

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES



**BACTERIOLOGICAL PROFILE OF LOCALLY
PREPARED FRESH FRUIT JUICES IN
HAWASSA TOWN, SOUTHERN ETHIOPIA**

BY

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BACTERIOLOGICAL PROFILE OF LOCALLY PREPARED FRESH FRUIT JUICES IN HAWASSA TOWN, SOUTHERN ETHIOPIA

A Thesis submitted to the School of Graduate studies Addis Ababa University in partial fulfillment of the requirements of Masters degree in Medical Microbiology

BY

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Definitions:

- **Aerobic:** Grows in the presence of atmospheric oxygen.
- **Aliquot:** The portion of food that is inoculated into a container of bacteriological medium in accordance with a specified method.
- **Analytical Unit:** The amount of product withdrawn from the sample unit for analysis
- **Coliform:** A gram-negative, facultative rod shaped bacterium that ferments lactose, producing gas.
- **Contamination:** The effect exerted by an external agent on food so that it does not meet acceptable food hygiene standards or is unfit for human consumption.
- **HACCP system:** An effective management tool for food safety assurance that can be applied to all sections of the food chain.
- **Indicator:** Historically, an organism itself non-pathogenic, but often associated with pathogens, used to portray a risk of the presence of pathogens for which feasible methods of detection were not generally available (sometimes called 'index organisms').
- **Lot:** A batch or production unit which may be identified by the same code. When there is no code identification, a lot may be considered as (a) that quantity of product produced under essentially the same conditions, at the same establishment and representing no more than one day's production; or, (b) the quantity of the same kind of product from one and the same manufacturer available for sampling at a fixed location.
- **Mesophile:** A microorganism with a growth optimum around 20° to 45°C.
- **Microbiological guidelines:** A microbiological criterion used by a manufacturer or regulatory agency to monitor a food, ingredient, process, or system; often used also to describe a microbiological criterion where no standard has been prescribed.
- **Pathogens:** Organisms that cause disease.
- **Sample Unit:** Usually a consumer size container of the product, and should consist of a minimum of 100 g (ml). A sample unit is often referred to as a subsample.

- **Sample:** The sample units (subsamples) taken per lot for analysis.
- **Total coliform counts (TCC):** The number of colony-forming units of gram-negative, facultative rod shaped and lactose fermenting bacteria present per gram or per ml in the analytical unit as determined by a standard method.
- **Total viable counts (TVC):** The number of colony-forming units of aerobic mesophilic bacteria present per gram or per ml in the analytical unit as determined by a standard method.

Abbreviations

- **ACMSF**- Advisory committee on the Microbiological Safety of food
- **BAM**-Bacteriological Analytical Manual
- **cfu**-colony forming units
- **CHF**- congestive heart failure
- **CPSC**- Coagulase positive Staphylococcus
- **CNSC**- Coagulase negative Staphylococcus
- **D.H.S.S.** - Department Of Health & Social Security.
- **MPN**- Most Probable Number
- **MSA**- Mannitol salt agar
- **TCC**- Total Coliform Count
- **TSS**- Total Staphylococcal count
- **TVC**-Total Viable Count
- **VRBA**-Violet Red Bile Agar

Abstract

Background: - 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 foodborne illness. Most of the juice venders in Hawassa prepare avocado, papaya, mango, and pineapple juices. Common bacterial illnesses associated with contaminated fruit juices are staphylococcal food poisoning, Salmonellosis, shigellosis and diarrhea associated with enterotoxogenic *E. coli*.

Objective: - The aim of the study was to assess the bacteriological quality of the locally prepared unpasteurized fruit juices and the hygienic conditions of preparation sites.

Method: - A cross sectional study was conducted from November 2010 to January 2011 in Hawassa town using structured Questionnaire to assess source of fruit and processing of fruit juices and Bacteriological analysis. i.e. the sufficient amount of the specimen of avocado, papaya, mango and pineapple juices were collected in aseptic manner and kept in ice box and transported to Hawassa University Health Science College Referral Hospital. Finally, the samples were appropriately diluted and inoculated on Nutrient agar to determine the total Viable Count, on Violet Red Bile Agar (VRBA) to determine total coliform count and on Mannitol salt agar to determine total staphylococcal count. Furthermore pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella*, and *E. coli* were isolated and identified.

Results: - A total of 120 locally prepared fresh fruit juice samples were collected. Among these juice samples, the total viable count of 38(31.67%) was found to be above Gulf region standards (5.0×10^5 cfu/ml) and, the total coliforms count of 93(77.5%) were shown to be above Gulf region standard (100 cfu/ml). Out of 98 growth on MSA, 11 (11.22%) were positive for *Staphylococcus aureus* and these positive samples were above the Australian standard. Seven of total samples were positive for thermotolerant *E. coli* and three of the total samples were shown to contain *Salmonella* species. Moreover, all venders obtained fruit from the open market and only one vender stored fruits in refrigerator.

Conclusion and recommendations: - According to the current study, the results may be attributed to contamination during either harvesting of fruits or processing and handling of fresh fruit juices. Therefore, regular supervision and training about harvesting fruit, safe processing, and handling of fruit juices and hygiene of venders can improve the quality of fresh fruit juices.

Key Words: Bacteria, contamination, locally prepared fresh fruit juice.

CHAPTER I: INTRODUCTION

1.1. Background

Statement of the problem

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. In many tropical countries they are common. 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). Nowadays, the demand for freshly squeezed juices in comparison to bottled or canned juices has increased, as unpasteurized juices are preferred by the consumer because of the “fresh flavor” and no addition of preservatives. Traditionally, juice is consumed more in the morning at breakfast time. Consumption of fresh fruits continues to increase in many countries owing to consumer preferences for fresher, more nutritious foods that also happen to meet the needs of busier lifestyles. Fresh fruit juices have no artificial color, sweetness is natural, and that is why they are preferred over bottled or canned juices (Addo *et al* 2008; Melbourne, 2005).

The rapid increasing number of juice vendors has extended consumption of juice products across Ethiopia specifically in Hawassa town. The farmers are attracted toward cultivating different kinds of fruits as well (Abadias *et al*, 2008). Therefore, economical contribution of fruit production by the study area is great enormous.

Additionally 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 (Ketema *et al*, 2008).

Freshly squeezed juices are simply prepared by extracting the liquid and pulp of mature fruit usually by mechanical means or Blenders. Prior preparation of fruit to avoid bitterness of skin or to remove large stone such as mango, avocado and pineapple followed by separation of juices and pulp by blender. The final product is an unfermented, unclarified, untreated juice, ready for consumption (Melbourne, 2005).

However, it is well known fact that food serves as very good medium for growth of microorganisms especially when the principles of hygiene and sanitation are not met the food becomes contaminated by pathogens from humans or from the environment during production, processing or preparation. Specifically local preparation of fruit juices has no processes that reduce pathogen levels, if contaminated, such as microbe killing step. Pathogenic organisms can enter fruits through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting and processing. A pathogen that has become internalized within a fruit must be able to survive in the product until it reaches the consumer in order to become a public health hazard. Most fruit juices are sufficiently acidic to inhibit the growth of pathogenic organisms (Melbourne, 2005).

