

**MICROBIOLOGICAL QUALITY AND SAFETY OF FRUIT JUICE
SERVED IN CAFES OF DIRE DAWA TOWN, EASTERN ETHIOPIA**

MSc. THESIS

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HARAMAYA UNIVERSITY, HARAMAYA

**Microbiological Quality and Safety of Fruit Juice Served in Cafes of Dire
Dawa Town, Eastern Ethiopia**

**A Thesis Submitted to the Department of Biology,
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**In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN BIOLOGY**

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HARAMAYA UNIVERSITY
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We hereby certify that I have read and evaluated this Thesis entitled Microbiological Quality and Safety of Fruit Juice Served in Cafes of Dire Dawa Town, Eastern Ethiopia prepared under our guidance by Abiyot Gergara. We recommend that it be submitted as fulfillment the thesis requirement.

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DEDICATION

This work is dedicated to my beloved mother W/ro Tsige Mekonin Gessesse who passed away when I was a third year undergraduate student. I wish she could see her son achieving this stage she was wishing for. May her souls rest in peace!

STATEMENT OF THE AUTHOR

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

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BIOGRAPHICAL SKETCH

The author was born on March 26, 1980 G.C in Holeta town, Oromiya rigion. He attended his primary education at Holeta General Primary and Secondary School and his secondary education at Haramaya High School in Haramaya town. Then he joined Education Faculty, Bahir Dar University, and graduated with BEd degree in Biology in June 2005. After that he served as a biology teacher for eleven years at Harar Medehanialem Comprehensive Secondary School, Harar Abadir Secondary School and recently he is serving as a biology teacher at Harar Preparatory Secondary School. Finally, he joined the Postgraduate Program Directorate at Haramaya University in July 2012 to pursue his studies leading to the Degree of Master of Sceince in Biology.

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ACRONYMS AND ABBREVIATIONS

BHI	Brain Heart Infusion
BGLBB	Brilliant Green Lactose Bile Broth
CFU	Colony forming units
DD	Dire Dawa
JH	Juice house
MCA	MacConkey Agar
MPN	Most Probable Number
MSA	Mannitol Salt Agar
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
SAC	<i>Staphylococcus aureus</i> Count
SFBC	Spore Forming Bacterial Count
TCC	Total Coliform Count
YMC	Yeast and Mold Count
TFCC	Total Fecal Coliform Count
SC	<i>Salmonella</i> Count
TVAMBC	Total Viable Aerobic Mesophilic Bacterial Count
XLDA	Xylose lysine Deoxycholate Agar

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Microbiological Quality and Safety of Fruit Juice Served in Cafes of Dire Dawa Town, Eastern Ethiopia

ABSTRACT

Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits. 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, mango 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 March 2016 to August 2016 in Dire Dawa town using structured Questionnaire to assess source of fruit and processing of fruit juices and to perform bacteriological analyses. aerobic mesophilic bacterial count, yeast and mold count, total coliform, faecal coliform, aerobic spore forming bacterial Count and the presence of pathogenic microorganisms such as Staphylococcus aureus, and Salmonella spp. were analyzed following standard methods. The results indicated that among the juices, higher total coliform(2.17×10^2 mpn/ml) and faecal coliform(6.43×10^1 mpn/ml) were observed in papaya juice than in avocado and mango juices. However, the aerobic mesophilic bacterial, spore forming bacterial counts and yeast and mold counts (7.21×10^5 cfu/ml 2.94×10^3 cfu/ml, and 1.08×10^4 respectively) were higher in avocado juice than in the other two types of juices. The proportion of avocado, mango and papaya juice samples positive for fungi were 70% (21/30), 76.67% (23/30), and 80% (24/30), respectively. Whereas the proportion of fruit juice samples that were positive for Staphylococcus aureus, Salmonella species and E. coli were 52.2%, 37.77%, and 11.1%, respectively. The higher prevalence of fecal coliforms indicated that the selected fruit juices were hygienically of lower standards. 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.

Key Words: Avocado, Bacterial contamination, Dire Dawa, Fresh fruit juice, Mango, Microbiology, Papaya

1. INTRODUCTION

Juices are the aqueous liquids expressed or otherwise extracted usually from one or more fruits or vegetables, purees of the edible portion of one or more fruits or vegetables, or any concentrates of such liquids or purees (Fraternal, 2011). Juice is prepared by mechanically squeezing or macerating fresh fruits without the application of heat or solvents. The consumption of fruit juices could have both positive and negative effect on the part of consumers. When processed under hygienic conditions, it could play an important role in enhancing consumers' health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection. Eating a diet rich in fruits and vegetables reduces risk of stroke, diabetes, certain cancers such as (mouth), heart diseases, developing kidney stone, and decreases bone loss. Fruit consumption reduces the risk of neural tube defects such as spina bifida and anencephaly during fetal development (Chumber *et al.*, 2007).

Food-borne diseases are harmful illnesses mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated foods or drinks (Tambekar *et al.*, 2009). In addition to their increasing popularity in consumption patterns, fresh fruits have also become increasingly important vehicles in food-borne disease statistics. There are reports of food-borne illnesses associated with the consumption of fruit juices at several places (Chumber *et al.*, 2007).

Contamination of fruit juices sold in restaurants, cafes and even road side stalls are sometimes unacceptable for human consumption and create significant health problems. Most of the sellers have on site facilities for extracting the juice from fresh fruits like orange, bananas, mangoes, papaya, watermelons and a mixture of these and other fruits that may be available (Al-Jedah and Robinson 2002). After preparation, the sellers then serve the juices to their thirsty customers. However, bearing in mind the sellers' method of extraction, an inevitable question arises over the microbial safety. For example the outside of the fruit may not be washed properly before it is placed in the juicer for extracting the juice and even if it is washed the total colony count may be exceeding 1.0×10^5 cfu/cm² (Lewis *et al.*, 2006).

Many factors can contribute to microbial contamination throughout the production and packaging of fresh produce. Pathogenic microorganisms can enter fruits through damaged surfaces that occur during growing or harvesting. But the main sources of contamination in fruit juices are untidy instruments and utensils, unhygienic water for dilution, dressing with contaminated ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust (Sandeep *et al.*, 2001). Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, *Salmonella* spp., *Shigella* spp., and *S. aureus*. Furthermore, natural juices, even when kept under refrigeration, have a short shelf life (Barro *et al.*, 2006).

Contamination of fruits with eggs and cyst especially those hawked by fruit vendors may also serve as a source of infection to consumers of such fruit items. These parasites includes: *Entamoeba histolytica*, *Giardia duodenace*, *Trichuris trichura*, *Ascaris Lumbricoides* and *Enterobius vermiculais* (Muinde and Kuria, 2005).

There are various reports on the outbreaks of illnesses in humans associated with consumption of unpasteurized fresh fruit juices. In 1995, unpasteurized fresh orange juice contaminated with *Salmonella* spp. was linked to an outbreak in Florida Theme Park, USA, where more than 60 visitors were affected (Sandeep *et al.*, 2001). There are also reports of food-borne illnesses associated with the consumption of fruit juices at several places in India and elsewhere (Vojdani *et al.*, 2008).

Until recent decades, it was generally accepted that high acid fruit juices (pH 3.0-4.0) could not support survival and growth of microbial pathogens. However, a number of outbreaks of human illnesses that occurred during the 1990s were associated with the consumption of unpasteurized fruit juices (Linton, 2004). Although growth is unlikely at low pH, it is well documented that pathogenic microorganisms may survive in fruit juices, become adapted to the acid environment, and cause outbreaks of food borne illnesses (Parish, 2009). Antimicrobial activities related to organic acids such as malic and citric acids have been reported Han and (Linton, 2004). They are commonly found in fruit juices or they may be added to low-acid foods as a preservation agent. In addition, refrigerated storage can considerably extend the survival of the pathogens in juices. At warmer temperatures, such as

room temperature, *E. coli* O157:H7 and *Salmonella* populations will be reduced rapidly, compared to those in refrigerated acid foods. This should be kept in mind when making decisions regarding the storage temperatures (El-Safey, 2013).

Quality in the context of fruit is the sum total of all those attributes which combine to make the fruit acceptable, desirable and nutritionally valuable as human food (Ha *et al.*, 2007;. Microbiological quality of drinks is ascertained in order to ensure the safety of the consumer. Traditionally, the detection and enumeration of indicator organisms rather than of pathogens have been used. The coliform is the principal indicator of the suitability of a particular drink for consumption. Large numbers of coliforms may cause gastroenteritis, and also streptococci may be a better indicator (Mutaku *et al.*, 2005). Indicator organisms in foods serve as tools to evaluate the microbial quality of the product. The aerobic plate count, with its inherent nutritional limitations which are due to the heterogeneous distribution of bacteria in foods, gives a useful measure of the quality of a product (Derlet, 2008).

Freshly squeezed fruit and vegetable juices have little or no process steps that reduce pathogen level, if contaminated, such as no kill step. Freshly squeezed juices are simply prepared by extracting, usually using mechanical means, the liquid and pulp of mature fruit or vegetable. The final product is an unfermented, untreated juice, ready for consumption (Jaeger *et al.*, 2009). During the process, contamination from raw materials, equipment or food handlers could be easily transferred to final product. If pathogens such as salmonellae were present in freshly squeezed juice, individuals may be exposed.

In Ethiopia, various foods were reported to carry pathogens or allow the growth of pathogens (Mezgebu and Mogessie, 1998; Wolde Aregay and Mogessie, 1998). Indeed, studies made by FAO (1997) in Africa, Asia and Latin America pointed out that the important aspect of fresh fruit juice is their safety. The study suggested that the problems associated with safety of fresh fruit juices are real and need to be addressed to protect consumers. There is, however, limited information (Mogessie, 1995 and Tsige *et al.*, 2008) on the microbial load and safety of fresh fruit juice, in spite of the widespread use of such food items in Ethiopia. On the other hand, bacterial food-borne diseases pose considerable problems in countries like Ethiopia and thus

call for a marked concern. Hence, there is strong need to generate data on the microbial load and safety of fresh fruit juice served in big cities such as Dire Dawa. Such information is useful to appreciate the safety problems related to fresh fruit juice so that regulatory agencies may take appropriate steps to improve safety and sanitation with respect to this economic sector.

General objective

The main objective of this study is to assess the microbiological quality and safety of freshly squeezed fruit juices consumed in Dire-Dawa town.

Specific objectives

1. To enumerate the major indicator microbial groups (aerobic mesophilic bacteria, coliforms, faecal coliforms, staphylococci, spore-forming bacteria, yeasts and molds) that allow us evaluate the quality and safety of freshly squeezed fruit juice in the study area.
2. To detect the presence of selected bacterial pathogens (*E. coli*, *salmonella* and *S. aureus*) in freshly squeezed fruit juices.
3. To determine the association between the major risk factors and the microbial loads of fresh fruit juices consumed at vending houses.

2. LITERATURE REVIEW

2.1. Juice

Juices are the aqueous liquids expressed or otherwise extracted usually from one or more fruits or vegetables, purees of the edible portion of one or more fruits or vegetables, or any concentrates of such liquids or purees (Fraternale, 2011).

Juice is intended for direct consumption, obtained by the mechanical process from sound, ripe fruits, preserved exclusively by physical means. Juices may be prepared from nearly all fruits, if desired; the most common ones include pineapple, orange, grapefruit, mango and passion fruit. Nevertheless, any fruits (e.g. banana) can be easily pureed, but it is more expensive to produce a clear juice from the pulp. The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice. The addition of sugars or acids can be permitted but must be endorsed in the individual standard. (FAO, 2006).

