Quality and Safety Assessment of Locally Processed Fresh Fruit Juices of Restaurants, Cafes and Juice Bars in Three Divisional Secretariats (Dehiwala, Rathmalana and Moratuwa) of Colombo District

By

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Thesis submitted to the University of Sri Jayewardenepura as the partial fulfillment requirement for the award of the degree of Masters of Science in Food Science and Technology.

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DECLARATION

This work describe in this thesis was carried by me as the partial fulfillment of the requirement for the Degree of Masters of Food science and Technology under the supervision of Professor Ranaweera K.K.D.S, Department of Food Science and Technology, university of Sri Jayewardenepura. Report of this thesis has not been submitted in whole or in part of any University or any Institute for another degree.

Date

L.P.N.S.Jayawardhana
This is to certify that above statement made by the candidate is true and suitable for submission to the University for the purpose of evaluation,

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25.11.2014

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DEFINITIONS

1. **Aerobic**: Grows in the presence of atmospheric oxygen.

2. **Aliquot**: The portion of food that is inoculated into a container of bacteriological medium in accordance with a specified method.

3. **Analytical Unit**: The amount of product withdrawn from the sample unit for analysis.

4. **Coliform**: A gram-negative, facultative rod shaped bacterium that ferments lactose, producing gas.

5. **Contamination**: The effect exerted by an external agent on food so that it does not meet acceptable food hygiene standards or is unfit for human consumption.

6. **HACCP system**: An effective management tool for food safety assurance that can be applied to all sections of the food chain.

7. **Indicator**: Historically, an organism itself non-pathogenic, but often associated with pathogens, used to portray a risk of the presence of pathogens for which feasible methods of detection were not generally available (sometimes called 'index organisms').

8. **Lot**: A batch or production unit which may be identified by the same code. When there is no code identification, a lot may be considered as (a) that quantity of product produced under essentially the same conditions, at the same establishment and representing no more than one day's production; or, (b) the quantity of the same kind of product from one and the same manufacturer available for sampling at a fixed location.

9. **Mesophile**: A microorganism with a growth optimum around 20°C to 45°C.

10. **Microbiological guidelines**: A microbiological criterion used by a manufacturer or regulatory agency to monitor a food, ingredient, process, or system; often used also to describe a microbiological criterion where no standard has been prescribed.

11. **Pathogens**: Organisms that cause disease.

12. **Sample Unit**: Usually a consumer size container of the product, and should consist of a minimum of 100 g (ml). A sample unit is often referred to as a subsample.
13. **Sample**: The sample units (subsamples) taken per lot for analysis.

14. **Total coliform counts (TCC)**: The number of colony-forming units of gram-negative, facultative rod shaped and lactose fermenting bacteria present per gram or per ml in the analytical unit as determined by a standard method.

15. **Total viable counts (TVC)**: The number of colony-forming units of aerobic mesophilic bacteria present per gram or per ml in the analytical unit as determined by a standard method.
ABSTRACT

Fresh fruits are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh fruits. However, many outbreaks of human infections have been associated with the consumption of contaminated fruit juices. During processing contamination from raw materials, equipment or food handlers could be easily transferred to the final product of fruit juices resulting foodborne illness. Common bacterial illnesses associated with contaminated fruit juices are staphylococcal food poisoning, Salmonellosis, shigellosis and diarrhea associated with enterotoxogenic E. coli. Most of the fruit juices being served had high microbial load. So that, these products could be the cause of health problems and potential vehicle of food borne outbreaks (Ketema et al, 2008). During this study it was focused on such type of hygienic problems that have impact on quality and safety of locally prepared fruit juices.

The aim of the present study was to assess the quality and safety of Locally Prepared unpasteurized fresh fruit juices sold for immediate consumption in restaurants cafes and Juice Bars in three Divisional Secretariats of Colombo district namely Dehiwala, Rathmalana and Moratuwa

Microbial quality and safety of four types of fruit juices (mangoes (*Mangifera indica*), oranges (*Citrus sinensis*), Papaya (*Carica papaya*) and pineapple (*Ananas comosus*) were determined by identifying, total viable count (ISO 4833:2003), total coliform count (ISO 4831:2006), total staphylococcal count (ISO 6888-1: 1999) and total salmonella count (ISO 6579:2002). At the same time using questionnaire, details on hygienic conditions of preparation sites, cleaning and sanitizing practices with their frequencies, fruit storage conditions, personnel hygiene practices and fruit preparation techniques prior to process also were assessed during December 2012 to June 2013. Sampling and testing were done by fully competent staff of QA department, GlaxoSmithKline pvt ltd, Sri Lanka.

The present study evaluated bacterial profile of locally prepared fresh fruit juices. And also through this study it was assessed the hygienic conditions of processing and handling of locally prepared unpasteurized juices. As a major achievement it was helpful to assess quality of locally prepared unpasteurized fresh juices in terms of basic hygienic problems associated with and in order to recommend remedial action for identified problems. Finally it enabled to identify juice types that are more susceptible to contamination.
CHAPTER 01 - INTRODUCTION

Fruit juices are well recognized for their nutritive values, minerals and vitamin contents. In many tropical countries they are common beverages and are sold at all public places and roadside shops which are widely consumed by millions of people. These juices provide a source of readily available and affordable source of nutrients to many sectors of the population. Unpasteurized juices are consumed owing to consumer preferences for fresher, more nutritious foods that also happen to meet the needs of busier lifestyles and hence, in recent times, their demand has increased.

Fresh fruit juices have no artificial color, sweetness is natural, and that is why they are preferred over bottled or canned juices (Addo et al 2008; Melbourne, 2005).

Freshly squeezed fruit juices have little or no process steps that reduce pathogen levels, if contaminated, such as no kill step. Freshly squeezed juices are simply prepared by extracting, usually by mechanical means, the liquid and pulp of mature fruits. The final product is an unfermented, unclarified, untreated juice, ready for consumption.

Fruits usually require some preparation before being fed into the juicer. Fruits such as, mangoes (Mangifera indica), oranges (Citrus sinensis), Papaya (Carica papaya) and pineapple (Ananas comosus) will need prior preparation due to the bitterness of the skin or to remove large pips and stones. Once prepared, the fruit can be fed into the juicer / mechanical blender. The juice will be extracted and any pulp will be simultaneously removed. During the process, contamination from raw materials, equipment or food handlers could be easily transferred to the final product.

There are differences in the handling of each type of fruit intended for juice. Overall, unpasteurized juice manufacturing includes several processing steps, such as receiving, storing, washing, grinding and extraction, separation/centrifugation, blending of ingredients, and storage. The first processing step is a receiving protocol which includes fruit inspection and grading. After fruits are received and hand-sorted on a conveyer belt, they are mechanically scrubbed and washed with a sanitizing solution, rinsed with water, and ground into a pulp that is consistency of sauce. There are many ways to extract juice depending on the type of
fruit and they include squeezing, pressing, grinding, etc. A hydraulic press squeezes/pressed the pulp to extract juice, which flows into refrigerated tanks. The pressing operation can range from manual to mechanical with complete automated system common in the juice industry. A simple example of a flow chart for juice processing can be seen in Figure 1.

Figure 1.1: Flow chart of Juice Processing

Source: Qualitative Microbiological Risk Assessment of Unpasteurized Fruit Juice and Cider (Biljana et al. 2013).

However, it is well known fact that food serves as very good medium for growth of microorganisms especially when the principles of hygiene and sanitation are not met the food becomes contaminated by pathogens from humans or from the environment during production, processing or preparation. Pathogenic organisms can enter fruits through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting and processing. A pathogen that has become internalized within a fruit must be able to survive in the product until it reaches the consumer in order to become a public health hazard. Most fruit juices are sufficiently acidic to inhibit the growth of pathogenic organisms (Melbourne, 2005).

Studies conducted on the survival, or growth of microorganisms in produce and juices have shown a number of pathogenic organisms can be present and survive in a wide range of
fruits. There have been documented outbreaks of illness in humans associated with the consumption of unpasteurized fruit juices and fresh produce. Pathogens responsible for these outbreaks include *Salmonella* and verotoxin producing *E coli*. In 1995, unpasteurized fresh orange juice contaminated with *Salmonella* was linked to an outbreak in a Florida Theme Park, USA. More than 60 visitors were affected. In Australia, 427 confirmed cases of salmonellosis were reported in 1999 after the consumption of unpasteurized orange juice. A total of 48 cases of *E coli* O157 were reported after drinking unpasteurized apple (*Malus domestica*) juice in Washington DC in 199612. *L. monocytogenes* has also been identified as a pathogen that is of concern in relation to these products as the bacteria are present on the surfaces of raw fruits. Outbreaks of listeriosis have been associated with ready to eat raw foods such as, fruit salad, and fresh juices. In Victoria, little information is available on the microbiological quality and associated illness of freshly squeezed juices. This survey investigates the microbiological quality of freshly squeezed fruit juices and the processes and food handling practices from retail businesses that freshly squeeze juices on request across the state of Victoria, over a given period of time. Therefore, any results reported in this survey are an empirical evaluation over a given time period and geographic region. (Melbourne 2005)

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. Even in developed countries, an estimated one-third of the population is affected by microbiological food borne diseases each year (Andargie et al, 2008). There are reports of food borne illness associated with the consumption of fruit juices of several places of India and elsewhere (Sandeep et al., 2001). Most of the fruit juices being served had high microbial load. So that, these products could be the cause of health problems and potential vehicle of food borne outbreaks (Ketema et al, 2008). Contamination of fruit juices sold in restaurants, cafes and juice bars are sometimes unacceptable for human consumption and create significant health problems (Lewis et al, 2006).

In response to the increasing number of food borne illnesses, governments all over the world are intensifying their efforts to improve food safety (Sudershan et al, 2009). *Salmonella* has been reported to survive and grow rapidly on cut surfaces of watermelon (*Citrullus lanatus*)
held at room temperature and the levels of contamination remained unchanged when the melons were held at refrigerated temperatures.

Several studies have demonstrated the survival of microorganisms, including human pathogens, in various juices. *E. coli* O157:H7 has been found to survive in apple juice for up to 24 days at 4°C and orange juice for 24 days at refrigeration temperatures with very little decrease in numbers. Although it has been shown that pathogens can survive in orange juice, *Salmonella* and *Listeria* do not grow when the pH is below 4.4 (Biljana *et al.*, 2013).

Until recent decades, it was generally accepted that high acid fruit juices (pH 3.0-4.0) could not support survival and growth of microbial pathogens. However, a number of outbreaks of human illness that occurred during the 1990s were associated with the consumption of unpasteurized fruit juices. Although growth is unlikely at low pH, it is well documented that pathogenic microorganisms may survive in fruit juices, become adapted to the acid environment, and cause outbreaks of food borne illnesses. In addition, refrigerated storage can considerably extend the survival of the pathogens in juices. At warmer temperatures, such as room temperature, *Escherichia coli* O157:H7 and *Salmonella* populations will be reduced rapidly, compared to those in refrigerated acid food. With respect to fresh fruit juices, pasteurization is very effective in reducing the number of viable pathogens so they are unlikely to cause illness. However a considerable amount of fresh fruit juice is purchased and consumed in an unpasteurized state and is, therefore, of concern with respect to foodborne illness. The high-acid tolerances of some pathogens add to this concern since juice acidity was once thought to be a major inhibitory barrier (Biljana *et al.*, 2013).

Between 1974 and 2012, numerous illness outbreaks associated with unpasteurized fruit juice and cider have been reported worldwide, involving approximately 2,527 cases. These were caused by *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Cryptosporidium* spp., *Trypanosoma cruzi*, and *hepatitis A*. Ten of these outbreaks were associated with orange juice, 17 implicated apple juice, and 5 involved other types of fruit juice, such as watermelon, sugarcane (*Saccharum arundinaceum*), and guava (*Psidium guajava*) juice. In addition to the more commonly associated pathogens mentioned above, another emerging issue is that of orally-acquired Chagas’ disease in South America, which has been associated with the consumption of a variety of unpasteurized juices contaminated with the parasite *Trypanosoma*
The availability in Canada of the unpasteurized juices associated with these South American outbreaks (e.g., sugarcane juice, guava juice) is not clear, so while the risk to Canadians is likely very low, it cannot yet be accurately estimated (Biljana et al, 2013).