In addition, during the process contamination from raw materials, equipment or food handlers, the pathogen could be easily transferred to the final product. If pathogens such as *Staphylococcus aureus*, *Salmonellae*, *Shigella*, *E. coli O157:H7*, etc were present in freshly squeezed juices, individuals may get health threat. The source of contamination could be improper washing of fruits, use of unhygienic water for dilution, prolonged preservation without refrigerator, unhygienic environment for juice preparation and poor handling of properly prepared juices (Lewis *et al*, 2006).

Foodborne or waterborne microbial pathogens are leading causes of illnesses in developing countries, killing an estimated 1.9 million people annually at the global level. Even in developed countries, an estimated one-third of the population is affected by microbiological foodborne diseases each year (Andargie *et al*, 2008). There are reports of food borne illness associated with the consumption of fruit juices of several places of India and elsewhere (Sandeep *et al.*, 2001). Most of the fruit juices being served in Jimma had high microbial load. So that, these products could be the cause of health problems and potential vehicle of foodborne outbreaks (Ketema *et al*, 2008). Contamination of fruit juices sold in restaurants, cafes and even road side stalls are sometimes unacceptable for human consumption and create significant health problems (Lewis *et al*, 2006).

Food safety has emerged as an important global issue with international trade and public health implications. In response to the increasing number of food borne illnesses, governments all over the world are intensifying their efforts to improve food safety (Sudershan *et al*, 2009). However, in Ethiopia no continuous survey/ assessment of food safety has been prepared in restaurants and cafes especially on locally prepared fruit juices. Therefore, this study has tried to determine the bacteriological safety and quality of locally prepared fruit juices in Hawassa town.

1.2. Literature Review

1.2.1 Fruit juices

Juice is defined in the most general sense as the extractable fluid contents 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).

1.2.2. 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).

1.2.3. Pasteurized Juice

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

1.2.4. Handling and processing

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

made them unsafe for drinking. It was concluded that 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).

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

1.2.5. Contamination

The most likely cause of the contamination is fruit coming in contact with animal faeces, or water, workers, containers or processing equipment contaminated with animal faeces. 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).

Unpasteurized juice products can be contaminated with harmful bacteria such as *Salmonella* and *E. coli*, viruses, and parasites like *Cryptosporidium*. Although fruits that are used to make juice do not naturally contain harmful bacteria, viruses 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).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting, handling and processing (Melbourne, 2005).

A pathogen that has become internalised within a fruit or vegetable must be able to survive in the product until it reaches the consumer in order to become a public health hazard. Most fruit juice is sufficiently acidic to inhibit the growth of pathogenic organisms. Studies conducted on the survival or growths of microorganisms in juices have showed a number of pathogenic organisms can be present and survive in a wide range of fruit and vegetables (FDA, 2008).

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

A research conducted in Ethiopia on microbial spoilage of market bulla and kotcho stated that when stored at room temperature in a loosely wrapped condition, both products resulted in undesirable odor, sticky consistency and dark coloration after 8 days. Drop in pH and a high degree of proliferation of aerobic mesophilic bacteria and molds were observed. Microorganisms active in starch hydrolysis, proteolysis and lipolysis were encountered in both products. The aerobic mesophilic (spoilage) bacterial flora was dominated by *Micrococcus* and *Bacillus* spp. About 33 percent of the products were lost due to such spoilage. Rural producers, vendors and urban consumers of bulla and kotcho use various methods to improve keeping quality. Wrapping the products with fresh enset

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).

1.2.6. Equipment

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

1.2.7. Water Supply

Water used in processing establishments must be potable 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 foodborne 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 *faecal coliforms* and *faecal streptococci*. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils and maintenance of the premises. The location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and overcrowding seem to add to the contamination. Such locations should be avoided for establishing a street vender juice shop. Lack of sanitary conditions in street vended juice shops and the occurrence of pathogenic *E. coli* O157:H7, *Shigella* and *S. typhimurium* is alarming

enough for an immediate action by the suitable agency. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogen outbreaks (Lewis *et al*, 2006).

A survey on the bacteriological quality of both drinking water and flavoured drinks from coin-operated vending machines explains that forty-four per cent of 25 drinking water samples examined contained coliforms and 84% had viable counts of greater than 1000 organisms ml at 30⁰C. Thirty-one flavoured drinks were examined; 6% contained coliforms and 39% had total counts greater than 1000 organisms per ml. It is suggested that the D.H.S.S. code of practice on coin-operated vending machines is not being followed. It is also suggested that drinking water alone should not be dispensed from such machines (Hunter *et al*, 1986).

1.2.8. Personnel

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

Without personal hygiene of food handlers, safe processing of fruit juices alone has no value to improve the community health. Therefore, all rounded safety precautions should

be applied by food handlers as well as during processing. According to study conducted in Gondar, Ethiopia Food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms (Andargie *et al*, 2008).

1.2.9. Fruit Storage Practices

Ideally, fruit should be pressed as soon as possible after picking to avoid increases of pH that would favor growth of pathogens during storage. The lower the pH, the worse the conditions will be for the growth and survival of pathogens. However, if fruit needs to be stored, rapid cooling to as close to 0°C as possible (0 to 4°C) and achievement of adequate storage conditions will maintain fruit condition. Storage facilities must be clean, secure from rodents and insects and suitable for storing food (Canada food agency, 2001).

A research conducted in Ethiopia stated that Papaya and avocado juices had initial pH values of >5.7 and allowed all test strains to reach numbers >10⁷ cfu/ml at ambient temperature holding. At refrigeration temperatures, at least no elimination was observed. In pineapple juice (pH 3.8), the *E. coli* test strains were eliminated at both holding temperatures within 16 h whereas slight increase in counts of *Salmonella* test strains was observed at ambient temperature holding. Orange juice (pH 3.1) did not allow the survival or growth of the test organisms at both holding temperatures (Yigeremu *et al*, 2001).

1.2.10. Outbreak associated with unpasteurized fruit juices

Three outbreaks of illness from *E. coli* O157:H7 in the United States in 1996 were linked to unpasteurized juice/cider. These incidents proved that harmful bacteria can survive in high acid products such as juice or cider, if contaminated. Until recently, scientists did not think this was possible. In the fall of 1998 in Ontario, 14 cases of food-borne illness including seven cases of confirmed *E. coli* O157:H7, were reported. Unpasteurized juice/cider was suspected in these cases. Local health officials identified one batch of

unpasteurized non-commercial, custom-pressed apple cider as the most likely source (Health Canada, 2006).

According to study conducted in the united state of America, unpasteurized orange juice from one company was the vehicle of a widespread outbreak of salmonellosis. Although the route of contamination is unknown, noncompliance with the juice Hazard Analysis and Critical Control Point regulation likely contributed to this outbreak. Pasteurization or other reliable treatment of orange juice could prevent similar outbreaks (Jain *et al*, 2005).

1.2.11. 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).