2.2. Unpasteurized Juice

Unpasteurized juice 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 may also be found on ice or in refrigerated display cases and in produce sections at grocery stores (Health Canada, 2006).

2.3. Pasteurized Juice

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

2.4. Juice Processing

Juice is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvents. For example orange juice is a liquid extract of one fruit of orange tree. Juice may be prepared in the home from fresh fruits and vegetables using variety of hand or juice extractor. Many commercial juices are filtered to remove fiber or pulp, but high pulp fresh orange juice is a popular beverage. Juice may be marketed in concentrated form, sometimes frozen, requiring the user to add water to reconstitute the liquid back to its original state. However, concentrates generally have a noticeably different taste than their comparable fresh squeezed versions. Other juices are reconstituted before packaging for retail sale. Common methods for preservation and processing of fruit juices include canning, pasteurization, freezing, evaporation and spray drying (Fasoyiro *et al.*, 2006).

2.5. General Composition of Fruit Juices

Fruit juices have the essential physical, chemical, organoleptical, and nutritional characteristics of the fruits from which they come. The relevant substance groups are carbohydrates, organic acids, minerals, polyphenols (tannins) including the colourful anthocyanins, water-soluble vitamins, amino acids, aroma compounds, carotenoids, fibers and other bioactive substances (Mahale *et al.*, 2008). Fruit juices are low in compounds such as sodium and fats which are believed to have negative health impacts when ingested in large amounts. Conversely, juices contain a variety of beneficial micronutrients, including significant quantities of minerals, like potassium as the major mineral (Mahale *et al.*, 2008), followed by calcium and magnesium.

The major component is, of course, water derived from the extra and intracellular fluid necessary for metabolic processes and maintenance of cell turgor. Water content can range from 97 percent in some wild berries to 70 percent in over ripe grapes and less than 50 percent in fruits drying naturally on the plant (FAO, 2006)

Many trace elements of fruits are also found in the corresponding fruit juices. The B-vitamin folate is present in orange, grapefruit, pineapple juices and some other tropical juices. Vitamin C, although sometimes added to fruit juices, is found in significant amounts in different fruit

juices. Examples are orange juice, grapefruit juice, blackcurrant juice, strawberry juice, or acerola juice. In recent years, the beneficial phytochemicals present in all the major fruits and fruit juices have been characterized and a database on the polyphenol food content is now available online (Neveu *et al.*, 2010).

2.6. Health Benefits of Fruit Juices

In recent years, juices have been included significantly as the diet of many communities, irrespective of age. They are nutritious drinks and can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits (FAO, 2006). The high potassium and low sodium characteristic of most juices help maintain a healthy blood pressure, furthermore the lack or near absence of fat in fruit juices is beneficial for the cardiovascular system. The fortification of juices with calcium and phytosterol provide some supplemental bone and cardiovascular benefits (Delichatsios and Welty, 2005).

Vitamins have a special role since they are essential for life and most are not produced by the body. Vitamin C (ascorbic acid), naturally presents to most juices, is necessary for the body to form collagen, cartilage, muscle, and blood vessels, and aids in the absorption of iron. More recently, the influence of vitamin C in gene modulation and biochemical pathways modifications has been shown, particularly in blood vessel endothelium (Wu *et al.*, 2007) and atherosclerosis (Frikke Schmidt and Lykkesfeldt, 2009). Among the vitamins found in fruit juices, folate from citrus and pineapple is essential for the prevention of spina bifida (Bell and Oakley, 2009) and premature birth (Bukowsky *et al.*, 2009). It also helps in maintaining a low level of the amino acid homocysteine, a marker of inflammation, that has been associated with a higher risk for heart disease, stroke, and heart failure (Sánchez- Moreno *et al.*, 2009).

The health benefits of minerals, vitamins, and micronutrients have been well characterized but many beneficial properties of juices have been shown to come from phytochemicals, mainly polyphenols, carotenoids and limonoids (Holst and Williamson, 2008).

2.7. Sanitation and Good Manufacturing Practices of Fruit Juices

Fruits are very perishable and, if care is not taken in their harvesting, handling and transport, they will soon decay and become unfit for human consumption. Estimates of production losses in developing countries are hard to judge, but some authorities put losses of sweet potatoes, plantain, tomatoes, bananas and citrus fruit as high as 50 percent, or half of what is grown (Chukuezi, 2010). This figure is even higher for underdeveloped countries. Reduction in these losses, particularly if they can be avoided economically, would be of great significance to growers and consumers alike.

The general purpose of quality and safety control of fruit juice is to ensure that a maximum amount of the product being processed reaches the desired level of quality and safety with minimum variation (Codex, 2001). Fresh juice can be contaminated with spoilage and pathogenic microorganisms. Thus, the safety of freshly squeezed fruit juices should have to be insured during harvesting and purchasing of fruits, washing of fruits, juice extraction, juice cooling, juice filling, juice storage, and juice serving to consumers.

The first washing of vegetables at harvest removes much of the adhering soil and dirt. However, it should be recognised that washing may also be a source of microbial contamination. Mangoes exported to the US from Brazil were found to be infecting consumers with *Salmonella* (Tauxe, 2007). The infection was traced to contaminated hot water used to kill fruit-flies in the mangoes. For the mangoes exported to Europe ethylene-bromide was used for the same purpose (Sivapalasingam *et al.*, 2000).

Common methods for preservation and processing of fruit juices include canning, pasteurization, freezing, evaporation and spray drying. Various disinfectants can be used to reduce the microbial load on fruits. However, the safety assessments of these substances and the legal requirements concerning such treatments also have to be taken into account. The purpose of using these agents is to control food pathogens or spoilage organisms (preserving additive). The effect of disinfectants on contaminants depends on many factors including the

concentration used, treatment time, temperature, pH and sensitivity of the target organism(s) (Parish, 2009).

Chlorine is the major compound used for disinfection of fresh produce. During sprouting of seeds chlorine can be used in the water to prevent growth of contaminating microorganisms. The most effective form is hypochlorous acid (HOCl) and chlorine concentration of 100 ppm is frequently used. However, the use of chlorine does not ensure elimination or even an efficient reduction in pathogen levels. Other substances may be used including organic acids, chlorine dioxide, hydrogen peroxide and ozone. Organic acids alone, or in combination with chlorine, have been shown in experimental designs to effectively reduce the number of pathogens for example, *Yersinia enterocolitica* and *Listeria monocytogenes* in parsley (Adesetan *et al.*, 2013).

Most food safety recommendations call for fruit juice to be pasteurized. Pasteurization is a heat treatment that kills the bacteria *Escherichia coli* O157:H7 that is a public health hazard. Most juice manufacturers are no longer willing to accept the liability risk of producing fruit juice that has not been pasteurized. Acid juices like citrus containing pH levels less than 4.0 can be pasteurized by heating the juice to 90°C for a few seconds (Mutaku *et al.*, 2005).

2.8. Factors that affect the Safety and Quality of Fruit Juices

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

Quality in the context of fruit is the sum total of all those attributes which combine to make fruit acceptable, desirable and nutritionally valuable as human foods (Ha *et al.*, 2007; Jaeger *et al.*, 2009; Schouten *et al.*, 2002). The quality of fruit juices is strictly maintained in developed countries under several laws and regulations but in many developing and underdeveloped countries, the manufacturer is not concerned about the microbiological safety and hygiene of fruit juices because of lack of knowledge and enforcement of the law. Thus the transmission of certain human diseases through juice and other drinks in recent years is a serious problem (Jaeger *et al.*, 2009).

Many factors can contribute to microbial contamination throughout production and packaging of fresh produce. Pathogenic microorganisms can enter fruits through damaged surfaces that occur during growing or harvesting. But the main sources of contamination in fruit juices are untidy instruments and utensils, unhygienic water for dilution, dressing with contaminated ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust. Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, species of *Salmonella*, *Shigella*, and *S. aureus*. Further more natural juices, even kept under refrigeration, have a short shelf life (Sandeep *et al.*, 2001; Barro *et al.*, 2006; Lewis *et al.*, 2006).

2.9. Microorganisms Affecting the Quality and Safety of Fruit Juices

Fruits undergo tremendous chemical changes once separated from the parent plant, until finally spoilage sets in as a result of attack from bacteria, yeasts and fungi (Chen *et al.*, 2010). Typical changes may show in texture, colour, flavour and respiratory activity which affect the processed fruits. Among the organisms specified as agents of bacterial food intoxication are *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococci* and with restriction, *Bacillus cereus* (Arnesen *et al.*, 2008). Non-safety-related spoilage of fruit juices and beverages is due to growth of fermentative yeasts, acidophilic bacteria, and/or filamentous fungi. Such growth usually results in deleterious effects on the sensory quality of the beverage. Juice may become contaminated by using fruit that is on the ground in contact with soil, water, sewage, or manure that harbor pathogens (Beuchat *et al.*, 2006).

2.9.1. Spoilage Microorganisms

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 (pH<4.5), 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*. However, yeasts are predominant because of their high acid tolerance and the ability of many of them to grow anaerobically. *Pichia*, *Candida*, *Saccharomyces* and *Rhodotorula* are the genera mainly involved in spoiled juices; the species frequently isolated are *Pichia membranifaciens*, *Candida maltosa*, *C. sake*, *Saccharomyces bailii*, *S. bisphorus*, *S. cerevisiae*, *S. rouxii*, *S. bayanus*, *Brettanomyces intermedius*, *Schizosaccharomyces pombe*, *Torulopsis holmii*, *Hanseniaspora guilliermondii*, *Schwanniomyces occidentalis*, *Dekkera bruxellensis*, *Torulasporea delbruckii*, *Zygosaccharomyces microellipsodes*, and *D. naardenensis*. A high level of yeast contamination in fruit juices may be indicative of poor plant hygiene. Amongst the spoilage yeasts, *P. membranifaciens* is considered as the target microorganism for the optimisation of thermal treatments of juices because it is resistant to heat as well as to moderate amounts of salt, SO₂, sorbic, benzoic and acetic acids (Robert and Boekhout, 2003 and Keller and Miller, 2006).

In 1982, a new type of spoilage bacterium emerged in a large scale spoilage incident in Germany, during which flat sour type spoilage with offensive smelling medicinal or antiseptic characteristics was noted in commercial pasteurized apple juice. The microbe responsible for the incident was a thermo-acidophilic, endospore-forming bacterium, later identified as *Alicyclobacillus acidoterrestris* (Vasavada, 2003). To date, 20 species and 2 subspecies that belong to this genus have been identified and more spoilage incidents have been reported in various fruit juices, fruit juice blends, carbonated fruit juice drinks, fruit pulps and lemonades, with apple juice as the product most often involved. *A. acidoterrestris* is the species primarily responsible for spoilage incidents, although other species, including *A. acidiphilus*, *A.*

pomorum, *A. hesperidum*, *A. herbarius*, *A. cycloheptanicus* and *A. acidocaldarius* have also been implicated due to their ability to produce taint compounds (Steyn *et al.*, 2011).

2.9.2 Pathogens

The ability of different pathogens to survive in low pH environments has been documented at length (Teeteh and Beuchat. 2003). Therefore, it is not surprising that unpasteurized fruit juices, such as orange and apple juices, have been identified as the vehicle of food borne pathogens in several outbreaks. In particular, unpasteurized orange juice has been linked to several outbreaks of disease caused by *Salmonella* (Krause *et al.*, 2001), *Shigella*, or viruses (Fleet *et al.*, 2000) in different countries. Although food borne pathogens can be destroyed by pasteurization, consumption of unpasteurized juice occurs frequently due to consumer preferences. Freshly squeezed juice may be an important source of pathogens if the fruit is contaminated. The relocation of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* from contaminated oranges onto all utensils used during orange crushing and into the juice (Martínez-Gonzales *et al.*, 2003).

