ASSESSMENT OF BACTERIAL QUALITY AND ASSOCIATED HANDLING PRACTICES OF UNPASTEURISED FRUIT JUICES VENDED IN DAR ES SALAAM CITY, TANZANIA

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ABSTRACT

Fresh fruit juices are essential components of human diet and there is considerable evidence of health and nutritional benefits associated with their consumption. However, during processing contamination from raw materials, equipment or food handlers could be easily transferred to the final product of the juices resulting to food-borne illness. A cross-sectional study was conducted to assess bacterial quality and associated handling practices of unpasteurised fruit juices vended in Dar es Salaam city, Tanzania. A total of 90 juice vendors were interviewed followed by collection of 90 juice samples for laboratory analysis. Physicochemical and bacterial qualities of the juices were analysed. The results showed that the pH of the juices ranged between 2.7 to 6.4 while the acidity ranged from 0.01% to 1.3%. The total soluble solids ranged between -1.5 to 18.04°Brix. Most juices had °Brix levels below Codex recommended minimum values and 67.8% were classified as weak and watery. The total plate counts (TPC) of the juices ranged between 2.32 to 8.54 (Log cfu/ml). About 72.2% juice samples had TPC above Codex recommended maximum levels ($5x10^3$ - 10^4cfu/ml). The prevalence of *Escherichia coli* in the juices was 80% with a range between -1.13 to 4.97 (Log MPN/ml). All samples were negative for *Salmonella* species. Risk factors for higher TPC and *E. coli* counts which were statistically significant ($P < 0.05$) included pH, type of juice, extraction methods, vending sites, storage containers and sex of the vendor. Generally, 78.9%; of preparation and vending premises were unhygienic and encouraged contamination of the juices. It is concluded that, the overall handling, preparation practices and bacterial quality of unpasteurised fruit juices vended in Dar es Salaam city are poor. The government should educate the vendors on food safety and hygiene as well as enforcing regular monitoring of the quality of street fruit juices.

DECLARATION

I, Edeltruds Simforian**,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF ABREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
CAC	Codex alimenterius commission
CFU/ml	Colon forming unit per millitre
CI	Confidence interval
et al	and others
FAO	International food and agriculture organization
g	gramme
g/ ml	gramm per millitre
ISO	International standards organization
^{o}C	Degree celsus
pH	Hydrogen ion concentration
spp.	species
StdEv	Standard deviation
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
µg/ml	microgram per millitre
%	Percent
&	and
<	Less than

>	Greater than
≤	Less or equal
≥	Greater or equal
®	Registered trade mark

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Codex Alimentarius defines juice as "the fermentable but unfermented juice, intended for direct consumption, obtained by the mechanical process from sound, ripe fruits, preserved exclusively by physical means (FAO, 2005). The juice must have the characteristic colour, flavour and taste typical of the fruit from which it comes. The juice may be concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice (FAO, 2005). The constituent of juice predominantly is water and also contains carbohydrate, sucrose, fructose, glucose, sorbitol and small amount of protein (Pao *et al*., 2001). Fruit juices have a low pH (2-5) because they are comparatively rich in organic acid (Tasnim *et al.*, 2010). The total acidity of fruit juices is due to presence of a mixture of organic acids, whose composition varies depending on fruit nature and maturity. The main acids encountered in fruits are tartaric, malic, citric, succinic, lactic and acetic acids. Organic acids are important for the characteristics and nutritive value of fruit juices and confer individual originality among natural beverages. Total soluble solids (TSS) contents are related directly to both the sugars and fruit acids. The TSS content is significantly influenced by the combined effect of stages of maturity and ripening conditions (Tasnim *et al.*, 2010).

Juices are nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health. Minerals and vitamins help in activation of enzymes and co-enzymes which work as catalysts in performing many chemical reactions that take place inside the human body. These enzymes help the body in digestion of food and absorption of important vitamins and minerals and transformation of food into the

body tissue. For example, orange juice is rich in vitamin C, an excellent source of bio-available antioxidant phytochemicals (Franke *et al.,* 2005) and significantly improves blood lipid profiles in people affected by hyper-cholesterolemia (Kurowska *et al.,* 2000). The anti-oxidant components of fruit juice have beneficial long term health effects, such as helping in the removal of free radicals and some other harmful materials from the body (Titarmare *et al*., 2009), promote detoxification (Deanna and Jeffrey, 2007), as well as decreasing the risk of cancer and heart diseases (Boyer and Liu, 2004; AICR, 2007).

Raw fruit juices are among the street foods that are vended in urban areas mostly in developing countries. They are prepared by low income vendors who have poor premises and facilities and lack basic needs such as portable water. Water for street food preparation is not enough resulting in vendors using little water for washing utensils hence hygiene is compromised (Mensah *et al.,* 2002; Muinde and Curia, 2005). Stalls for street foods preparation and vending are also poorly constructed, such that they can not give proper protection of the foods from dust and smoke from vehicles (Mensah *et al.,* 2002).

In spite of the potential benefits offered by street fruit juices, concerns over their safety and quality have been raised. Freshly squeezed fruit and vegetable juices have little or no process steps that reduce pathogen levels, if contaminated. Microorganisms like bacteria and fungi can enter fruits through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting and processing of fruits. The microorganisms may be the potential sources of fruit spoilage which are known to shorten shelf life of fruits (Chen *et al.,* 2010). However, the organisms that have become internalized within a fruit can be able to survive during processes to the final product until they reach the consumer (FDA, 2008). Such microorganisms may further

cause fast spoilage of the processed products like juices or if pathogenic may be the source of food-borne diseases.

Furthermore, water used for juice preparation can be a major source of microbial contaminants such as coliforms bacteria, faecal streptococci and other members of Enterobacteriaceae (Reddy *et al.*, 2009). In most cases, running water is not available at vending sites; hands and utensils washing are usually done in one or more buckets, and sometimes without soap. Wastewaters and garbage that are discarded nearby, favour multiplication and survival of insects and rodents. Some of the juices are not efficiently protected against flies, which may carry food borne pathogens. In addition, there is potential health risks associated with initial contamination of foods by pathogenic bacteria as well as subsequent contamination by vendors during preparation, handling, and cross contamination (Mosupye and van Holy, 2000). Another important issue influencing food quality and contributing to further increase in contamination is food storage temperature. Safe food storage temperatures are rarely applied to street vended foods. The preparation of food long before its consumption, storage at ambient temperature, inadequate cooling and reheating, contaminated processed food, and undercooking are identified as the key factors that contribute to food poisoning outbreaks (Omemu and Aderoju, 2008).

Unsanitary handling of street foods by some of the vendor has been commonly found to be the source of contamination (Muinde and Kuria, 2005; Sharmila, 2011). The vendors can be carriers of pathogens like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter* and *Staphylococus aureus* who eventually transfer these food borne hazards to the consumers. Pathogens like *Salmonella, Campylobacter* and *E. coli* can survive on finger tips and other surfaces for varying periods of time (Sharmila, 2011). A study conducted on hand rinses, stored water, and source waters in Bagamoyo Tanzania indicated that,

both water and hands are important means of bacterial pathogen transmission (Mattioli *et al.*, 2013).

Numerous serious safety problems associated with fruit juice consumption have been documented (Vojdani *et al.*, 2008). In the last decade in North America, over 1700 people have fallen ill after consuming juice and cider. Most of these outbreaks involved unpasteurized juices such as apple, orange, lemon, pineapple, carrot, coconut, cane sugar, banana, acai and mixed fruit juices (Bevilacqua *et al.,* 2011). The most common pathogens were *E. coli* O157:H7 and O111, *Salmonella* spp., *Cryptosporidium* and norovirus. A few other outbreaks were due to *Vibrio cholerae*, *Clostridium botulinum* and yeasts (Bevilacqua *et al.,* 2011). *E. coli* is incriminated as the common microbial contaminants of raw juices (Lewis *et al.,* 2006). Numerous dangerous strains of *E. coli* exist and are able to produce toxins of various types and toxicities that cause different diseases in humans. The enterohemorrhagic (EHEC) class is of most concern, due to its low infectious dose and its association with hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Keller and Miller, 2006; Vojdani *et al.,* 2008). In 2004, the US Center for Disease Control and Prevention reported a serious outbreak of 213 illnesses associated with untreated apple cider consumption in New York, due to Shiga toxin–producing *E. coli* O111 together with *C. parvum* (Keller and Miller, 2006). In a number of African countries, several devastating outbreaks of food-borne diseases such as cholera, salmonellosis, entero-haemorrhagic *E. coli* (EHEC), hepatitis A and acute aflatoxicosis have been reported (FAO, 2005).

Salmonella infections are commonly associated with animal-derived foods, such as meat, seafood, dairy, and egg products. However, outbreaks associated with fresh juice have

occurred as far back as 1922 in France (Bevilacqua *et al.,* 2011; Danyluk *et al.,* 2012). Early outbreaks resulting in typhoid fever were associated with poor hygiene by asymptomatic *S. typhi* shedding food handlers. In USA, more recent outbreaks of non-typhoidal salmonellosis in fresh juice have been attributed to fecal-associated contamination of fruit or poor processing practices (Jain *et al.,* 2009; Bevilacqua *et al.,* 2011; Danyluk *et al.,* 2012). In 2005, 152 cases of *S. typhimurium* infection associated with commercially distributed unpasteurized orange juice were reported in USA. Upon inspection by Food and Drug Administration (FDA), it was found that the production facility did not comply with the HACCP plan and that noncompliance likely contributed to this outbreak (Keller and Miller, 2006; Vojdani *et al.,* 2008).

In Tanzania, especially in cities like Dar es Salaam, there has been an increase in consumption of fresh extracted fruit juices because of availability of variety of fruits throughout the year (Ministry of Agriculture Food Security and Cooperatives, 2009). People have developed interest on freshly prepared fruit juices due to their fresh taste and also because of being scared of preservatives and other added ingredients in industrially processed juices. Most street fruit juices are prepared at homes, food stalls, or along vending sites such as roadsides, bus stands and markets. However, some are prepared in restaurants and food kiosk. Most of the preparation and vending sites are hygienically poor and basic needs such as portable water are inadequate. On the other hand, the water that is available is not safe. Mushi *et al*. (2012) used WHO Risk-of-Contamination (ROC) scoring procedure to study the quality of well water in Dar es Salaam city. The findings indicated that about 87% of the water was contaminated with *E. coli*.

Dar es Salaam city is characterized by a large population of about 4,364,541 million people with a growth rate of 5.6% (Census, 2012). The population is fuelled partly by an

influx of unemployed youth from the rural areas looking for better opportunities in urban areas. As a result, it has one of the highest proportions of informal-settlement households in East Africa, with 65% of households living in informal areas (Penrose *et al.,* 2010).

There has been a big number of food-borne diseases in the city including diarrhoea and cholera (Penrose *et al.,* 2010), but it is not known how much of these diseases have been contributed by the consumption of unpasteurised fruit juices. It was the aim of this study, to determine the microbial status and associated handling practices of the locally prepared and vended fruit juices in Dar es Salaam city. The baseline data of this work may help in associating the available incidences of food borne disease and contaminated juices. This will also be useful to public health officials in instituting control measures of food borne diseases in Dar es Salaam city and Tanzania at large.

1.2 Main Objective

To determine the bacterial quality of unpasteurised fruit juices and associated risk factors for their contamination in Dar es Salaam city, Tanzania.

1.2.1 Specific objectives

i. To assess sources and handling of raw materials for juice preparation;
ii. To assess the possible factors for bacterial contamination of unpasteurised fruit juice along the value chain;
iii. To establish the status of bacterial contamination of locally prepared and vended unpasteurised fruit juices.

1.2.2 Research questions

1. What are the sources of fruits and water used in the preparation of juices?
2. How are the fruits handled before they are subjected to juice extraction?
3. What methods are in use during preparation of the juices?
4. What equipment and utensils used in the process and how are they handled?
5. How are the juices handled and stored after preparation and during vending?
6. What is the status of bacterial contamination of the juices?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Street Foods in Developing Countries

The street food industry plays an important role in developing countries in meeting the food demands of the urban dwellers. Street foods feed millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. The street food industry offers a significant amount of employment, often to persons with little education and training (Latham, 1996). Besides offering business opportunities for developing entrepreneurs, the sale of street foods can make a sizeable contribution to the economies of developing countries. In India for example, the National Policy for Urban Street Vendors/Hawkers reported that street vendors constitute approximately 2% of the population of a metropolis (Bhowmik, 2005). According to FAO, street foods have significant nutritional implications for consumers, particularly from middle and low-income sectors of the population. According to studies done in Africa on street foods, their tremendous unlimited and unregulated growth has placed a severe strain on city resources, such as water, sewage systems and interference with the city plans through congestion and littering adversely affecting daily life (Canet and N'diaye, 1996). Street food vendors are often unlicensed, untrained in food hygiene and sanitation, and work under crude unsanitary conditions (FAO, 1997).

In Tanzania, urbanisation coupled with low wages offered to employees and labourers has led to proliferation of street food vendors who offer commercial meals but of high microbial contaminants due to poor hygiene and handling methods (Kinabo, 2003). In growing cities such as Dar es Salaam, street foods account for 70% of the total calorie intake of the urban low and middle income groups. They are available at all places of

work where they are required, such as factories, construction cites, offices, schools, transit points and market places (Kinabo, 2003). In a study to evaluate the microbiological quality of ready-to-eat foods in Pemba Island, thermotolerant coliforms were detected in 34% of sea-foods, 58% of household meals and 98% of milk samples; *Salmonella* spp. was exclusively found in 11% cow milk and *V*ibrio *alginolyticus* was isolated in 15% sea-foods (Vigano *et al.*, 2007).

2.2 Handling and Preparation of Street Foods

The conditions under which some street vendors operate are reported to be unsuitable for the preparation and selling of food (Barro *et al.*, 2006). The food is prepared either at home or at stalls, which are located on the street side and are made up of wood and polythene bags (Muinde and Kuria, 2005). The place of preparation is not always clean, well lit and not far from source of contamination. Preparation surfaces used by some vendors have remains of foods prepared earlier that can promote cross contamination. Most of these foods are not covered and are exposed to flies and dust, which may harbour food-borne pathogens (Mensah *et al.*, 2002). In 70–90% of the cases, presence of animals, insects and liquid wastes in food preparation areas have been reported (Sharmila, 2011). The two major sources from where the contaminants can enter the preparation area are improper food handling and waste disposal. Unsanitary handling of street foods by some of the vendor has been commonly found to be the source of contamination (Muinde and Kuria, 2005). The vendors themselves can be carriers of pathogens like *E. coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* who eventually transfer the pathogens to consumers through food. The hands of the food handlers are the most important vehicle for the transfer of organisms from environment (food surfaces), nose and skin to the food (Sharmila, 2011). This supports the reports of contamination of street vended food with toxigenic *S. aureus*, the major being suppurative lesions of human beings and the

environment (Mohapatra *et al.,* 2002). A study conducted on hand rinses, stored water, and source waters in Bagamoyo Tanzania indicated that, both water and hands are important means of bacterial pathogen transmission (Mattioli *et al.,* 2013).

Street vendors are mostly uninformed of good hygienic practices (GHP) and food safety knowledge (Muinde and Kuria, 2005; Sharmila, 2011). They are also not aware of causes of diarrheal diseases (Mersah *et al.,* 2002) which can increase the risk of street food contamination (Bhaski *et al.,* 2004). Practices towards food handling are poor and basic food safety hygiene is not adhered to hence, put the food at high risks of contamination. They are also unaware of food regulations as well as lacking supportive services such as water supply of good and adequate quality, waste disposal systems which enhance their ability to provide safe food.

2.3 Waste Disposal

Street food vendors tend to congregate in overcrowded areas where there are high numbers of potential customers, which usually provide limited access to basic sanitary facilities. Hence, the contamination of street foods is often linked to the waste generated by food processing, that is usually dumped near the vending sites. The lack of facilities for liquid drainage and wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganisms. Muinde and Kuria (2005) found that 85% of the vendors prepared foods like fish, fruit salads, roasted maize and chips in unhygienic conditions, given that garbage and dirty waste were conspicuously close to the stalls. In these areas large amounts of garbage accumulates which provide harborage for insects and animal pests that are linked to enteric disease transmission (Mosupye and Holy, 1999; Barro *et al.,* 2006; 2007).

2.4 Indicator Organism in Food

Routine examination of foods for a range of pathogenic microorganisms is impractical. In order to assess the microbiological safety from food-borne 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 (WHO, 2008).

The occurrence of pathogens and indicator organisms in groundwater and surface water sources depends on a number of factors, including intrinsic physical and chemical characteristics of the catchment area and the magnitude and range of human activities and animal sources that release pathogens to the environment (WHO, 2008). The indicator organism of choice for faecal pollution is *E. coli*. Water intended for human consumption and/or used for domestic activities should not contain indicator organisms.