1.2.12. Incidence of organism

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

A study conducted in Pakistan stated that microbiological quality of all the products was well outside the Gulf Standards for fruit juices, and coliform counts usually exceeded 1,000 cfu/ ml. In one sample of mixed fruit juice, the coliform count was above 1.0×10^6

cfu/ml, and both *Escherichia coli* and *Enterococcus faecalis* (1.0×10^7 cfu/ml) were detected. It is concluded that, while the practice of consuming fresh fruit juices with meals should be encouraged on nutritional grounds, steps must be taken to improve the microbial quality of the products (Al-Jedah *et al*, 2002).

According to a research conducted in Ethiopia, the total viable bacterial count of fresh cassava before cleaning ranged from 8.7×10^4 to 2.1×10^9 c.f.u / ml, whereas, in thoroughly cleaned product it was reduced to 10^6 c.f.u/ ml. Enterobacteriaceae and spore former bacteria mean count of 10^4 and 10^3 , respectively. The dominant bacteria group within mesophilic microflora were *Actinobacter spp* (29.1%), *Micrococcus spp* (17.4%) and *Enterobactercae* (16%). Bacterial spores, *pseudomonas*, *moraxella* and *aeromonas spp*. Were detected in small proportion. Fate of *staphylococcus aureus*, *Bacillus cerus* and *Listeria moncytogenes* in cassava juice was also evaluated. Except the *Bacillus cereus* the growth of bacterial strains was retarded at higher concentration (Desse *et al*, 2001).

Another study conducted in Ethiopia, most street food sample had aerobic mesophilic count more than 10^7 c.f.u per g. Nine “kitifo” and one “egg sandwich” yield salmonella. Shigella was isolated from three macaroni sample. It was concluded that street foods are heavily contaminated with microorganism and potential source of food borne infection. Health hazard from street foods may be significantly minimized consumption within four hours of preparation (Mulleta *et al*, 2001).

Study conducted in Ethiopia on manufacturing efficiencies and microbial properties of butter and Ayib - cottage cheese stated that Aerobic mesophilic bacterial count (AMC), counts of *enterobacteria*, and coliform bacterial count (CC) were performed. Average AMC, counts of *enterobacteria* and CC of butter samples were 8, 5.3 and 3.8 log cfu/g, respectively, while the counts for Ayib samples were 7.9, 5.1 and 4.4 log cfu/g, respectively. *Enterobacter*, *Escherichia*, *Klebsiella* and *Klyuvera* were the genera identified, while *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae* are the species commonly isolated from both products (Yilma *et al*, 2007).

1.2.13. Indicator organism

Routine examination of foods for a range of pathogenic microorganisms is impractical. In order to assess the microbiological safety from foodborne pathogens, widespread use of groups or species which are easily enumerated and whose presence in foods indicates exposure to conditions that might introduce hazardous organisms and/or allow their growth, are used. These groups are referred to as indicator organisms (Department of health directorate, South Africa, 1997).

1.2. 14. Colony Count

One of the methods for counting of viable bacteria in any fluid is viable colony count by diluting the fluid and culturing for bacteria. Counts of viable bacteria are commonly based on the number of colonies that develop in nutrient agar plates which have been inoculated with known amounts of diluted foods and then incubated under prescribed environmental conditions. Only those bacteria, which will grow under the chosen environmental conditions, can be counted. A wide variety of conditions can be obtained by changing the composition of the growth (agar) medium, the gaseous environment of incubation (presence or absence of O₂) and the time and temperature of incubation. The aerobic mesophilic count is most commonly used (Roberts *et al*, 2003).

1.3. Significance of the study

Poor handling, processing, and use of unsafe raw materials such as water, fruits etc and utensils for preparation of juices can result in health threat. Therefore, this study will be important in order to:

- Identify basic hygienic problems that affect quality of locally prepared unpasteurized fresh juices.
- Recommend remedial action for identified problems.
- Provide information for further study.
- Identify which juice types are easily contaminated

CHAPTER II: HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

The bacteriological profile of locally prepared fresh fruit juices could be similar to the previous studies in other developing countries.

2.2. Objective of the study

General objective:-

- To evaluate bacteriological profile of locally prepared fresh fruit juices and assess the hygienic conditions of fruit juice preparation sites in Hawassa town.

Specific objectives: -

- To determine bacteriological profile of locally prepared unpasteurized fruit juices.
- To isolate and identify selected pathogens associated with foodborne illnesses.
- To assess hygienic condition of processing and handling of locally prepared unpasteurized juices.

CHAPTER II: MATERIALS AND METHODS

3.1: Materials and Methods

Study Design

A cross-sectional study design was applied to evaluate the bacteriological profile of locally prepared fresh fruit juices in Hawassa town.

Study Area and Period

The study was conducted in Hawassa town from November 2010 to January 2011. Hawassa town is the capital city of SNNPR and it is located 275 km from capital city of Ethiopia, Addis Ababa. The altitude of the town is 1697 km above sea level with mean annual temperature and rainfall of 20.9 °C and 997.6 mm, respectively. In the town there are many Restaurants and Cafeteria that prepare unpasteurized fruit juices that can be consumed by visitors and people of the town.

Source of sample

Restaurants and Cafeteria that prepare unpasteurized fruit juices found in Hawassa town.

Sample Description

1. Unpasteurized Avocado juices
2. Unpasteurized Papaya juices
3. Unpasteurized Mango juices
4. Unpasteurized Pineapple juices

Sampling Technique

Stratified Sampling method

Sample Size

Total of one hundred twenty (120) fruit samples of four types (avocado, papaya mango and pineapple) from 10 sites (five restaurants and five cafés) were collected. Three samples of each type of fruit juices were collected from a single site. That is 30 samples of each juice type were collected.

Eligibility or Inclusion and exclusion criteria

In this study unpasteurized fruit juices were included and pasteurized juices were excluded.

Variables

a. **Dependent Variable**

- Quality of locally prepared unpasteurized fruit juices

b. **Independent Variable**

- Personal hygiene
- Environmental hygiene
- Water quality
- Educational status of juicer
- Health status of juicer
- Storage environment of juices

Data collection

Two basic data collection methods were used for study.

1. Questionnaire:

A questionnaire was used to obtain information on the demographic characteristics of the fruit juicers and servers, as well as, source of fruit and processing of fruit juices. All the personnel involved in the processing and/or serving of the fruit juices in the selected restaurants and cafés were included and type of the vender was determined.

2. Laboratory Procedure:

Laboratory procedures such as sample collection, sample processing, culture, microscopical examination and biochemical tests were used to determine colony count, isolation and identification of indicator organisms and selected pathogens.

Collection of samples:

From November, 2010 to January, 2011, a total of one hundred twenty (120) Samples of four types (Avocado, Papaya, Mango and Pineapple) of locally prepared unpasteurized fruit juices were collected randomly from Hawassa town. All the samples were collected on a voluntary basis from participating restaurant and cafes in wide mouth sterile (250 ml) containers aseptically, labeled and immediately transported to the laboratory in an ice-box where they were processed immediately.

At the time of sample collection, swabs were also collected from the blender machines in order to get strong evidence for source of contamination. The swabs were collected aseptically using sterile applicator cotton swab and inoculated in sterile bottle containing sterile nutrient broth.