This study was undertaken in three secretarial divisions (Dehiwala, Rathmalana and Moratuwa) of Colombo district, Sri Lanka, due to high popularity and their high demand on fresh fruit juice consumption. Along these lines of evidences, a prompt assessment of juices was undertaken in this study to assess their safety and quality for the sake of the better management of public health in three secretarial divisions (Dehiwala, Rathmalana and Moratuwa) of Colombo district, Sri Lanka, with achieving following objectives,

- To evaluate Bacterial profile of locally prepared fresh fruit juices.
- To assess the hygienic conditions of processing and handling of locally prepared unpasteurized juices.
- To assess quality of locally prepared unpasteurized fresh juices in terms of basic hygienic problems associated with and in order to recommend remedial action for identified problems.
- To identify juice types those are more susceptible to contamination.
2.1. **Fruit juices**

Juice is defined in the most general sense as the extractable fluid contents of cells or tissues (Bates and Crandall, 2001). It is the aqueous liquid expressed or extracted from one or more fruits or vegetables, purees of the edible portions of one or more fruits or vegetables, or any concentrates of such liquid or puree (FDA, 2002).

2.2. **Unpasteurized Juice**

Unpasteurized juice/cider does not undergo treatment. Often it can be purchased as freshly pressed from local orchards, roadside stands, farmers markets, country fairs and juice bars. Unpasteurized juice/cider may also be found on ice or in refrigerated display cases and in produce sections at grocery stores (Health Canada, 2006).

2.3. **Pasteurized Juice**

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

2.4. **Hygiene Condition of Equipment and Preparation area**

Equipment should be made of stainless steel as it is easier to clean, sanitize and maintain than equipment made from other materials. All lubricants and surfaces coming into contact with foods should be made of food grade materials. Galvanized buckets, pipes or sheeting should not be used. Equipment that comes into contact with fruit juice/cider should not be made of a material that could lead to undesirable or unacceptable migration or leaching of chemicals into juice/cider, for example, brass equipment should not be used since the acidity of the juice/cider could leach the copper out of the brass (Canada food agency, 2001).
A research conducted in Ethiopia on microbial spoilage of market bulla and kotcho stated that when stored at room temperature in a loosely wrapped condition, both products resulted in undesirable odor, sticky consistency and dark coloration after 8 days. Drop in pH and a high degree of proliferation of aerobic mesophilic bacteria and molds were observed. Microorganisms active in starch hydrolysis, proteolysis and lipolysis were encountered in both products. The aerobic mesophilic (spoilage) bacterial flora was dominated by Micrococcus and Bacillus spp. About 33 percent of the products were lost due to such spoilage. Rural producers, vendors and urban consumers of bulla and kotcho use various methods to improve keeping quality. Wrapping the products with fresh enset 7 leaves and burying them in pits are the most frequently used method by rural producers. They can store the products from two to three months using this method. Urban consumers could store the products only for 2-3 weeks (Ashenafi et al, 1996). The conditions of preparation area are main concern for consumer’s health. 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’s are discarded nearby, providing nutrients for insects and rodents. Some of the juices are not efficiently protected against flies, which may carry food borne pathogens. Safe food storage temperatures are rarely applied to street vended juice. In addition, there are 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).

According to the study carried out in Amarawati city, India, it was identified that all samples were found contaminated and 77 bacterial pathogens were isolated. The highest contamination was recorded in juice vended at Rajapeth (14%) followed by Sai Nagar (13%), Nawathe plot and Camp area (12% each), Gandhi square (10%), Dasara Maidan and Rukmini Nagar (9% each), Maltekadi road (8%), Panchavati square ((6%) and S.T. Stand (4%). Among isolated organisms; E.coli (40%) was most dominant followed by Ps. aeruginosa (25%), Salmomella spp. (16%), Proteus spp. (9%), S. aureus (6%), Klebsiella spp. (3%) and Enterobacter spp. (1%). The maximum contamination of E.coli was recorded in the study, similar findings were also recorded by Subbannayya et al., (2007) in locally prepared fresh juices and indicating possible risk of infection involved with drinking of such juices. The main source of Escherichia coli contamination might be through contaminated water supplies. The utensil
washed by contaminated water or water that used for dilution of juices is contaminated then the outbreak of Escherichia coli may occur. The presence of Escherichia coli and other coliform bacteria could be due to inadequate hand washing by food workers and the absence of good manufacturing practices (Tambekar et al., 2007).

The study carried out in Mumbai City, India, identified that, freshly squeezed juices of sugarcane, lime and carrot showed occurrence of high microbial loads consisting of number of pathogens like coliforms, fecal coliforms, E.coli, S.aureus and Vibrio cholerae. Sugarcane juice followed by carrot juice showed high microbial counts consistent with pH values of 5.4 and 6.2 which do not affect the survival of pathogens adversely. In contrast, lime juice with pH 2.3 showed not only much lower total viable count ranging between log 0-8.2, but also showed absence of coagulase positive Staphylococcus aureus and Vibrio cholerae. A number of factors are responsible for contamination of freshly squeezed fruit juices. Most fruit contains bacterial counts of $1 \times 10^5$ cfu/cm$^2$ on their surface (Splittstosser 1979; Harrigan 1998; Al-Jedah et al., 2002). Improper washing of fruits adds these bacteria to juices leading to contamination. In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads. These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis et al., 2006).

2.5. Handling and Processing

Freshly squeezed fruit and vegetable juices have little or no process steps that reduce pathogen levels, if contaminated, such as no kill step. Freshly squeezed juices are simply prepared by extracting, usually by mechanical means, the liquid and pulp of mature fruit or vegetables. The final product is an unfermented, unclarified, untreated juice, ready for consumption. Most mechanical juicers use centrifugal force to separate the juice and residue (pulp) automatically. This makes it possible to prepare a variety of juices with minimal preparation. Fruits usually require some preparation before being fed into the juicer. Fruits such as mangos (Mangifera indica), oranges (Citrus sinensis), papaya (Carica papaya) and pineapple (Ananas comosus) will need prior preparation due to the bitterness of the skin or to remove large pips and stones. Once prepared, the fruits can be feed into the juicer. The juice will be extracted and any pulp will be simultaneously removed by centrifugal force and ejected via an
outlet pipe. During the process, contamination from raw materials, equipment or food handlers could be easily transferred to the final product. If pathogens such as *Salmonellae*, were present in freshly squeezed juices, individuals may be exposed (Melbourne, 2005).

Poor handling and processing of fresh fruit juices are some of the main cause of food associated illness to the community who live in developing countries. In most case a number of pathogenic organisms are isolated and identified from locally prepared fruit juices. According to study conducted in Dhaka, Bangladesh, the total viable count of samples ranged from $3.00 \times 10^2$ to $9.60 \times 10^8$. Out of 114 freshly prepared fruit juices samples collected, 113 samples (99%) showed the presence of coliform and *Escherichia coli*. The other bacteria like *B. cereus*, *Staphylococcus aureus*, *Salmonella*, *Streptococcus* were found in 64.91%, 6.14%, 7.89% and 5.26% of the tested samples, respectively. The number and type of microorganisms recovered from the freshly squeezed fruit juices made them unsafe for drinking. It was concluded that due to unhygienic fruit handling in the unsanitary environmental conditions under which the vendors operate the juices become contaminated with harmful bacteria. The results of this study demonstrate the unhygienic quality of popular types of market vended freshly squeezed fruit juices and their risk to the consumers (Shakir et al, 2009).

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

According to the study carried out in Amarawati City, India, The fruits, which were already peeled out for juice preparation showed more contamination (61%) than fruits peeled on time (39%). The highest bacterial contamination was observed in sweet lemon (35%), pineapple (29%), pomegranate (*Punica granatum*), apple (*Malus domestica*), orange each with (12%) and mix fruit with a strain of bacteria showed (100%) contamination. Sweet lemon (*Citrus limetta*) and pineapple juices were highly contaminated because it is maximally consumed
and already peeled out quite before the juice preparation. In both the juices *E. coli* (40%) and *P. aeruginosa* (22%) were dominant organisms. The occurrence of *P. aeruginosa* might be due to improper personal hygiene, unhygienic surroundings, vehicular transmission, and sewage. The presence of *S. aureus* (20%) in pineapple showed severe contamination through handling. Least contamination in apple and pomegranate juices was recorded as these fruits were peeled on time of juice preparation (Internet Journal of Food Safety, Vol.10, 2009, p. 72-76).

In 2003, the Eastern Region Food Surveillance Group (ERFSG), Victoria undertook a study to determine the microbiological status of freshly squeezed juices and assess the risk of such products to public health. All samples (n=29) submitted were assessed to be satisfactory against the Food Standards Australia New Zealand guidelines. The number of samples submitted as part of the ERFSG study was low and made interpretation difficult and unreliable. However, a number of potential food handling problems were identified from information gathered. The problems identified in the ERFSG study were similar to the ones identified in this study. For example, not washing fruit and vegetables prior to use, not using sanitizers in the cleaning process for utensils and chopping boards.

Although poor food handling practices were identified in both the ERFSG and this study and issues are raised regarding food safety knowledge, it is difficult to make any conclusions from study results. A number of studies have shown that knowledge of food handling in general is not optimal and food handlers have difficulty in understanding some food safety procedures. For example, a study in 2002 reported only 77% of food handlers understood that detergent does not kill microorganisms and in a repeat study in 2004 reported that only 66% of the food handlers understood that detergent does not kill microorganisms. The results from the study questionnaire showed a number of premises did not wash the fruit or vegetables prior to juicing. Although it is not necessary to wash fruit with sanitizer, businesses should wash them thoroughly with potable water before juicing. If heavily contaminated the fruit and vegetables should be subjected to a double wash. Washing should occur on all fruits even if the rind, skin or peel is to be removed before juicing. The removal process of the rind, skin or peel could result in cross contamination of the edible portion. The results from the study questionnaire indicated some proprietors did not clean and sanitize equipment and
utensils on a regular basis or did not understand how to do it correctly. Operators should understand effective cleaning and sanitizing procedures as equipment can contaminate juice during processing. Temperature control of the prepared fruit and vegetables is important as growth of pathogenic organisms can occur on cut surfaces. The survival and growth of pathogenic organisms is not only dependant on temperature but also on pH and the type of organic acid present in the fruit and vegetables. Although it has been shown pathogens can survive in high acid fruits, such as lemon and oranges it is unlikely that the pathogens numbers will increase in these environments. However, growth of pathogenic organisms has been shown to occur in medium to low acid produce such as melons (Victoria, 2005).

2.6. Quality of raw materials

The study carried out in Victoria, Australia identified that food handlers did not have a full understanding of food safety and some poor food handling practices were identified suggests that the quality of the raw fruits used in the juice samples has a significant factor in the microbiological status of the final product. While *E. coli* is an indicator of fecal contamination and low levels were detected in a number of samples analyzed in this study. The detection of *E. coli* in these foods is not unexpected as the raw materials or produce may be exposed to environmental contamination during maturation and harvesting. For example fecal organisms can enter fruits through damaged surfaces, such as punctures, wounds, cuts and splits. Although not assessed in this study the quality of the raw materials used should be of a good quality and premises should sort and discard any fruits or vegetables that are badly damaged or bruised. The removal of badly damaged or bruised fruits will decrease the risk of microbial transfer from the raw materials to equipment and the final product. Apart from the detection of Coliform species, in some of the juice samples *staphylococcus* species, were also detected. The microbiological results from this study and scientific studies suggest further investigation is required to assess the risk of consuming juices that have a pH greater than 4.5. The number of samples submitted against these juice types in this study was low and no conclusions can be made about the safety of these juice types (Victoria, 2005).

A number of studies from different countries have shown that microbial quality of ice manufactured for use to cool foods and drinks could be a cause of concern. The microbial safety of commercial ice used in drinks was evaluated by Lateef et al. (2006) in Nigeria and it was
found that microbial loads of these ice samples ranged from 1.88-3.20 X 10^4 cfu/ml which was largely above the recommended loads of more than 500 and 1000 cfu/ml for ice obtained from manufacturing plants and retail outlets respectively.