2.5 Water Supply and quality

According to WHO (2008) guidelines for drinking water, the community should use safe water sources for drinking and food preparation. The guidelines are also applicable to packaged water and ice intended for human consumption. According to Codex general principles of food hygiene (*CAC/RCP 1-1969, Rev.4- 2003*) food processing establishments should have an adequate supply of potable water with appropriate facilities for its storage, distribution and temperature control, to ensure the safety and suitability of food (FAO, 2003). Contrary to this, most street food vendors do not have adequate and potable water for use in food activities resulting in using little water for their activities of food preparation and handling hygiene is compromised (Mensah *et al.*, 2002; Muinde and Curia, 2005). Without enough potable water, food safety and hygiene cannot be met. Reddy *et al.* (2009) studied the quality of water used to prepare unpasteurized fruit juices

in Bellary city India. The findings indicated that, water used in the preparation of fruit juices was highly contaminated with faecal coliforms. This served as the potential sources of juice contamination and posed dangers of food-borne diseases to the consumers.

Bacteriological water assessment conducted in Morogoro Tanzania indicated that all samples of raw water from the river and treated water points from the distribution system were contaminated with coliforms, faecal coliforms, faecal streptococci and *C. perfringens*. The study also revealed a recovery rate of 57 – 90% of injured coliform after passing through filtration process (Jiwa *et al.,* 1991). It was concluded that, recovery of injured coliforms suggests possibility of heavier contamination and might also point to deficiencies in filtration process and other preliminary safeguards or resistance to chlorination (Jiwa *et al.,* 1991). According to Robert (2011) findings, 75% of water supplies in Iringa region in Tanzania were contaminated with *E. coli.* Similarly, Mushi *et al.* (2012) investigated water wells in Dar es Salaam city and 87% of the water samples analysed was contaminated with *E. coli.*

Nevertheless, use of ice to cool foods and juices has been found to be the sources of contamination. If water used for ice production is of poor quality, harmful microorganisms may persist in the ice. Moreover, the process of freezing cannot destroy the organisms hence, when the ice is thawed, the surviving micro-organisms though may be injured, tend to recover their viability (Mahale *et al.,* 2008). In a study conducted in Uyo Metropolis, Nigeria which assessed the edible ice used by street vendors to cool the juice revealed that all edible ice samples analyzed had high microbial load of coliforms and *Staphylococcal* counts (Sunday *et al.,* 2011). It was also noted that in Uyo Metropolis, the major source of water was from bore holes and deep wells which were

prone to contamination by *E. coli,* coliforms and other pathogenic micro-organism. A major outbreak of *Hepatitis A* virus in Lampage and Chiang Rai, Thailand, affecting about 900 people has also been reported due to contaminated ice. Initial investigations pointed to ice factory which draw its water from contaminated artesian wells (APEC, 2005). A cholera epidemic in Pune city India was related to street vended sugarcane containing ice contaminated with *V. cholerae* (Mosupye *et al.,* 1999). It has also been noted in a similar study by Lateef *et al.* (2004) on microbiological safety of commercial ice used in Ogbomoso south west, Nigeria where it reported the presence of *Pediococcus* spp., *Bacillus* and *Streptococcus* spp.

2.6 Safety and Quality of Fruit Juices

Relatively large number of reports of food-borne illness associated with fruit juices indicates that unpasteurized juices pose a high public health risk. Several authors have reported that pathogens such as *Salmonella, Shigella, E. coli* O157:H7 or *L. monocytogenes* can survive for long periods in refrigerated juices and acidified culture media. The ability of different pathogens to survive in low pH environments has been documented at length (Eribo and Ashenafi, 2003; Teeteh and Beuchat, 2003; Zaika, 2001). Example, *Salmonella* are chemoorganotrophic and non-fastidious microorganisms with inherent ability to adapt to wide pH ranges of 4.0 to 9.0 and temperature ranges of 5°C to 47°C, respectively (Jay, 2000). In a study conducted in Mexico where pH values of the juice ranged from 3.0 to 4.8, with approximately one-third of the samples showing a pH of < 4.0, 13% of the samples were positive for *Salmonella* and *Shigella*. From these reports, it is clear that pathogens can survive at low pH (Castillo *et al*., 2009).

Furthermore, the juices with very low pH have been reported to have significant inhibitory effects against a wide range of bacteria. Zahra (2010) studied antagonism

activity of lemon and lime juices against some bacteria including *E. coli*, *Klebsiella pneumonia* and *Shigella flexneri*. The results indicated that, due to their inherent low pH both juices had antimicrobial effects against bacteria. The antagonism effects of these two juices were compared with the antagonism effects for some antibiotics. Rodrigues *et al.* (2000) and Castillo *et al.* (2000) reported the antimicrobial activity of freshly squeezed lemon juice against *V. cholerae.*

2.7 Sources of Fruit Juices Contamination

Fruit juices contain water, sugars, organic acids, vitamins, and trace elements thus providing an ideal environment for spoilage by microorganisms. On the other hand, they generally have a lower pH thus the common feature of their potential spoilage agents is that they must be acid-loving microorganisms. The most commonly encountered microbial genera are *Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymomonas,* and *Zymobacter* (Keller and Miller, 2006). However, yeasts are also common because of their high acid tolerance and ability to grow anaerobically. *Pichia*, *Saccharomyces* and *Rhodotorula* are the genera of fungi mainly involved in spoiled juices. However, other 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, Torulaspora delbruckii, Zygosaccharomyces microellipsodes*, and *D. naardenensis* (Vasavada, 2003). A high level of yeast contamination in fruit juices may be indicative of poor plant hygiene. Most spoilage yeasts are highly fermentative, forming ethanol and CO_2 from sugar, causing split cans and cartons, and explosions in glass or plastic bottles.

However, bacteria, virus and parasites with potential of causing infections in humans have also been reported (Eni *et al.,* 2010). Bacteria are the greatest concern in terms of serious illness and high numbers of persons are at risk of infection especially in developing countries. In North America, for example, *E. coli* O157 and O111, *Salmonella, Cryptosporidium* and norvirus were the most common pathogens which caused food-borne illnesses and deaths due to consumption of contaminated unpasteurized fruit juice and ciders (CDC, 2011). A few other outbreaks were due to *V. cholerae, C. botulinum,* yeast and *Hepatitis A* virus (CDC, 2011). In a study conducted in India reported *E. coli,* followed by *Pseudomonas aeruginosa*, *Salmonella* spp., *Proteus* spp., *Staphylococcus aureus*, *Klebsiella* spp. and *Enterobacter* spp. as the common bacterial pathogens of unpasteurised fruit juices (Tambekar *et al.,* 2009).

Most fruits contain bacterial counts of up to 1.0 X 10^5 cm^{-2} on their surfaces (Reddy *et al*., 2009). Improper washing and handling of fruits may facilitate the bacteria to contaminate the juice extract. Contamination from raw materials and equipment, additional processing conditions, improper handling, prevalence of unhygienic conditions have shown to contribute substantially to the entry of bacterial pathogens in the prepared juices (Oliveira *et al.*, 2006; Nicolas *et al.*, 2007). A study by Tambekar *et al.* (2009) found that, contamination of raw fruit juices is mainly due to poor quality of water used for dilution as well as unhygienic conditions related to washing of utensils, contaminated water and ice, poor personal and processing hygiene. Shops located alongside roads with heavy vehicle traffic or waste disposal systems, and over crowding further increase the degree of contamination.

2.8 Equipment Used in Food Preparation

Equipment for food preparation should be made of stainless steel as it is easier to clean, sanitize and maintain than equipment made from other materials. All lubricants and surfaces coming into contact with foods should be made of food grade materials. Equipment that comes into contact with fruit juice/cider should not be made of a material that could lead to undesirable or unacceptable migration or leaching of chemicals into juice/cider, for example, brass equipment should not be used since the acidity of the juice/cider could leach the copper out of the brass Canadian Food Inspection Agency (2010). In a study by Castillo *et al*. (2006) on bacterial quality of unpasteurized orange juice in Guadalajara, Mexico reported high counts. The study concluded that high bacterial counts was associated with cross-contamination from improperly sanitized utensils or contaminated oranges. Lakshmanan and Schaffner (2005) in the study of orange squeezing machines found that, some of the machines had scraps of oranges in internal tubing which were then reflected in the formation of bacterial biofilms.

2.9 Food-borne Outbreaks

Food-borne or waterborne microbial pathogens are leading causes of illnesses in developing countries, killing an estimated 1.9 million people annually at the global level (Schlundt *et al.,* 2004). Even in developed countries, an estimated one-third of the population is affected by microbiological food-borne diseases each year (Andargie *et al.*, 2008). The number of documented outbreaks of human infections associated with the consumption of raw fruits, vegetables, and unpasteurized fruit juices has increased in recent years (Buck *et al.,* 2003). There are reports of food borne illness associated with the consumption of fruit juices in several places (Sandeep *et al*., 2001). In Amravati city, India where there is high rate of consumption of fresh juice made of a variety of fresh fruits, such as oranges, grape, pomegranate, apple, pineapple, watermelon, papaya and

carrot, outbreaks of gastroenteritis due to pathogenic *E. coli*, *Salmonella* and *Shigella* are common (Mensah *et al.,* 2002; Burt *et al*., 2003; Bhaskar *et al.,* 2004; Lewis *et al.,* 2006). In the last decade, over 1700 people fell ill in North America after consuming juices and ciders. Most of these outbreaks involved unpasteurised juices and ciders such as apple cider, orange juice, lemonades, pineapple, carrot, coconut, cane sugar, banana, acai and mixed fruit juices (Bevilacqua *et al.,* 2011; CDC, 2011).

Table 1: Recorded outbreaks of food borne diseases associated with fruit juices

Product	Pathogen isolated	Year	Location	Cases (deaths)	Reference
Unpasteurized orange juice	*Shigella flexneri*	1995	South Africa	14	Thurston *et al*., 1998
Unspecified mixed Fruit	*Shigella sonnei*	2002	Canada, USA, UK,	78	CDC, 2011
Unspecified sugar cane	*Trypanosoma cruzi*	2005	Brazil	25 (3)	Pereira *et al.,* 2009
Unpasteurized apple	*E. coli* O157:H7	2005	Canada (ON)	4	LSDEPC, 2005
Mixed fruit (Açaí, sugarcane)	*T. cruzi*	2006	Brazil	94 (6)	Pereira *et al.,* 2009
Unspecified guava	*T.cruzi*	2007	Venezuela	103 (1)	Alarcón de Noya *et al*., 2010
Unpasteurized apple	*E. coli* O157:H7	2008	USA (IA)	7	CDC, 2011
Mamey frozen pulp	*S. typhi*	2010	USA	9	CDC, 2010
Unpasteurized apple	*E. coli* O157:H7	2010	USA	7	FDA, 2010
Unspecified mixed fruit	*Hepatitis A*	2007	USA (FL)	3	CDC, 2011

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

This study was conducted in Dar es Salaam City, Tanzania from September, 2012 to August, 2013. The city has three Municipalities namely Ilala, Kinondoni and Temeke (Fig. 1). The Municipalities are divided into 10 divisions, which are subdivided into 93 wards, 448 streets. The city lies along the western coast of Indian Ocean. It is situated between 6 and 7 degrees South of the Equator and between longitudes 33.33 and 39 degrees East of Greenwich Meridian. It borders Coast Region in the North, West and South while to the East, the Indian Ocean. The total surface area of Dar es Salaam city is 1397 km² which is equivalent to 0.15 percent of the entire Tanzania Mainland area.

The region experiences a modified type of equatorial climate. It is generally hot and humid throughout the year with an average temperature of 29^{o}C. The highest temperature season is from October to March during which temperatures rise up to 35^{o}C. It is relatively cool between May and August, with temperature around 25^{o}C.

Dar es Salaam is the commercial city of the country and one of the fastest growing cities in Africa. In 2012 census, it had a population of 4 364 541 with an inter-censual growth rate of 5.6% (2002 - 2012) (Census, 2012). The rate is above the national population growth rate of 2.7%. The higher population growth rate is mainly due to migration factor; however fertility and mortality rates have also played a significant factor in increasing the population in the City. Dar es Salaam city was chosen for this study due its large population and rapid increase of street foods including fruit juice vending activities.

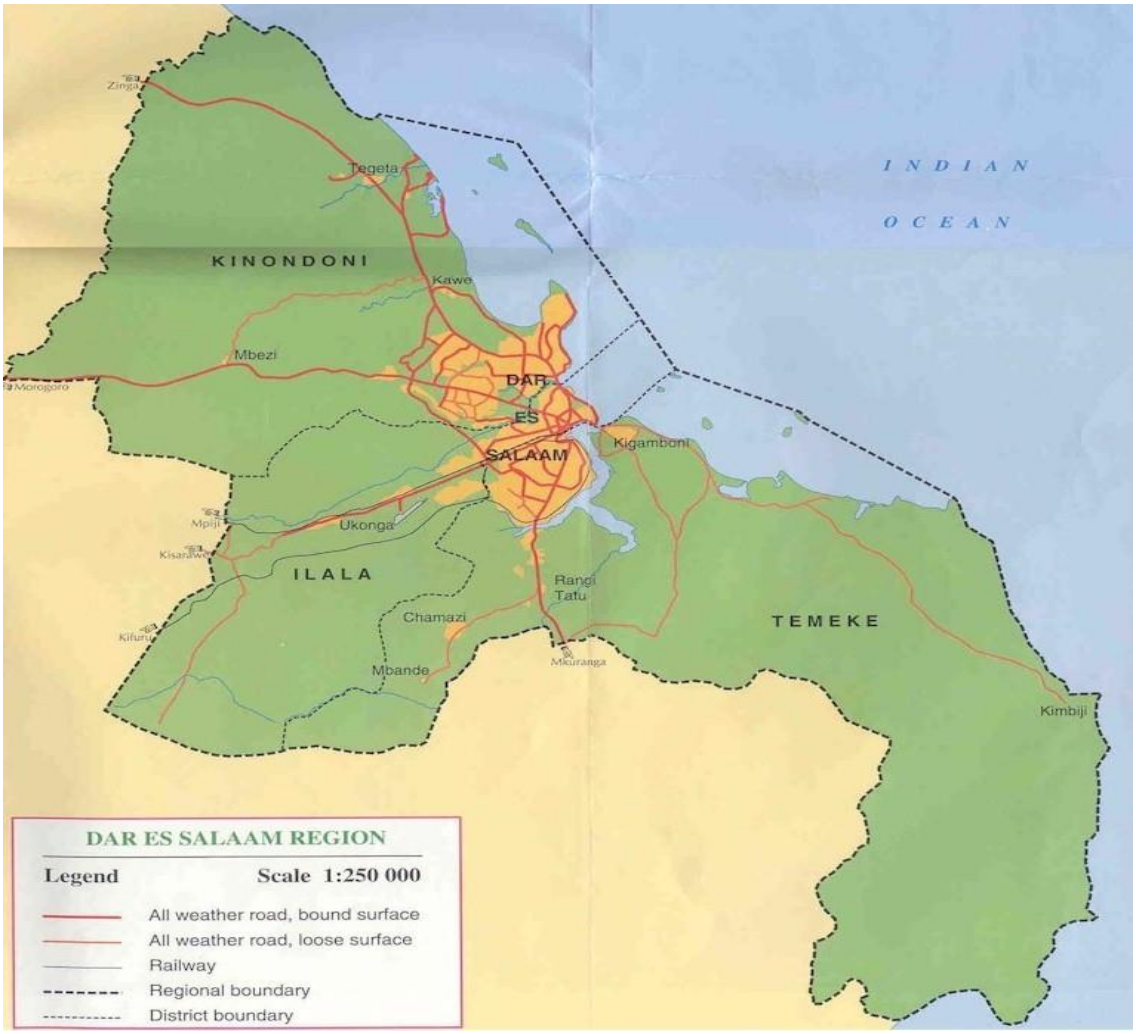

Figure 1: Map of Dar es Salaam city showing the Municipalities of Ilala, Kinondoni and Temeke

3.2 Study Design

A cross-sectional research design was conducted where sociological and laboratory data were collected. Randomly selected juice vendors, were administered with structured questionnaire and observational checklist and at the same time taking sample for laboratory analysis. The method was useful because of time limitation and resource constraints.

3.4 Study Population

Selected juice vendors (both males and females) who prepared and sell raw fruit juices in Ilala, Kinondoni and Temeke municipalities were selected for the study.

3.4.1 Inclusion criteria

Fruit juice vendors who were available at the time of data collection, willing to participate in the study and ready to give the required information were included in the study.

3.4.2 Exclusion criteria

Those vendors who were not willing to participate or not ready to give the required information were excluded in the study.

3.4.3 Sample size

The sample size was estimated by the formula for the unknown population (Kothari, 2004).

$$n = \frac{Z^2 SD^2}{e^2} \quad \text{.....(1)}$$

Where: n = size of sample, z = standard variate at 95% confidence level (1.96), SD = the standard deviation of population, and for the case of this study it was taken at 14% and e = acceptable error which was taken at 5% (0.05).

$$\text{The sample } n = \frac{[(1.96)^2\ (0.14)^2]}{(0.05)^2} \text{ x } 3 = 90 \quad \text{.....(2)}$$

Therefore a total of 90 respondents from the three municipalities (30 from Ilala, 30 from Kinondoni and 30 from Temeke) were involved in the study.

3.5 Ethical Consideration

Research permit was provided by the Vice Chancellor Sokoine University of Agriculture and permission letters were obtained from Dar es Salaam Region Administrative Secretary and Municipal Councils of Ilala Kinondoni and Temeke. Verbal consent was obtained from each vendor after explaining the purpose and importance of the study prior to commencement of interviews and sampling. Participation in the study was on voluntary basis. All the information collected from the participants and the laboratory results obtained after juice sample analysis were kept under the custody of the researcher as confidential.