Moreover, water samples were also collected from tap and container aseptically using sterile Duran bottle for determination of fecal coliform.

Sample processing:

After complete mixing of original juice sample, 10ml was measured and transferred to 90ml of peptone water and homogenized by Vortex machine (Biocote) in aseptic environment which was achieved by cleaning and disinfecting by different disinfectant as well as using Bunsen burner flame. A series of dilutions (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) were made by taking 1ml from homogenized sample and adding to sterile test tube containing 9ml sterile alkaline peptone water and mixed properly by vortex machine (Robers *et al*,2003).

Culture:

Colonial count

➤ Total viable count (TVC)

Pour plate

Serial decimal dilutions of each sample were made using peptone water solution as diluent. As a guide, with 'clean' products dilution to 10^{-3} may be sufficient whereas heavily contaminated products may require dilution to 10^{-6} .

Procedure

- a. Placed 1 ml of the dilution into each of two sterile Petri dishes.
- b. Added about 15 ml of molten clear nutrient agar, tempered to 44–47°C, to each plate.
- c. Mixed each plate well by moving it five times in a vertical, clockwise, horizontal and anticlockwise direction.
- d. Incubated all plates at 37°C for 24 hr

Calculation

Use the plates containing fewer than 300 colonies at two consecutive dilutions to calculate the results from a weighted mean. The number (N) of cfu/g or ml of test sample was calculated as follows:

$N = C/v (n1 + 0.1n2) d$ where: C is the sum of colonies on all plates counted

v is the volume applied to each plate

$n1$ is the number of plates counted at the first dilution

$n2$ is the number of plates counted at the second dilution

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

Round the result to two significant figures and express it as a number between 1.0 and 9.9 multiplied by 10^x where x is the appropriate power of 10 (Roberts *et al*, 2003).

➤ **Total coliform count (TCC):**

From each samples of previously prepared serial dilution, one ml was transferred into sterile Petri dishes and 15 ml of violet red bile agar (RVBA) medium (Oxoid company) previously sterile which was kept in water bath at 45°C was poured and swirled and finally incubated at 37°C for 24-48 hours. Purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of precipitated bile acids were counted using digital colony counter.

Procedure

- A. Placed 1ml of liquid sample or 10^{-1} homogenate into each of two Petri dishes; repeated with each dilution prepared.
- B. To each plate add 15 ml of molten VRBA cooled to 44–47°C. Mixed carefully and allowed to set. Overlaid each plate with a further 4–5 ml of molten, cooled VRBA.
- C. Allowed to set. Incubated the plates at 30°C or 37°C for 24±2h.
- D. Selected dishes that contain not more than 150 colonies and count purplish red.
- E. Colonies that have a diameter of 0.5 mm or greater, usually surrounded by precipitated bile acids were counted.
- F. Calculate the count per g or ml

➤ **Staphylococcal Count (SCC):**

From each samples of previously prepared serial dilution, one ml was transferred into sterile Petri dishes and 15 ml of Mannitol salt agar (MSA) medium(Oxoid company) previously sterile and kept in water bath at 45°C was poured and swirled and finally incubated at 37°C for 24-48 hours. Yellow and orange colonies surrounded by yellow zones due to mannitol fermentation were enumerated and further tested by coagulase test after overnight sub-culturing in nutrient agar plates.

Microscopic examination:

Microscopic investigation for Gram reaction and morphological features of suspected colony was determined using standard method of Gram's staining.

Detection of Salmonella:

A 0.5 ml quantity of each juice sample was aseptically transferred to a sterile bottle nutrient broth and incubated at 37°C for 24hrs. Then, 1.0ml of each pre- enriched sample was transferred into 10ml of selenite broth previously warmed to 37°C. It was incubated at 37°C for 48 hours. After incubation, one Petri-dish of Xylose lysine deoxycholate agar (XLD) was inoculated from each enrichment bottle incubated at 37°C for 24- 48 hours and examined for typical colonies of Salmonella.

Detection of Escherichia coli:

A 0.5 ml of each juice sample was inoculated on replicate MacConkey agar by spread plate method and incubated at 37°C and at 45°C for 24 hours in order to identify thermotolerant *E. coli*.

Biochemical Tests:

In order to identify enterobacteriaceae from the primary culture on XLD and MacConkey agar, the suspected colonies were sub cultured in to sterile nutrient broth and incubated until the broth gained cloudy appearance. Then organisms were sub cultured in to different biochemical tests such as Triple sugar iron agar, Simmons citrate agar, Lysine Iron agar, Urea broth, Motility test medium and Indol test.

Water analysis using multiple tube/most probable numbers (MPN):

Standard method of testing coliforms in water was used. 100 ml of water was aseptically collected in sterile Duran bottle to check water quality for preparation of fresh fruit juices. 100 ml water samples were distributed (five 10 ml amounts and one 50 ml amount) in bottles which contain Durham tube and sterile double strength MacConkey broth containing lactose and pH indicator (Oxoid company). After incubation at 44°C for 24 hours, the number of bottles with lactose fermentation and acid and gas production was counted. The lactose was fermented by the *coliforms* in the water. By reference to probability tables, the most probable number of *coliforms* in the 100 ml water sample was estimated (Cheesbrough *et al*, 2006).

pH Measurement:

pH of all undiluted samples was measured by pH meter immediately (TESTRONIX 511) after collection. It is important to determine the pH of the food sample before undertaking microbiological examination as this can influence the colony count and organisms sought. In general, in foods with a pH below 4.5 pathogens would not be expected to survive; the organisms present would be limited to yeasts, moulds and a few acid tolerant bacteria. Foods with a pH above 4.5 require full microbiological examination.

3.2: Quality Control

The Quality of the study was kept by training the data collector, preparing, and using standard operational procedures for laboratory investigation and media preparation. Structured Questionnaire was tested using pretest before conducting the study. Sample collection and processing were carried out using aseptic techniques. The samples were labeled properly. Culture and bacterial colony count were determined by experienced laboratory personnel. The performance and sterility test of prepared media were checked by incubating at 37°C and 44.5°C and inoculating with control strain organisms, respectively.

3.3: Data analysis

After completion of data collection, each measurement of different variables was recorded according to the work flow. Data entry and analysis was done using SPSS version 15.0 software. ANOVA was used to compare results among juices type and compared with the previous findings from the literature. P-value less than 0.05 were considered statistically significant.

3.4: Ethical considerations

The proposed work was ethically cleared by Department's Research and Ethical Committee of the Department of Microbiology, immunology and parasitology. Letter of support was also written from the department head to bureau of culture and tourism of SNNPRS. Only Volunteer Restaurants and/or cafes participated in this study. They were informed about the objectives and the nature of the study. Confidentiality was kept by using code number rather the name of vender as well as juicer. Finally the results will be displayed to concerned one.