2.10. Microbiological Concerns

The chief microbiological concerns associated with these products center around two types of microorganisms— psychrotropic and mesophilic pathogens—that could grow during extended refrigerated storage or temperature abuse. Psychrotrophs are bacteria, yeasts, and molds that grow, although slowly, at refrigeration temperatures (below 7°C) but grow optimally at temperatures above refrigeration, e.g., 25–30°C. Their maximum growth temperatures are 30–35°C (Kraft, 1992). Mesophilic pathogens could survive under refrigeration and grow during any temperature abuse of the food. Mesophiles grow well between 20–45°C with optimum growth between 30–40°C (Jay, 1996). The potential for psychrotrophic spoilage microorganisms to grow during the extended refrigerated storage period and decrease organoleptic quality or spoil the food product is also a concern (Fernandez- Trujillo *et al.*, 2009).

2.11. Source of Contamination

Various workers suggest that human beings shed from 10^3 to 10^4 viable organisms per minute. The numbers and type of organism shed is closely related to the subjects working environment. Apart from microbial invasion of plant tissues during various stages of fruit development, a second factor contributing to microbial contamination of fruits pertains to their post-harvest handling and through enzyme preparation for food processing (Fernandes, 2010).

Pathogens such as *Listeria monocytogenes*, *Clostridium botulinum*, and *Bacillus cereus* on fresh fruit, can be expected since such flora naturally occurs in the soil. However, contaminated water and raw or improperly composted manure are more likely to contaminate fresh fruit with *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., parasites and viruses (Beuchat and Ryu, 1997). Direct contact with livestock and other animals, or their faeces, can also result in the contamination of fruits with various pathogens. This is of particular concern with respect to drop fruit. Human handling and contact surfaces/equipment represent other potential sources of contamination throughout the preparation of fruit for consumption. This includes all phases: growing, harvesting, packing, processing, and shipping, as well as handling by food workers and consumers (Beuchat *et al.*, 1998).

Contamination of food stuffs during processing and/or during storage may have various causes. The most frequently occurring contaminants belong to the following classes: microbial toxins substances originating from food processing materials and fermentation products. Detergents, lubricants and any other contaminants may also be encountered (Lawley *et al.*, 2008).

But the main sources of contamination in fruit juices are poor personal hygiene, untidy instruments and utensils, unhygienic water for dilution, dressing with contaminated ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust (Sandeep *et al.*, 2001).

2.12. Prevention of Spoilage and Pathogenic Microorganisms in Fruit Juices

There are various techniques to prevent pathogenic as well as nonpathogenic microflora such as chilling, freezing, water activity, modified atmosphere packaging, pasteurization, non thermal physical techniques, and by addition of natural antimicrobials (Raybaudi-Massilia *et al.*, 2009). The most common method to inactivate microorganisms and enzymes for increasing the shelf life of fruit juices is by thermal processing; however, loss of original taste and flavor compounds occur in fruit juices. These negative effects have motivated a great interest in the development of new technology that offer advantages of using low processing temperatures, low energy consumption, and high retention of nutritional and sensory properties of the food and improving its microbiological quality (Mosqueda-Melgar *et al.*, 2008).

The quality of fruit juices is strictly maintained in developed countries under several laws and regulations but in many developing and underdeveloped countries, some of the vendors are not concerned about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law. Thus the transmission of certain human diseases through juices in recent years is a serious problem. Because of this, it is very difficult to evaluate how the consumer can be affected by taking freshly squeezed fruit juices. The better alternative is to monitor the proper management of raw materials and sanitation of the juice houses to prevent or minimize bacterial contamination of juices (Doyle *et al.*, 2001 and Tasnim *et al.*, 2010).

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).

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 such as *Escherichia coli*, *Shigella spp.* and *Salmonella spp.* (Department of health directorate, South Africa, 1997).

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 and Greenwood, 2003).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The present study was conducted in Dire Dawa town from March 2016 to August 2016. Dire Dawa is one of the two chartered cities (astedader akabibi) that lie in the eastern part of the nation, on the Dechatu River, at the foot of a ring of cliffs. The town is located at a latitude and longitude of 9°36' N and 41°52' E. Dire Dawa is one of the largest cities in Ethiopia and it is 40 kilometers away from Haramaya University to the west.

The climatic condition of Dire Dawa seems to be greatly influenced by its topography, which lies between 950 – 1250 meters above sea level, and which is characterized by warm and dry climate with a relatively low level of precipitation. The mean annual temperature of Dire Dawa is about 25.4°C. The aggregate average annual rainfall that the region gets from these two seasons is about 604 mm. On the other hand, the region is believed to have an abundant underground water resource.

Based on the 2016 Census conducted by the Central Statistical Agency of Ethiopia (CSA), Dire Dawa has a population of 607,321. In the town there are many Restaurants and cafeterias that prepare unpasteurized fruit juices which are consumed by visitors and people of the town (CSA, 2012).

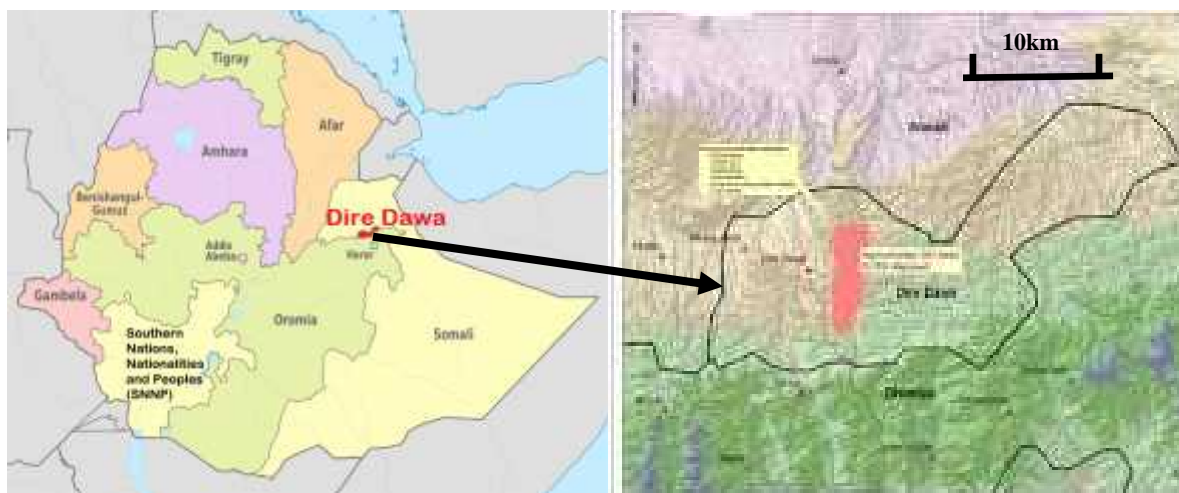


Figure 1: Location of the study site (www.shutterstock.com)

3.2. Study Design

A cross-sectional study design was employed to evaluate the microbiological quality and safety of locally prepared fresh fruit juices in Dire Dawa town. A questionnaire was developed and administered to assess the hygienic conditions of the workers as well as the utensils used for preparation of the juices and to identify handling methods and gather other related information. Identification of the bacterial species affecting the quality and safety of juice was carried out in the laboratory using the collected juice samples from cafes of the study area. Three types of fruit juice (i.e. papaya, avocado, and mango fruit juice) samples were collected and portions transferred to appropriate culture media. Indicator microbial groups were enumerated from cultures using standard methods. *Staphylococcus aureus* and *Salmonella* spp. were detected from samples (fruit juices). The bacteriological analysis was done in Haramaya University Pathology Laboratory. The results of all bacteriological analyses were compared with microbiological criteria for food stuffs (Gulf Standards, 2000).

3.3. Study Population

In the juice houses of the study area, avocado, mango, papaya, pineapple, guava, orange, and mixed fruit juices are common and available to consumers throughout the year. Among these, unpasteurized and freshly squeezed Papaya, avocado, and mango juices that were highly consumed by consumers in the study were purposively selected to investigate their microbial quality and safety to consumers since these three types of fruit juices are highly consumed by the consumers.

3.4. Sample Size

A total of nineteen (90) fruit juice samples of three types (avocado, papaya and mango) from 6 sites where there was high juice consumption were collected. Five samples of each type of fruit juices were collected from a single site every ten days. In short, 30 samples of each juice type were collected from 30 cafés and restaurants.

3.5. Sample Collection

The samples of juices were collected from March 2016 to August 2016. Purposive and random sampling techniques were used to choose sample juice houses in the study area.

A total of 90 fruit juice samples (30 juice samples for each type of fruit) were collected from 30 participating restaurants and cafes in wide mouthed sterile (250 ml) containers aseptically. The collected samples were labeled and immediately transported to Haramaya University Pathology Laboratory in an ice-box and were processed within a maximum of two hours after collection and arrival at the laboratory (Tsige *et al.*, 2008).

3.6. Questionnaire Survey

A structured questionnaire was prepared to gather information on source of fruit, source of water used, how the fruits are stored, hygienic conditions of the utensils used for producing juice and the working conditions, how the juices are produced, and other related information from workers. The questionnaire was pre-tested and adjusted as required. Purposive sampling was used to select the key informants (Appendix-1).

A questionnaire was administered to 15 vendors to obtain data on general information, and practices, behavior and personal hygiene were also included in connection to the food safety. The questionnaire contained three sections with 22 questions in which the first two sections had open and closed ended questions regarding general information about practices of street vendors. The last section dealt with personal hygiene and behavior of vendors. The questionnaire was administered to participants during the time of fruit juice sample collection.

3.7. Laboratory Procedure

3.7.1. Sample Processing

After complete mixing of original juice sample, 10ml was measured and transferred to 90ml of sterile peptone water and homogenized by Vortex mixer (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^{-2} , 10^{-3} , 10^{-4} and 10^{-5}) were then made by taking 1ml from homogenized sample and adding to sterile test tubes containing 9ml sterile alkaline peptone water each and mixed properly by vortex mixer (Roberts *et al.*,2003).

The sample dilutions prepared were plated on pre-dried surfaces of PCA for total viable aerobic mesophilic bacterial (TVAMB) counts and for coli forms. After enumerations were completed, different colonies on each agar media were taken and further purified by repeated plating on plate count agar (PCA). Examination of cell morphology, test for gram reaction, colony characterization, appropriate biochemical tests were carried out to identify the isolates.

The microbial analysis in this study included isolation and enumeration of potential pathogens using standard procedures. Samples were examined for the presence of *Staphylococcus aureus* and *Salmonella* spp. on manitol salt agar (MSA) xylose lysine deoxycholate (XLD) agar respectively. At the same time, total coliforms determined using MPN method and yeast and mold counts from samples were determined using media potato dextrose agar (PDA). The mean number of colonies counted was expressed as log colony forming units (cfu)/100 ml. The isolated bacteria from culture media were also characterized using appropriate biochemical tests to identify pathogenic bacteria.

3.7.2. Total Viable Aerobic Mesophilic Bacterial Count

For total viable aerobic mesophilic bacterial (TVAMB) counts, serial decimal dilutions of each sample were made using sterile peptone water solution as diluent and then were spread plated on nutrient agar (Ahmed *et al.*, 2009). In the present study, dilutions up to 10^{-6} were prepared and used for spread plating. The plates were then incubated at 37°C for 24-48 h. After incubation, plates with colonies between 30 to 300 were used for estimating total counts using appropriate formula (Mahale *et al.*, 2008).