2.7. Contamination

In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised. Freshly squeezed fruit juices have little or no process steps that reduce pathogen levels, if contaminated (Victorian Government Department of Human Services 2005).

1. Fruit can become contaminated with pathogens during growth, harvesting, transportation and storage as well as during processing, packaging and distributing. If this occurs, the pathogens of concern can survive in the acidic juice for various times depending upon juice pH and temperature. If contaminated fruits are used, there is a greater chance that the final product will be contaminated with pathogens such as *Escherichia coli* O157:H7 and Salmonella spp. Juicing and further processing methods that are presently practiced, do not guarantee the absence of pathogens, should the raw juices be contaminated (Biljana et al., 2013).

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

Unpasteurized juice products can be contaminated with harmful bacteria such as *Salmonella* and *Escherichia coli*, viruses, and parasites like *Cryptosporidium*. Although fruits that are used to make juice do not naturally contain harmful bacteria, viruses or parasites, they can become contaminated in the farm environment, through handling, processing or transportation. Contaminated unpasteurized juice and cider can potentially pose a health risk to consumers (Health Canada, 2006).

2. Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during har-
vesting, handling and processing. Pathogenic organisms can enter fruits through damaged surfaces, such as punctures, wounds, cuts, and splits. This damage can occur during maturation or during harvesting and processing. A pathogen that has become internalised within a fruit must be able to survive in the product until it reaches the consumer in order to become a public health hazard. Most fruit juice is sufficiently acidic to inhibit the growth of pathogenic organisms. Studies conducted on the internalization, survival, or growth of microorganisms in produce and juices have shown a number of pathogenic organisms can be present and survive in a wide range of fruits. Several studies have demonstrated the survival of microorganisms, including human pathogens, in various juices. *Escherichia coli* O157:H7 has been found to survive in apple juice for up to 24 days at 4°C and orange juice for 24 days at refrigeration temperatures with very little decrease in numbers. Although it has been shown that pathogens can survive in orange juice, *Salmonella typhimurium* and *Listeria* do not grow when the pH is below 4.4 (Melbourne, 2005).

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

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

A research conducted in Ethiopia on microbial spoilage of market bulla and kotcho stated that when stored at room temperature in a loosely wrapped condition, both products resulted in undesirable odor, sticky consistency and dark coloration after 8 days. Drop in pH and a high degree of proliferation of aerobic mesophilic bacteria and molds were observed. Microorganisms active in starch hydrolysis, proteolysis and lipolysis were encountered in both products. The aerobic mesophilic (spoilage) bacterial flora was dominated by Micrococcus and Bacillus spp. About 33 percent of the products were lost due to such spoilage. Rural producers, vendors and urban consumers of bulla and kotcho use various methods to improve keeping quality. Wrapping the products with fresh enset 7 leaves and burying them in pits are the most frequently used method by rural producers. They can store the products from two to three months using this method. Urban consumers could store the products only for 2-3 weeks (Ashenafi et al., 1996).

According to the International journal of Qualitative Microbiological Risk Assessment of Unpasteurized Fruit Juice and Cider (Biljana et al., 2013), it was identified that likelihood of microbial growth is most concern in contamination. The ability of *Escherichia coli* O157:H7 or *Salmonella* spp. to survive on fruit surfaces during juicing and storage raises concerns about the way fruit and juice are handled. Contamination of the interior can occur through surface bruises, cuts or orifices. Because handling is unavoidable, the extent of microbial attachment will depend on the sanitation conditions in the manufacturing environment that reduce microbial build-up. However, the likelihood of actual growth upon intact fruit surfaces (peels) is minimal, provided procedures to process fruits after harvesting are immediate or storage conditions are adequate. For example, the decision to use drop-fruits (apples or oranges, for example) carry the risk of contamination by *Salmonella* spp., *Escherichia coli* and other pathogens, directly from raw or improperly composted manure, contaminated irrigation water, soil or contact with animals and insects. Therefore, drop-fruits tend to be processed immediately, and before any surface bacterial growth can occur. Damaged fruit surfaces have sometimes been shown to protect attached bacteria from washing and sanitizing operations. For example, the stem-scar area of ‘Valencia’ oranges can contain bacterial
loads that can be difficult to remove. This is due to the roughness of this area where organisms could be shielded by entrapped air, debris and plant surface structures. In addition, bruised and punctured surfaces of fruit can permit the entry and growth of bacteria. The blossom end of whole apples can allow the uptake of bacterial pathogens from wash waters into the outer core regions inside the fruit. However, research has shown that wash water is less likely to be drawn into the core area if the apple or orange is colder than the wash water. There is little information available on the survival of Cryptosporidium spp. oocysts specifically on fruits. A recent study found that C. parvum oocysts attached to apples can remain viable and possibly infectious during prolonged storage (i.e., 6 weeks of cold storage). It is generally considered that fruits such as berries with moist, irregular surfaces likely afford some protection to contaminating parasite cysts or oocysts from dessication. An important distinction to be made between Cryptosporidium spp. and bacterial pathogens is that the former does not grow outside the host, so no multiplication will take place on contaminated fruits regardless of the environmental conditions. Acidic fruit juices were generally believed to be unusual vehicles of transmission for human pathogens. Pathogenic organisms survive rather than grow in such adverse pH conditions. For example, Escherichia coli O157:H7 remains viable (without apparent proliferation) for extended periods in refrigerated apple cider (turbid, non fermented apple juice containing pulp). The pH of apple juice is typically between 3.3 and 4.1. Research conducted at the University of Tennessee shows that bacteria can survive in apple cider for up to 15 days at pH 4.1. In fact, Escherichia coli O157:H7 was shown to be very resistant to the low pH values of both apple cider and orange juice, when held at either 5 or 25°C. Growth of this pathogen actually occurred in one brand of apple cider with a pH of 3.98. Zhao et al., (1993) also found that Escherichia coli O157:H7 can survive at 8°C for up to 31 days in apple cider (pH 3.1 to 3.7), with no apparent growth. Eleftheriadou et al. (1998) reported that S. Typhimurium survived in apple juice of pH 3.6 for at least 30 days. Salmonella serotypes and Cryptosporidium spp. are also resistant to low pH. Although the average pH level of Florida orange juices is 3.7 (range: 3.4 to 4.0), they have been implicated in Salmonella outbreaks. The unpasteurized orange juice that was implicated in the Florida theme park outbreak of 1995 was less acidic than expected - a mean pH of 4.3. The associated salmonellae pathogens were able to survive in detectable numbers up to 27
days at pH 3.5, 46 days at pH 3.8, 60 days at pH 4.1 and 73 days at pH 4.4 (Biljana et al., 2013).

2.8. Water Supply

Water used in processing establishments must be potable unless it is used solely for fire protection, or auxiliary services and there must be no connection between the system for that water and the system for potable water. Potable water, hot and cold under pressure, should be provided (Canada food agency, 2001).

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

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

According to the study carried out in Mumbai city, India, it was identified that, ice samples obtained from vendors also showed high total viable count (log 5-8.5). 70% of the ice sam-
pies analyzed showed presence of Total coliforms, Total faecal coliforms and *Vibrio cholerae*. A number of studies from different countries have shown presence of *E.coli*, coliforms and a variety of microorganisms like *Streptococcus pyogenes*, *Streptococcus equi*, *Pseudomonas aeruginosa*, *Staphylococcus* spp, *Micrococcus* spp etc (Lateef et al., 2006; Moyer et al., 1993; Vieira et al., 1997; Nichols et al., 2000). This is an indication of unsanitary conditions, unhygienic practices during or after production and poor quality of source of water used. If the source water used is of poor quality, harmful microorganisms may persist in ice since the process of freezing cannot destroy them. When ice is thawed the surviving microorganisms though may be injured, tend to recover their viability so that when the ice melts into the juices, they may be able to survive these too (FEHD 2005).

### 2.9. Personnel

All workers must be free from communicable diseases. They should be trained not only for their task, but also to keep the vendors clean and to practice personal hygiene. Written requirements for personal hygiene should be available. Workers must have ready access to clean washrooms and proper hand washing (hot water and soap) facilities with disposable towels and closed trash containers. All persons must wash their hands upon entering food handling areas, before starting work, after handling contaminated materials, after breaks, and after using toilet facilities. Where necessary to minimize microbiological contamination, employees should use disinfectant hand dips. Washrooms must be segregated from production and storage areas. Employees having open cuts or wounds must not handle food or food contact surfaces unless the injury is completely protected by a secure waterproof covering (e.g. rubber gloves). All persons entering food handling areas should remove jewellery and other objects which may fall into or otherwise contaminate food. Protective clothing, hair covering, footwear and/or gloves, appropriate to the operation in which the employee is engaged should be worn and maintained in a sanitary manner (Canada food agency, 2001). Without personal hygiene of food handlers, safe processing of fruit juices alone has no value to improve the community health. Therefore, all rounded safety precautions should be applied by food handlers as well as during processing. According to study conducted in Gondar, Ethiopia Food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms (Andargie et al, 2008).
According to the study carried out in Amarawati City, India, Juices from the crowded sites were more contaminated (55%) than that of the less crowded places (45%). The juice collected in the evening showed more contamination (60%) while samples collected at morning showed less contamination (40%). The dominant organism found in the samples collected at evening was *Proteus* spp. (86%). It might be due to the overcrowding and more polluted environment or dust in the evening than in morning. Out of 77 microorganisms, 42 (61%) microorganisms were found in the monsoon and 35 (39%) were found in post monsoon period samples. The most dominating organism in monsoon season was *E. coli* (55%). The presence of *Escherichia coli* also gets reduced to 45% in post monsoon period. Presence of *Escherichia coli* may occur due to human sewage or contaminated water. In monsoon the fecal matters get mixed with water and causing contamination of *Escherichia coli* (Tambekar et al., 2008).

Where there is only one servant or only owner, the degree of contamination in the juices was high (47%) as compare to having two servants (32%) or three servants (21%). It might be due to, a single servant or owner, who is doing all the works right from peeling, preparation juices, cleaning of glasses and dishes and serving, while doing these work, he is not washing or cleaning his hands frequently and contaminate the prepared juice. Personal hygiene plays an important role in spread of infection. Poor personal hygiene of vendor showed (55%) contamination than fair (45%). The servants in shops with dirty clothing showed (55%) contamination in street vended juices (Table 3). Presence of *S. aureus* (60%) may be due to dirty clothing and contaminated hands of vendor indicating lack of knowledge of hygienic practices and safety of food products. Hygienic surrounding of vending site also plays an important role in contamination of juices. More contamination was observed in the juices that were at poor hygienic vending site (74%) than fair hygienic conditions (26%). Unhygienic surroundings like sewage, improper waste disposal system, inadequate water supply causes contamination of food. Also houseflies and fruit flies due to sewage may contaminate juices as juices attract the flies (Subbannayya et al., 2007).

### 2.10. Fruit Storage Practices

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

A research conducted in Ethiopia stated that Papaya and avocado (*Persea Americana*) juices had initial pH values of >5.7 and allowed all test strains to reach numbers >10^7 cfu/ml at ambient temperature holding. At refrigeration temperatures, at least no elimination was observed. In pineapple juice (pH 3.8), the *Escherichia coli* test strains were eliminated at both holding temperatures within 16 h whereas slight increase in counts of *Salmonella* test strains was observed at ambient temperature holding. Orange juice (pH 3.1) did not allow the survival or growth of the test organisms at both holding temperatures (Yigeremu *et al.*, 2001).

### 2.11. Outbreaks associated with unpasteurized fruit juices

Three outbreaks of illness from *Escherichia coli* O157:H7 in the United States in 1996 were linked to unpasteurized juice/cider. These incidents proved that harmful bacteria can survive in high acid products such as juice or cider, if contaminated. Until recently, scientists did not think this was possible. In the fall of 1998 in Ontario, 14 cases of food-borne illness including seven cases of confirmed *Escherichia coli* O157:H7, were reported. Unpasteurized juice/cider was suspected in these cases. Local health officials identified one batch of 10 unpasteurized non-commercial, custom-pressed apple cider as the most likely source (Health Canada, 2006).