CHAPTER IV: RESULTS

4.1. Questionnaire Result

A total of thirty seven juice maker were interviewed to obtain data on fruit juice processing, source of fruits and storage of fruits from randomly selected restaurants and cafes. Among 37, 26 (70%) were females and 11 (30%) were males. 22 (59.5%) of the respondents had completed high school grade and fifteen were attending in elementary school. None of the fruit juice makers had professional training related to safe handling and processing of fruit juices. Moreover, none of fruit juice makers was experienced using of antiseptics material prior to preparation of fruit juices. Among establishment of juice makers, five were restaurants and five were cafes. All of the venders were using tap water for dilution of fruit juices. Except one, all of venders did not boil water for juice dilution. Concerning to the source of fruits, all of the venders had bought from open markets. Only one vender was using refrigerator for temporary storage of fruit but the rest were storing on shelves and in baskets (Table1).

Table 1: Source of fruits and their storage in randomly selected venders in Hawassa town from November 2010 to January 2011.

Source and Storage	No of Venders	Percent
<i>Source</i>		
Open Market	10	100
<i>Storage</i>		
Shelf	8	80
Basket	1	10
Refrigerator	1	10

4.2. Swab from blender

A total of thirty swabs (three from each blender) were collected from ten vendors (five restaurants and five cafes) for bacteriological analysis during study period. Among these, three swabs from one vendor yielded for *Staphylococcus aureus*. Twenty seven swabs from nine blenders were free from any pathogen associated food poisoning.

4.3. Water Analysis

A total of sixty (60) water samples (30 from the containers and 30 directly from taps) from 10 vendors were analyzed for bacteriological quality of water which was used for preparation of unpasteurized fruit juices and washing of glasses and other equipments. Among these, the most probable number of water from the tap of each vendor was zero (0 MPN/ml) whereas the most probable numbers (MPN/100ml) of water samples from the container of each vendor were in range from one (1MPN/100ml) to nine (9 MPN/100ml) (Table 6) .

4.4. Standard Plate Count (SPC)

From each of 120 locally prepared fresh fruit juice representative portion was homogenized, serially diluted with alkaline peptone water and inoculated on Nutrient agar, Violet red bile agar, and Mannitol salt agar using pour plate method and incubated at 37°C for 24-48 hours to determine Total Viable Count, Total *Coliform* Count, and Total *Staphylococcal* count respectively.

4.4.1. Total Viable Count

As depicted in table 2, a total of one hundred twenty (120) locally prepared fresh fruit juices samples were cultured for total viable count (TVC). The overall mean of the total samples was $4.06 \log \text{ cfu/ml}$. The mean of total viable count of Avocado was highest ($4.76 \log \text{ cfu/ml}$) whereas the mean of Papaya, Mango and Pineapple were $4.57 \log \text{ cfu/ml}$, $3.57 \log \text{ cfu/ml}$, and $3.34 \log \text{ cfu/ml}$ respectively. The means of the fruit juices have statically significant difference ($P=0.00$).

Table 2: Total viable count (log cfu/ml) of locally prepared fresh fruit juices Hawassa twon, 2011.

Juice type	N	Mean(log g TVC)	Std. Deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower boundary	Upper boundary		
Avocado	30	4.76	1.23	0.22	4.31	5.22	1.74	6.74
Papaya	30	4.57	1.11	0.20	4.15	4.99	1.45	6.48
Mango	30	3.57	0.98	0.18	3.21	3.93	1.34	5.45
Pineapple	30	3.34	0.97	0.18	2.98	3.70	1.23	4.97
Total	120	4.06	1.23	0.11	3.84	4.28	1.23	6.74

TVC: total viable count

4.4.2. Total coliform count

Similarly a total of one hundred twenty (120) fresh fruit juices were cultured in order to determine total coliform count (TCC). The mean count of total *coliform* of all samples was $3.15 \log \text{ cfu/ml}$ and likewise Avocado with the highest mean ($3.98 \log \text{ cfu}$) over papaya, mango and pineapple which were with 3.61 , 2.54 , and $2.45 \log \text{ cfu}$, respectively. The means of total coliform count among juice type showed statically different ($p=0.00$) (Table 3).

Table 3: Total coliform count (log cfu/ml) of locally prepared fresh fruit juices in Hawassa twon, 2011.

Juice type	No of sample	Mean(log TVC)	Std. Deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower boundary	Upper boundary		
Avocado	30	3.98	1.23	0.22	3.52	4.44	1.30	5.73
Papaya	30	3.61	1.33	0.24	3.11	4.11	1.04	5.95
Mango	30	2.54	1.01	0.18	2.17	2.92	1.23	5.20
Pineapple	30	2.45	0.98	0.18	2.09	2.82	1.00	4.54
Total	120	3.15	1.31	0.12	2.91	3.38	1.00	5.95

TCC: total coliform count

4.4.3. Total Staphylococcal count

Out of one hundred twenty specimens of fresh fruit juices, ninety eight showed colonial growth on *mannitol salt agar (MSA)* for *staphylococcus species*. The mean count of all types of juice was $2.99 \log \text{ cfu/ml}$ with maximum mean of $3.48 \log \text{ cfu/ml}$ (Avocado) and minimum mean of $2.33 \log \text{ cfu/ml}$ (Pineapple). The total staphylococcal count among the juice type were statically significant ($P=0.01$) (Table 4).

Table 4: Total staphylococcal count (log cfu/ml) of locally prepared fresh fruit juices in Hawassa town, 2011.

Juice type	N	Mean(log TSC)	Std. Deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower boundary	Upper boundary		
Avocado	30	3.48	1.44	0.26	2.96	4.02	1.08	5.96
Papaya	26	3.09	1.58	0.32	2.44	3.74	1.11	5.96
Mango	30	2.89	0.74	0.17	2.54	3.24	1.30	4.00
Pineapple	23	2.33	0.73	0.15	2.02	2.65	1.36	4.48
Total	98	2.99	1.28	0.13	2.73	3.25	1.08	5.96

TSC: total staphylococcal count

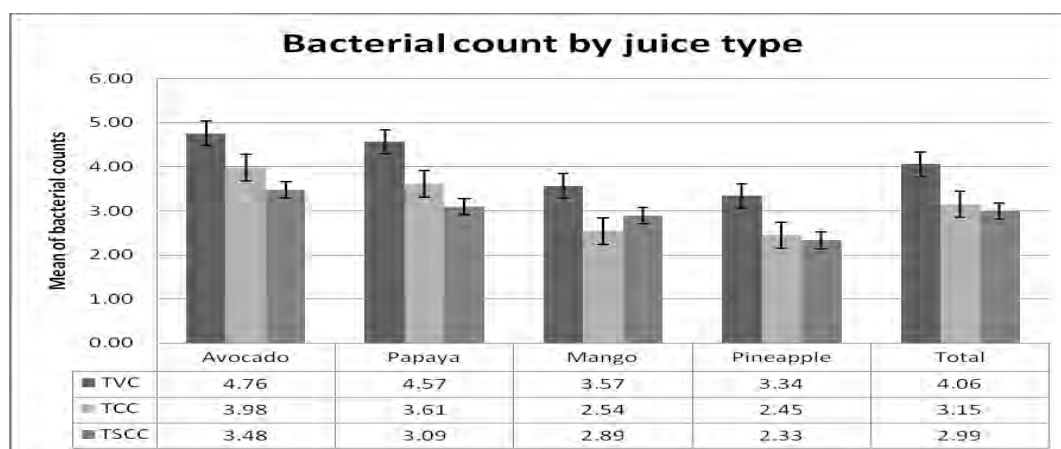


Figure 1: The mean bacterial count (log cfu/ml) of locally prepared fresh juices against fruit juice types in Hawassa town, 2011.