Counting was done manually with the aid of a colony counter. Each time, two critical dilutions per each sample were counted. A plate was divided into quarters using a marker-pen and colony forming units were counted on at least two critical dilution plates.

Two consecutive plates with less than 300 colonies were considered for record (ISO 4833-1:2013). The number (N) of cfu/g or ml of test sample was calculated as follows:

$$N = \frac{C}{V(n_1 + 0.1n_2)d}$$

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.

The results obtained are rounded off to two significant figures and expressed as a number between 1.0 and 9.9 multiplied by 10^x, where x is the appropriate power of 10 (Roberts and Greenwood, 2003).

3.7.3. Enumeration of Total Coliforms and Total Faecal Coliforms

The enumeration of total coliforms in samples was done using the Most Probable Number (MPN) method. The MPN of coliforms was determined as described by Adams and Moss (2000), and American Public Health Association (APHA, 1985). After making serial dilutions of the samples as described in section 3.8.1., 0.1 ml of each dilution (10⁻³, 10⁻⁴ and 10⁻⁵) was inoculated into three test tubes of Lauryl sulphate Tryptose Broth (LTB). After 24 hours of incubation at 35⁰C, the number of tubes in each set of three that showed positive result for acid and gas production was counted and checked against standard probability table that gives the MPN for the sample. For confirmed and completed tests of Total Coliforms, a loopful from each gas positive tube was transferred to a separate tube of Brilliant Green Lactose Bile Broth (BGLBB) and incubated at 35⁰C for 48 hrs. On the other hand, for confirmed test of Faecal Coliforms, a loopful of sample from each gas positive tube was transferred to a separate tube of EC broth and incubated 45.7⁰C. Calculation of MPN was done from the completed test result table as the ratio of the number of tubes positive in each of the three dilutions gives the MPN ratio. (Mahale *et al.*, 2008; Titarmare *et al.*, 2009; and Aneja, 2002).

In order to detect the presence of *E. coli*, one loopful of each positive EC broth culture was inoculated in to tryptone water and incubated for 48 ± 2 hrs at 44°C after incubation period 0.5ml of Kovac's indole reagent was added. The tube were subjected to gentle shaking and examined for red colour in the surface of layer within 10 minutes. A red ring on top of the tube indicated indole positive reaction (ISO 7251:2005).

3.7.4. Analysis of *Staphylococcus aureus*

Enumeration, isolation and characterization of *Staphylococcus aureus* were done using standard methods and procedures. Appropriate dilutions of sample fruit juices were spread plated on Mannitol Salt Agar (MSA) and plates were incubated at $36 \pm 1^{\circ}\text{C}$ for 18-24 (to 48) hours. Then, each plate was observed at 24 to 30 h for possible overgrowth; presumptive colonies were counted at this time but the count was verified at 48 ± 4 h (Mahle *et al.*, 2008). Yellow and orange colonies surrounded by yellow zones due to mannitol fermentation were enumerated and further tested by coagulase test. The number of live bacteria (or Colony Forming Units [CFU]) per ml of original culture was calculated.

For confirmation of *Staphylococcus aureus*, coagulase test was performed. To do this, inoculum from each presumptive colony from MSA was transferred to a separate tube of Brain Heart Infusion (BHI) broth and was incubated at 35°C for 18-24h under aerobic condition. Then, 0.2 ml of BHI broth culture was transferred into sterile 13 x 100 mm tubes containing 0.5 ml certified coagulase plasma and mixed thoroughly. The mixture was incubated at 35°C and examined after 1 h and 4 h. A firm clot which does not move when the tube is tipped on its side (coagulase reaction) was considered a positive test for *Staphylococcus aureus*. Gram staining technique was also being done for confirmation of deep yellow opaque colonies of *Staphylococcus aureus*.

3.7.5. Detection of *Salmonella* spp.

Using a sterile pipette, 25 ml of each juice sample was transferred into a conical flask containing 225 ml of Buffered Peptone water (BPW) and incubated at 37°C for 24hrs. Then, 1.0 ml of each pre-enriched sample was transferred into 10 ml of Tetrathionate broth (Müller-

Kaufmann) (tube I) or 0.1ml was transferred into 10ml Rappaport-Vassiliadis Soy peptone (RVS) broth (tube II). Tube I was incubated at $37.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and tube II at $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ overnight (18-24 hours). A 10 μl loopfull from the inoculated and incubated Tetrathionate broth (I) and RVS broth (II) was spread on XLD agar plates and incubated at 37°C overnight (18-24 hours). A typical *Salmonella* colony that has a slightly transparent zone of reddish colour and a black centre was seen with a pink-red zone in the media surrounding the colony.

In order to identify *Salmonella* from the primary culture on XLD agar, the suspected colonies were sub cultured into sterile nutrient agar plates and incubated. Then the organisms were subjected- to different biochemical tests such as Triple Sugar Iron Agar, Urea broth, and Indol test.

From a pure culture on nutrient agar plates, the media was inoculated into TSI (a straight wire was used to stab the butt and streak the agar surface), and a 1 μl loop full of the media was inoculated into Urea broth, L-lysine decarboxylation medium, LDC control medium, ONPG medium, VP medium and Tryptone/tryptophane medium for indole. All biochemical tests were incubated at 37°C for 18 to 24 hours except for VP, which need 48h of incubation.

In order to carry out the -galactosidase test, a loop full of the suspected colony was suspend in a tube containing 0.5 ml of ONPG medium and incubated at 37°C . A positive reaction was seen already after 3-4 hours. VP test was also carried out by adding four drops of the creatine solution, six drops of the ethanolic solution of 1-naphthol and four drops of the potassium hydroxide solution to the medium. It was shaken well after each reagent was added. And finally Indole test was carried out when 1 ml of the Kovacs reagent was added to the medium (ISO 6579:2002).

3.7.6. Spore-Forming Bacterial Count

For spore-forming bacterial count, 1:10 dilutions of the sample were prepared in test tubes using distilled water and heated to 80°C for 10 minutes. The sample tube was immediately cooled and 1ml of the heat shocked sample was transferred to a Petri dish containing dextrose

tryptone agar with the added bromocresol purple. After mixing, the culture was incubated at a temperature of 55°C for 48 hrs. (ASFAM, 1982).

3.7.7. Yeast and Mold Count

Serial dilutions were prepared for each juice sample first by taking 50 ml of juice sample and mixing with 450 ml of sterile peptone water. Each sample was then serially diluted 10 fold (i.e. 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) in sterile peptone water. An aliquot of 1ml of each sample was plated out on potato dextrose agar (PDA) and incubated at ambient temperature (26-28°C) for 48-72 hours. The colonies were counted, multiplied by the inverse of corresponding dilutions and reported as yeast and mold count (YMC) per ml (Samson and Varga, 2007). During enumeration yeast were distinguished from mould by using microscope. Thus, smooth (non-hairy) colonies and without extensions at periphery (margin) were quantified as yeasts, whilst big, spreading and hairy colonies at margin and surface were counted as moulds.

3.8. Data Analysis

All the data including the associations between prevalence of food-borne pathogens and practices of fruit juice vendors on food safety were analyzed with the Statistical Package for Social Sciences version 20.0 for Windows (SPSS Inc.; Chicago, IL, USA) software. The analysis of mean microbial counts in sample fruit juices were interpreted using one way ANOVA. Differences between means were considered as statistically significant with 95% confidence at $p < 0.05$.

4. RESULTS AND DISCUSSION

4.1 Microbiological loads of freshly squeezed fruit juices

In this study freshly squeezed juices of avocado, mango, and papaya served in the town were assessed for their microbial quality and safety. The results of the analysis are shown in Table 1. As shown from the table, the mean aerobic mesophilic bacterial count in avocado (7.21×10^5) were higher than in papaya (5.26×10^5) and mango juice (4.95×10^5). However, there were no statistically significant difference ($P=0.858$) among these values. These results were in agreement with the mean counts of AMB in avocado (5.71×10^6), papaya (4.54×10^6) and mango (4.47×10^6) reported from Harar town (Nurhasen, 2012). On the other hand, the results of the current study were not in agreement with findings from Jimma town, Ethiopia for avocado (8×10^6) and papaya (3.1×10^7) (Tsige *et al.*, 2008). The probable reason for the discrepancy between these results may be geographical variation, time of sample collection, hygiene of the worker, the nature of the fruits used for juice making, and the water used for dilution of the juice.

The mean value of TCC was 1.73×10^2 mpn/ml (Table 1). There was no statistically significant difference ($P = 0.558$) among the mean values of coliform count in papaya (2.17×10^2 mpn/ml), mango juice (1.83×10^2 mpn/ml), and avocado (1.23×10^2 mpn /ml), and it was observed that papaya juice contained slightly higher mean count than mango and avocado juices. This mean value indicate that papaya juice followed by mango and avocado juice showed huge contamination by coliforms.

The fecal coliform count of the avocado and mango fruit juice samples range from 30.0 mpn/ml to 92.0 mpn /ml but the fecal coliform count of papaya fruit juice samples ranged from 30.0 mpn/ml to 150 mpn /ml (Appendix 7.4). The mean fecal coliform counts of avocado, mango and papaya juice in the present study were 4.06×10^1 mpn/ml 6.27×10^1 mpn/ml 6.43×10^1 mpn/ml, respectively, without any statistically significant difference ($P = 0.368$) (Table 1).

Table 1: Microbiological loads of freshly squeezed fresh fruit juices in DD town.

Microbial Indicators	Statistic	Microbial Loads of the Three Types of Juice Samples			p-value	Gulf Standards (2000)	
		Avocado	Mango	Papaya		Max. Anti	Max. Perm
AMBC (cfu/ml)	Mean	7.21x10 ⁵	4.95 x10 ⁵	5.26 x10 ⁵	0.858	5x10 ³	1x10 ⁴
	Std. Dev.	1.82 x10 ⁶	1.61 x10 ⁶	1.70 x10 ⁶			
TCC (mpn/ml)	Mean	1.23 x10 ²	1.83 x10 ²	2.17 x10 ²	0.558	10	1x10 ²
	Std. Dev.	1.15 x10 ²	2.52 x10 ²	2.86 x10 ²			
TFCC (mpn/ml)	Mean	4.06 x10 ¹	6.27 x10 ¹	6.43 x10 ¹	0.368	0	0
	Std. Dev.	2.28 x10 ¹	2.42 x10 ¹	3.90 x10 ¹			
SAC (cfu/ml)	Mean	9.07 x10 ³	3.62 x10 ³	1.69 x10 ⁴	0.468	10 ²	10 ³
	Std. Dev.	2.41 x10 ⁴	4.84 x10 ³	4.45 x10 ⁴			
TSC (cfu/ml)	Mean	5.71 x10 ²	4.60 x10 ²	6.23 x10 ²	0.872	ND	-
	Std. Dev.	4.44 x10 ²	5.74 x10 ²	1.01 x10 ³			
SFBC (cfu/ml)	Mean	2.94 x10 ³	1.94 x10 ³	1.52 x10 ³	0.528	10 ²	10 ³
	Std. Dev.	5.76 x10 ³	2.73 x10 ³	1.81 x10 ³			
YMC (cfu/ml)	Mean	1.08 x10 ⁴	8.94 x10 ³	7.42 x10 ³	0.363	10 ²	10 ³
	Std. Dev.	2.10 x10 ⁴	1.14 x10 ⁴	2.97 x10 ⁵			

AMBC = Aerobic Mesophilic Bacterial Count, TCC = Total Coliform Count, TFCC = Total Fecal Coliform Count, SAC = *Staphylococcus aureus* Count, Stat = Statistic, TSC = total *Salmonella* Count, SFBC = Spore Forming Bacterial Count, YMC = Yeast and Mold Count, ND = not detected, Max. Anti= maximum anticipated, Max. perm = maximum permissible

The mean *Staphylococcus* count of papaya (1.69 x10⁴cfu/ml) in this study was higher than those of avocado (9.07 x 10³cfu/ml) and mango (3.62 x10³cfu/ml) although there was no statistically significant difference among the three values difference (P = 0.468). This finding was not, however, supported by the study conducted in Jimma town where the mean *Staphylococcus* count of avocado was higher than those of papaya and mango (Tsige *et al.*, 2008). Similar results were also observed for *Salmonella* count (SC) that showed higher contamination in papaya juice (6.23x10²cfu/ml) than in avocado (5.71x10²cfu/ml) and mango

(4.60×10^2 cfu/ml) juices without any significant statistical difference ($p=0.872$). The presence of more *S. aureus* and *Salmonella* spp. in papaya juice than in others may be due to contamination during processing of the fruit, cross-contaminations at the time of storage, and physical conditions of the fruits used for juicing.