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

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

In response to the increasing number of food borne illnesses, governments all over the world are intensifying their efforts to improve food safety (Sudershan et al, 2009). Salmonella has been reported to survive and grow rapidly on cut surfaces of cantaloupe, watermelon, and honeydew melon held at room temperature and the levels of contamination remained unchanged when the melons were held at refrigerated temperatures.

Several studies have demonstrated the survival of microorganisms, including human pathogens, in various juices. E coli O157:H7 has been found to survive in apple juice for up to 24 days at 4°C and orange juice for 24 days at refrigeration temperatures with very little decrease in numbers. Although it has been shown that pathogens can survive in orange juice, Salmonella and Listeria do not grow when the pH is below 4.4 (Biljana et al, 2013).

Until recent decades, it was generally accepted that high acid fruit juices (pH 3.0-4.0) could not support survival and growth of microbial pathogens. However, a number of outbreaks of human illness that occurred during the 1990s were associated with the consumption of unpasteurized fruit juices. Although growth is unlikely at low pH, it is well documented that pathogenic microorganisms may survive in fruit juices, become adapted to the acid environment, and cause outbreaks of food borne illnesses. In addition, refrigerated storage can considerably extend the survival of the pathogens in juices. At warmer temperatures, such as room temperature, Escherichia coli O157:H7 and Salmonella populations will be reduced rapidly, compared to those in refrigerated acid food. With respect to fresh fruit juices, pasteurization is very effective in reducing the number of viable pathogens so they are unlikely to cause illness. However a considerable amount of fresh fruit juice is purchased and consumed in an unpasteurized state and is, therefore, of concern with respect to foodborne illness. The high-acid tolerances of some pathogens add to this concern since juice acidity was once thought to be a major inhibitory barrier (Biljana et al, 2013).
Between 1974 and 2012, numerous illness outbreaks associated with unpasteurized fruit juice and cider have been reported worldwide, involving approximately 2,527 cases (Table 1). These were caused by *Escherichia coli* O157:H7, *Salmonella* species., *Shigella* species., *Cryptosporidium* species., *Trypanosoma cruzi*, and *hepatitis A*. Ten of these outbreaks were associated with orange juice, 17 implicated apple juice, and 5 involved other types of fruit juice, such as watermelon, sugarcane and guava juice. In addition to the more commonly associated pathogens mentioned above, another emerging issue is that of orally-acquired Chagas’ disease in South America, which has been associated with the consumption of a variety of unpasteurized juices contaminated with the parasite *Trypanosoma cruzi*. The availability in Canada of the unpasteurized juices associated with these South American outbreaks (e.g., acai juice, sugarcane juice, guava juice) is not clear, so while the risk to Canadians is likely very low, it cannot yet be accurately estimated (Biljana et al, 2013).

### Table 2.1: Food-borne outbreaks traced to unpasteurized fruit juice and cider (1974 – 2012).

<table>
<thead>
<tr>
<th>Year</th>
<th>Pathogen</th>
<th>Number of cases</th>
<th>Vehicle</th>
<th>Location</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1974</td>
<td><em>Salmonella typhimurium</em></td>
<td>296</td>
<td>Apple cider</td>
<td>New Jersey, USA</td>
<td>Manure used as fertilizer; drop apples</td>
</tr>
<tr>
<td>1980</td>
<td>Most likely <em>Escherichia coli</em> O157:H7</td>
<td>14</td>
<td>Apple cider</td>
<td>Toronto, Ontario, Canada</td>
<td>Not reported</td>
</tr>
<tr>
<td>1991</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>23</td>
<td>Apple</td>
<td>Massachusetts, USA</td>
<td>Drop apples; no washing; cattle raised</td>
</tr>
<tr>
<td>Year</td>
<td>Pathogen</td>
<td>Count</td>
<td>Product</td>
<td>Location</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>--------------------------</td>
<td>-------</td>
<td>------------------</td>
<td>------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1992</td>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
<td>6</td>
<td>Orange juice</td>
<td>India</td>
<td>Roadside vendors selling fresh squeezed juice</td>
</tr>
<tr>
<td>1993</td>
<td><em>Cryptosporidium</em> Species</td>
<td>160</td>
<td>Apple cider</td>
<td>Maine, USA</td>
<td>Drop apples</td>
</tr>
<tr>
<td>1993</td>
<td><em>Salmonella</em> species</td>
<td>18</td>
<td>Watermelon juice</td>
<td>Florida, USA</td>
<td>Home-made watermelon juice</td>
</tr>
<tr>
<td>1995</td>
<td><em>S. harford, Gaminara and Rubislaw</em></td>
<td>63</td>
<td>Orange juice</td>
<td>Florida theme park, USA</td>
<td>Local processing plant producing for a large Florida theme park; inadequately sanitized processing equipment; unclean facility</td>
</tr>
<tr>
<td>1995</td>
<td><em>Shigella flexneri</em></td>
<td>14</td>
<td>Orange Juice</td>
<td>South Africa</td>
<td>Contamination of the hands of staff squeezing the oranges to make juice</td>
</tr>
<tr>
<td>1996</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>14</td>
<td>Apple cider</td>
<td>Connecticut, USA</td>
<td>Drop apples</td>
</tr>
<tr>
<td>1996</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>6</td>
<td>Apple cider</td>
<td>Washington State, USA</td>
<td>Made for local church event from local orchard; apples were washed in a chlorine solution</td>
</tr>
<tr>
<td>1996</td>
<td><em>C. parvum</em></td>
<td>20 confirmed, 11</td>
<td>Apple cider</td>
<td>New York, USA</td>
<td>Drop apples; orchard adjacent to dairy farm.</td>
</tr>
<tr>
<td>Year</td>
<td>Pathogen</td>
<td>Suspected</td>
<td>Source</td>
<td>Additional Details</td>
<td></td>
</tr>
<tr>
<td>------</td>
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<td>-----------</td>
<td>---------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>70</td>
<td>Apple juice</td>
<td>Drop apples; improper use of sanitizers; deer and cattle in close proximity; distribution through fresh juices, shakers and energy bars.</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>6</td>
<td>Apple cider</td>
<td>All cases visited a local apple orchard and cider pressing operation</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>14</td>
<td>Apple cider</td>
<td>Total of 4 trees; some in a cattle pasture; drop apples used; apples not washed; distribution to family and friends from 2 local farms</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td><em>S. typhimurium</em></td>
<td>500</td>
<td>Orange juice</td>
<td>Oranges were source of contamination</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td><em>S. muenchen</em></td>
<td>200</td>
<td>Orange juice</td>
<td>Juice distributed as both frozen and liquid; for commercial use in restaurants and hotels; products include ‘smoothies’; detected in samples taken from blenders and dispensers.</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Pathogen</td>
<td>Number</td>
<td>Product</td>
<td>Location</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>----------------</td>
<td>--------</td>
<td>-----------</td>
<td>---------------------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1999</td>
<td>S. anatum</td>
<td>4</td>
<td>Orange juice</td>
<td>Sarasota County, Florida, USA</td>
<td>Contamination most likely occurred during the manufacturing process</td>
</tr>
<tr>
<td>1999</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>7</td>
<td>Apple cider</td>
<td>Tulsa, Oklahoma, USA</td>
<td>Contamination most likely occurred at apple orchard or cider pressing operation</td>
</tr>
<tr>
<td>1999</td>
<td>S. typhimurium</td>
<td>16</td>
<td>Mamey frozen puree</td>
<td>Florida, USA</td>
<td>Import from Guatemala and Honduras</td>
</tr>
<tr>
<td>2000</td>
<td>Salmonella species.</td>
<td>14</td>
<td>Orange Juice</td>
<td>Colorado, California, Nevada, USA</td>
<td>Unpasteurized citrus products produced by juice company in California</td>
</tr>
<tr>
<td>2003</td>
<td>C. parvum</td>
<td>144</td>
<td>Apple cider</td>
<td>Ohio, USA</td>
<td>Ozone treatment was insufficient to inactivate pathogens</td>
</tr>
<tr>
<td>2004</td>
<td><em>Escherichia coli</em> O111 and C. parvum</td>
<td>213</td>
<td>Apple cider</td>
<td>New York, USA</td>
<td>Retail establishment</td>
</tr>
<tr>
<td>2004</td>
<td>Hepatitis A</td>
<td>351</td>
<td>Orange juice</td>
<td>Egypt</td>
<td>Juice contaminated during manufacturing</td>
</tr>
<tr>
<td>2005</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>4</td>
<td>Apple cider</td>
<td>Ontario, Canada</td>
<td>Juice produced and sold at a small local retail outlet</td>
</tr>
<tr>
<td>2005</td>
<td>Trypanosoma cruzi</td>
<td>25</td>
<td>Sugarcane</td>
<td>Brazil</td>
<td>Juice sold at a roadside kiosk; infected triatomine bugs and opos-</td>
</tr>
<tr>
<td>Year</td>
<td>Pathogen</td>
<td>Cases</td>
<td>Juice Type</td>
<td>Location</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>-------</td>
<td>------------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>2005</td>
<td><em>T. cruzi</em></td>
<td>27</td>
<td>Açaí juice</td>
<td>Brazil</td>
<td>All cases consumed juice from a single sales outlet.</td>
</tr>
<tr>
<td>2005</td>
<td><em>S. typhimurium</em> and <em>Saintpaul</em></td>
<td>157</td>
<td>Orange juice</td>
<td>Multistate, USA</td>
<td>'Fresh-squeezed' orange juice; outbreak was identified in 24 states.</td>
</tr>
<tr>
<td>2007</td>
<td><em>T. cruzi</em></td>
<td>103</td>
<td>Guava juice</td>
<td>Venezuela</td>
<td>Outbreak occurred at a school in Caracas; juice may have become contaminated with triatomine bugs during overnight storage outside.</td>
</tr>
<tr>
<td>2007</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>9</td>
<td>Apple cider</td>
<td>Massachusetts, USA</td>
<td>Not reported</td>
</tr>
<tr>
<td>2008</td>
<td><em>S. panama</em></td>
<td>15</td>
<td>Orange juices</td>
<td>The Netherlands</td>
<td>The causative Salmonella strain was able to survive under low pH conditions, such as those in the human stomach</td>
</tr>
<tr>
<td>2008</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>7</td>
<td>Apple cider</td>
<td>Iowa, USA</td>
<td>Fair, festival; cider purchased from a temporary booth.</td>
</tr>
<tr>
<td>2010</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>7</td>
<td>Apple cider</td>
<td>Maryland, USA</td>
<td>Retail establishment</td>
</tr>
</tbody>
</table>
2.12. Incidence of organism

Even in developed countries, an estimated one-third of the population is affected by microbiological foodborne diseases each year. According to a study conducted across Victoria, Australia, collected 291 juice samples between March 2004 and May 2004 from retail businesses. All samples submitted were analyzed for *Salmonella* spp, *Escherichia coli*, *Listeria monocytogenes* and coagulase positive staphylococci; sample pH was also determined. *Salmonella* was not detected in any juice samples. However, *Escherichia coli* was detected in seven juice samples, two of which had levels greater than 100 cfu/ml. *Listeria* spp. were detected in nine juice samples; *L. monocytogenes* was detected in one of these at a level of 25,000 cfu/ml and was assessed as being potentially hazardous. All juice samples analyzed for coagulase positive staphylococci contained less than 100 cfu/ml and were assessed as satisfactory. Overall, the microbiological quality of the juice samples submitted in this study was good despite the one sample that was assessed as being potentially hazardous (Melbourne, 2005).

A study conducted in Pakistan stated that microbiological quality of all the products was well outside the Gulf Standards for fruit juices, and coliform counts usually exceeded 1,000 cfu/ml. In one sample of mixed fruit juice, the coliform count was above $1.0 \times 10^6$ cfu/ml, and both *Escherichia coli* and *Enterococcus faecalis* ($1.0 \times 10^7$ cfu/ml) were detected. It is concluded that, while the practice of consuming fresh fruit juices with meals should be encouraged on nutritional grounds, steps must be taken to improve the microbial quality of the products (Al-Jedah et al., 2002).