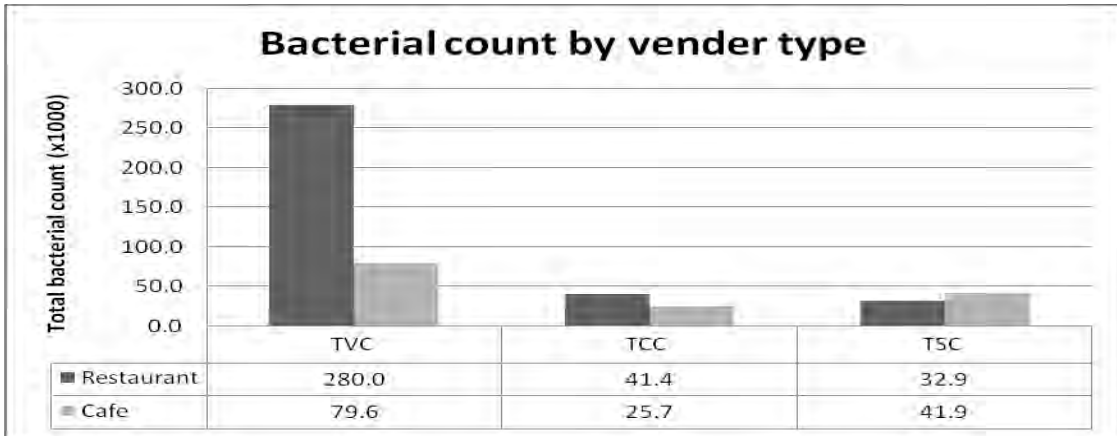


Figure 2: Bacterial count (cfu/ml) of locally prepared fresh juices against vender type in Hawassa town, 2011.

4.5. pH measurement

A total of one hundred twenty fresh fruit juices were analyzed for their pH measurement. The mean value of total fruit juices was 4.76 with range of 3.09 to 6.57. The pH of both Mango and Pineapple were 3.86, and 3.67 respectively and more acidic than Avocado and Papaya (6.22, and 5.28, respectively).

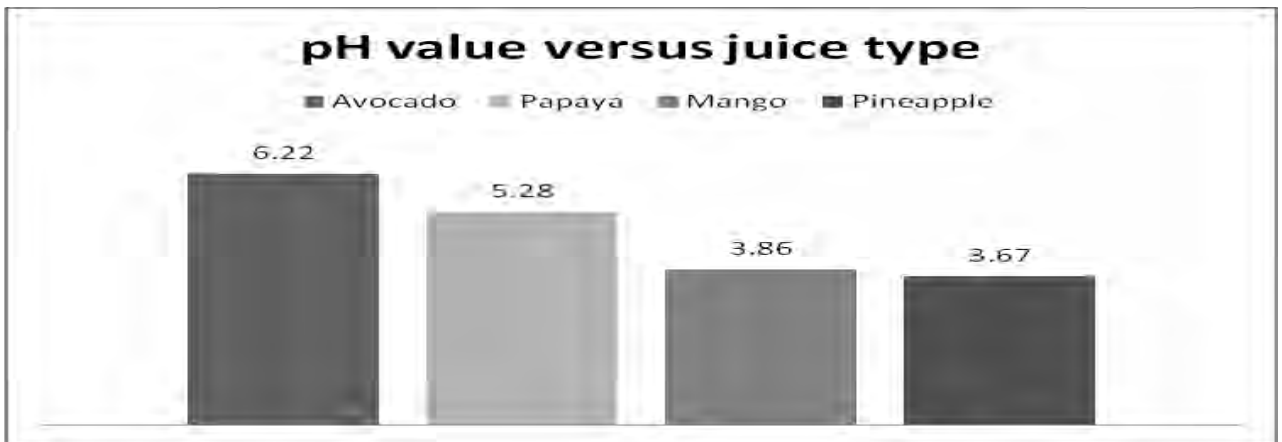


Figure 3: The mean pH measurement of locally prepared fresh juices among juice types in Hawassa town, 2011.

4.6. Biochemical test result for Enterobacteriaceae

Biochemical analysis was performed on isolated colony from locally prepared unpasteurized fruit juices sample for different Enterobacteriaceae. Out of one hundred thirty nine (139) isolates, the predominant organism was *Citrobacter species* with 36.7%(51/139) followed by *Klebsiella species*, *Enterobacter species*, *Escherchia coli*, *Serratia species*, *Proteus species* and *Salmonella species* with 25.2%(35/139), 18.0%(25/139), 9.4(13/139), 5.0%(7/139), 3.6%(5/139) and 2.2%(3/139), respectively.

Table 5: Bacteriological classification of locally prepared fresh fruit juice samples in Hawassa town, 2011.

Type of pathogen	N	No of total satisfactory samples	No of Unsatisfactory samples	No of Hazardous Samples
<i>S . aureus</i>	120	109(90.8%)	0	11(9.2%)
<i>E. coli*</i>	120	113(94.2%)	7(5.8%)	0
<i>Salmonella species</i>	120	117(97.5%)	0	3(2.5%)
<i>Shigella species</i>	120	120(100%)	0	0

*Thermotolerant *E. coli*

Table 6: Bacteriological quality of water used to juice preparation and equipment washing in Hawassa town, 2011.

Source of water	No of sample	Results of water analysis	
		Mean of MPN/100ml	Range of MPN/100ml
Tap	30	0	0
Container	30	1.8	1-9

CHAPTER V: DISCUSSION

5.1 Discussion of finding

Fruit juices specially unpasteurized one are well recognized by consumer for their fresh flavor, vitamins content and nutritive value. Therefore, the number of the juice venders is significantly increasing in order to not only give services for consumers, but also to create job opportunities to community. On the other hand, unpasteurized fruit juices can pose problems for human health specifically to children, pregnant women, elderly people and people with a weakened immune system due to poor handling, processing and storage of fruit and fruit product (Health Canada, 2006). Ethiopian culture of taking fresh fruit juices from local venders is relatively higher than preserved or pasteurized fruit juices.