3.5 Sociological Data Collection

3.5.1 Data collection tools

Data collection tools were structured questionnaires and observational check lists.

3.5.1.1 Structured questionnaires

Structured questionnaires (Appendix 1) were used to collect information from the juice vendors. Most questionnaires were made with pre-coded response choices (closed-ended questions), while a few were made of open-ended questions. The questionnaires were used to collect sociological information from the respondents as regard to socio-demographic information, type of fruits and sources, water sources and treatment, fruit handling and juice preparation methods, storage, serving equipment and vending sites.

3.5.1.2 Observational checklists

The observational checklist (Appendix 2) was made with some of the Codex recommended general principles (*CAC/RCP 1-1969, Rev.4- 2003*) of food hygiene (FAO, 2003) for food preparation settings, washing processes, general hygiene of the

vendor and premises, waste management and general upkeep of the juices. On the other hand, the checklist was provided with “YES” and “NO” sections for each parameter for the researcher to fill in the observations.

3.5.1.3 Pre-testing of the research tools

Before commencing data collection, pre-testing of questionnaires and observational checklist was done at Mbezi Luis ward in Kinondoni Municipality where 10 juice vendors were involved. The aim was to check the clarity of questions and instructions to the respondents, applicability of the questions and observational checklists as well as estimating the time required to collect sociological data for each respondent. After testing the questionnaires, they were revised and arranged in a better chronology. The revised version of the questionnaires that was used in the study was translated into ‘Kiswahili’, the national language understood by majority of Tanzanians.

3.5.1.4 Recruitment of research assistants

One research assistant from each ward with adequate knowledge and skills on health issues was recruited and trained on how to collect information and samples from the vendors. The assistants were recruited from ward health committee to assist in identifying the locations within the wards that were famous for fruit juices business as well as data collection activity. All research assistants were informed about the purpose and objectives of the research as well as data collection strategies. However, in each stage of data collection from the field, the researcher fully participated and closely supervised the research assistants.

3.5.2 Selection of wards and juice vendors

i) Selection of wards

Purposive sampling technique was used to select the study wards. By the help of municipal food inspectors, locations famous for juice preparation and vending were identified and ranked in accordance with the number of juices vendors available. Then, the first four wards were selected and included in the study. The selected wards included Mtongani, Miburani, Tandika and Sandali wards in Temeke municipality; Mchikichini, Ilala, Vingunguti and Buguruni wards in Ilala municipality and Mwananyamala, Hananasif, Kinondoni and Makumbusho wards in Kinondoni municipality.

ii) Selection of juice vendors

Simple random technique was employed to select juice vendors for the study. Firstly, juice vendors were identified and through good communication they understood the purpose of the study and were ready to participate. Seven to eight vendors from each ward were chosen randomly and were enrolled in the study.

3.6 Sample Collection for Laboratory Analysis

Juice sampling was done after questionnaire interview and observations were completed. One juice sample was bought from each vendor. Samples were collected directly from the storage containers used by juice vendors in which case at least 250 ml of juice samples was collected, put into a sterile glass bottle and stored in a cool box with ice parks. For those who packed the juice in re-used plastic water bottles, the samples were collected with their original containers, and were put in the same cool box. After the field work, the samples were immediately transported to Tanzania Food and Drugs Authority (TFDA) laboratory for analysis. The Laboratory is located in Kinodoni Municipality.

3.6.1 Laboratory Analysis

Two levels of laboratory analyses of samples were undertaken. The first was analysis of physicochemical parameters of the juice such as pH, titratable acidity and total soluble solids (°Brix). The second was determination of microbial contamination. This involved analysis for Total Plate Counts (TPC) by using ISO 4833:2003(E), *Escherichia coli* using ISO 7251:2005(E), and *Salmonella* using ISO 6579:2002(E).

3.6.2 Determination of physicochemical parameters of juices

Immediately after arriving to the laboratory, the juice samples were removed from the cool box and placed on the lamina floor, where aliquots for determination of physicochemical parameters were taken. Three aliquots of the juice sample, each 30 ml were drawn by using a sterile pipette and dispensed into separate beakers for the determination of pH, °Brix and titratable acidity.

3.6.2.1 Determination of pH

The pH of the samples was measured by using Jenway 3540 pH & Conductivity Meter (Bibby Scientific Ltd, Staffordshire, UK) which was first calibrated using standard buffer solutions of pH 7.0 and pH 4.0 (ISO 7218:2007). The pH of each sample was measured by immersing two probes of the meter (one for pH and the other for temperature) into the sample. After a few seconds, the reading on the screen of the meter stabilized and it was recorded. After measuring the pH of each sample, the probes were cleaned thoroughly by using distilled water and dried with a soft tissue before using them in measuring the next sample. The same procedure was repeated for all samples.

3.6.2.2 Determination of Total Soluble Solids (°Brix)

Total soluble solids TSS (°Brix) were determined using a RFM 340 refractometer (Bellingham and Stanley Ltd., London, U.K). Before measuring the °Brix of the samples, the refractometer was standardized by using distilled water at 0 °Brix and sucrose solution at 30 °Brix. The prisms of the refractometer was cleaned with distilled water and dried with a soft tissue before each reading. The juice sample was thoroughly mixed, then by using a sterile pipette an aliquot of a sample (approximately 3 drops) was drawn and applied to the refractometer prism. The prism was covered and the start button was pressed to initiate the measuring. After 2 - 3 seconds, the reading of °Brix and temperature appeared on the screen of the refractometer. The reading was recorded accordingly. The procedure was repeated for all the samples.

3.6.2.3 Determination of Titratable acidity

Total titratable acidity of the samples was established using the recommended method by AOAC, (1999). The solution of Sodium hydroxide (NaOH) was standardized to 0.1 Normality (N). The solution was put into a 25 ml Buret with 0.1 ml graduation and Teflon® stopcock. The juice sample was thoroughly mixed, and then 10 g was measured into 250-ml glass flasks. About 100 ml of distilled water was added and mixed well, and then 4 - 5 drops of phenolphthalein solution were added and mixed thoroughly. A magnetic stirrer was put into the solution to aid stirring during titration. The solution was titrated with the standardized NaOH until the solution showed a faint discernible pink color that persisted for 30 seconds (end point pH 8.2). The volume of the NaOH was recorded and was used to calculate acidity of the sample. For each sample two sets of titrations were conducted to obtain two titre values which were finally used to compute an average volume. The acidity of the sample was determined by the formula:

$$\%Acid\,(W/W) = \frac{(Net\,ml\,Titrant)\,(NTitrant)(0.064)}{Sample\,weight}\,X\,100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where: 0.064 = the acid factor for citric acid, Net ml Titrant = titre value of NaOH and NTitrant = Normality of Ttrant (NaOH)

3.6.2.4 Brix / Acid Ratio

The Brix / acid ratio was obtained by dividing the total soluble solids (°Brix) by the total titratable acid (% Acid, w/w) as follows:

$$Brix/Acid\,Ratio = \frac{^0\,Brix}{\%Acid,\,(w/w)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

3.7 Microbiological analysis

3.7.1 Media preparation

3.7.1.1 Buffered peptone water

The Buffered Peptone Water (BPW) (OXOID® Ltd., Basingstoke, U.K.) is composed of; 10 g Peptone, 5.0 g Sodium chloride, 3.5 g Disodium phosphate and 1.5 g Potassium dihydrogen phosphate. The medium was prepared according to manufacturer's instructions by adding 20 g of the powdered medium into 1 litre of distilled water. It was mixed well and distributed into final containers, then was sterilised by autoclaving at 121°C for 15 minutes. The media was cooled to about 20°C before use.

3.7.1.2 Muller-kauffmann tetrathionate-novobiocin broth

Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn) (OXOID® Ltd., Basingstoke, U.K.) is composed of 4.3 g of Meat extract, 8.6 g Enzymatic digest of casein, 2.6 g Sodium chloride, 38.7 g Calcium carbonate, 30.5 g Sodium thiosulphate (anhydrous), 4.78 g Ox bile, and 0.0096 Brilliant green. The prepared medium in its final

form is added with iodine/iodide solution + Novobiocin Selective Supplement. The medium was prepared according to manufacture's instructions by suspending 89.5 g of the powder in 1 litre of distilled water, mixed well and brought to the boil. It was then cooled to below 45°C. Immediately before use 20 ml of iodine-iodide solution were added. Also, the contents of four vials of Oxoid Novobiocin Selective Supplement were added. The medium was mixed well and aseptically dispensed into sterile screw-capped bottles.

3.7.1.3 Rappaport-vassiliadis soya peptone broth

Rappaport-Vassiliadis Soya Peptone Broth (RVS Broth) (OXOID® Ltd., Basingstoke, U.K.) is composed of 4.5 g Soya peptone, 7.2 g Sodium chloride, 1.26 g Potassium dihydrogen phosphate, 0.18 g Di-potassium hydrogen phosphate, 13.58 g Magnesium chloride (anhydrous) and 0.036 g Malachite green. The medium was prepared according to manufacture's instructions by suspending 26.75 g in 1 litre of distilled water and heated gently to dissolve. Then, 10 ml volumes were dispensed into screw-capped bottles and were sterilised by autoclaving at 115°C for 15 minutes. The bottles with the medium were placed on the lamina floor to cool to about 20°C prior to use.

3.7.1.4 Xylose-Lysine-Desoxycholate Agar

Xylose-Lysine-Desoxycholate (X.L.D) Agar (OXOID® Ltd., Basingstoke, U.K.) is composed of 3.0 g Yeast extract, 5.0 g L-Lysine HCl, 3.75 g Xylose, 7.5 g Lactose, 7.5 Sucrose, 1.0 g Sodium desoxycholate, 5.0 g Sodium chloride, 6.8 g Sodium thiosulphate, 0.8 g Ferric ammonium citrate, 0.08 g Phenol red and 12.5 g Agar. The medium was prepared according to manufacture's instructions by **s**uspending 53 g of powdered medium in 1 litre of distilled water. The medium was heated with frequent agitation until it boiled. Care was taken not to overheat it. The medium was transferred immediately to a

water bath at 50°C. As soon as the medium had cooled to around 44 – 47 °C it was poured into sterile Petri dishes placed on the lamina floor and was left to solidify.

3.7.1.5 Triple Sugar Iron Agar (TSI)

Triple Sugar Iron Agar (OXOID® Ltd., Basingstoke, U.K.) is composed of 3.0 g `Lab-Lemco' powder, 3.0 g Yeast extract, 20.0 g Peptone, 5.0 g Sodium chloride, 10.0 g Lactose, 10.0 g Sucrose, 1.0 g Glucose, 0.3 g Ferric citrate, 0.3 g Sodium thiosulphate, 0.024 g Phenol red and 12.0 g Agar. The medium was prepared by suspending 65 g of powdered medium in 1 litre of distilled water. It was boiled to dissolve completely, mixed well and distributed in to final containers. The medium was sterilised by autoclaving at 121°C for 15 minutes. After sterilization was completed, the medium was allowed to set in sloped form with a butt about 1 inch deep.

3.7.1.6 Lysine decarboxylase broth

Lysine decaboxylation broth (OXOID® Ltd., Basingstoke, U.K.) is composed of 3.0 g Yeast extract, 1.0 g Glucose, 5.0 L-lysine and 0.016 g Bromocresol purple. The medium was prepared according to manufacture's instructions by adding 1 tablet to 5 ml of distilled water in a ¼ oz screw-capped bottle. It was then sterilised by autoclaving at 121°C for 15 minutes.

3.7.1.7 Motility-indole medium (SIM)

Motility-indole medium (OXOID® Ltd., Basingstoke, U.K.) is made of 20.0 g Tryptone, 6.1 g Peptone, 0.2 g Ferrous ammonium sulphate, 0.2 g Sodium thiosulphate and 3.5 g Agar. The medium was prepared according to manufacture's instructions by suspend 30 g of the dehydrated medium in 1 litre of distilled water. It was then boiled to dissolve the

medium completely. The medium was dispensed into final containers and sterilised by autoclaving for 15 minutes at 121°C.

3.7.1.8 Urea Agar base

Urea agar (OXOID® Ltd., Basingstoke, U.K.) is made of 1.0 g Peptone, 1.0 g Glucose, 5.0 g Sodium chloride, 1.2 g Disodium phosphate, 0.8 g Potassium dihydrogen phosphate, 0.012 g Phenol red and 15.0 g Agar. The medium was prepared according to manufacture's instructions by suspending 2.4 g in 95 ml of distilled water and was boiled to dissolve completely. It was then sterilised by autoclaving at 115°C for 20 minutes. The medium was cooled to 50°C and aseptically introduced 5 ml of sterile 40% Urea Solution. It was mixed well, and then 10 ml amounts were distributed into sterile containers and allowed setting in the slope position.

3.7.1.9 Plate Count Agar (PCA)

Plate Count Agar (OXOID® Ltd., Basingstoke, U.K.) consists of 5.0 g Tryptone, 2.5 g Yeast extract, 1.0 g Glucose and 9.0 g Agar. The medium was prepared according to manufacture's instructions by adding 17.5 g of the powdered medium to 1 litre of distilled water. It was dissolved by bringing to the boil with frequent stirring, mixed and distributed into final containers. It was then sterilised by autoclaving at 121°C for 15 minutes. Finally it was cooled in a water bath to around 44 - 47°C prior to use.

3.7.1.10 Phosphate Buffered Saline

Phosphate Buffered Saline (PBS) (OXOID® Ltd., Basingstoke, U.K.) is made of Oxoid Dulbecco `A' Tablets. The tablets are composed of 8.0 g Sodium chloride, 0.2 g Potassium chloride, 1.15 g Disodium hydrogen phosphate and 0.2 g Potassium dihydrogen phosphate. The PBS solution was prepared according to manufacture's

instructions by dissolving 10 tablets (Dulbecco `A') in 1 litre of distilled water and autoclaved for 10 minutes at 115°C. The solution was then quite free from insoluble matter.

3.7.1.11 Lauryl Tryptose Broth

Lauryl Tryptose Broth (LSB) (OXOID® Ltd., Basingstoke, U.K.) is composed of 20.0 g Tryptose, 5.0 g Lactose, 5.0 g Sodium chloride, 2.75 g Dipotassium hydrogen phosphate, 2.75 g Potassium dihydrogen phosphate and 0.1 g Sodium lauryl sulphate. The medium was prepared according to manufacture's instructions by dissolving 35.6 g in 1 litre of distilled water and distributed into test tubes with Durham tubes. For double strength medium the weight of the medium was multiplied by two. The medium was sterilised by autoclaving at 121°C for 15 minutes. After sterilization, it was cooled to 20 °C on the lamina floor.

3.7.1.12 EC broth

EC Broth (OXOID® Ltd., Basingstoke, U.K.) consists of 20 g Tryptone, 5.0 g Lactose, 1.5 g Bile salts No. 3, 4.0 g Di-potassium phosphate, 1.5 g Mono-potassium phosphate and 5.0 g Sodium chloride. EC broth was prepared according to manufacture's instructions by dissolving 37g of powdered medium in 1 litre of distilled water. It was then dispensed into final containers and sterilized by autoclaving at 121°C for 15 minutes. Finally, it was cooled to 20 °C on the lamina floor before inoculation.

3.7.1.13 Tryptone water

Tryptone Water (OXOID® Ltd., Basingstoke, U.K.) is made of 10.0 g Tryptone and 5.0 g Sodium chloride. The medium was prepared by dissolving 15 g of the powder in 1 litre of

distilled water and distributed into final containers. It was then sterilised by autoclaving at 121°C for 15 minutes. Finally, it was cooled to 20 °C on the lamina floor.

3.7.2 Laboratory procedures

3.7.2.1 Total plate counts

Sample preparation and incubation

A total of 10 sterile test tubes were dispensed with 9 ml of sterilized Phosphate Buffered Saline (BPS). Ten- fold serial dilution from 10^{-1} to 10^{-10} in sterile Phosphate Buffered saline solution was done, using disposable pipettes. One (1 ml) of the juice sample was added into 9 ml of PBS (10^{-1} dilution). Then, 1 ml of the dilution was transferred into a second tube containing 9 ml of PBS (10^{-2} dilution); the procedure was repeated for further dilutions as shown in Fig. 2.