According to a research conducted in Ethiopia, the total viable bacterial count of fresh cassava before cleaning ranged from $8.7 \times 10^4$ to $2.1 \times 10^9$ c.f.u/ml, whereas, in thoroughly cleaned product it was reduced to $10^6$ c.f.u/ml. Enterobacteriaceae and spore former bacteria mean count of 104 and 103, respectively. The dominant bacteria group within mesophilic microflora were *Actinobacter* spp (29.1%), *Micrococcus* spp (17.4%) and *Enterobacteriaceae* (16%). Bacterial spores, *pseudomonas, moraxella* and *aeromonas* spp. Were detected in small proportion. Fate of *staphylococcus aureus, Bacillus cerus* and *Listeria monocytogenes* in cassava
juice was also evaluated. Except the *Bacillus cereus* the growth of bacterial strains was retarded at higher concentration (Desse *et al.*, 2001).

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

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

As per the Risk assessment study carried out in Canada, it was identified that, the microbial hazards associated with unpasteurized Locally prepared fruit juices. According to them the identified hazards are described as follows,

3. *Escherichia coli*

*Escherichia coli* classes considered of most concern with respect to unpasteurized juices, especially apple belong to enterohemorrhagic *Escherichia coli* (EHEC) (e.g., the *Escherichia coli* O157:H7 serotype), and enterotoxigenic *Escherichia coli* (ETEC). These cause distinct syndromes of diarrheal disease in humans. Animals are the primary asymptomatic reservoir for *Escherichia coli* O157:H7. Cattle, in particular, serve as important reservoirs for the organism. Drop apples can become contaminated by coming into contact with manure from infected animals. In an outbreak of *Escherichia coli* O157:H7 infections in Massachusetts, USA in 1991, a cider press operator also raised cattle, which grazed in a field adjacent to the mill. The presence of animals near a cider mill can result in manure inadvertently contacting
apples, equipment, or workers' hands. In addition, apples can become contaminated if transported or stored in areas that contain manure, or if rinsed with contaminated water. In another outbreak in Ontario, Canada in 1998, apple cider was most likely contaminated by the use of apples collected from the ground. In this case, a farmer kept his heifers in the orchard, but only until late July (i.e., a few weeks before apple harvest). However, studies have shown that Escherichia coli can survive in soil for > 20 weeks. Run-off from the nearby sheep pasture on a nearby farm could also have been a source of contamination. Another consideration is that Escherichia coli O157:H7 is acid-tolerant and can survive at low pH for up to 4 weeks. Therefore, transmission is possible through contaminated apple cider or juice, which has typical pH values of between 3 and 4. In addition to the food borne route, the transmission of Escherichia coli O157:H7 through contaminated water supplies, the person-to-person route, and direct animal-to-human transmission, have all been documented. ETEC is not considered a serious food-borne disease hazard in countries having high sanitary standards and practices. Contamination of water by human sewage can, however, lead to contamination of foods. Infected food handlers may also contaminate foods (Biljana et al, 2013).

The Canadian Public Health Agency reports, published in 2009 and 2010, established that at least 85% of the pathogenic Escherichia coli isolates from human cases were of serovar O157, while less than 15% were non-O157. Non-O157 Escherichia coli infections are likely being underreported in Canada due to inadequate laboratory surveillance. A decrease in vertoxigenic Escherichia coli cases was noticed between 2004 and 2009. In Canada, the incidence of pathogenic Escherichia coli shows a distinct seasonal trend, with increased rates in the summer and fall. Although most provincial rates were stable between 2000 and 2004, a slight decrease was noticed in the Eastern provinces, including Ontario. Of the three most frequently reported notifiable enteric pathogens, E.coli is third following Campylobacter spp. and Salmonella spp. Nevertheless, pathogenic Escherichia coli cases showed the highest hospitalization rate, while Salmonella infections resulted in the largest number of deaths overall. Of the reported cases in Canada, there is a strong indication that foods of animal origin are an important source of Escherichia coli O157:H7 infections. Household settings represent the largest number of reported outbreaks, while community settings resulted in higher outbreak related case counts. Several outbreaks occurred in non-residential institutions including daycare settings. About 10% of patients with hemorrhagic colitis develop hemolyt-
ic uremic syndrome (HUS), characterized by acute renal failure, hemolytic anemia and thrombocytopenia. The disease can lead to permanent loss of kidney function. On average, 2-7% of HUS cases are fatal, but the mortality rate in the elderly can be as high as 50%. All people are believed to be susceptible to hemorrhagic colitis, but the young children, elderly and immune compromised are more sensitive. Infants in underdeveloped countries, and travelers to these regions, are most at risk of infection with ETEC. At least 25 foodborne outbreaks of ETEC have been documented in the U.S. between 1998 and 2011, but none of them appear to be linked to juice consumption.

4. Cryptosporidium spp.

Cryptosporidium is a single-celled protozoan and an obligate intracellular parasite. There are numerous species and genotypes of Cryptosporidium found in a large number of different hosts, including humans, worldwide. The infectious stage of the organism, known as oocysts, are 4-5 micrometers in diameter and are shed with the hosts faeces. Cryptosporidium spp. oocysts are more resistant than bacteria to most chemical disinfectants such as chlorine, but are susceptible to drying. Currently, no data are available to support oocyst reduction from washing fruits and vegetables. A relatively small number of oocysts can cause illness characterized by watery diarrhea, abdominal pain, nausea, vomiting, fever and other symptoms. These symptoms are self-limiting and generally last 1-2 weeks. Severe and chronic symptoms of cryptosporidiosis are reported among immunocompromised individuals, and are potentially life-threatening. Transmission of cryptosporidiosis is facilitated by the ability of oocysts to survive for weeks to months in the environment. Routes of transmission include ingestion of oocysts in drinking or recreational water, direct person-to-person (e.g., daycares, institutionalized settings), and zoonoses, particularly through direct contact with cattle and other livestock. Foodborne outbreaks have also been reported. Fresh produce, in particular, may become contaminated pre-harvest (e.g., contaminated irrigation/washing water, infected farm workers, use of manure as fertilizer, contaminated equipment), or postharvest (e.g., packaging, storage, transport, food-handlers and consumers).

The relative frequency of the disease in the North American population is reported to be approximately 2%. Serological studies indicate that 80% of the population has been exposed to
Cryptosporidium. The very young and elderly may be at a higher risk of disease as a result of Cryptosporidium infection. Recently, it was estimated that 8% of domestically acquired cases of cryptosporidiosis in the U.S. are foodborne. (Biljana et al, 2013).

5. *Salmonella*

The antigenic scheme for classifying salmonellae recognizes more than 2300 serovars and, while all can be considered human pathogens, only about 200 are associated with human illness. Animal husbandry practices used in the poultry, meat and fish industries, and the recycling of offal and inedible raw materials into animal feeds, has favored the continued prominence of *Salmonella* in the global food chain (D.Aoust, 1997). There are reports of human salmonellosis linked to cantaloupe (Ries et al., 1990) and sprouts produced from alfalfa seeds (Mahon et al., 1996) imported to the United States. Hygienic conditions during the production, harvesting, transport and distribution of raw fruits and vegetables from some countries may not always meet minimum hygienic requirements, thus facilitating contamination on arrival in another country. Application of night soil, untreated sewage sludge or effluents, or irrigation water containing untreated sewage to fields and gardens can result in contamination of fruits and vegetables with *Salmonella* and other pathogens. Washing fruits and vegetables with contaminated water and handling of produce by infected workers, vendors and consumers in the marketplace helps the spread of pathogenic microorganisms, including *Salmonella*. Salmonellae have been isolated from many types of raw fruits and vegetables (Beuchat, 1996b; Wells and Butterfield, 1997). Outbreaks of salmonellosis have been linked to a diversity of fruits and vegetables, including tomatoes (Centers for Disease Control and Prevention, 1993; Hedberg et al., 1993; Wood et al., 1991), bean sprouts (Mahon et al., 1996; O.Mahony et al., 1990; Van Venedey et al., 1996), melons (Blostein, 1991; Centers for Disease Control and Prevention, 1979;1991; Gaylor et al., 1955; Ries et al., 1990), unpasteurized orange juice (Cook et al., 1990) and apple juice (Centers for Disease Control and Prevention, 1975). The pathogen can grow on the surface of alfalfa sprouts (Jaquette et al., 1996), tomatoes (Zhuang et al., 1995) and perhaps on other mature raw fruits and vegetables, making it imperative to use hygienic practices when handling them (WHO/FSF/FOS/98.2).
6. *Staphylococcus aureus*

*Staphylococcus aureus* is known to be carried in the nasal passages of healthy food handlers and has been detected on raw produce (Abdelnoor *et al*., 1983) and ready-to-eat vegetable salads (Houang *et al*., 1991). However, enterotoxigenic *S. aureus* does not compete well with other microorganisms normally present on raw fruits and vegetables, so spoilage caused by nonpathogenic microflora would probably precede the development of the high populations of this pathogen that would be needed for production of staphylococcal enterotoxin (Biljana *et al*., 2013).

### 2.13. Indicator Organism

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

### 2.14. Colony Count

One of the methods for counting of viable bacteria in any fluid is viable colony count by diluting the fluid and culturing for bacteria. Counts of viable bacteria are commonly based on the number of colonies that develop in nutrient agar plates which have been inoculated with known amounts of diluted foods and then incubated under prescribed environmental conditions. Only those bacteria, which will grow under the chosen environmental conditions, can be counted. A wide variety of conditions can be obtained by changing the composition of the growth (agar) medium, the gaseous environment of incubation (presence or absence of O2) and the time and temperature of incubation. The aerobic mesophilic count is most commonly used (Roberts *et al*., 2003).
CHAPTER 03 - METHODS AND MATERIALS

3.1. Study Design
A cross-sectional study design was applied to evaluate the bacteriological profile of locally prepared fresh fruit juices in Dehiwela, Rathmalana and Moratuwa secretarial divisions of Colombo district, Sri Lanka.

3.2. Study Area and Period
The study was conducted in three secretarial divisions (Dehiwala, Rathmalana and Moratuwa) of Colombo district, Sri Lanka from December 2012 to June 2013. In these areas there are many Restaurants, Cafeteria and juice bars that prepare unpasteurized fruit juices that can be consumed by visitors and people of the town. As highly populated and having high demand on locally prepared unpasteurized fruit juices, these three divisions created clear picture on problems associated with quality and safety of the locally prepared fruit juices.

3.3. Source of samples
Restaurants, Cafeteria and Juice Bars that prepare unpasteurized fruit juices found in Dehiwela, Rathmalana and Moratuwa secretarial divisions of Colombo district, Sri Lanka.

3.4. Sample Description
1. Unpasteurized Orange (Mangifera indica) juices
2. Unpasteurized Papaya (Carica papaya) juices
3. Unpasteurized Pineapple (Ananas comosus) juices
4. Unpasteurized Mango (Mangifera indica) juices

3.5. Sampling Technique
Stratified Sampling Method was used as sampling technique.
Total target population into different subgroups, and then randomly selected the final samples proportionally from the different subgroup.

3.6. Sample Size:
Total of one hundred twenty (120) fruit Juice samples of four types from 30 sites including restaurants, cafes and juice bars were collected.
Juice types collected;
Orange (*Citrus sinensis*) Juice.
Papaya (*Carica papaya*) Juice.
Pineapple (*Ananas comosus*) Juice.
Mango(*Mangifera indica*) Juice.

3.7. Eligibility or Inclusion and exclusion criteria
In this study unpasteurized fruit juices were included and pasteurized juices were excluded.

3.8. Variables
a. Dependent Variable
   Quality of locally prepared unpasteurized fruit juices
b. Independent Variable
   Personal hygiene
   Environmental hygiene
   Water quality
   Educational status of juicer
   Health status of juicer
   Storage environment of fruits and juices

3.9. Data collection
Two basic data collection methods were used for study.
3.9.1. Questionnaire:

A questionnaire was used to obtain information on the demographic characteristics of the fruit juicers and servers, as well as, source of fruit, storage condition of fruits, preparation details of fruits prior to process and processing of fruit juices. All the personnel involved in the processing of the fruit juices in the selected restaurants cafés and Juice bars were included and type of the vendor was determined.

3.9.2. Laboratory Procedure:

3.9.2.1. Collection of samples:

From December 2012 to June 2013, a total of one hundred twenty (120) Samples of four types (Avocado, Papaya, Mango and Pineapple) of locally prepared unpasteurized fruit juices were collected randomly from Dehiwala, Rathmalana and Moratuwa Secretarial Divisions of Colombo District. All the samples were collected on a voluntary basis from participating restaurant cafés and juice bars in wide mouth sterile (250 ml) containers aseptically, labeled and immediately transported to the laboratory in an ice-box where they were processed within an hour from sampling time.

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

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

3.9.2.2. Sample processing:

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

3.9.2.3. Culture:

**Total viable count (TVC)**
Plate count method was used to get total viable count of each sample. The method used here was Aerobic plate count in food ISO 4833:2003 (Appendix B – part 1).

Plates containing colonies between 30 – 300 at two consecutive dilutions were used to calculate the results. The results were recorded as cfu/g or ml of test sample.

**Total coliform count (TCC):**
3- Tube Most Probable Number (MPN) method was used for Total Coliform Count Test. The method used was ISO 4831:2006 (Appendix B – part 2), Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms - Most probable number technique. With reference to MPN table, the results were reported as MPN/g.

**Staphylococal Count (SCC):**
Direct Plate count technique was used in Staphylococcal Count test. ISO 6888:1999, Staphylococcal in food (Appendix B – part 3) method was used. Plates containing fewer than 300 colonies at two consecutive dilutions were used to calculate the results. The results were recorded as cfu/g or ml of test sample.

**Detection of Salmonella:**
ISO 6579:2002, Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp. (Appendix B – part 4) method was used to detect salmonella in each sample. The results were recorded as cfu / 25g or ml of test sample.

**Water analysis:**
Water analysis for fecal coliform was done using 3MTM PetrifilmTM Coliform Count Plates.
Qualitative analysis was performed to confirm presence of fecal coliforms. Procedure used as per the method of AFNOR validated method 3M 01/2 -09/89C (all food types). Product details of this analysis are given in Appendix B – part 5.

Swab Analysis:
Swab analysis was done for fruit juice contacting surfaces like blenders/ juices and utensils. ISO 18593:2004(E), Microbiology of food and animal feeding stuffs - Horizontal methods for sampling techniques from surfaces using contact plates and swabs (Appendix B – part 6), was used as the method for analysis. Results were recorder as cfu/cm².

3.9.2.4. pH Measurement:

pH of all undiluted samples was measured by pH meter immediately using digital pH meter (Nig 333, Naina Solaris LTD, India) after collection. It is important to determine the pH of the food sample before undertaking microbiological examination as this can influence the colony count and organisms sought. In general, in foods with a pH below 4.5 pathogens would not be expected to survive.

3.9.2.5. Quality Control:
The Quality of the study was kept by training the data collector, preparing, and using standard operational procedures for laboratory investigation and media preparation. Structured Questionnaire was tested using pretest before conducting the study. Sample collection and processing were carried out using aseptic techniques. The samples were labeled properly. Culture and bacterial colony count were determined by experienced laboratory personnel. The performance and sterility test of prepared media were checked by incubating (Memmert IF30) at 37°C and 44.5°C and inoculating with control strain organisms, respectively.

3.10. Data analysis:

After completion of data collection, each measurement of different variables was recorded according to the work flow. Data entry and analysis was done using MINI TAB version 16.0 software. ANOVA was used to compare results among juices type and compared with the previous findings from the literature.
CHAPTER 04 - RESULTS AND DISCUSSION

4.1. Results obtained from Questionnaire

Data was collected from 30 food premises on juice processes, sample storage and sample preparation using a set questionnaire (appendix A). In general, when questioned most food handlers understood what was required to produce and handle freshly squeezed juices safely. Even when poor practices were identified the microbiological results of the samples taken from these premises did not appear to reflect these practices. The information collected from the one sample that was assessed as being potentially hazardous was prepared and processed in a reasonable manner when compared to other premises assessed in the study. Two other samples analyzed from the same premise were assessed as being satisfactory.

All the thirty fruit juice makers interviewed were males and [60% (n=18/30)] of them were younger than 30 years. Although [83.3% (n=25/30)] of them had completed or were attending high school education, none of the fruit juice makers had any exposure to professional training related to their current career.

4.1.1. Cleaning and Sanitizing Practices

In terms of cleaning and sanitizing practices, overall only [6.7% (n=8/120)] of the samples analyzed were deemed unsatisfactory and therefore, it is difficult to identify a relationship between handling practices and microbiological quality. [60% (n=18/30)] of the premises had a cleaning and sanitizing process for juicers documented in the premises food safety program.

4.1.2. Cleaning and sanitizing frequency for juicer/blender.

The cleaning and sanitizing frequency of juicers was reflected by premise type. Restaurants generally cleaned and sanitized juicers/blenders on a more frequent basis when compared to cafes or juice bars. Restaurants, when questioned, cleaned the juicers/blenders at a frequency greater than once a day [(80%) n=8/10)] with the majority of the juice bars cleaning the juicers/blenders between 2-3 times per day or after each use. In mixed handling premises the cleaning frequency decreased when compared to juice bars. For example, Cafés only [30% (n=3/10)] cleaned the juicers more than once per day. The cleaning frequency of juicers may
reflect the number of juices being sold from the premises rather than the actual necessity to clean the juicer. While cleaning frequency differed by premise type there was no significant difference between premise types when questioned on how often they dismantled and sanitized the juicer. In total, 93.3% (n=28/30) of the premises dismantled and sanitized the juicer at least once per day or more than once per day. The remaining 2 premises only dismantled and sanitized the juicer 2 or 3 times a week. Figure 02, 03 and 04 depicts the frequencies of juice/blender cleaning and sanitizing of cafes, restaurants and juice bars respectively.

Figure 4.1: Frequency of Juicer/blender cleaning and Sanitizing - Cafes

Figure 4.2: Frequency of Juicer/blender cleaning and Sanitizing – Restaurants
4.1.3. Cleaning and sanitizing frequency for utensils

The frequency for cleaning utensils, including chopping boards, knives, spoons, peelers across all premise types related to the frequency of use and the majority of premises cleaned utensils after each use [73.3% (n=22/30)] or more than once per day [20% (n=6/30)]. Two premises [6.7% (n=2/30)] stated that they only cleaned their utensils once per day. When premises were asked how often they sanitized utensils [93.3% (n=28/30)] of premises did so only once per day (figure 05). One premises only sanitized the utensils twice per week and one premises only sanitized the utensils once a month. Two samples were submitted and analyzed from this premises and were both assessed as being satisfactory.

Figure 4.3: Frequency of Juicer/blender cleaning and Sanitizing – Juice Bars

Figure 4.4: Cleaning and sanitizing frequency for utensils
4.1.4. Understanding of cleaning and sanitizing practices

All premises were asked to describe the cleaning and sanitizing procedure for juicers and utensils. All premises (n=30) responded to the question with varying degree of detail. Premises 60% (n=18/30) used sanitizers when cleaning and sanitizing utensils. Most of the premises that sanitized used Chlorine, tri-sodium phosphate, quaternary ammonium compounds and household brand sanitizers. A small number of premises [20% (n=6/30)] used dishwashers as part of the cleaning and sanitizing procedure (figure 06). The remaining premises [20% (n=6/30)] cleaned with water and detergent/soap but did not sanitize. The cleaning and sanitizing procedure of the dismountable parts of the juicer mirrored the procedures described by the premises for the cleaning and sanitizing of utensils.

![Procedures used for cleaning & sanitizing](chart)

Figure 4.5: Understanding of cleaning and sanitizing practices

4.1.5. Preparation methods

When questioned on sample preparation prior to juicing the majority of premises appeared to prepare the fruit prior to juicing in a safe manner. Premises were asked if they washed the fruit prior to juicing [70% (n=21/30)] reported they washed the produce before preparation and juicing. The majority of these premises rinsed the produce in running water or rinsed and scrubbed the produce in water. Sanitizer was used on the fruit prior to juicing for only three premises. Some of the fruits [30% (n=9/30)] used in the juice samples were reported as not being washed prior to preparation or juicing. All of these samples, 120 were produced from fruits that require some form of preparation and the removal of the skin or peel before con-
sumption. Although the practice of not washing the fruit and is not desirable, the microbiological results did not reflect the absence of washing. The samples that were reported total coliform count and total staphylococcal count were comparatively high (potentially hazardous) were washed under running water.

During this study from juice handlers were asked how they prepared the fruits that were used in the juice samples. Preparation of the fruit depended on the fruit type. For example, most premises peeled the orange by hand and then cut the orange with knives before being juiced. Some of the premises placed oranges whole into the juicing machine and the orange juice sample that was assessed as being marginal was cut using a machine. The sample that was assessed as potentially hazardous was prepared and cut manually. Figure 07 depicts the preparation methods of each premises used prior to process.

![Preparation methods used prior to process](image)

**Figure 4.6: Preparation methods prior to use**

4.1.6. **Source of fruits**

Moreover, the fruits used for juice preparation were all bought from open markets in with preference to the ripened fruits. No premises were bought fruits direct from farms/producers.

4.1.7. **Temporary storage site of fruit / fruit pulp**

Storage of fruits prior to use was generally good across all premises. [70% (n=21/30)] reported that the temporary storage site of fruits prior to use was shelf. Six premises [20%
(n=6/30)) used refrigerators and three premises [10% (n=3/30)] used baskets as temporary storage of fruits.

The majority of Premises prepared the fruit for immediate use or prepared the fruit in bulk for the same day use. [90% (n=27/30)] of the samples were produced from fruits that were prepared for immediate use or prepared in bulk for the same day use. The sample that was assessed as potentially hazardous was prepared and stored in this manner. Only [10% (n=3/30)] of the premises stored bulk prepared fruit in for more than 2 days.

One of the objectives of this study was, to assess the hygienic conditions of processing and handling of locally prepared unpasteurized juices. Based on the questionnaire results of the study it was identified that following parameters are to be considering to assess hygienic conditions of processing and handling of locally prepared unpasteurized juices.

- Cleaning and Sanitizing Practices of the premises.
- Cleaning and sanitizing frequency for juicer/blender.
- Cleaning and sanitizing frequency for utensils.
- Understanding of cleaning and sanitizing practices.
- Preparation methods used, prior to process.
- Source of Fruits.
- Temporary storage site of fruits / fruit pulp.

With the limitations of this study of having low number of samples, it is recommended further studies with high number of samples, to achieve this objective.

4.2. Analytical Results

4.2.1. Ranges of pH of Juice Samples

A total of one hundred twenty fresh fruit juices were analyzed for their pH measurement. The pH value ranges from 3.11 to 5.76. The mean pH of Orange, Pineapple and Mango were, 3.80, 3.72 and 4.10 respectively and more acidic than Papaya (5.50) (Table 02).

<table>
<thead>
<tr>
<th>Juice Type</th>
<th>No of samples</th>
<th>pH Range</th>
<th>pH Mean</th>
<th>pH Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>30</td>
<td>3.11</td>
<td>3.80</td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>30</td>
<td>3.76</td>
<td>3.72</td>
<td></td>
</tr>
<tr>
<td>Mango</td>
<td>30</td>
<td>4.10</td>
<td>4.10</td>
<td></td>
</tr>
<tr>
<td>Papaya</td>
<td>30</td>
<td>5.50</td>
<td>5.50</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Ranges of pH of Juice Samples
<table>
<thead>
<tr>
<th></th>
<th>Analyzed</th>
<th>pH Range</th>
<th>Mean</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>30</td>
<td>3.11 - 4.48</td>
<td>3.80</td>
<td>3.79</td>
</tr>
<tr>
<td>Papaya</td>
<td>30</td>
<td>5.14 - 5.76</td>
<td>5.50</td>
<td>5.47</td>
</tr>
<tr>
<td>Pine Apple</td>
<td>30</td>
<td>3.35 - 4.03</td>
<td>3.72</td>
<td>3.73</td>
</tr>
<tr>
<td>Mango</td>
<td>30</td>
<td>3.62 - 4.90</td>
<td>4.10</td>
<td>3.94</td>
</tr>
</tbody>
</table>

**Figure 4.7: pH values vs. Juice type**

Fruit juices have a low pH because they are comparatively rich in organic acid. The overall range of pH is 2 to 5 for common fruits with the most frequent figures being between 3 and 4. In this study, the pH of the fruit juices varied from 3.11 to 5.76. The highest pH was shown by Papaya juice (mean value of 5.50) (Figure 08) and it was in a range that support the growth of most bacteria. In this study it was identified that comparatively high bacterial count in papaya juices.