Fruits should be inspected for clean, dry, and intactness by juice makers who should be trained in inspection and personal hygiene. Only sound whole fruit should be used for fruit preparation. Decayed, wormy, damaged, soiled fruit should be sorted and discarded to prevent contamination of juice product. All fruit should be subjected to effective washing, brushing and rinsing. Water supplies for fruit cleaning must be potable. All workers must be free from communicable diseases. They should be trained not only for their task, but also to keep the venders clean and to practice personal hygiene (Canadian Food Inspection Agency, 2010). However, none of juice maker that participated to the current study was experienced to training relevant to juice processing and personal hygiene. Ideally, all fruit/food handlers must be inspected by health personells for any communicable disease

Generally, the overall assessment of the fruit samples analyzed bacteriologically indicated high counts. Most of the samples showing high bacterial counts were Avocado followed by papaya. The profile of total aerobic mesophilic bacteria count as shown in table 2, the mean total viable count of Avocado and papaya were $4.76 \log cfu/ml$ and $4.57 \log cfu/ml$, respectively. Among all of the samples, the total viable count of 38(31.67%) were found to be above Gulf region standards ($5.0 \times 10^5 cfu/ml$) (Table 7). Regarding to type of juices, Avocado was predominant to be beyond the Gulf region standard with

19(63.33%) and the rest papaya, mango and pineapple were 14(46.67%), 1(3.33%) and 4(13.33%) respectively. The results of the current study were not in agreement with findings which were reported from Jimma town for Avocado (6.0 log/ml) and Papaya (6.6 log/ml) (Ketema *et al*; 2008). The probable reason for the discrepancy may be Geographical variation, seasonal variation, time of sample collection, hygiene, and incubation time. On the other hand the total viable count of both Mango and Pineapple were significantly different ($P=0.00$) than Avocado (3.57 log cfu/ml) and Papaya (3.34 log cfu/ml) in the current study. This may be attributed to pH value, oxidation- reduction potential, and nutritional constituent of fruits (Yigeremu *et al*; 2001).

The mean quantitative analysis of fruit juices for Total coliform count was 3.15 log cfu/ml and likewise Avocado with the highest mean (3.98±1.23 log cfu) over papaya, mango and pineapple were 3.61, 2.54, and 2.45 log cfu respectively. Out of 120 unpasteurized fruit juices, the total *coliforms* count of 93(77.5%) were shown to be above gulf region standard (100 cfu/ml). Out of thirty Avocado samples, majority (86.69%) were beyond the Gulf region standard by their total *coliforms* count. Moreover majority of the other fruit juice like papaya (83.33%), mango (70.00%) and pineapple (77.50%) were also above gulf region standard. The total coliform count of current study for Avocado, Mango and Pineapple were comparable with similar study conducted in Qatar (3.97log/ml, 2.91log/ml and 3.3log/ml respectively) and in Nagpur city, India (3log to 4log/ml in all type of juices) (Titarmare *et al*, 2009). The mean Total coliform count of Mango and Pineapple were significantly different ($P=0.00$) from both Avocado and Papaya. The probable reason for this again similar with the one mentioned for the Total viable count.

The presence of *staphylococci* is usually indicative of contamination from food handlers. Inadequately cleaned equipment or raw animal products may also be sources of contamination. The presence of large numbers of *stapylococcus* is in general an indication of poor hygiene and temperature control (Department of health, South Africa). In the present study *staphylococci* count, the overall mean of all unpasteurized fruit juices was 2.99 log cfu/ml. And among the type of juice, Avocado was above the others to show high number of *staphylococcal species*. Twenty two (22) of total sample were beyond the

Australian standard (10^4 cfu/ml) (Appendix 2). Out of ninety eight, eleven (11.22%) were positive for *Staphylococcus aureus* and these entire positive sample were above the standard. According to study conducted in Jimma the highest number of *staphylococcus species* (5.41 log/ml) was observed in Avocado juices. Even though the type of juices to show high number of *staphylococcus species* was similar in both study, the magnitude of *staphylococcus species* was relatively less in this study. The difference in colonial count between the two studies may attribute to different factors such as Geographical variation, seasonal variation, hygiene, incubation time, sample transportation time, handling and processing, and storage.

Regarding to pH of the fruit juices sample, both Avocado (6.22) and Papaya (5.28) were shown the pH range that support growth of most bacteria (Figure 3). This may be the possible reason for the high number of both Total viable and total coliform count in Avocado and Papaya juices (Table 2). The pH result obtained from the current study was comparable with study conducted in Jimma, Ethiopia and Nagpur City, India (Ketema *et al*, 2008; Titarmare *et al*, 2009). However study conducted in Visakhapatnam City, India (Lewis *et al*, 2006) showed that there was great difference in pH measurement of Mango (4.5-5.6) and Pineapple (5.2-6.6). The probable reason may be inappropriate storage of fruit and over dilution of fruit juices.

In the present study 9.2% (11/120) of the total samples were positive for *Staphylococcus aureus* and which was categorized as potentially hazardous to human health according to the Australia Guideline for microbiological examination of Ready to eat food (Australian Guideline, 2001). The occurrence of *Staphylococcus aureus* in fruit juices may be attributed to contamination as a result of improper handling, processing and storage of fruit juice. In addition, equipment that used for processing may contribute to increase not only to Staphylococcal count but also both Total viable and total coliform count values. Reports from studies conducted from Amravati city, India (6%) (Tambekar *et al*, 2009) and Dhaka city, Bangladesh (7.89%) (Shakir *et al*, 2009) are comparable with present study.

The presence of termotolerant *E.coli* in 5.8% (7/120) of locally prepared unpasteurized fruit juices samples indicate evidence of fecal contamination which may be attribute to

poor hygienic practice of juice makers or from fruit farm fields during harvesting of fruits. Moreover preparation site may also contribute its own part to incidence of organism in juice sample. Of 7 finding of *E.coli*, 3 were concomitantly identified with pathogenic bacteria. These finding strongly indicated the fecal contamination of juice sample. Similar research conducted in India reported that predominant bacteria were *E.coli* (40%). Another study conducted in Bangladesh showed that all of the samples such as Papaya (100%), Mango (100%) and Pineapple (100%) were positive for *E.coli* (Shakir *et al*, 2009). However survey conducted in Ireland shown that only 0.2% of the unpasteurized juice contained *E.coli* (Melbourne, 2005). The incidence of *E.coli* in the current study was inconsistent with the above mentioned studies. The probable reason for this difference may be number of factors such as geographical variation, seasonal variation, sanitation habit, collection and transportation of sample, and procedure of inoculation.

According to current study, the incidence of *Salmonella species* was 3 (2.5%) out of 120 fruit juice samples (two from Avocado and one from Papaya). Even though the number of positive samples is very small, it can affect large number of individuals who consumed these contaminated juices. Similar study in Bangladesh showed that the overall finding of *Salmonella species* was 7.89% in unpasteurized fruit juices (Shakir *et al*, 2009). Another study conducted in India reported that 50% of fruit and vegetables juices were positive for *Salmonella species* (Titarmare *et al*, 2009). Similar research conducted in India again documented 38.8% of fruit juices were identified as *Salmonella species* positive (Lewis *et al*, 2006). The incidence of *Salmonella species* was 16% in street vended fruit juices samples (Tambekar *et al*, 2009). Incidence rate of current study was lower than study conducted in Asian countries. The probable reason for difference may be attributed to fruit type, geographical variation, seasonal variation, sanitation habit and variation in diagnosis.

Present study also identified members of *Enterobacteriaceae* other than *E. coli* and *salmonella species* such as *Citrobacter species*, *Klebsiella species*, *Entrobacter species*, *Seracia species* and *Proteus species*. However finding of these organisms in fruit juices

would not surprise because the surface of different fruit is colonized by this organism normally (European commission health, 2002).