The present study also attempted to determine the spore forming bacterial count. The mean counts of these spore forming bacteria in avocado juice were higher (2.94×10^3 cfu/ml) than in mango and papaya juices which were with 1.94×10^3 cfu/ml and 1.52×10^3 cfu/ml, respectively. But there was no statistically significant difference among the juice types in terms of spore forming bacterial counts ($p=0.528$).

The yeast and mold counts of the fruit juice samples ranged from 1.50×10^2 to 6.40×10^4 cfu/ml (for avocado), 1.00×10^2 to 3.50×10^4 cfu/ml (for mango) and 1.00×10^2 to 1.40×10^6 cfu/ml (for papaya) (Appendix 7.4). The mean counts obtained from mango (8.94×10^3 cfu/ml) and avocado (1.08×10^4 cfu/ml) juice samples in this study were analogous to those reported from avocado (4×10^4 cfu/ml) and mango (3.2×10^4 cfu/ml) in Doha, Qatar (Al-Jedha and Robinson, 2002). The results of this study were also comparable to those reported from Jimma town (Tsige *et al.*, 2008) for Avocado (4.5×10^5 cfu/ml) and papaya (6.2×10^3 cfu/ml).

The microbial loads contained in juice samples of the present study were higher than the maximum loads recommended by the Gulf standards (Gulf Standards, 2000) for foods described in Table 1. This implied that, these freshly squeezed fruit juices had poor microbiological quality.

The presence of high loads of these microbes in foods can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing and dilution, cross contamination from rotten fruits and vegetables, or the use of dirty processing utensils like knife and trays. It is contended that contamination is mainly due to poor quality of water used for dilution as well as the prevailing unhygienic conditions related to improper washing of fruits, vegetables, and utensils, inadequate storage of these at ambient temperatures in unhygienic places, maintenance of premises and personal hygiene of vendors.

4.1.1 Aerobic Mesophilic Bacterial Counts

A total of 90 juice samples from Dire Dawa town were cultured for Aerobic Mesophilic Bacterial Count (AMBC). It was found that 100% (n=90) of the samples had aerobic bacterial growth. The AMBC ranged from 2.30×10^2 -- 9.00×10^6 cfu/ml. The highest bacterial load (9.00×10^6 cfu/ml) and lowest bacterial load (2.30×10^2 cfu/ml) for fresh fruit juice samples were found in papaya juice collected from Taiwan (Appendix 7.5). The mean AMBC in this study was found to be 5.81×10^5 cfu/ml which is higher than the maximum recommended level (5×10^3 cfu/ml) by the Gulf Standard (2000). Almost all fruit juice samples showed much higher viable bacterial counts than the permitted count.

Table 2: AMBC of freshly squeezed fresh fruit juices in DD town by site of juice production. (n=5 for each juice sample)

J	Stat	Microbial Loads of the Three Types of Juice Samples						p-value
		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala	
A	Mean	7.26×10^4	5.13×10^3	3.38×10^4	3.73×10^4	2.38×10^6	1.80×10^6	0.106
	Std. Dev.	1.33×10^5	1.93×10^3	4.13×10^4	3.27×10^4	2.16×10^6	3.48×10^6	
M	Mean	1.69×10^4	3.14×10^4	5.10×10^3	7.40×10^4	1.05×10^6	1.80×10^6	0.375
	Std. Dev.	2.55×10^4	3.89×10^4	4.55×10^3	1.37×10^5	1.66×10^6	3.54×10^6	
P	Mean	3.78×10^4	1.61×10^5	2.50×10^3	6.98×10^5	2.01×10^6	2.46×10^5	0.422
	Std. Dev.	7.95×10^4	1.66×10^5	4.75×10^3	1.29×10^6	3.93×10^6	3.09×10^5	

Key: A= avocado, M= mango, P= papaya, F= frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic

The overall results indicated that most (74.4 %) of the juice samples handled had higher AMBC than the maximum recommended level. Comparison of AMBC between different sites showed that on the average more fruit juice samples from Taiwan (2.38×10^6 cfu/ml) for avocado juice and Megala (1.80×10^6 cfu/ml) for avocado and mango juice had higher AMBC than the other four sites (Table 2). The variation in AMBC of the three types of fruit juice samples may be due to the unhygienic conditions practiced in the preparation and handling of the juices. However, the difference was not statistically significant ($P > 0.05$).

4.1.2. Total coliform count

A total of 90 fresh juice samples (those used in the AMBC analysis) were used for coliform count. The MPN results showed that 41 out of 90 fresh juice samples had coliforms and the mean value was calculated for the positive samples. Eighteen of the positive samples (43.9% of the total samples) were found to be unsafe and unfavorable for consumption because they exceeded the limit of coliforms recommended by Gulf Standards (2000).

Table 3: TCC of freshly squeezed fresh fruit juices in DD town by sites of juice production. (n=5 for each juice sample)

J	Stat	Site from which the juice sample was collected						p-value
		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala	
A	F	3	4	1	2	2	2	0.591
	Mean	1.17×10^2	8.17×10^1	2.00×10^2	1.20×10^2	5.10×10^1	2.52×10^2	
	Std. Dev	9.02×10^1	7.99×10^1	0	1.27×10^2	2.97×10^1	2.52×10^2	
M	F	3	2	3	2	1	1	0.447
	Mean	4.68×10^2	4.55×10^1	9.27×10^1	1.45×10^2	7.40×10^1	6.20×10^1	
	Std. Dev	4.25×10^2	2.19×10^1	4.70×10^1	7.07	0	0	
P	F	3	2	2	2	2	2	0.605
	Mean	2.99×10^2	5.40×10^2	8.55×10^1	1.70×10^2	4.60×10^1	1.20×10^2	
	Std. Dev	3.91×10^2	5.52×10^2	3.46×10^1	8.48×10^1	2.26×10^1	1.27×10^2	

Key: A= avocado, M= mango, P= papaya, F= frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic

As can be seen from Table 3, large number of coliforms was found in this study. The highest and lowest mean coliform counts for fresh fruit juice samples were 5.40×10^2 mpn/ml (for papaya fruit juice collected from Dechatu) and 4.55×10^1 mpn/ml (for mango fruit juice collected Dechatu) respectively. It was also observed that the juice vending houses of Greek Camp had the highest percentage of coliform positive samples ($9/90 = 10\%$).

4.1.3. Total fecal coliform count

Even though the presence of fecal coliforms in any fruit juice sample is not permitted (Gulf Standards, 2000), the data in the present study indicate that 23.33% (21/90) of the fresh fruit juice samples collected in the town showed contamination with faecal coliforms (Table 4). It is known that presence of faecal coliforms suggests faecal contamination which is normally associated with poor hygiene in juice processing and juice handling. The contamination of juices may be due to the use of unhygienic conditions of water, water storage and use of unclean utensils and unhygienic physical and biological contaminants.

Out of 21 positive samples, samples collected from Greek Camp, Kezira and Dechatu accounted for most of the contaminations (76%=16/21) with fecal coliforms. The highest percentage (28.6%=6/21) of contamination among the contaminated juice samples were recorded from juice samples collected from Greek Camp. The second highest contamination was observed in Dechatu and Kezira which account for 23.81% (5/21) for each. The least (9.52%=2/21) contamination was observed in juice samples collected from Taiwan and Megala with a fecal coliform count of 30 mpn per ml for each. The means total fecal coliform count of avocado fruit juice sample among different site showed statically different ($p=0.023$).

The results of the present study clearly indicated the presence of fecal coliforms in 21 of the 90 fruit juice samples. The presence of fecal coliforms in the juice samples may suggest the possibility of finding enteric pathogens such as *Enterobacter aerogenes* and some strains of *E. coli* that may cause serious diseases in human beings (Matthew *et al.*, 2007 and Samonis *et al.*, 2009). The relatively higher prevalence of fecal coliform bacteria in this study could be due to unhygienic surroundings, prolonged preservation without refrigeration, and use of unhygienic water for dilution, inadequate hand washing and poor processing practices by food workers. In general, the observation of fecal coliforms in fresh juice samples is a serious public health problem and needs prompt attention in correcting the dilemma (Tambekar *et al.*, 2007). Several researchers had conducted similar types of investigations in different places with different street vended fruit and vegetable juices. Sandeep *et al.*, (2004) have detected coliform counts in three samples of carrot juices and Kinnow-mandarin juices obtained from two different areas of the Patiala city. Moushumi *et al.*, (2004) explained the presence of

faecal coliforms in fresh squeezed carrot juices and explained the possible entry points of bacterial pathogens in carrot at several points during course in the distribution chain and hence into carrot juice.

Table 4: TFCC of freshly squeezed fresh fruit juices in DD town by sites of juice production. (n=5 for each juice sample)

J	Stat	Site from which the juice sample was collected						p-value
		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala	
A	F	2 (9.52%)	2 (9.52%)	1(4.76%)	0	1(4.76%)	1(4.76%)	0.023
	Mean	3.30 x10 ¹	3.30 x10 ¹	9.20 x10 ¹	0	3.00 x10 ¹	3.00 x10 ¹	
	Std. Dev	4.24	4.24	0	0	0	0	
M	F	2 (9.52%)	1(4.76%)	2 (9.52%)	1(4.76%)	0	0	0.312
	Mean	7.30 x10 ¹	3.00 x10 ¹	5.40 x10 ¹	9.20 x10 ¹	0	0	
	Std. Dev	1.41	0	2.54 x10 ¹	0	0	0	
P	F	2 (9.52%)	2 (9.52%)	2 (9.52%)	2 (9.52%)	0	0	0.341
	Mean	3.30 x10 ¹	6.65 x10 ¹	5.20 x10 ¹	1.06 x10 ²	0	0	
	Std. Dev	4.24	7.79	3.11 x10 ¹	6.29 x10 ¹	0	0	

Key: A: avocado, M: mango, P: papaya, F: frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic

4.1.4 *Staphylococcus aureus*

A total of 90 fresh juice samples (those used in the AMBC analysis) were plated to determine for staphylococcal count. Out of these, 47 (52.2%) of the fresh juice samples showed growth of *Staphylococcus aureus*. The highest value of *Staphylococcus aureus* count was 1.90 x 10⁵ cfu/ml which was found in papaya juice sample collected from Sabiyan. On the contrary, the least *Staphylococcus aureus* count (5.00x10¹ cfu/ml) was obtained in avocado juice samples collected from Dechatu, and in mango and papaya juice samples collected from Kezira. The mean *staphylococcus aureus* count in mango fruit juice sample among different site showed statically different (p=0.000) (Appendix 7.6).

Table 5: SAC of freshly squeezed fresh fruit juices in DD town by sites of juice production.
(n=5 for each juice sample)

J	Stat	Site from which the juice sample was collected						p-value
		Greek Camp	Dechatu	Kezira	Sabiyana	Taiwan	Megala	
A	F	2	2	3	2	2	3	0.397
	Mean	8.35×10^2	1.78×10^3	6.33×10^2	3.80×10^3	2.70×10^3	3.80×10^4	
M	Std. Dev	9.40×10^2	2.44×10^3	4.04×10^2	5.23×10^3	1.13×10^3	4.65×10^4	0.000
	F	3	2	2	2	4	2	
P	Mean	1.27×10^2	4.10×10^3	1.75×10^2	1.45×10^4	3.48×10^3	1.25×10^3	0.127
	Std. Dev	6.66×10^1	1.27×10^3	1.77×10^2	2.12×10^3	1.94×10^3	1.06×10^3	
P	F	2	4	2	3	4	3	0.127
	Mean	2.80×10^2	1.2×10^3	7.75×10^2	8.30×10^4	4.00×10^3	1.09×10^4	
P	Std. Dev	3.11×10^2	1.22×10^3	1.02×10^3	9.28×10^4	2.47×10^3	1.48×10^4	0.127

Key: A: avocado, M: mango, P: papaya, F: frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic

There were about 68.88% (62/90) samples among the three types of juice samples within the Gulf standard. From this percent, sample contained no staphylococcal count and sample meet the standard limit of total staphylococcal count were 43/90 and 19/90 respectively. It was also observed that the juice vending house of Taiwan had the highest percent (10/15) of *Staphylococcus aureus* positive sample than the others and the juice vending house of Greek Camp, Kezira and Sabiyana had the least percentage (7/15 for each) of *Staphylococcus aureus* positive sample.

It is also observed that the mean count of *Staphylococcus aureus* of papaya juice samples collected from Sabiyana was the highest (8.30×10^4 cfu/ml) over avocado juice samples collected from Megala which were the second highest (3.80×10^4 cfu/ml) and mango juice sample collected from Sabiyana which were the third highest (1.45×10^4 cfu/ml). The minimum mean count (1.27×10^2 cfu/ml) was observed in mango fruit juice collected from Greek Camp (Table 5).

The presence of large numbers of *Staphylococcus aureus* is in general an indication of poor hygiene and temperature control in the juice house (Ghosh *et al.*, 2007). It is usually indicative of contamination from food handlers, inadequately cleaned equipment or raw animal products. Coagulase positive staphylococci may cause human diseases through the production of toxins. Effective levels of toxin formation require a large number of microorganisms (approximately 10^5 - 10^6 cfu/ml of food) (Health Canada, 2006). A few reports have shown the prevalence of staphylococci in fruit juice samples (Ahmed *et al.*, 2009).

4.1.5. *Salmonella* spp.

A total of 90 fruit juice samples were examined for the presence of *Salmonella* spp. The prevalence of *Salmonella* species was 34 (37.77%) out of 90 fruit juice samples (eleven from avocado juice, ten from mango juice and thirteen from Papaya). Even though the number of positive samples is small (that is 34 positive samples out of the total 90 samples), it is possible that it might have affected a large number of individuals who had consumed these contaminated juices. Similar research conducted in India documented 38.8% of fruit juices were identified as *Salmonella* species positive (Lewis *et al.*, 2006).

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.

The data in Table 6 also showed that among all the fruits analyzed, papaya juice samples collected from Kezira were the most heavily contaminated with *Salmonella* spp showing a mean count of 2.02×10^3 cfu/ml even though the site had the least frequency (4/15). Mango juice sample from Greek Camp with 1.16×10^3 cfu/ml and avocado juice sample from Kezira 1.45×10^3 cfu/ml were the second and the third most heavily contaminated samples.

Table 6: TSC of freshly squeezed fresh fruit juices in DD town by sites of juice production.
(n=5 for each juice sample)

J	Stat	Site from which the juice sample was collected						P-value
		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala	
A	F	2	2	1	2	2	2	0.099
	Mean	5.10×10^2	7.20×10^2	1.45×10^3	1.35×10^2	2.40×10^2	8.50×10^2	
	Std. Dev	6.15×10^2	1.69×10^2	0	4.95×10^1	0	1.06×10^2	
M	F	2	2	1	2	1	2	0.687
	Mean	1.16×10^3	4.40×10^2	3.40×10^2	1.25×10^2	2.40×10^2	2.85×10^2	
	Std. Dev	1.19×10^3	4.74×10^2	0	7.07	0	2.12×10^1	
P	F	3	1	2	3	2	2	0.512
	Mean	9.60×10^1	4.70×10^2	2.02×10^3	5.23×10^2	4.10×10^2	4.50×10^2	
	Std. Dev	2.52×10^1	0	2.66×10^3	2.13×10^2	1.21×10^2	4.73×10^2	

Key: A: avocado, M: mango, P: papaya, F: frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic

The least contaminated were the papaya juice samples collected from Greek Camp that showed a mean count of 9.60×10^1 cfu/ml.

It was also observed that compared the frequency of detection of *Salmonella* between sites, juice vending houses of Greek Camp and Sabiyan had the same highest percent (7/15) of *Salmonella* positive samples than the others and the juice vending house of Kezira had the least percentage (3/15) of *Salmonella* positive samples. The finding of *Salmonella* in the fruit juice might indicate that these pathogens come originally from contaminated fruits. However, due to poor sanitation often observed during this study, the source of these pathogens remains uncertain.

Despite the common belief that low-pH foods are safe, which does not have any scientific support, the relatively large number of reports of foodborne illness associated with fresh fruit

juices indicates that unpasteurized juices pose a high public health risk. Several authors have reported that pathogens such as Salmonella, or *E. coli O157:H7* can survive for long periods in refrigerated juices and acidified culture media (Parish *et al.*, 2004). From these reports, it is clear that pathogens can survive at low pH, maybe in low numbers, and still be infective.

4.1.6. Aerobic Spore forming bacteria

A total of 90 fresh juice samples (those used in the AMBC analysis) were used for spore forming bacteria count. The results showed that 61.11 % of fresh juice samples had Spore forming bacteria. When the mean count of these bacteria between different site were compared, large no (9.20×10^3 cfu/ml) of spore forming bacteria count were found in avocado juice sample collected from Dechatu and the lowest mean spore forming bacteria count were 5.00×10^1 cfu/ml (in mango fruit juice collected from Sabiyan). It was also observed that the juice vending house of Sabiyan had the lowest percent 40% (6/15) of spore forming bacteria positive sample than the other sites (Table 7). The mean aerobic spore forming bacteria count in mango and papaya fruit juice sample among different site showed statically different ($p=0.038$ and 0.034 respectively).

Spore forming bacteria that are present in foods are important because the formation of the spore by the bacterium allows it to be resistant to heat, freezing, chemicals, and other adverse environments that our food undergoes during processing and preparation. Although the vegetative cell is killed by these conditions, the spores can survive and need harsher conditions to be inactivated. Spores are found in soil, water, and intestinal contents of humans and animals; hence, they can find their way into foods. As a consequence of the persistence of spores and the ubiquity of spore formers in many different environments, spores are common contaminants of foodstuffs and if not dealt with appropriately in food processing may “return to life” via spore germination and outgrowth and then contribute to food spoilage and food poisoning. In addition to food poisoning (*B. cereus*, *C. perfringens*, *C. botulinum*), there are a number of other human illnesses in which spores play a causative role including, wound infections (Montville and Matthews, 2005).

Table 7: ASFBC of freshly squeezed fresh fruit juices in DD town by sites of juice production. (n=5 for each juice sample)

J	Stat	Site from which the juice sample was collected						p-value
		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala	
A	F	4	4	3	4	2	4	0.211
	Mean	7.50×10^2	9.20×10^3	4.40×10^3	8.50×10^2	3.10×10^2	9.90×10^2	
	Std. Dev	1.06×10^3	1.13×10^4	4.02×10^3	9.45×10^2	2.54×10^2	1.54×10^3	
M	F	3	3	3	2	2	3	0.038
	Mean	6.80×10^2	3.10×10^3	5.80×10^3	5.00×10^1	3.50×10^2	4.70×10^2	
	Std. Dev	9.72×10^2	2.53×10^3	3.50×10^3	2.83×10^1	2.12×10^1	2.89×10^2	
P	F	4	3	2	3	2	4	0.034
	Mean	8.80×10^2	1.70×10^3	5.20×10^3	4.90×10^2	7.60×10^2	1.35×10^2	
	Std. Dev	1.55×10^3	1.41×10^3	2.83×10^2	2.72×10^2	1.55×10^2	1.93×10^3	

Key: A= avocado, M= mango, P= papaya, F= frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic, ASFBC= aerobic spore forming bacterial count

4.1.7. Yeast and mold count

Generally, the overall assessment of the fruit samples analyzed indicated high yeast and mould counts. From the total (90) samples of avocado, mango and papaya fruit juices, yeast and mould were found in 73.33% (66/90) samples which is the second highest percentage next to AMBC. Juices from Megala were more contaminated 100% (15/15) than the other sites and still the highest mean count (3.20×10^5 cfu/ml) was also encountered in this site (Table 8). The second and the third most contaminated sites were Greek Camp and Kezira (73.33% for each) and Dechatu and Sabiyan (66.67% for each). The mean yeast and mould count in mango fruit juice sample among different site showed statically different ($p=0.038$).

Table 8: YMC of freshly squeezed fresh fruit juices in DD town by sites of juice production.
(n=5 for each juice sample)

J	Stat	Greek Camp	Site from which the juice sample was collected					p-value
			Dechatu	Kezira	Sabiyan	Taiwan	Megala	
A	F	3	3	4	3	3	5	0.094
	Mean	3.90×10^4	3.30×10^3	5.20×10^2	2.20×10^3	2.73×10^2	1.75×10^4	
	Std. Dev	3.29×10^4	1.93×10^3	6.50×10^2	1.79×10^3	1.36×10^2	2.64×10^4	
M	F	4	4	3	3	4	5	0.038
	Mean	2.37×10^4	3.80×10^3	1.46×10^2	3.90×10^3	6.10×10^3	1.16×10^4	
	Std. Dev	1.64×10^4	1.39×10^3	5.03×10^1	5.30×10^3	6.38×10^3	1.14×10^4	
P	F	4	3	4	4	2	5	0.622
	Mean	2.41×10^3	2.06×10^2	2.06×10^4	2.40×10^3	9.50×10^3	3.20×10^5	
	Std. Dev	1.89×10^3	1.15×10^1	4.03×10^4	1.14×10^3	4.50×10^3	6.14×10^5	

Key: A: avocado, M: mango, P: papaya, F: frequency, J= juice, Std. Dev = standard deviation, Stat = Statistic

Among all of the samples, the total fungal count of 44(48.89%) were found to be above Gulf region standards (10^3 cfu/ml) (Table 2). Out of these 44 samples, 16, 15 and 13 were found in mango, papaya and avocado juice samples respectively. Regarding to sites, Megala was predominant to be beyond the Gulf region standard with 86.67% (13/15) and the other Greek Camp and Sabiyan were 60.0% (9/15) and 53.33% (8/15) 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 juices compared to bacteria because of the physical and chemical properties of the fruit juices (Obire *et al.*, 2008). However the presence of fungi in many of the juice may be due to the practice involved with the handling and processing of the juice. Though the fungal isolates were not identified, their greater load showed the probability of the presence of strains that could pose spoilage of the juice (Al-Hindi *et al.*, 2011 and Obire *et al.*, 2008)

4.2. Pathogens Identified from the Juice Samples

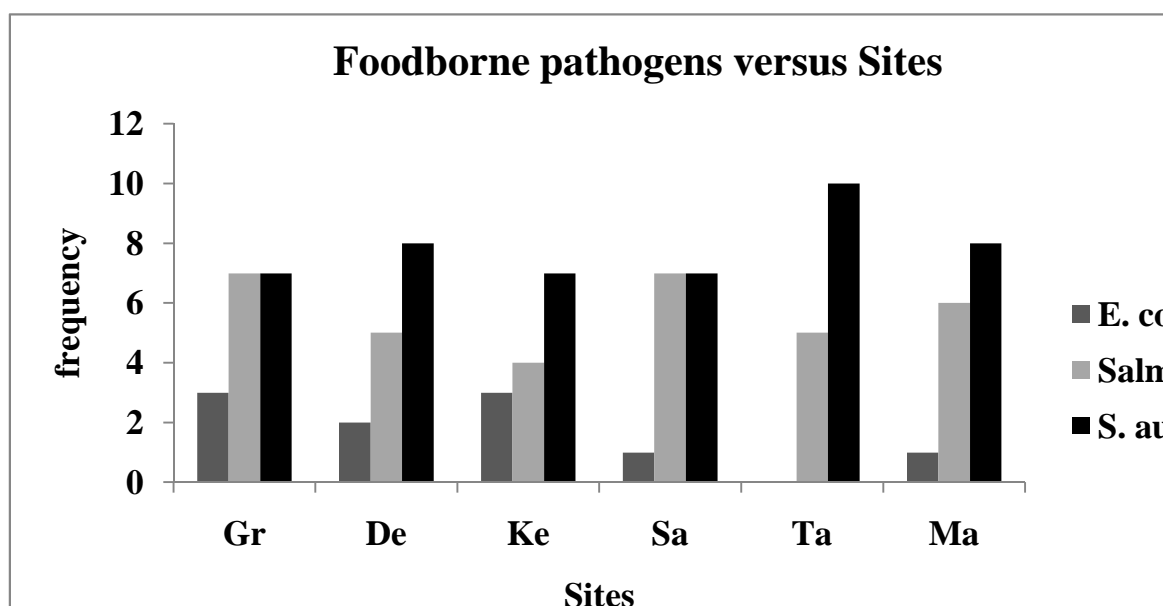
Biochemical analyses for different pathogens were performed on isolates of locally prepared unpasteurized fruit juice samples. Out of ninety two (92) isolates, 47, 34 and 10 isolates belonged to *S. aureus*, *Salmonella species* and *E. coli*, respectively (Table 9).

Table 9: Bacterial pathogens detected from avocado, mango and papaya fruits juices collected from DD town

Pathogens	Juice	N	Positive sample	Total	Percent	P-value
<i>S. aureus</i>	avocado	30	14	47	52.2	0.779
	mango	30	15			
	papaya	30	18			
<i>E. coli</i>	avocado	30	3	10	11.1	0.368
	mango	30	4			
	papaya	30	3			
<i>Salmonella species</i>	avocado	30	11	34	37.77	0.264
	mango	30	10			
	papaya	30	13			

As can be seen from the table 10, freshly squeezed juice samples of avocado, papaya, and mango showed occurrence of pathogens like *Salmonella* spp. *E.coli*, and *S.aureus*. Papaya (34%) followed by mango (30%) and Avocado (28%) juice showed high microbial counts consistent with pH values of 5.28, 3.87, and 5.81 respectively (figure 2), which do not affect the survival of pathogens adversely. This study demonstrated that papaya (34%) juice was more contaminated. This may be due to its pH than other fruit juices. The mean pH-value of the study for avocado and papaya is in agreement to similar study conducted in Jimma town, whose pH value were 5.84 and 5.23 for avocado and papaya respectively (Nurhasen, 2012 and Tsige *et al.*,2008).

Fruit juices from all six sites have shown contamination with either one or the other foodborne pathogens (Figure 2). These six sites are generally polluted due to poor hygienic conditions and the consumers are at risk of contacting food borne infections. In this study Greek Camp was more contaminated than the other sites which were almost equally contaminated with food borne pathogens.



Key: Gr=Greek Camp, De=Dechatu, Ke= Kezira, Sa=Sabiyan, Ta=Taiwan, Me=Megala

Figure 2: Distribution of food borne pathogens in six sites of DD town.

4.3. Associations between food borne pathogens and risk factors

Thirty juice makers from cafes of Dire-Dawa were used as respondents of questionnaires administered to obtain data on the source of fruits and the handling practices of fruits used for juice making. The same respondents were also used to assess the level of awareness about food safety, hygienic conditions of the fresh fruit juice processing equipment and sites.

4.3.1. Fruit juice preparation and utensil sanitation practices

The source of fruits used for the processing of juice was primarily from the open market (80%) while some juice makers (20%) got their fruits directly from the producers who were their routine suppliers.

The temporary storage sites of fruits were baskets (60%), shelves (33.3%) and refrigerators (6.7%). It was also observed that 86.7% and 13.3% of the juice makers had the habit of preparing fruit juices using tap water and bottled drinking water for washing fruits prior to making juices and for dilution of the prepared fruit juices, respectively.

Table 10: Fruit Preparation, Processing and Sanitation of Utensils for making juice.

Parameter	Gr	De	Ke	Sa	Ta	Me	Total	%	P
Source of fruit:									
Open market	4	4	4	4	3	5	24	80	0.776
Directly from producers	1	1	1	1	2	0	6	20	
Temporary storage site of fruit:									
Shelf	2	1	2	1	3	1	10	33.3	0.694
Refrigerator	0	0	1	1	0	0	2	6.7	
Basket	3	4	2	3	2	4	18	60	
Water source for juice preparation:									
Tap water	5	5	3	4	5	4	26	86.7	0.329
Packaged drinking water	0	0	2	1	0	1	4	13.3	
Preparation of fruits are prepared prior to juicing:									
Peeled by hand	2	1	2	1	1	2	9	30	0.921
Cut by hand with knives	3	4	3	4	4	3	21	70	
Method of cleaning utensils before preparing the juice:									
With water only	1	1	0	1	0	0	3	10	0.649
With water and soap	4	4	5	4	5	5	27	90	
Frequency of juicer cleaning and sanitization:									
After each use	3	3	3	1	4	2	16	53.3	0.509
Between different juice types	2	2	2	4	1	3	14	46.7	

Gr = Greek Camp, Me = Megala, Ke = Kezira, Sa = Sabiyan, Ta = Taiwan, De = Dechatu

The data in Table 10 indicates that the majority of juice makers (70%) peel and cut fruits by hand with knives prior to juicing and 30% Peel the fruits by hand in order to prepare the juice

but for these fruit juices which are of higher demand, vendors tend to peel and keep them in an uncovered container before time of consumption. The table also showed that 53.3% of juicers cleaned and sanitized after each use whereas 46.7% of them between different juice types.

Most of the juice houses clean utensils used during preparation of juice between different juice types and after each bulk preparation. In addition it was observed that juice makers store fruit in the basket and cut the fruit by holding them in their bare hands which may result further contamination. The study was comparable to similar study conducted in Amravati city, India, More contamination was observed in the juices that were at poor hygienic vending site than fair hygienic conditions (Tambekar *et al.*, 2009). Unhygienic surroundings like sewage, improper waste disposal system, inadequate water supply causes contamination of food. Also houseflies and fruit flies due to sewage may contaminate juices as juices attract the flies (Subbannayya *et al.*, 2007).

4.3.2. Juice Handling Practices and Personal Hygiene

In the present study, poor personal hygiene and some of the key risk behaviors of vendors which are major contributors for the contamination of fruit juices were studied (Table 11). About 40% of vendors wore untidy clothes, 16.7% had long finger nails and 23.3 % of them wore hand jewelries while preparing juice. All the vendors were handling the fruit juices with bare hands.

The findings of this study also showed that all of the vendors were handling money while serving fruit juice, a practice which has been also reported from Nigeria (Chukuezi, 2010). About 36% of the vendors were frequently wiping hands to their clothes. The results of the study also demonstrated that 56.7% of vendors wore apron and out of these, 58.9%, 23.5%, and 17.6% have changed their aprons two times per week, once per week and once per day, respectively. Table 11 also shows that 73.3% and 36.7% of juice makers had the habit of cleaning themselves using tap water only and using tap water and soap, respectively. They were also washing hands before preparation of fruits and juice between different juice types (50%), before each juicing process (30%) and 2-3 times per day (20%), respectively.

Table 11: Juice handling practices and personal hygiene of juice makers

Parameter	Gr	De	Ke	Sa	Ta	Me	Total	%	P
Vendors cloth:									
Neat	3	3	4	3	3	2	18	60	0.893
Untidy	2	2	1	2	2	3	12	40	
Do you have any training on how to prepare and handle juice:									
Yes	0	0	0	0	0	0	0	0	--
No	5	5	5	5	5	5	30	100	
Do you wear aprons while serving juice:									
Yes	2	2	4	3	3	3	17	56.7	0.805
No	3	3	1	2	2	2	13	43.3	
How often do you change your apron:									
Once per day	0	0	2	0	0	1	3	17.6	0.535
Once per week	1	1	0	1	0	1	4	23.5	
Two times per week	1	1	2	2	3	1	10	58.9	
Long finger nails:									
Yes	1	1	2	0	1	0	5	16.7	0.538
No	4	4	3	5	4	5	25	83.3	
Wear hand jewelries while preparing juice:									
Yes	1	1	2	1	2	0	7	23.3	0.674
No	4	4	3	4	3	5	23	76.7	
Frequency of washing hands before preparation of fruits and juice:									
Before each juicing process	1	2	1	2	2	1	9	30	0.706
Between different juice types	2	2	4	3	1	3	15	50	
2-3 times per day	2	1	0	0	2	1	6	20	
How do you wash your hands:									
With tap water only	3	4	4	4	3	4	22	73.3	0.928
With water and soap	2	1	1	1	2	1	8	36.7	

Gr = Greek Camp, Me = Megala, Ke = Kezira, Sa = Sabiyan, Ta = Taiwan, De = Dechatu, P = probability, Neat = free from dirt, untidy = not neat

A significant association (Table 12) was found between *E. coli* and some of the practices like no apron wear while serving juice (P = 0.040) and Wear hand jewelries while preparing juice (P = 0.042). *E. coli* is widely disseminated in the environment through the feces of humans and other animals. This, coupled with the ability of the bacterium to survive for months external to the colon makes its presence ubiquitous. Fruit juices generally attract houseflies because of their sweet odour which act as vectors of bacterial pathogens like *E. coli* (Sheri *et al.*, 2007). There was also an association (trend) found between having long finger nails and presence of fecal coliforms in fruit juices (p = 0.042). There was also another significant association where washing hands with tap water only (P = 0.007) with *S. aureus* and also changing apron two times per week (P=0.024) and washing hands between different juice type (P=0.025) with *salmonella spp.*. Among all isolated foodborne pathogens in this study *salmonella spp.* was considered as high risk pathogen because of its low infective dose (100 - 1000 organisms) (Public health agency of Canada, 2010) which has shown significant association with changing apron two times per week.

Table12: Association between hygiene practices and microbial contamination

Parameter	Types of juice	Food borne pathogen	Mean (cfu/ml)	P value
no apron wear while serving juice	Papaya	<i>E. coli</i>	6.43 x10 ¹	0.040*
Changing apron two times per week	Avocado	<i>Salmonella</i>	5.71 x10 ²	0.024*
Long finger nails	Mango	Faecal Coliform	6.27 x10 ¹	0.042*
Wear hand jewelries while preparing juice	Mango	<i>E. coli</i>	6.27 x10 ¹	0.042*
Washing hands between different juice type	Papaya	<i>Salmonella</i>	6.23 x10 ²	0.025*
Washing hands with tap water only	Avocado	<i>S. aureus</i>	9.07 x 10 ³	0.007**

**Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

The present study revealed that street vended fruit juices are contaminated with foodborne pathogens like *S. aureus* and *salmonella spp.* The indicator organisms such as fecal coliforms and *E. coli* had a high prevalence which indicates that these fruit juices are hygienically of low standards. These findings indicated that targeted food safety education and training to vendors is essential to create awareness among vendors and translate into practice to avoid possible sources of microbial hazards.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

Summary and conclusion

Present study exhibited that microbial status of available fresh fruit juices served in cafeteria to ensure food safety for a precise control over public health risk. A total of 90 avocado, mango and papaya fruit juice samples from the cafeteria of Dire Dawa town were cultured for microbial analysis. It was found that 100% (n=90) of the samples had bacteria growth. In this study, generally most of total fruit samples were found to show higher microbial counts above the specification set of Gulf region standard. Among the type of juice, high bacterial and fungal load was observed in Papaya juice than Avocado and Mango juice except that of total viable bacterial and spore forming bacterial counts which are higher in avocado juice with the mean 7.21×10^5 cfu/ml and 2.93×10^3 cfu/ml respectively. According to fungal count of total positive sample, 70% (21/30) were Avocado, 76.67% (23/30) were mango and 80% (24/30) were Papaya fruit juices sample. Furthermore, 52.2%, 37.77%, and 11.1% of total fruit samples were positive for *Staphylococcus aureus*, *Salmonella* species and *E. coli*, respectively. The indicator organisms such as fecal coliforms had a high prevalence which indicates that these fruit juices are hygienically of low standards.

Based on these data of the assessed fruit juices, it could be hard to claim that, consumption of fresh juices is safe because almost all types of fresh juice samples collected from different areas of the city were not satisfactory as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, were detected in large numbers from samples.

Also some of the key risk behaviors which are major contributors for contamination of fruit juices were observed. Specific food safety practices regarding preparation of street vended fruit juices such as washing hands with tap water only, no apron wear while serving juice, long finger nails, and wear hand jewelries while preparing juice have shown significant associations with foodborne pathogens. Since the product is usually consumed within a short period of time after squeezing, pathogens present in contaminated juice can survive and cause infection.

The study indicated that all fruit juices served in cafeteria of many parts of Dire Dawa town were contaminated and potential sources of contamination such as lack of training (orientation) on the proper storage of fruit juices, processing of fruit juices and also on personal hygienic practices were identified. Juice vendors in the town are generally unaware of food regulations and have no training in food-related matters. They also lack supportive services such as rubbish disposal systems, which hamper their ability to provide safe food. A combination of regular monitoring and proper training could be an appropriate choice to minimize the health risks. Since the practice of consuming fresh fruit juice cannot be stopped based on unhygienic grounds, and neither can the vendors be prohibited from selling such items which provide them with a source of livelihood, the Government Health Agencies must put in place measures to educate the vendors on food safety and hygienic practices and enforce adequate guidelines for food vending. In addition to this, not only government authorized institution but also some strongly active administrative organization court should be given more authorization to undertake pre-emptive investigations to check the microbial and chemical quality of fruit juices. Besides, government and non-government institutions should create public awareness about the contamination of fruit juices more intensely with the help of mass media. Regular monitoring of the quality of fruit juices for human consumption should be introduced to avoid infection while shop locations that predispose juices to contamination should be avoided.

Recommendations

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

- National microbiological standard for food stuff and guidelines for juice processing should be prepared in order to interpret the result at national level.
- Training on the importance of personal hygiene, proper storage of fruit, should be given by government officials or non-governmental body to people involved in preparing and handling fruit juices.

- In order to assure the safety of fruit juices for consumers, there should be regular inspection and monitoring of the quality and safety of fruit juice for human consumption should be made.
- Data from this study may be useful for further risk assessment of *E. coli*, *Salmonella*, and *S. aureus* in freshly squeezed juice.
- Since current study was conducted on small sample size, the researcher also recommends further study by increasing sample size.

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10. What types of fruits are used for juice making?

Unripe Ripened Over ripened

11. Do you wash fruits before making juices?

Yes No

12. If your answer is yes for 11 above, how?

With water only with water and soap Other _____

13. How are the fruits prepared prior to juicing?

Peeled by hand Cut by hand with knives
Cut using machine placed the whole into juicing machine

14. Are the utensils cleaned before preparing the juice?

Yes No

15. If your answer is 'yes' for Q 14, how was it practiced?

With water only with water, soap, and antiseptic

With water and soap

16. How often are the juicers cleaned and sanitized?

After each use

Between different juice type

Once per day

Other specify _____

III. JUICE HANDLING PRACTICE AND PERSONAL HYGIENE

17. Do you have any training on how to prepare handle juice: Yes No

18. Do you wear aprons while serving juice Yes No

19. Handle juice with bare hand: Yes No

20. Handle money while serving: Yes No

21. Wear hand jewelries while preparing juice: Yes No

22. If your answer is 'yes' for Q18, how often do you change your apron

Once per day twice per day

Once per week two times per week

Other (please specify) _____

23. Do you wash your hands before preparation of fruits and juice

Yes No

24. If your answer is yes to Q23, how often do you wash

Before each juicing process

Between different juice typing

Once per day

2-3 times per day

Other (specify please) -----

25. How do you wash your hands

With tap water

With water and soap

Other please specify -----

Thank you for your cooperation

7.2. Sample Collection Format

Site	Sample No.	Type of sample	Finding						
			TVAMBC	TCC	TFCC	SAC	SC	SFBC	TFC

Key: TVAMBC= Total Viable Aerobic Mesophilic Bacteria Count, TCC= Total Coliform Count, TFC= Total Fecal Coliform Count, SAC= *S. aureus* Count SC= Salmonella Species Count, SFBC= Spore Forming Bacteria Count, TFCC= Total Fungi Count

7.3. Biochemical Characteristics of *Salmonellas*

Test	Positive or negative reaction
TSI glucose (acid formation)	+
TSI glucose (gas formation)	+
TSI lactose	-
TSI sucrose	-
TSI hydrogen sulfide	+
Urea splitting	-
Lysine decarboxylation	+
-Galactosidase reaction	-
Voges-Proskauer reaction	-
Indole reaction	-

7.4. Microbiological loads of freshly squeezed fresh fruit juices in DD town.

Microbial		Microbial Loads of the Three Types of Juice Samples		
Indicators	Statistics	Avocado	Mango	Papaya
AMBC (cfu/ml)	Range	5.60x10 ² -8.00x10 ⁶	2.80x10 ² -8.10x10 ⁶	2.30 x10 ² -9.00 x10 ⁶
TCC (mpn/ml)	Range	3.00x10 ¹ -4.30x10 ²	3.00x10 ¹ -9.30x10 ²	3.00 x10 ¹ -9.30 x10 ²
TFCC (mpn/ml)	Range	3.00x10 ¹ -9.20x10 ¹	3.00x10 ¹ -9.20x10 ¹	3.00 x10 ¹ -1.50 x10 ²
SAC (cfu/ml)	Range	5.00x10 ¹ -9.10x10 ⁴	5.00x10 ¹ -1.60x10 ⁴	5.00 x10 ¹ -1.9 x10 ⁵
TSC (cfu/ml)	Range	8.00x10 ¹ -1.45x10 ³	1.10x10 ² -2.00x10 ³	7.00 x10 ¹ -3.90 x10 ³
ASFBC (cfu/ml)	Range	1.00 x10 ² -2.50x10 ⁴	3.00x10 ¹ -9.80 x10 ³	3.00 x10 ¹ -5.40 x10 ³
YMC (cfu/ml)	Range	1.50 x10 ² -6.40x10 ⁴	1.00x10 ² -3.50x10 ⁴	1.00 x10 ² -1.40 x10 ⁶

AMBC = Aerobic Mesophilic Bacterial Count, TCC = Total Coliform Count, TFCC = Total Fecal Coliform Count, SAC = *Staphylococcus aureus* Count, TSC = total *Salmonella* Count, ASFBC = Aerobic Spore Forming Bacterial Count, YMC = Yeast and Mold Count

7.5. AMBC (cfu/ml) of freshly squeezed freshfruit juices in DD town by site of juice production.

J		Microbial Loads of the Three Types of Juice Samples					
Stat		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala
A	Mini	2.00x10 ³	2.65 x10 ³	5.60 x10 ²	3.80 x10 ³	2.80 x10 ⁵	2.30 x10 ³
	Max	3.10x10 ⁵	7.00 x10 ³	9.00 x10 ⁴	8.10 x10 ⁴	5.40 x10 ⁶	8.00 x10 ⁶
M	Mini	2.80x10 ²	3.00 x10 ³	5.20 x10 ²	2.00 x10 ³	2.60 x10 ⁴	3.00 x10 ²
	Maxi	6.10x10 ⁴	9.00 x10 ⁴	1.20 x10 ⁴	3.20 x10 ⁵	4.00 x10 ⁶	8.10 x10 ⁶
P	Mini	2.30x10 ²	5.50 x10 ³	3.00 x10 ²	1.7 x10 ⁴	1.90 x10 ³	6.60 x10 ³
	Max	1.80x10 ⁵	3.60 x10 ⁵	1.10 x10 ⁴	3.00 x10 ⁶	9.00 x10 ⁶	7.20 x10 ⁵

Key: A= avocado, AMBC= Aerobic Mesophilic Bacterial Count, M= mango, P= papaya, , Mini= minimum, Max= maximum, J=juice, Stat= statistic

7.6. SAC (cfu/ml) of freshly squeezed fresh fruit juices in DD town by sites of juice production.

J	Stat	Site from which the juice sample was collected					
		Greek Camp	Dechatu	Kezira	Sabiyan	Taiwan	Megala
A	Mini	1.70×10^2	5.00×10^1	2.00×10^2	1.00×10^2	1.90×10^3	2.00×10^3
	Max	1.50×10^3	3.50×10^3	1.00×10^3	7.50×10^3	3.50×10^3	9.10×10^4
M	Mini	7.00×10^1	3.20×10^3	5.00×10^1	1.30×10^4	1.20×10^3	5.00×10^2
	Maxi	2.00×10^2	5.00×10^3	3.00×10^2	1.60×10^4	5.50×10^3	2.00×10^3
P	Mini	6.00×10^1	3.50×10^2	5.00×10^1	2.50×10^4	1.00×10^3	1.60×10^3
	Max	5.00×10^2	3.00×10^3	1.50×10^3	1.90×10^5	6.40×10^3	2.80×10^4

Key: A= avocado, M= mango, P=papaya, Mini= minimum, Max= maximum, J=juice, Stat= statistic, SAC= *Staphylococcus aureus* Count