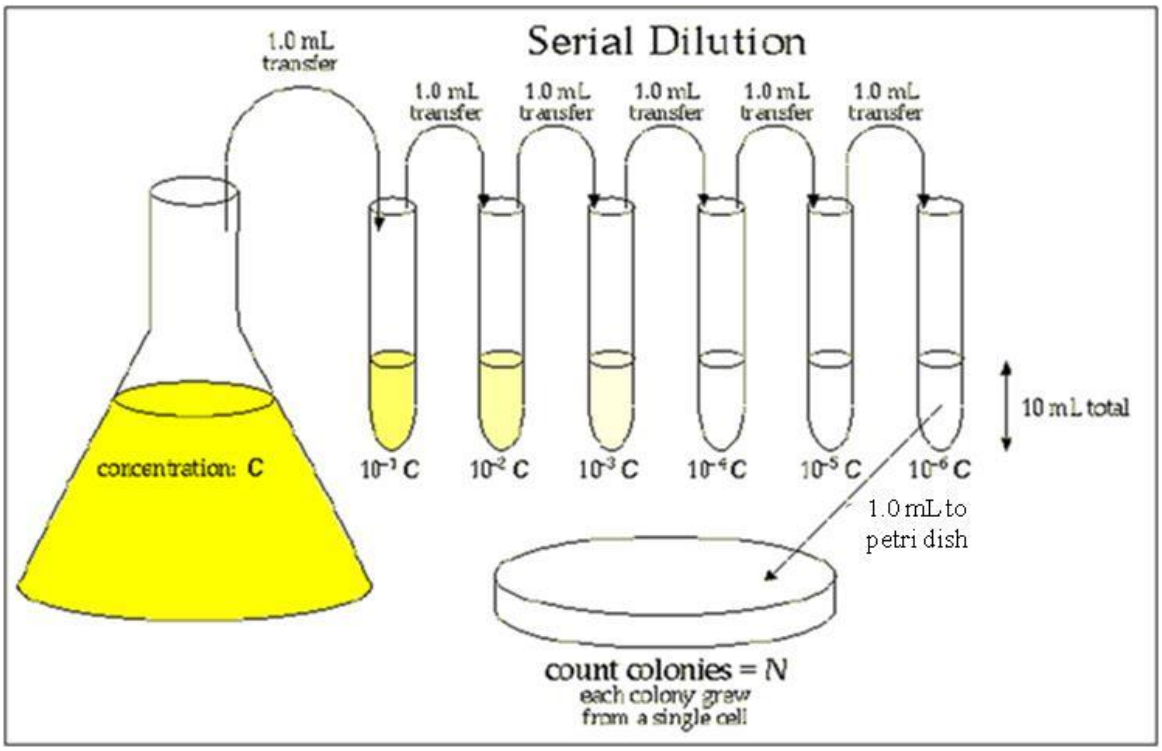

Figure 2: Serial dilutions of juice samples in tubes containing 9 ml of Phosphate Buffered saline

From each dilution, two sterile Petri dishes were each inoculated with 1 ml followed by the addition of 20-25 ml of Plate Count Agar at 44 - 47^{o}C onto the Petri dishes. The inoculums and the medium were carefully mixed by rotating the Petri dishes. It was then allowed to solidify by leaving the Petri dishes standing on the horizontal surface of the lamina floor. Parallel with the test samples, two controls were also involved and were prepared by pouring the same media in to two Petri-dishes at the same time and same analysis environment. One was used for checking the sterility of the medium (sterility check) while the other was used to check the quality of preparation environment (air settlement plate). After complete solidification, all the Petri dishes were inverted and placed in the incubator at 30^{o}C ± 1^{o}C for 72 ± 3 hours.

From the results it was learnt that, for mango and mixed fruit juices, critical dilutions of 10^{-3} to 10^{-7} were best for countable range of 15 - 300 colony forming units per plate (cfu/plate), while for passion and tamarind juices critical dilutions of 10^{-2} to 10^{-5} were the best.

Counting of colonies

After the incubation, the numbers of colony forming units were counted on at least two critical dilution plates by the aid of colony counter. Two consecutive plates with 15 to 300 colonies were considered for record (ISO 4833:2003(E)).

Expression of results

The countable colonies from two consecutive plates of each sample were converted into colony forming units per milligram (cfu/ml) using a formula $N=\sum C/v*1.1*d$, where N = the number of bacteria counted, C = sum of colony counted in two successful dilutions, v= volume of sample and d = dilution in the first plate counted (ISO 7218:2007(E)).

3.7.2.2 *Escherichia coli*

Initial suspension was prepared as recommended by ISO 6887-1:1999; by adding 10 ml of the juice into 90 ml of Buffered Pepton Water and mixed well. Inoculation of the initial suspension to the fermentation tubes proceeded immediately. Detection and enumeration of *E. coli* was done by using ISO 7251:2005(E) protocol through the following stages:

Stage 1: Inoculation and incubation of the selective medium (Laury sulfate broth)

A series of three tubes for each dilution was used. Using a sterile pipette, 10 ml of the initial suspension was transferred to each of the three tubes of double strength selective medium. Then, using a fresh sterile pipette, 1 ml of the initial suspension was dispensed into each of the first three tubes of the single-strength selective enrichment medium. Under the same conditions, another three tubes of the single strength medium were inoculated with 1 ml of decimal dilutions of the initial suspension. For each of the further dilutions the procedure was repeated as above by using a new sterile pipette for each dilution. The inoculums and the medium were carefully mixed. The inoculated tubes of the double-strength and single-strength selective enrichment medium were incubated at 37 ± 1°C for 24 ± 3 hours.

Stage 2: Subculture and incubation of the selective medium (EC broth)

For each tube of double-strength medium incubated in stage 1, showing opacity, cloudiness or any visible gas, and each tube of single-strength medium incubated according to stage 1 showing any visible gas were sub-cultured to a tube containing EC broth by using a sterile sampling loop. The inoculated tubes were incubated at 44 °C for 24 ± 2 hours.

Stage 3: Inoculation and incubation of the tryptone water

Each tube incubated in stage 2 and showing any visible gas, was inoculated in a tube of tryptone water, preheated to 44°C by using a sampling loop. It was then incubated at 44 °C for 48 ± 2 hours.

Stage 4: Examination for indole production

About 0.5 ml of indole reagent was added to the tubes of Tryptone water incubated according to stage 3. It was mixed well and was examined after 1 minute. A red ring in the alcoholic phase indicates the presence of indole Fig. 3.

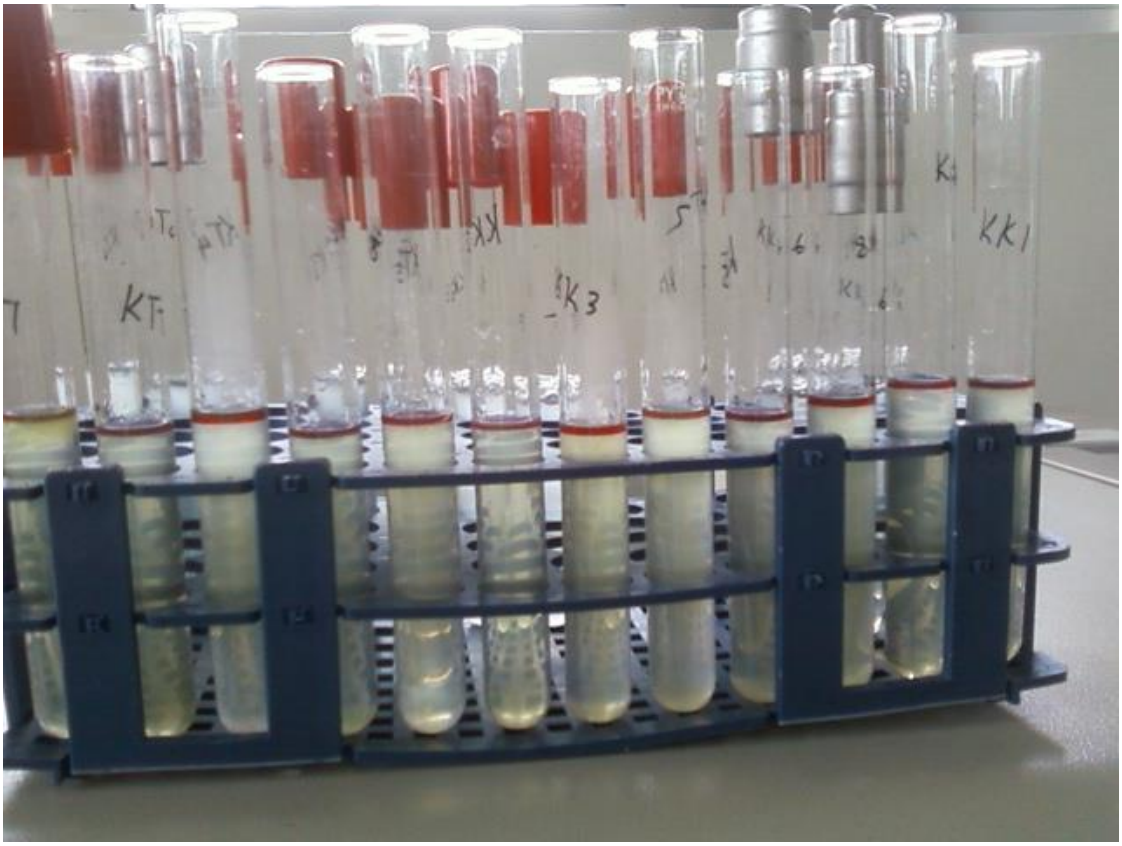

Figure 3: Red rings in the alcoholic phase showing indole positive reactions

Stage 5: Interpretation

Each tube of double-strength or single strength of selective enrichment medium incubated according to stage1 that had given rise to any visible gas in the tube of EC broth and to indole production in the tube of tryptone water, were considered positive. For each dilution, the number of positive result tubes of double-strength and single-strength medium were counted.

(i) Detection method

In accordance with the interpretation of results in stage 5, presence or absence of presumptive *Escherichia coli* per ml of the test sample was recorded.

(ii) Enumeration method

The MPN index of the number of presumptive *Escherichia coli* per ml of the sample was recorded from the MPN Table for three tubes method.

3.7.2.3 Determination of Salmonella spp

Determination of *Salmonella* spp. in juice samples was done by using ISO 6579:2002(E) protocol. It was done through the following stages:

Stage 1: Pre-enrichment in non-selective liquid medium

Pre-enrichment in Buffered Peptone Water (BPW) was done by adding 10 ml of the sample into 90 ml of sterilized BPW. Then it was incubated at 37 ± 1°C for 18 ± 2 hours.

Stage 2: Enrichment in selective liquid medium

About 0.1 ml of the culture obtained in stage 1 was inoculated in 10 ml of Rappaport-Vassiliadis medium with soya (RVS) broth (OXOID® Ltd., Basingstoke, U.K.). About 1

ml of the same culture was inoculated into 10 ml of Muller-Kaufffmann tetrathionate/novobiocin (MKTTn) broth (OXOID® Ltd., Basingstoke, U.K.). The RVS broth was incubated at 41.5°C for 24 ± 3 hours and the MKTTn broth at 37 ± 1°C for 24 ± 3 hours.

Stage 3: Plating out and identification

The cultures obtained in stage 2 were streaked onto Xylose lysine deoxycholate (XLD) agar (OXOID® Ltd., Basingstoke, U.K.) where the surface of the agar plate was divided in to two halves; one half was streaked by the culture from the RVS broth and the other half was streaked by the culture from MKTTn broth. The dishes were inverted so that the bottom is uppermost and were incubated at 37 ± 1°C for 24 ± 3 hours. After incubation, the cultures were examined for typical colonies of *Salmonella* and atypical colonies that may be *Salmonella*. Typical colonies of *salmonella* grown on XLD agar have a black center and a light transparent zone of reddish colour due to change of the phenol red indicator.

Stage 4: Confirmation of *Salmonella*

Suspected *Salmonella* colonies were picked by means of sterile inoculating loop and streaked onto the surface of nutrient agar (OXOID® Ltd., Basingstoke, U.K.) plates to allow well development of isolated colonies. The inoculated plates were incubated at 37 ± 1°C for 24 ± 3 hour. The resulting pure culture was used in the biochemical tests.

3.7.2.4 Biochemical tests for Salmonella

i. Triple Sugar Iron (TSI)

By means of an inoculating loop, each of the pure culture was inoculated to the agar slant surface and stubbed the butt of the agar. The inoculated medium was incubated at 37°C for 24 hours and after the incubation the changes in the medium were interpreted as follows:

Butt

- Yellow = glucose positive (Glucose fermented)
- Red or unchanged = Glucose negative (glucose not fermented)
- Black = Production of hydrogen sulfide
- Bubbles or cracks = gas formation from glucose

Slant surface

- Yellow = lactose and/sucrose positive (lactose and/or sucrose fermented)
- Red or unchanged = lactose and sucrose negative (neither lactose nor sucrose was fermented)

Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and in 90% of the cases produce hydrogen sulfide detected by blackening of the agar. When lactose-positive *Salmonell*a is isolated, the TSI slant is yellow.

ii. Urea agar

By means of an inoculating loop, each of the pure culture was inoculated on the agar slant surface. The inoculated agar was incubated at 37 ± 1°C for 24 ± 3 hours and examined at interval. If the reaction is positive, splitting of urea liberates ammonia, which changes the

color of phenol red to rose-pink and later to deep cerise. The reaction is often apparent after 2 to 4 hours.

iii. L-Lysine decarboxylation medium

A portion of pure colony was inoculated just below the surface of the liquid medium and was incubated at 37 ± 1°C for 24 h ± 3 h. Turbidity and a purple color after incubation indicates a positive reaction. A yellow colour indicates negative reaction.

iv. Medium for indole reaction

A tube containing 5 ml of the tryptone medium was inoculated with the *salmonella* suspected colonies and was incubated at 37 ± 1°C for 24 ± 3 hours. After incubation, 1 ml of Kovacs reagent was added. The formation of a red ring indicates a positive reaction. A yellow-brown ring indicates negative reaction.

3.8 Data Analysis

The data was analyzed by using EPI INFO version 7 statistical packages (Coulombier *et al.,* 2001) The Chi-squire and confidence intervals was used to compare proportions at probability $P > 0.05$. Descriptive statistics was used to compute means, standard deviations, median and range. Bacterial counts were normalized by log transformation. Analysis of variance (ANOVA) was adopted to compare differences in means of continuous variables.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic Characteristics of the Respondents

The demographic characteristics of the study juice vendors (respondents) are presented in Table 2. The study involved 30 respondents from each of the three municipalities making a total of 90 juice vendors. The results indicated a significant difference ($P < 0.05$) between the number of male 60% (n=54) and female respondents. The overall age profile showed that, those with age ranging from 25 – 34 years constituted the largest proportion of vendors (30%; n = 27) and respondents with the age above 55 years were few (3.3%; n = 3). The age differences across the municipalities were statistically insignificant ($P > 0.05$). Majority of the juice vendors had primary school education (56.7%; n=51) while those with college education formed the lowest group (3.3%; n=3). Some vendors had been in fruit juice vending business for long period of time ranging from one to more than 10 years. However, the overall pattern showed that, majority (30%; n=27) were transient vendors who vended fruit juices during the peak fruits seasons (Table 2).

Table 2: Socio-demographic characteristics of the respondents

Respondent Parameter	Category	Number (%) of respondents			Total n (%)	P- value
		Ilala	Kinondoni	Temeke		
Gender	Female	5 (16.7)	16 (53.3)	15 (50.0)	36 (40)	0.0059
	Male	25 (83.3)	14 (46.6)	15 (50.0)	54 (60)	
Age range	15 - 24	12 (40.0)	8 (26.7)	1 (3.3)	21 (23.3)	0.0646
	25 - 34	7 (23.3)	11 (36.7)	9 (30.0)	27 (30.0)	
	35 - 44	5 (16.7)	6 (20.0)	9 (30.0)	20 (22.2)	
	45 – 54	5 (16.7)	5 (16.7)	9 (30.0)	19 (21.1)	
	≥ 55	1 (3.3)	0 (0.0)	2 (6.7)	3 (3.3)	
Education level	Non formal	3 (10.0)	2 (6.7)	3 (10.0)	8 (8.9)	
	Primary	19 (63.3)	16 (53.3)	16 (53.3)	51 (56.7)	0.8227
	Secondary	8 (26.7)	10 (33.3)	10 (33.3)	28 (31.1)	
	College	0 (0.0)	2 (6.7)	1 (3.3)	3 (3.3)	
Vending duration	< 1 year	11 (36.7)	7 (23.3)	9 (30.0)	27 (30.0)	
	1-2 years	5 (16.7)	7 (23.3)	10 (33.3)	22 (24.4)	0.2561
	3-5 years	5 (16.7)	10 (33.3)	3 (10.0)	18 (20.0)	
	6-9 years	3 (10.0)	4 (13.3)	4 (13.3)	11 (12.2)	
	≥ 10 years	6 (20.0)	2 (6.7)	4 (13.3)	12 (13.3)	

4.2 Sources of Raw Materials and Methods of Preparation of Juices

The sources of raw materials and juice preparation methods are presented in Table 3. Fruits for juice extraction were mainly sourced from local markets namely; Sterio, Buguruni, Kariakoo, Tandale and other small markets within each locality. Majority of vendors from Ilala (76.7%; n=23) reported to obtain fruits from Buguruni market, while the majority in Temeke (86.7%; n=26) reported to obtain fruits from Sterio market.

The major sources of water for washing and for dilution of the juices were deep wells and tap water. All juice vendors in Temeke obtained water from deep wells, while most vendors in Kinondoni (93.3%; n=28) obtained water from tap where as vendors from Ilala obtained water from either deep wells or tap.

The juices were extracted by three methods mainly blending by use of blenders, boiling in water and squeezing by simple manual machines. Most vendors (80%; n=72) reported to use a simple blender in extraction of juices of mango, passion and most mixed fruit (mango, passion and avocado). However, tamarind juices were extracted through boiling the fruits in water while the mixed fruit juices that involved lemon and sugar cane were extracted by manual squeezing machine.

4.2.1 Juice pasteurization

As regard to juice pasteurization, none of the juice vendors declared to pasteurize the juice after preparation.

Table 3: Sources of raw materials and methods of juices preparation

Respondent parameter	Category	Number (%) of respondents			Total n (%)
		Ilala	Kinondoni	Temeke	
Source of fruits	Sterio	1 (3.3)	1 (3.3)	26 (86.7)	28 (31.1)
	Buguruni	23 (76.7)	9 (30.0)	0 (0.0)	32 (35.6)
	Kariakoo	1 (3.3)	5 (16.7)	1 (3.3)	7 (7.8)
	Tandale	3 (10.0)	1 (3.3)	0 (0.0)	4 (4.4)
	Others	2 (6.7)	14 (46.7)	3 (10.0)	19 (21.1)
Sources of water	Deep wells	16 (53.3)	2 (6.7)	30 (100.0)	48 (53.3)
	Municipal supply	14 (46.7)	28 (93.3)	0 (0.0)	42 (46.7)
Water treatment	Treated	11 (36.7)	23 (76.7)	16 (53.3)	50 (55.6)
	Not treated	19 (63.3)	7 (23.3)	14 (46.7)	40 (44.4)
Extraction methods	Blending	21 (70.0)	26 (86.7)	24 (80.0)	71 (78.9)
	Boiling	6 (20.0)	4 (13.3)	4 (13.3)	14 (15.6)
	Squeezing	3 (10.0)	0 (0.0)	2 (6.7)	5 (5.6)

4.2.2 Juice handling practices

Juice handling practices as regards to vending, storage containers, cold storage, selling utensils and cleaning methods are presented in Table 4. Common juice storage containers included buckets, used bottles and cool boxes. Majority of the vendors (78.9%; n=71) stored the juices in buckets.

The juices were either cooled in deep freezers or by use of ice cubes which were reported to be obtained from ice manufacturing factories. Most vendors (67.8%; n=61) used deep freezers to keep the juices while 17.8% (n=16) and14.4% (n=13) kept the juice in buckets with ice cubes and cool boxes with ice cubes respectively. The cool boxes were either directly filled with the juice or the juices were packed in plastic bottles and stored in them. In either case, ice cubes were added in the cool boxes to aid cooling.

As regard to juice selling equipment, commonly used utensils were glass cups, reused plastic bottles and disposable cups. Majority of vendors (67.8%; n=61) reported to serve the juices with glass cups, (15.6%; n=14) reused plastic bottle and (4.4%; n=4) disposable cups (Table 4).

Table 4: Juice handling practices

Parameter	Variable	Number (%)
Juice storage containers	Buckets	71 (78.9)
	Used bottle	14 (15.6)
	Ice box	5 (5.6)
Cold storage	Deep freezer	61 (67.8)
	Bucket with ice	16 (17.8)
	Ice box	13 (14.4)
Sell equipment	Glass cups	61 (67.8)
	Used plastic bottles	14 (15.6)
	Glass cups & used bottles	8 (8.9)
	Disposable cups	4 (4.4)
	Glass cups & disposable cups	3 (3.3)
Vending site	Restaurant	36 (40.0)
	Food kiosk	10 (11.1)
	Roadside	17 (18.7)
	Bus stand	19 (11.1)
	Market	7 (7.8)
Means of cleaning serving utensils	Cold water and soap	33 (36.7)
	Hot water and soap	25 (27.8)
	Cold water alone	24 (26.7)
	Hot water alone	4 (4.4)
	No washing	4 (4.4)

4.2.3 Observed practices of vendors towards preparation and vending of juices

Results of observational checklists are presented in Table 5. Most preparation settings did not meet basic standards for a food preparation premises. Majority of premises (78.9%; n=71) showed to encourage or introduce contamination to the juices.

Washing of utensils were poorly done, using cold water, without detergents or reusing of water which had been used several times. About 76.7 % (n=69) of the juice vendors were observed to predispose the juice and utensils to contamination through poor methods of washing.

With regard to waste disposal, about 22.2% (n=20) did not have waste receiving bins, hence haphazard dumping of wastes. During the survey, piles of dirty were seen in the juice preparation and vending areas. On the other hand, 48.9% (n=44) of the vendors used uncovered waste bins which were observed to encourage pests like flies and cockroaches in the premises. Indeed, most premises (83.3%; n=75) were observed with pests mainly house flies 96% (n=72) and 4% (n=3) with both house flies and cockroaches.

Table 5: Recorded observations on practices of the juice vendors

Parameter assessed	Category	Number (%) of juice vendors
Preparation setting minimize contamination	No	71 (78.9)
	Yes	19 (21.1)
Washing minimize contamination	Poorly	69 (76.7)
	Fairly	21 (23.3)
Vendor cleanliness	Poor	41 (45.6)
	Fair	49 (54.4)
Vendor had uniform	No	79 (87.8)
	Yes	11 (12.2)
Waste receiving bins present	No	20 (22.2)
	Yes (uncovered)	44 (48.9)
	Yes (covered)	26 (28.9)
Pests present in the preparation/ vending sites	No	15 (16.7)
	Yes	75 (83.3)
Type of pests	Flies	72 (96.0)
	Cockroaches & flies	3 (4.0)
Juice protected from sources of contamination	No	68 (75.6)
	Yes	22 (24.4)
Cold storage facility effective	No	44 (48.9)
	Yes	46 (51.1)

4.3 Physicochemical Characteristics of the Juice

4.3.1 Juice pH and acidity

The physicochemical characteristics of the juices are provided in Table 6. The pH value of the juices ranged from 2.7 to 6.4 depending on the type of juices. Mixed fruit and mango juices had higher pH values than Passion and Tamarind juices. Tamarind juices had the lowest pH. The differences in their pH values were consistent with high titratable acidities.

Table 6: Physicochemical characteristics of the juices

Type of juice	Samples (n)	Physicochemical Parameter	Mean ± S.D	Median	Range	Recommended values
Mixed fruit	45	pH	4.0 ± 0.7	4.0	3.1 – 6.4	-*
		°Brix	8.5 ± 3.6	8.6	-1.5 – 14.5	-*
		Acidity	0.2± 0.2	0.2	0.01 – 0.9	-*
		Brix-acid ratio	89.9 ± 137.9	41.1	-3.9 – 780.0	-*
Mango	23	pH	4.0 ± 0.4	4.0	3.3 – 4.9	3.5 – 4.0
		°Brix	8.1 ± 4.1	9.2	1.3 – 15.1	13.5
		Acidity	0.2 ± 0.1	0.2	0.1 – 0.4	0.25 – 0.5
		Brix-acid ratio	55.8 ± 42.4	39.4	11.8 – 139.6	30.0 – 50.0
Passion	8	pH	3.2 ± 0.3	3.1	2.9 – 3.9	2.5 – 3.0
		°Brix	8.1 ± 4.1	10.2	4.4 – 14.1	12.0
		Acidity	0.4 ± 0.1	0.4	0.01 – 0.9	0.8 – 1.5
		Brix-acid ratio	29.1 ± 15.0	23.7	10.8 – 60.5	8.0 – 15.0
Tamarind	14	pH	2.8 ± 0.1	2.9	2.7 – 3.4	2.4 – 3.0
		°Brix	8.0 ± 6.1	6.8	0.7 – 18.0	13.0
		Acidity	0.4 ± 0.3	0.3	0.2 – 1.3	0.5
		Brix-acid ratio	19.6 ± 17.2	10.8	3.5 – 65.2	27.0

Source for normal values: (Bates *et al*., 2001; FAO, 2005), -* = recommended values not found

The acidity of the juices had a range from 0.01 to 1.3. Both passion and tamarind juices had high acidities as compared to mixed fruit and mango juices ($P < 0.001$). The tamarind juices were more acidic than passion juices (Fig. 4). According to FAO, the juices

containing more than ~1.2% acid are sour, independent of °Brix/Acid (Bates *et al*., 2001). About 98.9% (n=89) of the juice were within the recommended range (≤1.2% acid).

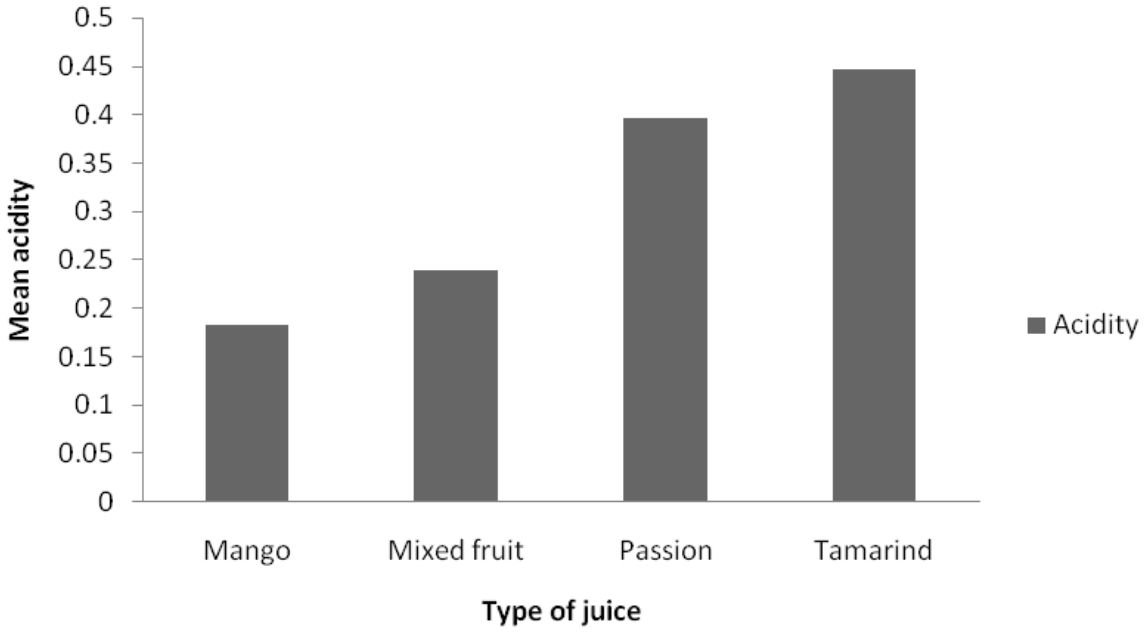

Figure 4: Shows relationship between types of juices and titratable acidity

4.3.2 Total soluble solids

The total soluble solids (°Brix) of the juices ranged from -1.5 to 18.04 °Brix. Majority of the juices had brix levels below the recommended standards (CODEX STAN 247:2005) for fruit juices and nectars (FAO, 2005) (Table 6 and Fig. 5). Neither type of juices nor location showed any significant difference in the values of °Brix ($P > 0.05$). However, juice blends or beverages with less than 7 °Brix are categorized as weak and watery (Bates *et al.*, 2001). Of all the samples assessed, 67.8% (n=90) were classified as weak and watery.

Figure 5: Percentage of juices with satisfactory or unsatisfactory values of ^{0}Brix

4.3.3 Microbial status of juices vended in Dar es Salaam

4.3.3.1 Total Plate Counts

The status of Total Plate Counts of the juices is summarized in Table 7. Results showed that the TPC ranged from 2.32 to 8.54 (Log cfu/ml). The means for mango and mixed fruit juices were relatively higher ($P < 0.001$) than those for passion and tamarind juices (Table 7 and 9). According to Codex standard (CX/NEA 03/16: 2002) the total viable count of any fruit juices should not exceed 5 x10^{3} - 10^{4} cfu/ml (FAO, 2002). Referring this standard, 72.2% (n=65) of the juice samples analyzed for TPC had bacteria counts beyond the recommendations (Table 7 and 9).

Table 7: Total Plate Counts (Log cfu/ml) of locally prepared fruit juices in Dar es Salaam city

Type of juice	n	Mean	Std. Dev.	Median	Minimum	Maximum
Mixed fruit	45	5.79	1.05	5.51	3.45	8.52
Mango	23	5.59	1.21	5.40	3.36	8.54
Passion	8	4.10	1.11	4.29	2.32	5.28
Tamarind	14	3.93	0.85	3.35	3.17	5.48

n = number of samples, Std. Dev. = Standard deviation

4.3.3.2 *E. coli* contamination

The status of *E. coli* contamination of the juices is presented in Table 8. The MPN counts for *E. coli* ranged from -1.13 to 4.97 (Log MPN/ml). Mango and mixed fruit juices were significantly more contaminated than passion and tamarind juices ($P < 0.001$) (Fig. 6). According to Tanzania Standard (TZS 585:2003), *E. coli* must not be present in ready to drink beverages (TBS, 2003). Majority of the juices 80% (n=72) were positive for *E. coli.*

Table 8: *E. coli* counts (Log MPN/ml) of locally prepared fruit juices in Dar es Salaam city

Type of juice	n	Mean	Std. Dev.	Median	Minimum	Maximum
Mixed fruit	45	2.30	1.78	2.07	-0.37	4.97
Mango	23	3.01	1.51	3.04	-0.03	4.97
Passion	8	0.99	0.77	0.90	0.18	1.97
Tamarind	14	-0.13	-*	-1.13	-1.13	-1.13

N = Number of samples, Std. Dev. = Standard deviation, -* = No value because only one sample in tamarind was positive

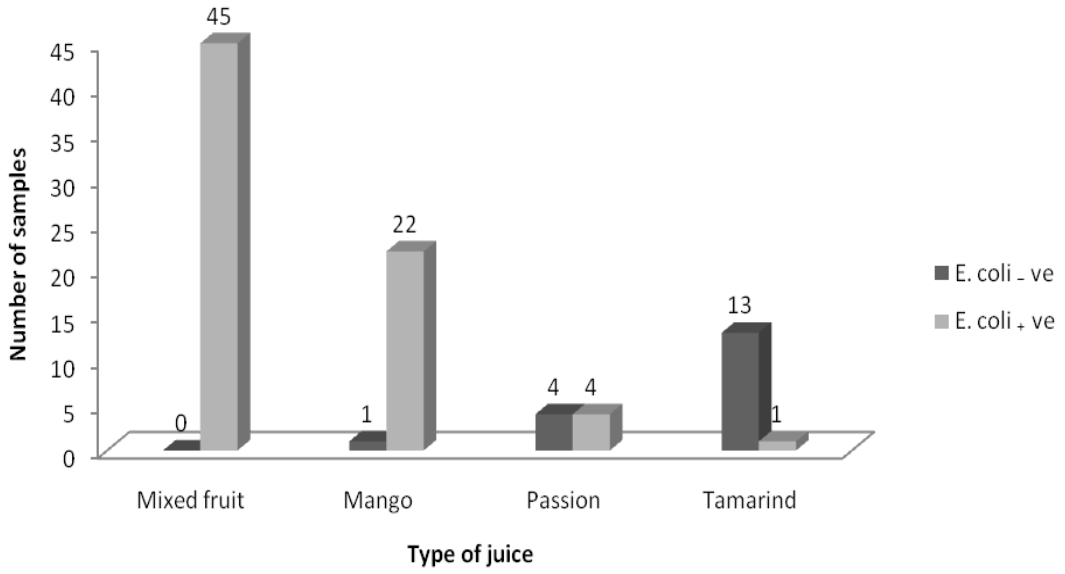

Figure 6: *E. coli* contamination in relation to type of juices

4.3.3.3 *Salmonella* spp.

A total of 90 juice samples were analyzed for *Salmonella* species and were all negative.

4.4 Risk Factors for Microbial Contamination of Juices

The status and rates of microbial contamination in relation to their risk factors are presented in Table 9. Juice type, juice pH, methods of extraction, vending sites, sex of the vendor and storage containers were considered to be risk factors for microbial contamination of the juices.

4.4.1 Type of juices

The type of juice showed to be a significant factor ($P < 001$) for contamination of the juices in both cases; TPC and *E. coli*. The magnitude of unsatisfactory levels of TPC in mango (82.6% n=19) and mixed fruit juices (88.9% n=39) were significantly higher compared to passion and tamarind juices (Table 9). Likewise, the prevalence of *E. coli* in the juices was significantly higher ($P < 0.001$) in mixed fruit and mango juices as compared to passion and tamarind.

Table 9: Risk factors for microbial contamination of the juices

Risk Factors assessed	Category	Number	Total Plate Counts			*Escherichia coli*		
			Log (TPC cfu/ml) (Means ± SD)	Unsatisfactory Number (%)	P- value	Log(MPN/ml) (Means ± SD)	Prevalence Number (%)	P-value
Juice	Mixed fruit	45	5.6 ± 1.3	39(86.7)	0.0000	2.3 ± 1.8	45 (100.0)	0.0000
	Mango	23	5.5 ± 1.3	19(82.6)		3.01 ± 1.5	22 (95.7)	
	Passion	8	4.1 ± 1.1	5(62.5)		1.0 ± 0.8	4 (50.0)	
	Tamarind	14	3.9 ± 0.9	2(14.3)		-1.13	1 (7.1)	
pH	≤ 3.1	20	4.0 ± 1.0	5(25.0)	0.0000	0.2 ± 1.3	4 (20)	0.0000
	> 3.1	70	5.7 ± 1.1	60(85.7)		2.5 ± 1.7	68 (97.1)	
Location	Ilala	30	5.1 ± 1.6	19(63.3)	0.19205	2.7 ± 1.8	21 (70.0)	0.25068
	Kinondoni	30	5.4 ± 1.0.	23(76.7)		2.02 ± 1.8	26 (86.7)	
	Temeke	30	5.6 ± 1.2	23(76.7)		2.6 ± 1.7	25 (83.3)	
Extraction Methods	Blending	71	5.6 ± 1.2	58(81.7)	0.0000	2.5 ± 1.7	66 (93.0)	0.0000
	Squeezing	5	5.9 ± 1.0	5(100.0)		1.9 ± 1.8	5 (100.0)	
	Boiling	14	3.9 ± 0.9	2(14.3)		-1.13	1 (7.1)	
Vending site	Restaurant	37	5.3 ± 0.9	26(70.3)	0.01343	2.5 ± 1.8	30 (81.1)	0.03950
	Bus stand	19	5.2 ± 1.5	13(68.4)		2.2 ± 2.04	15 (78.9)	
	Roadside	17	5.5 ± 1.1	14(82.4)		1.8 ± 1.1	14 (82.4)	
	Food shop	10	4.5 ± 1.4	5(50.0)		3.0 ± 1.8	6 (60.0)	
	Market	7	7.2 ± 1.2	7(100.0)		3.1 ± 1.9	7 (100.0)	
Sex	Females	36	5.9 ± 1.3	29(80.6)	0.15692	2.6 ± 1.6	34 (94.4)	0.00560
	Males	54	5.0 ± 1.1	36(66.7)		2.2 ± 1.8	38 (70.4)	
Storage containers	Buckets	71	5.1 ± 1.1	48(67.6)	0.04961	2.3 ± 1.8	55 (77.5)	0.18748
	Ice box	5	7.5 ± 1.0	5(100.0)		3.5 ± 1.6	5 (100.0)	
	Used bottles	14	5.8 ± 1.3	12(85.7)		2.4 ± 1.4	12 (85.7)	

4.4.2 Juice pH

Assessment of juice pH showed a strong statistical significance ($P < 0.001$) in the contamination of the juices with both TPC and *E. coli*. Juices with higher pH (> 3.1) were highly contaminated with *E. coli* (97.1% n=68) and high TPC (85.7% n=60). On the other hand, juices with low pH (≤ 3.1) were less contaminated with both categories (Table 9).

4.4.3 Methods of extraction of juices

Methods of extraction were found to be a strong risk factor for the contamination of juices with both *E. coli* and TPC ($P < 0.001$). Juice extracted trough squeezing by a simple manual machine were all contaminated with *E. coli* and had high TPC levels. Blending method by use of simple blender equipment also showed to contribute to high contamination of the juices as 93% (n=66) were positive for *E. coli* while 81.7% (n=58) had unsatisfactory values of TPC. On the other hand, those juices which were extracted through boiling the fruits in water (mainly tamarind) showed the lowest contamination in both categories.

4.4.4 Vending sites

Vending sites as risk factors for microbial contamination was assessed. Significant differences were observed for both TPC and *E. coli*. Juices vended in markets were all contaminated as both had high TPC and *E. coli* proportions. This was followed by juices vended at roadsides, then restaurants, bus stands and finally food shops. Juices vended in food shops were least contaminated in this category as up to 50% (n=5) and 60% (n=6) had high TPC and *E. coli* respectively (Table 9).

4.4.5 Sex

Considering sex as a risk factor for microbial contamination, juices from female vendors had significantly higher *E. coli* contamination rate ($P < 0.05$) (94.4%; n=34) as compared to male vendors (70.4%; n=38). Differently, juice vended by female vendors had higher TPC (mean count 5.87 Log cfu/ml) as compared to juice from male vendors (mean count 5.00 Log cfu/ml) but the difference was not statistically significant ($P > 0.05$) (Table 9).

4.4.6 Juice storage containers

Assessment of juice storage containers as risk factors for high TPC showed significant differences ($P < 0.05$). The juices stored directly in ice boxes were all found with unsatisfactory levels of TPC, followed by those stored in used plastic bottles (85.7%; n=14) (Table 9). However, with regard to *E. coli* counts, the differences in level based on different storage containers did not show any statistical significance ($P > 0.05$).

4.4.7 Location

Comparison of municipalities did not show any significant trend in terms of contamination proportions ($P > 0.05$) (Table 9). The rate of unsatisfactory TPC levels were 63.3% (n=19) for Ilala and 76.7 (n=23) for both Kinondoni and Temeke. The prevalence of *E. coli* were 70% (n=21), 86.7% (n=26) and 83.3% (n=25) for Ilala, Kinondoni and Temeke respectively.

CHAPTER FIVE

5.0 DISCUSSION

The current study was conducted to determine bacterial quality and handling practices that predispose unpasteurized fruit juices to contamination in Dar es Salaam city. The general finding showed that, the juices were prepared and served in unhygienic environments. This was supported by the laboratory results which showed significantly high total plate counts (TPC) and *E. coli* in ready to drink juices. With such kind of findings, it suggests that, low and middle income people who are the main customers of these locally vended juices are at risk of acquiring food-borne diseases.

5.1 Fruits Handling, Preparation and Vending of the Juices

In the current study, fruits used in the preparation of the juices were all obtained from open markets. The fruits were delivered in these markets by trucks from regions famous for fruit farming such as Morogoro and Tanga. In the chain of production through middlemen to the markets and to the final consumers, there are risks of contamination of the fruits in each stage. The safety of the juices prepared from these fruits depended greatly on the strict hygiene measures that were applied by the juices vendors on practices towards the fruits handling, preparation, storage and the hygiene of the vendors and premises. In the current study, the general practices of the vendors towards fruit handling, juice preparation and display for sell were observed to be poor. The general hygiene of the vendors and premises were also poor and were observed to encourage contamination of the juices in the aspects of washing of fruits and cleaning of utensils, preparation methods, storage of the juices and the general hygiene.

This is contrary to Codex general requirements for food hygiene which recommend that, a place for food preparation should be kept clean at all times and should be far from any source of contamination (rubbish, waste waster, dust and animals) (FAO, 1995). Such similar observations were also recorded elsewhere by Muinde and Kuria (2005) and Chukwu *et al.* (2010). Such situation is likely to be contributed by limited education on hygiene and inadequate food and premises inspections by health and food inspectors who would otherwise stop such food mishandling practices. Further more, a significant proportion of vendors stored the juice in un-recommended temperatures i.e. cooling by use of ice cubes which could not provide adequate and long lasting cooling hence providing good conditions for microbial growth and proliferation. Also the safety of the ice cubes used to cool the juices is questionable. The vendors reported to obtain the ice cubes from ice manufacturing factories. In case of mishandling of the ice cubes or the water that was used to manufacture the ice cubes was contaminated; then it could also result in contamination of the juices. On the other hand, the utensils were washed using the same water several time before it was replaced. Such dirty water probably further added contamination to the utensils and consequently to the juice. The findings are in agreement with the findings of Cardinale *et al.* (2005) in Senegal and Tambekar *et al.* (2009) in India where in both studies unhygienic practices were reported.

5.2 Physicochemical Characteristics of the Juice

The study explored the pH, total soluble solids (°Brix) and acidity so as to assess the quality of the juice in terms of the maturity status of the fruits used, organoleptic values and microbial status. The pH of the juices in this study ranged from 2.68 to 6.38 slightly lower than that reported by Poonam (2013). Tamarind and passion juices had lower pH as compared to mixed fruit and mango juices. Passion and tamarind had lower pH values because they are rich in organic acids (Tasnim *et al.,* 2010). On the other hand, most

mixed fruit juices were made of non citrus fruits such as avocado, sugar cane, mangoes and/or small portion of citrus fruit, hence had low acids and higher pH values. Mangoes at fully maturity and ripening have significantly more ºBrix than acids hence they are generally sweet and that accounted for higher pH.

In the current study, lower pH of the tamarind and passion fruit showed to inhibit microbial contamination of the juices significantly ($P < 0.001$) while on the other hand, higher pH of mixed fruit and mango juices showed to support growth of microbes as majority of the juices were contaminated with *E. coli* and had high TPC counts. Low pH of the fruit and juices has inhibitory effects to microbial growth. Zahra (2010) studied antagonism activity of lemon and lime juices against some bacteria. The results indicated that, low pH to both juices had inhibitory effects against bacteria. Castillo *et al.* (2000) and Rodrigues *et al.* (2000) reported the antimicrobial activity of freshly squeezed lemon juice against *V. cholerae* which was associated with low pH. The effects of pH against microbial contamination have also been reported by Titarmare *et al*. (2009), Mesfin, (2011) and Sunday *et al.* (2011).

The acidity values of the juices ranged from 0.01 to 1.3%. According to FAO, juices with more than ~1.2% acid are sour, independent of °Brix/Acid (Bates *et al.,* 2001). Fortunately, 99% (n=89) of the juices were within recommendations. This may indicate that the fruits used in the preparation of the juices which were analysed during this study were at the state of fully maturity and ripening. The acidity values reported in this study were in agreement with those reported by Wissanee and Renu (2007), Oranusi *et al.* (2012); and Rizzon and Miele (2012).

The total soluble solids of the juices were found to have a wide range of variation from -1.54 to 18.04 ºBrix. The total soluble solids of juices originate from the fruit, and may also be added during processing to meet the consumers preference. However, according to FAO, juice blends or beverages with less than 7ºBrix are deemed weak and watery (Bates *et al.*, 2001). About 67.8% of the juices in the current study were found to be weak and watery. Low values of ºBrix observed in this study may be due to over dilution of the juices. On the other hand, the decreased ºBrix levels could be attributed by the onset of fermentation due to improper storage temperatures of some of the juices. Similar findings were reported by Wissanee and Renu (2007) who also found that, the acidity of orange juice kept at 4°C, began to increase in 3 - 6 days and indicated fermentation of the juice. On the other hand, the levels of ºBrix did not seem to affect the microbial quality of the juices ($P > 0.05$) although Pilo *et al.* (2009) reported increase of microbial counts as the value of ºBrix increased.

5.3 Microbial Quality of Juice

Microbial quality of food is a very important measure of the safety of the food for human consumption. Presence of certain microbes such as *Salmonella, E. coli* O157:H7 and O111, S*higella* and *C. botulinum* indicate that the food is hazardous and should not be used for human consumption. On the other hand, presence of non pathogenic microbes in food does not necessitate unfitness for consumption, but may indicate the hygiene status of the preparation and processing. However, certain levels may indicate serious case of poor hygiene and the food becomes unfit for consumption. Generally, the quality of juice in the current study findings showed to be poor. The overall assessment of the juices indicated high total plate counts ranging from 2.32 to 8.54 (log cfu/ml). This range is

comparable to the findings reported by Shakir *et al.* (2009); it is however, lower than those reported by Kumar *et al*. (2006), Mahale *et al.* (2008) and Chukwu *et al.* (2010).

It was further observed that the highest bacterial contamination was in mixed fruit and mango. The mean TPC for mixed fruit (5.79 log cfu/ml) and mango (5.59 log cfu/ml) juices were significantly higher than the means for passion and tamarind juices which were 4.10 (log cfu/ml) and 3.93 (log cfu/ml) respectively. The high contamination of mixed fruit and mango juices could partly be linked to their high pH values which were favourable for microbial growth. Poonam, (2013) found that high pH coupled with high ambient temperatures (> 28°C) appeared to favour the bacterial growth and reduce the shelf life of juice. Similar findings were also reported by Yigeremu *et al.* (2001) and Mesfin (2011). The quality of fresh juice therefore is essentially depending on careful fruit handling and strict processing sanitation. Steven and Davis, (2001) studied transfer rates of microorganisms during juice extraction process and found that, about 1.7 - 2.6% of total aerobic organisms and 2.3 - 2.6% of aciduric organisms from the washed fruits were introduced into the fresh juice. Based on the Codex standard (CX/NEA 03/16:2002) the allowable TPC levels for fruit juices and drinks should be within $5x10^3$ to 10^4 cfu/ml (FAO, 2002). Majority of the juices (72.2%) in the current study had TPC levels above the recommended maximum levels indicating that the juices were of poor quality and therefore could predispose the consumer to food-borne diseases.

5.4 *E. coli* Contamination in the Juices

In the current study, *E. coli* contamination of the juices was generally high with a prevalence of 80%. This prevalence is however lower than that reported by Mahale *et al.* (2008) in Mumbai India where almost all the samples tested were positive for *E. coli* and Shakir *et al*. (2009) in Dhaka Bangladesh who reported that 99% of the fruit juices

studied was contaminated with *E. coli*. In the current study, the highest contamination was observed in mixed fruit and mango juices. Mixed fruit and mango juices were more contaminated probably because of the higher pH which showed to support bacterial growth. Similar findings were reported by Subbannayya *et al.* (2007) and Tambekar *et al.* (2009) where *E. coli* was found to be the predominant bacteria in the street vended raw juices. However, the results of the current study were in disagreement with a study conducted in Hawassa town, Ethiopia which showed that only 5.8% of the unpasteurized fruit juice was contaminated with *E. coli* (Mesfin, 2011).

The presence of *E. coli* in the juice samples indicates evidence of faecal contamination or poor hygiene. The main source of *E. coli* contamination might be through contaminated water supplies or the utensil washed by contaminated water or water that was used for dilution of juices. Also the presence of *E. coli* could be due to inadequate hand washing by food vendors and the absence of good manufacturing practices (Tambekar *et al.*, 2009). Based on observation, majority of vendors in the current study (76.7%) prepared and sold juices in unhygienic conditions. A significant proportion of vendors did not have waste bins and quite a big proportion had open waste bins. This made the surrounding environment filth, and attracted pests such as house flies which may have increased the chance of juice contamination. Most of the juice preparation, extraction and serving equipment were exposed to house flies and dust. *E. coli* is a normal inhabitant of the intestinal track of human and other warm blooded animals. Pathogenic strains cause diarrhea, urinary infections, pyogenic infections and septicemia among others (Matthew *et al.,* 2007; Samonis *et al.,* 2009).

Diarrheagenic *E. coli* (DEC) is the most important etiologic agent of childhood diarrhoea and represents a major public health problem in developing countries (Nataro and Kaper

1998). For example, in Dar es Salaam city, Tanzania, 280 children with diarrhoea were studied to determine the etiologic agent. The results indicated that 64 (22.9%) cases were due to Diarrheagenic *E.coli* out of which 14.6% were Enteroaggregative *E. coli* (EAEC), 4.6% Enteropathogenic *E. coli* (EPEC) and 3.6% Enterotoxigenic *E. coli* (ETEC) (Moyo *et al.,* 2007). This shows that *E. coli* is a bacterium of importance in Dar es Salaam city since it is among the major causes of diarrhoea especially in children under five years of age.

According to studies conducted in Tanzania, the level of contamination of food and water by *E. coli* was also significantly high. According to Robert (2011) report, 75% of water supplies in Iringa region were contaminated with *E. coli.* Mushi *et al.,* (2012) investigated water wells in Dar es Salaam and reported that 87% were contaminated by *E. coli.* According to Tanzanian Specifications (TZS 585:2003), *E. coli* must not be present in ready to drink beverages (TBS, 2003). The prevalence observed in the current study indicates that most of the raw unpasteurized fruit juices vended in Dare es Salaam are not fit for human consumption.

5.5 *Salmonella* spp. Contamination of the Juices

Generally, *Salmonella* infections are commonly associated with animal-derived foods, such as meat, seafood, dairy, and egg products, rather than juices (Bevilacqua *et al.,* 2011). However, a number of *Salmonella* outbreaks have been reported involving fruit juices especially unpasteurized ones in several places around the world (Keller and Miller, 2006; Vojdani *et al.,* 2008 and Poonam, 2013). In Tanzania, a study in Pemba to assess microbial quality of food samples found that *Salmonella* was exclusively found in 11% of milk sample (Vigano *et al.,* 2007). Several studies have revealed presence of *Salmonella* along with other pathogens including *E. coli* O157:H7 and O111,

Cryptosporidium and norovirus (Vojdani *et al.,* 2008) in the juices. However, in the current study, all 90 juice samples analyzed none of them was positive for S*almonella*. Similar results were reported by Victorian Government Department of Human Services (2005); Souza *et al.* (2008); Mahale *et al.* (2008) and Arijit *et al.* (2010). This may show that the bacterium was not present in the juice samples analysed in Dar es Salaam city or the juice which was sampled was not contaminated with S*almonella*. This is in consistent with the fact that no outbreak related to food poisoning caused by *Salmonella* has been reported in Dar es Salaam city. Nevertheless, the conventional bacterial isolation from food materials which was used in the current study is not that much sensitive as compared to other methods like molecular analysis. Therefore, there can be some *Salmonella* contamination in the juice samples but were missed by the isolation method. Before concluding with confidence that there is no *Salmonella* contamination in locally vended juice in Dar es Salaam, further studies are recommended using more sensitive screening methods and involving large sample size.

5.6 Risk Factors for Microbial Contamination of the Juices

Several factors were observed to predispose the juice to microbial contamination. The type of juice, pH, methods of extraction, vending sites, storage containers and sex of the vendor were shown to have a direct relationship with the microbial status in the juices.

Bacterial quality of the juices greatly depended on the type of juices. Mixed fruit and mango juices were more contaminated with both TPC and *E. coli* than passion and tamarind juices. Probably this was due to their inherent low acidity and higher pH values. Several studies have also reported the relationship between pH and microbial contamination where the common findings showed that as the pH of the juice increases

the higher the microbial contamination (Mahale *et al.,* 2008; Ashraf, 2009; Mesfin, 2011; and Sunday *et al.,* 2011).

Most bacteria do not thrive in high acid foods and that is why previously fruit juices were perceived as pathogen free foods due to their low pH values. However, as food safety issues continue to rise, several studies have reported a number of pathogen contaminations in acidic foods. In this study, all juices with low pH ($pH \leq 3.1$) were less contaminated as compared to those with high pH (> 3.1). Similar findings were reported by Yigeremu *et al.* (2001), Mesfin (2011) and Poonam (2013). High pH appeared to favour the bacterial growth and reduce the shelf life of the juice.

Extraction methods of the juice showed to strongly contribute to the juice contamination. In the three methods assessed that is blending, use of simple manual machine, and boiling fruits in water, showed a significant association ($P < 0.001$) on how each play part on the contamination of the juices. Extraction by using blenders and simple manual machines showed to increase the contamination of the juices at a great extent. The reasons may be due to improper washing of the equipment. Some of the equipment were observed to be used to extract the juice for the whole day without regular cleaning. Lakshmanan and Schaffner (2005) in the study of orange squeezing machines found that, some of the machines had scraps of oranges in internal tubing which were then reflected in the formation of bacteria biofilms. Tambekar *et al.* (2009) found that street fruit juices could often prove to be a public health threat due to their quick methods of cleaning utensils, handling and extraction.

Furthermore, juices vended in the markets had high TPC and *E. coli* followed by those which were vended along the road side, restaurants, bus stand and finally food kiosk.

Juices vended in markets showed to be extremely contaminated probably because markets are busy places with large population and limited sanitary service. On the other hand, road side and bus stand juices were found to be highly contaminated probably due to moving vehicles as well as dust particles, smoke generated and crowds of people. The findings of the current study are in agreement with findings reported by Tambekar *et al.* (2009), Shakir *et al.* (2009) and Poonam (2013). Juices sold in restaurants were also found to be highly contaminated this could probably be due to cross contaminations. In restaurants there are more activities involved with other types of foods along with the preparation of juices. High contamination of juices sold in restaurants was also reported by Lewis *et al.* (2006), Ketema *et al.* (2008) and Mesfin (2011).

The study further found that, juices prepared and sold by female vendors were more contaminated than the juices vended by male vendors. The reasons are not clear but probably females had many activities other than preparation of the juices. Most female vendors were also cooking and selling cooked foods at the same time preparing and selling juices hence more chances for contamination. In addition, the behaviour of women keeping long nails, and decorative equipment may have increased the rate of contamination of the juice. Ketema *et al.* (2008) and Mesfin (2011) also reported high contamination of the fruit juices in Jimma and Hawassa towns where majority of vendors were females.

Type of food storage containers are very important factor in the safety and quality of food. In this study, the common juice storage containers included buckets, cool boxes and reused plastic bottles. Juices stored in cool boxes and reused plastic bottles most of them had more TPC. Cool boxes were directly filled with the juices and some ice cubes were added in the juice to aid cooling. The juice in this type of storage could be affected by

these un-recommended temperatures because ice cubes can not provide effective and long lasting cooling. Martín-Diana *et al.* (2009) reported a significant increase of aerobic counts over storage time. Further more, if the ice cubes used in the cooling process were contaminated, it could also result in the contamination of the juice. Suday *et al.* (2011) assessed microbiological safety of edible ice used by street juice vendors to refrigerate juice in Nigeria and found that all the ice samples were heavily contaminated with pathogenic bacteria. Similar findings were also reported by Falcão *et al.* (2002), Lateef *et al.* (2004) and Agbaje *et al.* (2006). On the other hand, reused plastic bottles are not proper containers for storage of fruit juices because they can not be easily sterilized using hot water and may pose a risk of contamination of the juice. According to Codex general principles of food hygiene *(CAC/RCP 1-1969, Rev.4- 2003)* food containers/packaging materials should provide adequate protection for products to minimize contamination, prevent damage, and should not pose a threat to the safety and suitability of food under the specified conditions of storage and use and where appropriate, reusable packaging should be suitably durable, easy to clean and, where necessary, disinfect (FAO, 2003).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings of the current study it can be concluded that:-

i. Handling practices toward juice preparation, extraction methods and washing of equipment were observed to be very poor.

ii. General hygiene of vendors and premises were poor.

iii. Poor waste management within preparation and vending sites encouraged contamination of the juices.

iv. Majority of the unpasteurised fruit juice had total soluble solids levels below the recommended Codex specifications.

v. Majority of unpasteurised fruit juice were contaminated with *E. coli* and had higher TPC above Codex specification.

vi. Mixed fruit and mango juices were more contaminated than passion and tamarind juice.

vii. Low pH of some unpasteurised fruit juices inhibited bacterial contamination.

viii. High pH of majority of unpasteurised fruit juices supported bacterial contamination.

ix. Unpasteurised fruit juices vended at markets and roadside were significantly more contaminated.

x. There was no *Salmonelle* isolated in unpasteurised fruit juices vended in Dar es Salaam city suggesting that this is not a threat to juice consumers.

6.2 Recommendations

From the conclusions drawn, it is therefore recommended that:-

i. Presence of *E. coli* and high levels of TPC indicates fecal contamination. This provides circumstantial evidence that there is poor disposal of faeces and that proper hand washing among vendors is not practiced. It is important there fore to provide education to the vendors on proper hygiene and sanitation.

ii. Since the current study focused only on bacterial quality, it is recommended more studies on fungi and protozoa contamination in unpasteurised fruit juices.

iii. Prohibition of street foods including fruit juices can prove to be difficult considering the role they play in feeding a significant proportion of urban dwellers. It is therefore recommended that the government should educate both vendors and consumers on food safety and hygiene.

iv. Regular monitoring of the quality of street fruit juices for human consumption must also be enforced.

REFERENCES

Agbaje, L., Julius, K., Oloke, E.B., Gueguim, K. and Esther, P. (2006). The Microbiological Quality of Ice Used to Cool Drinks and Foods in Ogbomoso Metropolis, Southwest. *Nigeria Internet Journal of Food Safety* 8: 39-43.

AICR (America Institute for Cancer Research) (2007). Food, nutrition, physical activity, and the prevention of cancer: a global perspective [www.dietandcancerreport.org.] site visited on 9/7/2013.

Alarcón de Noya, B., Díaz-Bello, Z., Colmenares, C., Ruiz-Guevara, R., Mauriello, L., Zavala-Jaspe, R., Suarez, J.A., Abate, T., Naranjo, L., Paiva, M., Rivas, L., Castro, J., Márques, J., Mendoza, I., Acquatella, H., Torres, J. and Noya, O. (2010). Large urban outbreak of orally acquired acute chagas disease at a school in Caracas, Venezuela. *Journal of Infectious Disease* 201: 1308–1315.

Andargie, G., Kassu, A., Moges, F., Tiruneh, M., and Huruy, K. (2008). Prevalence of Bacteria and Intestinal Parasites among Food-handlers in Gondar Town, Northwest Ethiopia. *Journal of Health Population and Nutrition* 26: 451-455.

AOAC (Association of Official Analytical Chemists) (1999). *Official Methods of Analysis*. (15th Ed.), Washington D.C. pp. 992-995.

APEC (Asia-Pacific Economic Cooperation) (2005). Thailand: Embargo on ice production after hepatitis outbreak. [http://depts.washington.edu.einet] site visited on 24/12/2012.

Arijit, D.G.S., Nagananda, S.B. and Shilp, B. (2010). Microbiological quality of street-vended Indian Chaats sold in Bangalore. *Journal of Biological Sciences* 10(3): 255-260.

Barro, N., Bello, A.R., Aly, S., Ouattara, C.M.T., Iiboudo, A.J. and Traaore, A.S. (2006). Hygienic status assessment of dish washing waters, utensils, hands and pieces of

money from street food processing sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology* 5(11): 1107–1112.

Barro, N., Razack, B.A., Yollande, I., Aly, S., Tidiane, O.C.A., Philippe, N.A., Comlan, D.S. and Sababenedjo, T.A. (2007). Street vended foods improvement: contamination mechanisms and application of food safety objective strategy: critical review. *Pakistan Journal of Nutrition* 6(1): 1–10.

Bates, R.P., Morris, J.R. and Crandall, P.G. (2001). Principles and practices of small and medim -processing. *FAO Agricultural Services Bulletin* 146: 93-99.

Bevilacqua, A., Corbo, M.R., Campaniello, D., D'Amato, D., Gallo, M., Speranza, B. and Bhaskar, J., Usman, M., Smitha, S. and Bhat, G.K. (2004). Bacteriological profile of street foods in Mangalore. *Indian Journal of Medical Microbiology* 22: 197-197.

Bhowmik, S.K. (2005). Street vendors in Asia: a review. Economic and Political Weekly, pp 2257–2264.

Boyer, J. and Liu, R. (2004). Apple Phytochemicals and their health benefits. *Nutrition Journal* 3: 1-15.

Buck, J.W., Walcott, R.R. and Beuchat, L.R. (2003). Recent trends in microbiological safety of fruits and vegetables. Online. Plant Health Progress doi: 10.1094/PHP-2003-0121-01-RV.

Burt, M.B., Volel, C. and Finkel, M. (2003). Safety of vendor prepared foods: Evaluation of 10 processing mobile food vendors in Manhattan. *Public Health Reports* 118: 470-476.

Canadian Food Inspection Agency (2010). Code of Practice for the production and distribution of unpasteurized apple and other fruit juice/cidar in Canada. [www.inspection.gc.ca/english/flavege/processed/codee.shtml]site visited on 2/4/2013.

Cardinale, E., Claude, J.D., Tall, F., Gueye, E.F. and Salva, G. (2005). Risk factors for contamination of ready-to-eat street vended poultry dishes in Dakar, Senegal. *International Journal of Food Microbiology* 103: 157–165.

Castillo, M.C., Allori, C.G., Gutierrez, R.C., Saab, O.A., Fernandez, N.P., Ruiz, C.S., Holgado, R.A.P. and Nader, O.M. (2000). Bactericidal activity of Lemon juice and Lemon derivatives against Vibrio cholerae. *Biological and Pharmaceutical Bulletin* 23:1235-1238.

Castillo, A., Villarruel-Lo´ Pez, V., Navarro-Hidalgo, N.E. Marti´nez, G. L. and Torres-Vitela, M.R. (2009). Salmonella and Shigella in freshly squeezed orange juice, fresh oranges, and wiping cloths collected from public markets and street booths in Guadalajara, Mexico: Incidence and comparison of analytical routes. *Journal of Food Protection* 69(11): 2595–2599.

CDC (Centers for Disease Control) (2011). Foodborne Outbreak Online Database (FOOD). [http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx.] site visited on 4/10/ 2011.

Chen, S.J., Zhang, M. and Wang, S.J. (2010). Physiological and quality responses of Chinese 'Suli' pear (Pyrus bretschneideri Rehd) to 1-MCP vacuum infiltration treatment. *Journal of Science, Food and Agriculture* 90: 1317–1322.

Chukwu, C.O.C., Chukwu, I.D., Onyimba, I.A., Umoh, E.G., Olarubofin, F. and Olabode, A.O. (2010). Microbiological quality of pre-cut fruits on sale in retail outlets in Nigeria. *African Journal of Agricultural Research* 5(17): 2272 - 2275.

Danyluk, M.D. Goodrich-Schneider, R.M. Schneider, K.R. Harris, L.J. and Worobo, R.W. (2012). Outbreaks of Food-borne disease associated with fruit and vegetable juices, 1922–2010. Food Science and Human Nutrition Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. [http://edis.ifas.u!.edu.] site visited on 3/2/2013.

Deanna, M. M. and Jeffrey, S. B. (2007). Acid-alkaline balance: Role in chronic disease and detoxification. *Alternative Therapy* 13(4): 62 – 65.

Eni, A.O., Oluwawemitan, I.A. and Solomon, O.U. (2010). Microbial quality of fruits and vegetables sold in Sango, Ota, Nigeria. *African Journal of Food Science* 4(5): 291- 296.

Eribo, B., and Ashenafi, M. (2003). Behavior of Escherichia coli O157:H7 in tomato and processed tomato products. *Food Research International* 36: 823–830.

Falcão, J.P., Dias, A.M.G., Correa, E.F. and Falcão, D.P. (2002). Microbiological Quality of Ice Used to Refrigerate Foods. *Food Microbiology* 19: 269-276.

Frank, A.A., Cooney, R.V. Henning, S.M. and Custer, L.J. (2005). Bioavailability and Oxidant effects of Orange juice components in humans. *Journal Agriculture Food Chemistry* 53(13): 5170 – 5178.

FAO (Food and Agriculture Organisation) (1995). Condex Alimentrarious, General requirements (food hygiene) FAO, Rome. pp. 188-192.

FAO (Food and Agriculture Organisation) (1997). Street food: small entrepreneurs, big business [Http://www.Fao/NEWS/1997/97/970408-e.htm.] site visited on 22/6/2011.

FAO (Food and Agriculture Organisation) (2002). Working paper on elaboration of a region standard for microbiological levels in food. Joint FAO/WHO Food standards programme Codex coordinating for the Near East. [www.doh.gov. za/.../Codex%20working%20paper%2.] site visited 7/6/2013.

FAO (Food and Agriculture Organisation) (2003). Recommended International Code of practice general principles of food hygiene. (CAC/RCP 1-1969, Rev.4- 2003) [www.foodsafety.govt.nz/.../industry/codex-guidelines.pdf] site visited on 26/7/2013.

FAO (Food and Agriculture Organisation) (2005). General Standard for Fruit Juices and Nectars; Codex Alimentarius Commission. [www.codexalimentarius.org/.../standards/.../CXS_247]. site visited 10/6/2013.

FDA (Food and Drugs Administration) (2008). Internalization of microorganisms into fruits and vegetables. Literature review of common food safety. [wwwhttp:// www.cfsan.fda.gov.html] site visited on 10/7/2012.

FDA (Food and Drugs Administration) (2010). DHMH Issues Consumer Alert Regarding Recall of Baugher's Apple Cider. [http://www.fda.gov/safety/recalls/ ucm232878.htm.] site visited on 4/10/ 2011.

ISO (International Standards Organisation) (2002). ISO 6579:2002, Microbiology of food and animal feeding stuffs- Horizontal method for the detection of *Salmonella* spp.

ISO (International Standards Organisation) (2003). ISO 4833:2003, Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of microorganisms Colony-count technique at 30ºC.

ISO (International Standards Organisation) (2006). ISO 7251:2005, Microbiology of food and animal feeding stuffs- Horizontal method for the detection and enumeration of presumptive *Escherichia coli* – Most probable number technique.

Jain, S., Bidol, S. A., Austin, J. L., Berl, E., Elson, F., Williams, M.L., Deassy III, M., Moll, M.E., Rea, V., Vojdani, J.D., Yu, P.A., Hoekstra, R.M., Braden, C.R. and Lynch, M.F. (2009). Multistate outbreak of *Salmonella typhimurium* and Saintpaul infections associated with unpasteurized orange juice—United States, 2005. *Clinical Infectious Diseases* 48:1065–1071.

Jay, J.M. (2000). *Modern Food Microbiology*. (6thEd.) Spen Publishers Inc., Gaithersburg, MA. 625pp.

Jiwa, S.F.H., Mugula, J.K. and Msangi, M.J. (1991). Bacteriological quality of potable water sources supplying Morogoro municipality and its outskirts: a case study in Tanzania. *Epidemiology of Infection* 107: 479-484.

Kinabo, J. (2003). Impact of globalization on food consumption, health and nutrition in urban areas: a case study of Dar es Salaam, United Republic of Tanzania. [ftp://ftp.fao.org/es/esn/food_systems/kinaboF.pdf] site visited on 7/2/2012.

Keller, S.E., and Miller, A.J. (2006). Microbiological safety of fresh citrus and apple juices. In: Sapers, G.M., Gorny, J.R.and Yousef, A.E. (Eds) (2006). Microbiology of fruit and vegetables. Boca Raton, FL: CRC Press Taylor and Francis Group. pp. 211-224.

Ketema, T., Gaddisa, T. and Bacha, K. (2008). Microbiological safety of fruit juices served in cafes/restaurants, Jimma town, southwest Ethiopia. *Ethiopian Journal of Health sciences* 18(3): 98-100.

Kothari, C.R. (2004). Research Methodology Methods & Technique. New Age International (P) Limited, Publishers. 2:177-178.

Kumar, M., Agarwal. D., Ghosh, M. and Ganguli, A. (2006). Microbiological safety of street vended fruit chats in Patiala city. *Indian Journal of Medical Microbiology* 24 (1): 75-81.

Kurowska, E., Spence, J., Jordan, J., Wetmore, S., Freeman, D., Piche, L. and Serratore, P. (2000). HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. *American Journal of Clinical Nutrion* 72: 1095–1100.

Lateef, A.J.K., Oloke, E.B., Kana, E.P. and Guegium, E. (2004). The microbiological quality of ice used to cool drinks and foods in Ogbomoso Metropolis Southwest, Nigeria. *Internet Journal of Food Safety* 8: 39-43.

Lakshmanan, C. and Schaffner, D. W. (2005). Understanding and controlling microbiological contamination of beverage dispensers in university foodservice operations. *Food Protection Trends* 26: 27-31.

Latham, M.C. (1997). Human nutrition in tropical Africa. FAO, Rome. pp. 329-437.

Lewis, J.E. Patrina, T., Rao, B.V.V.B.N., Kalavati, C. and Rajanna, B. (2006). Human bacteria in street vended fruit juices: A case study of Visakhapatnam city, India. *Internet Journal of Food Safety* 8: 35 - 38.

LSDEPC, (Laboratory Surveillance Data for Enteric Pathogens in Canada) (2005). Annual summary. [http://www. nml-lnm.gc.ca/NESP-PNSME/index-eng.htm.] Site visited on 19/4/ 2010.

Mahale, P.D., Ranjana, G.K. and Varsha, K.V. (2008). Microbiological analysis of street vended fruit juices from Mumbai City, India. *Internet Journal of Food Safety* 10: 31-34.

Matthew, E., Falagas, P.I., Rafailidis, D.K., Simona V., Fotini, C.C., Vassiliki, P., Sofia M., George, S. and Argyris, M. (2007). Risk factors of carbapenem-resistant *Klebsiella pneumonia* infections: a matched case-control study. *Journal of Antimicrobial Chemotherapy* 60: 1124-1130.

Mattioli, M.C., Pickering, A.J., Gilsdorf, R.J., Davis, J. and Boehm, A.B. (2013). Hands water as vectors of diarrheal pathogens in Bagamoyo, Tanzania. *Environmental Science and Technology* 47(1): 355 – 363.

Martín-Diana, A.B., Rico, D., Barat, J.M., and Barry-Ryan, C. (2009). Orange juices enriched with chitosan: Optimisation for extending the shelf-life. *Innovative Food Science and Emerging Technologies* 10: 590-600.

Mensah, P., Yeboah-Manu, D., Owusu- Darko, K. and Ablordey, A. (2002). Street foods in Accra, Ghana: how safe are they? Bulletin of the World Health Organization. *The International Journal of Public Health* 80(7): 546 – 553.

Mesfin, W. (2011) Bacteriological profile of locally prepared fresh fruit juices in Hawassa town, southern Ethiopia. Addis Ababa University Libraries Electronic Thesis and Dissertations Database, pp.1- 44.

Ministry of Agriculture Food Security and Cooperatives (2009). Agriculture sector Review and public expenditure review 2008/09. [www.agriculture.go.tz/.../ Agricultutre%20Sector%20Review%20and%20Pu...] site visited on 22/7/2013.

Mohapatra, A.D., Rath, C.C., Dash, S.K. and Mishra, R.K. (2002). Microbiological evaluation of street foods in Bhubaneshwar. *Journal of Food Science and Technology* 39(1): 59 – 61.

Mosupye, F.M. and Holy, V.A. (2000). Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. *International Journal of Food Microbiology* 61: 137 – 145.

Mosupye, F.M. and Holy, V.A. (1999). Microbiological quality and safety of ready to-eat Street-vended foods in Johannesburg, South Africa. *Journal of Food Protection* 62: 1278–1284.

Moyo, S. J. Maselle, S.Y., Matee, M.I., Langeland, N. and Mylvaganam, H. (2007). Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. *BMC Infectious Diseases* 90(7): 1-7.

Muinde, O.K. and Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, 5(1): 1-13.

Mushi, D., Denis, B., Alexander, K.T.K., Robert, L.M., Kirschner, K. B. and Andreas, H.F. (2012). Sanitary inspection of wells using risk-of-contamination scoring indicates a high predictive ability for bacterial faecal pollution in the peri-urban tropical lowlands of Dar es Salaam, Tanzania. *Journal of water and Health* 10(2): 236-243.

Nataro, J.P. and Kaper, J.B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews* 11(1): 142-201.

Nicolas, B., Razack, B.A., Yollande, I, Aly, S., Tidiane, O.C.A., Philippe, N.A., De Souza, C. and Sababénédjo, T.A. (2007). Street-Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective Strategy: Critical Review. *Pakistan Journal of Nutrition* 6(1): 1-10.

Oliveira, A.C.G., Seixas, A.S.S., Sousa, C.P. and Souza, C.W.O. (2006). Microbiological evaluation of sugarcane juice sold at street stands and juice handling conditions in São Carlos, São Paulo, Brazil. *Cadernos de Saúde Pública* 22(5): 1111-1114.

Omemu, A.M. and Aderoju, S.T. (2008). Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. *Food Control* 19: 396 – 402.

Oranusi, U.S., Braide, W., and Nezianya, H.O. (2012). Microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria. *Greener Journal of Biological Sciences* 2 (1): 001-006.

Pao, S., Fellers, P.L., Brown, G.E. and Chambers, M. (2001). Formulation of Fresh Squeezed Unpasteurized Citrus Juice Blend. *Fruit Processing Journal* 7: 268-271.

Penrose, K., Castro, M.C.D. Werema, J. and Ryan, E.T. (2010). Informal urban settlements and cholera risk in Dar es Salaam, Tanzania. *PLoS Neglected Tropical Diseases* 4(3): 631 – 635.

Pereira, K.S., Schmidt, F.L., Guaraldo, A.M.A., Franco, R.M.B., Dias, V.L. and Passos, L.A.C. (2009). Chagas' disease as a food-borne illness. *Journal of Food Protection* 72: 441–446.

Pilo, F.B., Pereira, N.O., Frietas, L.F.D., Miranda, A.N.D., Carmo, L.S., Gomes, F.C.O., Nardi, R.M.D. and Rosa, C.A. (2009). Microbiological testing and physical and chemical analysis of reconstituted fruit juices and coconut water. *Alimentose Nutricao* 20(4): 523-532.

Poonam, U.S. (2013). Bacteriological analysis of street vended fruit juices available in Vidarbha. *International Journal of Current Microbiology and Applied Science* 2(5): 178-183.

Reddy, U. B., Chandrakanth, N., Indu, P. S., Venkata, N. R. and Usha, K. B. (2009). Isolation and characterization of faecal coliforms in street vended fruit juices and its safety evaluation: A case study of bellary city, india. *Internet Journal of Food Safety* 11: 35 – 43.

Rizzon, L.A. and Miele, A. (2012). Analytical characteristics and discrimination of Brazilian commercial grape juice, nectar and beverages. *Ciencia e Tecnologia de Alimentos* 32(1): 93-97.

Robert, H.M. (2011). Water point testing in Iringa region, Tanzania mainland. Enabling community involvement in water quality monitoring. California State University at Sacramento [www.worldwaterweek.org/.../2011/.../Water-Point-Te…] site visited on 12/7/2013.

Rodrigues, A., Sandstrom, A.C.T. Steinsland, H., Jensen, H. and Aaby, P. (2000). Protection from cholera by adding lime juice to food-results from community and laboratory studies in Guinea-Bissau,West Africa. *Tropical Medicine and International Health* 5: 418-422.

Samonis, G., Karageorgopoulos, D.E., Kofteridis, D.P., Mattaiou, D.K., Sidiropoulou, V., Maraki, S. and Falagas, M.E. (2009). Citrobacter infections in a general hospital: characteristics and outcomes. *European Journal of Clinical Microbiology and Infectious Diseases* 28(1): 61-68.

Sandeep, M., Diwakar, A. and Abhijit, G. (2001). Microbiological Analysis of Street Vended Fresh squeezed Carrot and Kinnow-Mandarian Juices in Patiala City, India. *Internet Journal of Food safety* 3: 1-3.

Schlundt, J., Toyofuku, H., Jansen, J. and Herbst, S.A. (2004). Emerging food-borne zoonoses. *Revue Scientifique et Technique* 23(2): 513-33.

Shakir, M., Ahmed, U., Nasreen, T., Feroza, B. and Parveen, S. (2009). Microbiological Quality of Local Market Vended Freshly Squeezed Fruit Juices in Dhaka City, Bangladesh. *Bangladesh Journal of Science and Industrial Research* 44: 421-424.

Sharmila, R. (2011). Street vended food in developing world: Hazard analyses. *Indian Journal of Microbiology* 51(1): 100-106.

Souza, M.C.C., Toledo, M.B., Renata, F.A.M. and Rui, S.S.F.S. (2004). Stability of unpasteurized and refrigerated orange juice. *Brazilian Archives of Biology and Technology* 47(3): 391-397.

Steven, P. and Davis, C.L. (2001). Transfer of natural and artificially inoculated microorganisms from orange fruit to fresh juice during extraction. *Lebensmittel-Wissenschaft and Technologie* 34: 113–7

Subbannayya, K., Bhat, G. K., Shetty, S. and Junu, V. G. (2007). How safe is sugarcane juice? *Indian Journal of Medical Microbiology* 25: 73 – 74.

Sunday, P. U., Nyaudoh, U. N. and Etido, J. U. (2011). Microbiological quality and safety evaluation of fresh juices and edible ice sold in Uyo Metropolis, South-South, Nigeria. *Internet Journal of Food Safety* 13: 374-378.

Tambekar, D.H., Jaiswal, V.J., Dhanorkar, D.V., Gulhane, P.B. and Dudhane M.N. (2009). Microbial quality and safety of street vended fruit juices: A case study of Amravati city. *Internet Journal of Food Safety* 10: 72-76.

Tasnim, F., Anwar, H.M., Nusrath, S., Kamal, H. M., Lopa, D. and Formuzul, H.K.M., (2010). Quality assessment of industrially processed fruit juices available in Dhaka City, Bangladesh. *Malaysian Journal of Nutrition* 16(3): 431 – 438.

Tanzania Bureau of Standards (TBS), (2003). Ready to drink beverages (non – carbonated) – Specifications. [www.tbs.go.tz/tbtdb/standards.] site visited on 2/5/2013].

Thurston, H., Stuart, J., McDonnell, B., Nicholas, S. and Cheasty, T. (1998). Fresh orange juice implicated in an outbreak of *Shigella flexneri* among visitors to a South African game reserve. *Journal of Infectious Diseases* 36: 350.

Titarmare, A., Dabholka, P. and Godbole, S. (2009). Bacteriological analysis of street vended fresh fruit and vegetable juices in Nagpur City, India. *Internet Journal of Food Safety* 11: 1-3.

Vasavada, P.C. (2003). Beverage quality and safety, In Microbiology of fruit juice and beverages. (Eds Foster, T. and Vasavada, C.P.), CRC Press. Boca Raton, FL, pp. 95-123.

Victorian Government Department of Human Services, (2005). Microbiological survey of fresh squeezed juices from retail businesses across Victoria. Food Safety, Unit Melbourne,Victoria. [http://www.health.vcc.gov.an/foodsafety.] site visited on 12/5 2012.

Viganò, A., Pellissier, N., Hamad, H.J., Ame, S.A. and Pontello, M. (2007). Prevalence of E. coli, thermotolerant coliforms, Salmonella spp. and Vibrio spp. in ready-to-eat foods: Pemba Island, United Republic of Tanzania. *Annal di igiene Medicinal ed Comunita* 19(5): 395-403.

Vojdani, J.D., Beuchat, L.R. and Tauxe, R.V. (2008). Juice-associated outbreaks of human illness in the United States, 1995 through 2005. *Journal of Food Protection* 71: 356–364.

WHO (World Health Organisation) (2008). *Guidelines for drinking-water quality.* (3rd ed). World Health Organization, Geneva. 668pp.

Wissanee, S. and Renu, P. (2007). Physical, chemical and microbiological changes during storage of orange juices cv. Sai Nam Pung and cv. Khieo Waan in Northern Thailand. *International Journal of Agriculture and Biology* 9(5): 726-730.

Yigeremu, B., Bogale, M. and Ashenafi, M. (2001) Fate of *Salmonella* species and *E. coli* in Fresh-Prepared Orange, Avocado, Papaya and Pine Apple Juices. *Ethiopian Journal of Health Science* 11: 89-94.

Zahra, M.A. (2010). Antagonism activity of citrus fruit juices on some pathogenic bacteria. *Journal of Kerbala University* 8(3): 123 – 128.

Zaika, L.L. (2001). The effect of temperature and low pH on survival of Shigella flexneri in broth. *Journal of Food Protection* 64: 1162–1165.

APPENDICES

Appendix 1: Questionnaires

A. Personal particulars of the respondents

Respondent No.----------

Name of location---------- Date----------

Name of interviewer ----------

1. Sex ---------- 1. Male 2. Female
2. Marital status

   a) Single b) Married c) widow/widower d) Divorced
3. Age range

   a) 15 – 24 b) 25- 34 c) 35 – 44 d) 45 – 54 e) 55 and above
4. Level of education

   a) No formal education b) Primary education c) Secondary d) College

   e) Vocational training
5. How long have you been vending raw juice? ----------

B. Sources of fruits and water used for juice preparation

6. What types of fruits do you use to prepare the juice?

   a) Mangoes b) Pineapples c) Baobab fruits (Ubuyu) d) Mixture. Specify ---------

   e) Others: Specify ----------
7. Where do you get fruits used to prepare the juice from?

   a. From the market. Specify ---------- b) From my own farm

   c) From farmers d) From hawkers

8. What time do you buy fruits for juice preparation?

   a) The day before preparation day b) Early in the morning on the day of preparation

   c) At the time of preparation of juice

   d) Others: Specify ----------

9. What do you use to carry the fruits?

   a) Polythene bag b) Sacks c) Basket d) Others: Specify ----------

10. Methods of preservation prior juice processing

    a) In a refrigerator b) On the floor c) On an open space d) In a bucket

    e) Others: Specify ----------

11. Where do you get water used for washing of fruits?

    a) From tap b) From wells c) Rain water d) Bottled water

    e) Others: Specify ----------

12. Where do you get water used for dilution of the juice?

    b) From tap b) From wells c) Rain water d) Bottled water

    f) Others: Specify ----------

13. Do you wash the fruits before you start preparation of the juice? 1. YES 2. NO

    If YES;

14. How do you treat the water before use in washing the fruits

    a) I don't treat b) I boil c) I add disinfectant d) I filter

    e) Others: Specify ----------

15. Have there been some incidences of fruit decaying before extraction of juice?

    YES ----------NO----------

    If YES,

16. What do you do with the decayed fruits?

    a) Just use them b) Trim and use them c) Throw them away d) Others:----------

C. Fruits handling before extraction of juices

17. What is the first procedure done on the fruits

    a) Peal b) Chop c) Wash d) Sterilize. How ----------

18. How do you remove the outer skin of fruits

    a) Peal with knife b) I do not remove c) I soak in hot water then peal

    d) Others: Specify ----------

19. What do you use to chop the fruits before extracting the juice?

    a) By using a bush knife b) By using a clean knife c) By use of a chopping machine d) I do not chop e) Others: Specify ----------

20. Where do you put the fruit chopped ready for juice extraction?

    a) In a clean sauce pan b) I n a basin c) On the table d) In a bucket

21. How long does it take from fruits preparation to extraction of the juice?

    a) One hour b) Two hours c) One day d) I extract immediately after chopping

D. Methods of juice extraction

22. What do use to extract the juice?

    a) I squeeze by hands b) I use squeezing machine c) I use a homogenizer

    c) Others: Specify ----------

23. Treatment of water before us for dilution of the juice

    a) I don't treat b) I boil c) I add disinfectant d) I filter e) Others: Specify ----

24. Do you pasteurize the juice after extraction/squeezing? 1. YES 2. NO If YES

25. How do you pasteurize?

    a) Boil in a saucepan b) In a kettle c) In a pot d) Use a pasteurizer

    e) Others: Specify ----------

E. Juices handling and storage after preparation and during vending

26. Where do you keep the juice after preparation

    a) In a jag b) In a squeezing machine c) In a refrigerator d) In a cool box with ice parks e) Others: Specify ----------

27. How often do you clean the storage facility mentioned above?

    a) Every day b) After use c) Once per week d) Once per months

    e) Others: Specify ----------

28. Vending place/site

    a) In a restaurant b) along the road/ bus stand c) In a market d) along schools. Specify ---------- e) Others: Specify ----------

29. Serving equipments

    a) Glasses b) Plastic cups c) Disposable equipments d) Plastic bottles. Specify: 1. Re-used bottles 2. New bottles

30. How do you wash the equipments after serving and before serving to another customer?

    a) With cold water without soap b) With cold water and soap

    c) With hot water without soap d) With hot water and soap e) I do not wash

31. Do you face problems of juice going bad after preparation? 1. YES 2. NO

    If YES How often?

    a) Regularly b) Once c) Twice d) Others. Specify ----------

32. What do you do with the juice which has gone bad?

    a) I sell at a low price b) I feed my family c) I mix with a fresh juice to improve the taste d) I dispose it

33. Where do you dispose the unwanted materials (left over, fruit peelings and residuals)

    a) In a pit b) In a sack c) In a polythene bag d) In an open bin

    f) In a covered bin

34. Have you ever done medical check up? 1. YES 2. NO

If YES

35. How often do you do medical check up??

a) Twice a year b) Every year c) When I feel sick d) Others: Specify ----------

Appendix 2: Observational Checklist

Respondent No. ---------- Location ----------

No.	ITEM	YES	NO
1	Does preparation setting minimize cross-contamination?	Effectively, Moderately, poorly	
2	Washing process; Does it minimize contamination?	Effectively, Moderately, poorly	
3	General cleanliness of the handler	Very good, Good, Poor	
4	Vendor has working uniform	Fully, Partial	
3	Hand washing equipments and disinfectant present		
4	Waste receiving receptacle present	Covered, Uncovered	
5	Presence of pests (flies, cockroaches)	Specify ----------	
6	Is the juice protected from sources of contamination?		
7	Is the storage facility effective for the intended purpose?		