This study also tried to address source of contamination by analyzing water sample that used to dilute or prepare fruit juices. Hence water was analyzed both from tap and container. According to the finding water from container was showed contamination (1-9MPN/100ml) whereas water from tap showed to be free from *coliforms* (0 MPN/100ml). This finding assures that the possible contamination of juices would be poor processing and handling of fruit juices. Furthermore swabs were collected from blenders of participant venders. Only from one blender *Staphylococcus aureus* isolated. This may suggests that equipment can also contribute its own for contamination of fruit juices.

5.2 Limitation of the Study

Some of deficiencies of current study as follow:

- As it is known, not all *Staphylococcus aureus* are associated with staphylococcal food poisoning. 50% *Staphylococcus aureus* strain produce heat stable enterotoxins. Therefore current study cannot address strains that secrete toxin as well as preformed toxin.
- Similarly present study identified thermotolerant *E. coli*. However, strains that associated with food poisoning (*EHEC O157: H7*) and preformed toxin were not determined.
- The reliability of the finding of any study depends on the sample size of the study subjects. However, this study conducted on small size of study subject due to various reasons. As stated above, it would have been better to identify *S. aureus* isolate up to strain level which would not be done in the present study due to time limits and absence of facility. Therefore, we cannot state with confidence whether the isolates were enterotoxogenic or not.
- Since our study focus only on bacterial quality, the effects of fungus on unpasteurized fruit juices were not stated.
- Our study findings were interpreted with standards from other parts of world rather than our country.

5.3 Conclusion

In this study 31.67% and 77.5% of total fruit samples were found to show higher total viable count and total coliform counts, respectively above the specification set of Gulf region standard. According to pH measurement, both Mango and Pineapple are acidic than Avocado and Papaya. Furthermore high bacterial load was observed in Avocado and Papaya than Mango and Pineapple. Moreover, 9.2%, 5.8%, and 2.5% of total of fruit samples were positive for *Staphylococcus aureus*, *Thermotolerant E. coli*, and *Salmonella species* respectively. Among total positive sample, 70.6% (12/17) were Avocado and 23.5% (4/17) were Papaya fruit juices sample. Bacteriological analysis of water sample, which was used for dilution of fruit juices, from tap and container were determined. As the result indicated water from tap was safer than water from containers. In addition swabs from the blender of ten vendors were examined for pathogenic organisms. However, only one was positive to *Staphylococcus aureus*. Therefore, based on these data of the assed fruit juices, Avocado and Papaya were found to be heavily contaminated with bacteria that could pose health problems. Probably the low pH value reported in Mango and Pineapple juices may favor these two juices to be safe for consumption. Moreover Lack of training (orientation) on the proper storage and processing of fruit juices may attributed to contamination of fruit during harvesting or poor processing and handling of fruit juices.

5.4 Recommendation

Based on the findings of the present study and the above mentioned limitations the following recommendations are made:

- The first and most important concern is national specification sets or microbiological standard for foodstuff should be prepared in order to interpret the result at national level.
- The importance of personal hygiene, storage of fruit at cold temperature, using boiled water for diluting the juice/cleaning equipment should be informed to people involved in preparing and handling fruit juices.
- In order to assure safe unpasteurized fruit juices for consumers, there should be regular inspection and routine Microbiological analysis should be made.
- Since current study was conducted on small sample size, the researcher also recommends further study by increasing sample size.
- Sereotyping and antimicrobial sensitivity should be carried out on identified pathogens in order to know resistance pattern to different antibiotics.

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APPENDEX-1

QUESTIONNAIRE

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

**DEPARTMENT OF MEDICAL MICROBIOLOGY, PARASITOLOGY AND
IMMUNOLOGY**

Name of data collector: _____

Type of vender: _____

Questionnaire format sheet to asses safety and quality of locally prepared fruit juices to be filled by juice makers.

1. Educational status of juice maker

- Illiterate

-Elementary

-Highs cool and above

2. Type of vender

- Restaurant

- Caf 

3. Type of fruit juices prepared in vender

- Avocado

- Papaya

- Mango

- Orange

- Pineapple

- Others

4. Source of fruit

- Open market

- Directly from producers

5. Temporary storage site

- Shelf

- Basket

- Refrigerator

- Others

6. Water source for juice preparation

- Tap

- Well

-spring

7. Cleaning habit of juice maker during processing

- Yes

- No

8. If yes, how you practiced?

- With Water only

- With Water and soap

- With water, soap and antiseptic

9. Do you wash fruit before making juices?

- Yes

- No

10. If yes, how _____

Sample collection Forma

Sample No	Type of vender	Type of sample	Finding						
			TVC	TCC	TSC	CPSC	E.coli	Salmonella	Shigella

KEY:

TVC- total viable bacterial colony count

TCC- total coliform count

CPSC- coagulase positive Staphylococcus

APPENDIX-2

Guidelines for the microbiological examination of ready - to - eat foods (December 2001)

Table 1. Guideline levels for determining the microbiological quality of ready-to-eat foods.

Test	Microbiological Quality (CFU per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
<i>Standard Plate Count</i>
Level 1	$< 10^4$	$< 10^5$	Greater than or equal to 10^5	.
Level 2	$< 10^6$	$< 10^7$	Greater than or equal to 10^7	.
Level 3	N/A	N/A	N/A	.
<i>Indicators</i>
<i>Enterobacteriaceae*</i>	$< 10^2$	10^2-10^4	Greater than or equal to 10^4	.
<i>Escherichia coli</i>	< 3	3 – 100	Greater than or equal to 100	**
<i>Pathogens</i>
Coagulase +ve staphylococci	$<10^2$	10^2-10^3	10^3-10^4	Greater than or equal to 10^4 SET +ve
<i>Clostridium perfringens</i>	$<10^2$	10^2-10^3	10^3-10^4	Greater than or equal to 10^4
<i>Bacillus cereus</i> and other pathogenic <i>Bacillus</i> spp	$<10^2$	10^2-10^3	10^3-10^4	Greater than or equal to 10
<i>Vibrio parahaemolyticus</i> #	<3	$<3 -10^2$	$10^2 -10^4$	Greater than or equal to

				10
<i>Campylobacter</i> spp	Not detected in 25g	-	-	detected
<i>Salmonella</i> spp	Not detected in 25g	-	-	detected
<i>Listeria monocytogenes</i>	Not detected in 25g	detected but <10 ² ++	-	Greater than or equal to 10 ² ##

* *Enterobacteriaceae* testing is not applicable to fresh fruits and vegetables or foods containing these.

**Pathogenic strains of *E. coli* should be absent.

V. parahaemolyticus should not be present in seafoods that have been cooked. For ready-to-eat seafoods that are raw, a higher satisfactory level may be applied (<10² cfu/g). The potentially hazardous level of *V. parahaemolyticus* relates to Kanagawa-positive strains.

. Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g.

The detection of *L. monocytogenes* in ready-to-eat foods prepared specifically for .at risk population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous.

N/A .SPC testing not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls)