% resistance to cefepime and vancomycin and *S. aureus* had high rates of resistance to cefepime. Yeast and molds also had 100% resistance to cefepime. Based on the data from the experiment on fruit juices of papaya and avocado both of them was found to be heavily contaminated with bacteria that could cause health problems. Generally, the results in this study clearly indicate the poor hygienic conditions of fruit juices which make consumers susceptible for the risk of food borne infections. Lack of training on juices or food hygiene and safety including improper storage and preparation of fruit juices may aggravate the contamination.

RECOMMENDATIONS

Based on the current finding, the following recommendations are forwarded: The concerned body should give training on the importance of food quality, safety and hygiene to people involved in harvesting and handling fruit and enforces adequate guidelines for fruit vehicles. Handlers must have awareness of microbiological contamination can occur during the harvest, by workers, from the soil, harvesting equipment, containers and transport vehicles. Imported fruit should be analyzed both chemically and microbiologically to find out whether they are fit or not for human consumption.

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