Since Orange (mean value of pH 3.8), Pineapple (mean value of pH 3.72) and Mango (mean value of pH 4.1) juices more acidic in nature it was identified that comparatively low bacterial count in these types (Table 02).

**4.2.2. Water Analysis**

A total of thirty (30) water samples were analyzed for bacteriological quality of water which was used for preparation of unpasteurized fruit juices and washing of glasses and other
equipments. Out of thirty, 25 were reported as absence of fecal coliforms. Only 5 samples were reported as presence. Out of those presences five three were from cafes and two were from juice bars. In restaurants no samples reported as presence. Out of 30 samples 25 were from the containers and 5 were directly from taps. Water from the containers of each vender was reported as absence and the reported 5 fecal coliform presence samples were directly from taps.

**Figure 4.8: Water analysis – Fecal Coliform**

Type of water used during juice preparation and washing activities has major impact on quality and safety of locally prepared fruit juices. During this study it was identified that absence of fecal coliforms in all the container water purchased from retail vendors. This was mainly due to their compliance with local standards on drinking water quality. But it was reported that the 5 vendors used tap water were presence of fecal coliforms though water is treated by government municipality according to local standards on portable water. So according to these presence results it is questioned that the hygiene practices performing inside the premises, the personnel hygiene standards of the juice preparation staff and the cleaning quality of the utensils and juicers/blenders.

As depicted in figure 09 comparisons was done with premises type. It was reported that all restaurants water samples were reported as absence of fecal coliforms. Among 5 samples resulted as presence of fecal coliforms, were drawn 3 from cafes and 2 from juice bars. So
cafes and Juice Bars were in question with their hygiene practices and cleaning/sanitizing practices.

4.2.3. Total Viable Count

As depicted in Table 03, a total of one hundred twenty (120) locally prepared fresh fruit juices samples were cultured for total viable count (TVC). The overall mean of the total samples was 5.18 log cfu/ml. The mean of total viable count of papaya was the highest (6.77 log cfu/ml) whereas the mean of Orange, Pineapple and Mango were 4.83 log cfu/ml, 4.54 log cfu/ml, and 4.59 log cfu/ml respectively.

Table 4.2: Total viable count (log cfu/ml) of locally prepared fresh fruit juices.

<table>
<thead>
<tr>
<th>Juice Type</th>
<th>No. of Samples</th>
<th>Mean (log TVC)</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% confidence interval for mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower boundary</td>
<td>Upper boundary</td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td>30</td>
<td>4.83</td>
<td>0.80</td>
<td>0.15</td>
<td>4.52</td>
<td>5.14</td>
<td>3.41 6.5</td>
</tr>
<tr>
<td>Papaya</td>
<td>30</td>
<td>6.77</td>
<td>0.82</td>
<td>0.15</td>
<td>6.46</td>
<td>7.08</td>
<td>5.08 7.91</td>
</tr>
<tr>
<td>Pineapple</td>
<td>30</td>
<td>4.54</td>
<td>0.76</td>
<td>0.14</td>
<td>4.25</td>
<td>4.83</td>
<td>3.25 5.84</td>
</tr>
<tr>
<td>Mango</td>
<td>30</td>
<td>4.59</td>
<td>0.86</td>
<td>0.16</td>
<td>4.26</td>
<td>4.92</td>
<td>3.11 5.94</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>5.18</td>
<td>1.23</td>
<td>0.11</td>
<td>4.96</td>
<td>5.40</td>
<td>3.11 7.94</td>
</tr>
</tbody>
</table>

Most fruit contains bacterial counts of $1 \times 10^5$ cfu/cm$^2$ on their surface (Splittstosser 1979; Harrigan 1998; Al-Jedah et al., 2002; Durgesh et al., 2008). Improper washing of fruits adds these bacteria to juices leading to contamination (Durgesh et al.,2008). In addition lack of appreciation of basic safety issues by vendors contribute to augmentation of the microbial loads (Durgesh et al., 2008). These include use of crude stands and carts, unavailability of running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust (Lewis et al., 2006; Durgesh et al.,
During this study it was identified that most of the fruit juice samples showed much higher viable bacterial count (Table 03). The highest viable count (7.91 log cfu/ml) for locally prepared fruit juice sample was found in a Papaya juice, collected from a Restaurant, and the lowest viable count was (3.11 log cfu/ml )found in mango juice also collected from a Restaurant. Therefore there is no higher relationship of Total viable count was identified with the premises type. On the other hand, it was identified the relationship of TVC with the pH value of the fruit juice. Being higher in pH value, and the pH value range is being favorable range for bacterial growth comparatively higher TVC were reported in Papaya juice (Figure 4.11).

Variations in TVC of the all types of fruit juices may be due to the unhygienic maintenance during preparing the juice. 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’s are discarded nearby, providing nutrients for insects and rodents. Some of the juices are not efficiently protected against flies, which may carry food borne pathogens. Safe food storage temperatures are rarely applied to juices. In addition, there are potential health risks associated with initial contamination of foods by pathogenic bacteria as well as subsequent contamination by vendors during preparation, handing, and cross contamination (Mosupye and van Holy, 2000).

Rahman et al. (2011) reported that total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice, as the highest count was found as 2.4x10^4 cfu/ml and 3.2x10^3 cfu/ml in fresh and packed juice, respectively which was found to be lower than this study. Tasnim et al. (2010) also found the load of viable bacteria in locally prepared fresh juice samples within the standard limit in the average of 10^3 cfu/ml. Bagde and Tumane (2011) found that total bacterial counts in juice samples ranged between 2.0 x 10^6 to 1.0 x10^5 cfu/ml in Nagpur, India.

The result obtained from this study showed that the total viable count of the fresh fruit juice samples ranged from 3.41 to 6.5 log cfu/ml (for orange juice), 5.08 to 7.91 log cfu/ml (for papaya juice), 3.25 to 5.84 log cfu/ml (for pineapple juice) and 3.11 tp 5.94 log cfu/ml (for mango juice). Therefore most of the fruit juices being served in these three divisional secretarial divisions in Colombo district (Dehiwala, Rathmalana, Moratuwa) had higher mi-
crobial load than the specification set for fruit juices in some parts of the world. When compare with past study in Ethiopia, High loads of different microbial groups, including coliforms and other Enterobacteriaceae were recorded from the fruit juices examined in the study. The range of microbial counts recoded in the fruit juices analyzed in this study (6.2x10³ - 3.1x10⁷ CFU/ml) was relatively higher than the microbial load (10²- 10⁵ CFU/ml) reported in some earlier works. To the authors’ knowledge, there is no specification set for the permissible level of microbes in fruit juices being served in Ethiopia. But the counts were comparatively same as this study results.

Since unavailability of SLS standard on Locally prepared fresh Fruit juices, results were compared with respect to SLS standard, Specification for Ready to Serve Fruit Drinks (SLS 729:2010, 1st revision), Sri Lanka. The satisfactory limit was set below 50 cfu/ml on TVC in this standard. So according to this standard all the fruit juices being served in these three divisional secretarial divisions were unsatisfied. But since all of these restaurants, cafes and juice bars were not being certified for SLS standard, it was not introduced these samples were hazardous or not suitable for consumption. As these products could be the cause of health problems and potential vehicle of food borne outbreaks, high level of workers hygiene should be enforced and the use of disinfectant better practiced to improve the microbial quality, safety, and shelf-life of the final product. Figure 10 depicts the distribution pattern of Total viable count of each juice type.
fruit juices in general practices. But in this study it has been reported that more than 50% of samples were positive for coliforms. Coliforms are indication of unsanitary conditions, unhygienic practices during or after production and poor quality of source of water used (Durgesh et al., 2008). This level of contamination could be due to the unhygienic production practices as the preparation involves manual mixing of the salt, sugar and probable non-potable water collected from streams nearby when municipal water supply is interrupted (Feglo and Sakyi, 2012).

Though coliforms were not reported in fruit juice samples examined in this study, it has been reportedly associated with tap water popularly consumed in some premises. A number of studies from different countries have shown presence of Escherichia coli, coliforms and a variety of other microorganisms like Streptococcus pyogenes, Streptococcus equi, Pseudomonas aeruginosa, Staphylococcus spp, Micrococcus spp in locally prepared fresh fruit juices (Moyer et al., 1993; Vieira et al., 1997; Nichols et al., 2000; Lateef et al., 2006; Durgesh et al., 2008). If the source water used is of poor quality, harmful microorganisms may persist in ice since the process of freezing cannot destroy them (Durgesh et al., 2008). When ice is thawed the surviving microorganisms though may be injured, tend to recover their viability so that when the ice melts into the juices, they may be able to survive these too (FEHD 2005; Durgesh et al., 2008).

Other previous studies have also reported that most ready-to-eat foods were contaminated with enteric bacteria and other potential food poisoning organisms with bacterial counts higher than the acceptable levels. In a study in Kumasi, Ghana, Feglo and Sakyi (2012) reported that the mean bacterial count of locally prepared fruit juice analyzed was 6.16 log10 cfu/ml much higher than the national acceptable reference of <5.0 log10 cfu/ml (Feglo and Sakyi, 2012). The generally observed high microbial counts in this study could be attributed to the influence of environmental factors on the microbial populations, which have been shown to play a significant role in affecting the quality of fresh fruit juices as these products were handled in an open air environment (Abdullahi et al., 2005; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008; Oyeyi and Lum-nwi, 2008; Wada-kura et al., 2009; Kawo and Abdulmumin, 2009).
Most of the fruit juices in our study were found to be unfavorable for consumption because many of them showed the presence of coliforms. The presence of coliform in fruit juice is not allowed by safe food consumption standard (Andres et al., 2004). The highest coliform count for fresh fruit juice during the study was 2.04 log MPN/g. In Bangladesh, Ahmed et al. (2009) showed the presence of Escherichia coli ranging from 43 to >2400/100 ml in different types of freshly squeezed fruit juices in Dhaka city. In India, the fruit juices were heavily contaminated by Escherichia coli (Bagde and Tumane, 2011).

Since unavailability of SLS standard on Locally prepared fresh Fruit juices, results were compared with respect to SLS standard, Specification for Ready to Serve Fruit Drinks (SLS 729:2010, 1st revision), Sri Lanka. The satisfactory limit was set as absent of coliforms per ml or g on TVC in this standard. So according to this standard, 8 samples out of 10 (80%) Orange juice of restaurants, 8 samples out of 10 (80%) Papaya juice of restaurants, one sample out of 10 (10%) papaya juice of cafes, 2 samples out of 10 (20%) papaya juice of juice bars, 10 samples out of 10 (100%) pineapple juice of restaurants, 8 samples out of 10 (80%) mango juice of restaurants and one sample out if 10 (10%) mango juice of juice bars were reported as satisfied. As a whole 38/120 samples were reported as satisfied (31.7%). According to the results, 34/38 (89.5%) of satisfied samples were collected from restaurants. Therefore it was identified as the hygiene practices, cleaning/sanitizing practices and frequency of cleaning/sanitizing practices of restaurants were shown satisfactory level then cafes and juice bars of these three (Dehiwala, Rathmalana and Moratuwa) divisional secretarial divisions. Figure 11 depicts the distribution patterns of total coliform counts of each juice type.

4.2.5. Total Staphylococcal count

Out of one hundred twenty specimens of fresh fruit juices, twenty four samples (20%) showed staphylococcus count. The mean count of all types of juice was 0.61 log cfu/ml with maximum mean of 0.71 log cfu/ml in pineapple juice and minimum mean of 0.46 log cfu/ml in orange juice (Table 4.4).

Table 4.4: Total staphylococcal count (log cfu/ml) of locally prepared fresh fruit juices

<table>
<thead>
<tr>
<th>Juice Type</th>
<th>No. of Samples</th>
<th>Mean TSC (log cfu/ml)</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% confidence interval for mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papaya</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cafe</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juice Bar</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

51
Coagulase-positive staphylococci may cause human disease through the production of toxins. Effective levels of toxin formation require a high number of microorganisms (approximately 10^5-10^6 micro-organisms per ml of food) (IDF, 1994). A few reports have shown the prevalence of staphylococci in fruit juice samples (Ahmed et al., 2009; Tambekar et al., 2009). In this study, staphylococci were found in 24 out of 120 tested samples. The highest total staphylococcal count for fresh fruit juice sample (4.66 log cfu/ml) was found in a pineapple juice collected from a café. And TSC not detected samples were also reported from all the three types of juice vending premises. Therefore there was no correlation was observed, in TSC with juice premises type. The presence of coagulase positive S. aureus in fresh juices can mainly be attributed to contamination via handlers. Although it is unlikely for the introduced S. aureus to survive in juices having low pH, it is possible that they may do so in juices having pH values more than 4 (Mudgil et al., 2004). But during this study it was identified that even in low pH juices like Orange, Pineapple also reporting TSC. its mainly due to contamination during preparation and through utensils used for the process. As per the standard SLS 729:2010, 1st Revision, Specification for Ready to Serve fruit Drinks, it was identified as all the TSC positive samples are unsatisfied. Therefore 26/120 (21.67%) samples were identified as not suitable for consumption and 94/120 (78.33%) were identified as suitable for consumption as per this standard. As these products could be the cause of health problems and potential vehicle of food borne outbreaks, high level of workers hygiene should be enforced and the use of disinfectant better practiced to improve the microbial quality, safety, and shelf-life of the final product.
4.2.6. **Total Salmonella Count**

No coliform bacteria were observed in all fruit juice samples. Salmonella is generally only a problem in fresh, unpasteurised fruit juices, due to its low thermal tolerance. There have been three major outbreaks of Salmonellosis, all from unpasteurised orange juice, in the United States, Canada and Australia, involving 62, 298 and 400 reported cases in 1995 and 1999 (Bell and Kyriakides, 2001). The main risk factors were associated with the fertilization of agricultural crops. Crops were grown in orchards where sheep grazed, manuring the soil. Fallen fruit were often used, allowing soil and fecal contamination. In addition, poor decontamination of fruit occurred in the factory, poor quality washing water was used, pest control within the factory was poor; and the cleanup of fruit boxes and conveyors was poor. During this study it was observed that there was no identical relationship with premises type also since all the samples reported as salmonella negative per 25g.

As per the standard SLS 729:2010, (1st revision), Specification for Ready to Serve Fruit Drinks, the acceptable limit was set as zero /25g of the sample. Therefore it was identified as all the samples were satisfied and suitable for consumption with respect to total salmonella count.

4.2.7. **Microbiological Results by Juice Type**

![Figure 4.11: Microbial results by juice type](image)

<table>
<thead>
<tr>
<th>Counts</th>
<th>Orange</th>
<th>Papaya</th>
<th>Pineapple</th>
<th>Mango</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVC</td>
<td>4.83</td>
<td>6.77</td>
<td>4.54</td>
<td>4.59</td>
</tr>
<tr>
<td>TCC</td>
<td>0.42</td>
<td>0.38</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>TSC</td>
<td>0.46</td>
<td>0.7</td>
<td>0.71</td>
<td>0.55</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 4.11: Microbial results by juice type
No firm correlation can be made between the microbiological quality and juice types, as depicted in figure 12. It was showed that all the papaya juice samples were high in TVC since the pH range of papaya juice was favorable for bacterial growth. Other juice types (Orange, Pineapple and Mango) were showed comparatively low TVC counts as their pH range were low and they were acidic in nature. With respect to TVC all the samples were in unsatisfactory category as per the standard SLS729:2010, Specifications for Ready to Serve Fruit Drinks, as the counts were reported as above from set acceptable limit (50 cfu/ml).

With respect to juice type there was no firm correlation made with TCC. As per the standard SLS 729:2010, Specification for ready to Serve Fruit Drinks 38/120 samples were reported as satisfied. Among these 38 satisfies samples 8 samples were Orange Juice (21.05%). Out of these 38 satisfies samples 11 were papaya juice (28.95%). 10 samples out of satisfied 38 (26.31%) samples were Pineapple and 9 samples out of 38 satisfied samples were mango (23.69%). Further studies are recommended with high number of samples to conclude juice type with TCC relationship more clear way.

According to the SLS 729:2010, Specification for Ready to Serve Fruit Drinks, it was identified that 94 samples out of 120 samples (78.33%) were satisfied and suitable for consumption, 26 out of 120 samples (21.67%) were in unsuitable for consumption category. Out of these unsuitable for consumption samples, 5 were orange juice, 8 were pineapple juice, 7 were Pineapple juice and 6 were mango juice. Therefore there is no clear correlation between juice type and TSC of the samples.

With the results of this study it was not clearly identified any relationship between Total salmonella count with juice type, as all the samples were recorded negative for total salmonella count.

One of the objectives of this study was, to identify juice types those are more susceptible to contamination. Based on the results of the study it was identified as there was no clear correlation between bacterial count and juice type with respect to TCC, TSC and Total salmonella count. But with respect to TVC, it was identified that papaya juice has slightly high susceptibility to be contaminated with mean value of 6.77 log cfu / ml. it was due to the pH range 5.14 – 5.76 which is the preferable pH for bacterial growth. With the limitations of this study
of having low number of samples, it is recommended further studies with high number of samples to, achieve this objective.

4.2.8. Microbiological Results by Premise Type

Figure 4.12: Bacterial counts vs. premises type

As per the Standard SLS 729:2010, specification for Ready to Serve Fruit Drinks, it was identified as all the samples were not in satisfactory level. So as depicted it was shown that high results on TVC were recorded in samples collected from cafes and juice bars than samples collected from restaurants. It was identified that cleaning /sanitizing practices and
frequency and personnel hygiene practices of the restaurants were optimum than cleaning/sanitizing practices and frequency and personnel hygiene practices of cafés and juice bars in these three (Dehiwala, Rathmalana and Moratuwa) divisional secretarial divisions of Colombo district, Sri Lanka.

With respect to TCC of the juice samples there was an identical relationship observed with the premises type and juice type. 34/40 (85%) Samples collected from restaurants were reported as absence of TCC, and 1/40 (2.5%) and 3/40 (7.5%) samples were reported as absence of TCC, collected from cafes and juice bars respectively. And it was shown that the cleaning/sanitizing practices and frequency and personnel hygiene practices of the restaurants were acceptable than cafés and juice bars.

As per the results reported during this study it was clearly identified correlation between premises type and TSC of the samples. As per the SLS 279:2010, Specification for Ready to Serve Fruit Juice, 26/120 (21.33%) samples were identified as unsatisfactory samples. Out of these unsatisfied 26 samples, 6 samples were collected from restaurants (23.10%), 12 samples were from cafes and 8 samples were collected from juice bars (0.31%) (Figure 13). Therefore it was shown that samples collected from cafes and juice bars had more susceptibility to be contaminated with respect to TSC of the samples. Assurance on hygiene and cleaning/sanitizing practices of restaurants were given through TSC of fruit samples as per the study results.

Since Total salmonella count was zero in all the fruit juice samples and recorded as suitable for consumption with respect to total salmonella count, it was not clearly identified any correlation between premises type and total salmonella count.

4.2.9. Swab Results – Juicer/Blender and Utensils

It was observed that the Swab results of the Juicer/Blender and utensils including chopping boards, knives and spoons were in satisfactory levels. Out of 30 13 samples of each category showed zero counts and other were also not more than 2.48 log cfu/cm². Swab results showed direct correlation with premises type. Swab samples collected from juicers/blenders of Restaurants, only two samples out of 10 were shown counts (20%) and other 8 samples were shown zero counts (80%). It’s indicated that the good cleaning practices and sanitizing
practices of restaurants. And the frequency of cleaning and sanitizing were also in acceptable level when consider with swab counts of juicers/blenders. Swab collected from Utensils of restaurants also showed 2 samples out of 10 (20%) with counts and other 8 (80%) were with zero counts. This results were indicated that good cleaning practices and good personnel hygiene practices practiced in restaurants other than Cafes or juice bars. The frequency of cleaning and sanitizing of utensils also are in acceptable level with considering the swab results.

The swab results of Juicers /blenders of Cafes and juice bars showed almost same pattern, and for cafes 6 samples out of 10 (60%) were shown bacterial counts and for Juice bars 5 samples out of 10 (50%) were shown bacterial counts. The swab results of utensils of cafes were shown as 5 plates with bacterial counts and 5 plates with zero counts (50%), where as juice bars were shown 4 plates with bacterial counts (40%) and 6 plates with zero counts. These results indicated that, the cafes and juice bars have much poor cleaning and sanitizing practices for juicers / blenders with low frequencies. As well as personnel hygiene practices and the handling and processing (Good manufacturing practices -GMP) were in questioned in these two premises.

According to the results of this study it was identified as the restaurants have good hygiene practices, personnel hygiene practices and good handling and processing practices with acceptable level of effective cleaning and sanitizing practices.
CHAPTER 05- CONCLUSIONS

Present study exhibited the microbiological status of available locally prepared unpasteurized fruit juices of restaurants, cafes & juice bars in three divisional secretarial divisions (Dehiwala, Rathmalana & Moratuwa) of Colombo district, to ensure quality & safety of juices for a precise control over public health risk.

As first objective of this study, it was helpful to evaluate Bacterial profile of locally prepared fresh fruit juices. With respect to total viable count, microbial growth was found more frequently in all types and the microbial load in most cases were still above the standard limit for consumption as per the SLS 279:2010 specification for ready to serve fruit drinks. Total coliform count and total staphylococcal count was common in most of the juice samples collected from cafes and juice bars rather than restaurants. Total salmonella count was zero for all the samples tested during the study. From the data presented, it can be concluded that the microbiological quality of most of the locally prepared fresh fruit juice samples collected from three divisional secretarial divisions on Colombo district were not satisfactory as coli-

form, Staphylococcus spp. were detected from the samples and on the basis of total viable count, 100% of the samples recorded were in not acceptable range based on the SLS standard, 279:2010 specification for ready to serve fruit drinks.

One of the objectives of this study was, to assess the hygienic conditions of processing and handling of locally prepared unpasteurized juices. Based on the questionnaire results of the study it was identified that following parameters are to be considering to assess hygienic conditions of processing and handling of locally prepared unpasteurized juices.

- Cleaning and Sanitizing Practices of the premises.
- Cleaning and sanitizing frequency for juicer/blender.
- Cleaning and sanitizing frequency for utensils.
- Understanding of cleaning and sanitizing practices.
- Preparation methods used, prior to process.
- Source of Fruits.
- Temporary storage site of fruits / fruit pulp.
With the limitations of this study of having low number of samples, it is recommended further studies with high number of samples, to achieve this objective. Among three types of premises according to the study results it was identified that restaurants have more effective cleaning sanitizing and procedures with acceptable limits than cafes and juice bars. Since all the types of Bacterial counts were shown less, comparatively with other two premises, restaurants created clear picture on hygienic condition and personnel hygiene practices during the process in restaurants. Additionally, the swab testing results detected the effectiveness of cleaning and sanitizing practices in premises. Questionnaire used to gather information on hygiene practices at premises added new insight to the existing knowledge of juice handling employees on hygiene practices. The lack of knowledge on safe fruit juice preparation as well as the contamination sources can contribute to the elevation of pathogens in prepared juices. It is therefore, essential for the people who handle and prepare juices, to be properly trained on safe fruit handling technique. Regular monitoring of the quality of fruit juices for human consumption is recommended to avoid any future bacterial pathogen outbreak.

It was set as third objective to assess quality of locally prepared unpasteurized fresh juices in terms of basic hygienic problems associated with and in order to recommend remedial action for identified problems. Throughout this study, following problems were identified as problems associated with quality of locally prepared fresh fruit juices.

- Using over ripened fruit for preparation of juice
- Using contaminated water for juice preparation.
- Cleaning and sanitizing of juicers/blenders and utensils were not acceptable.
- Cleaning and sanitizing procedures were not acceptable with frequencies.
- Using not suitable fruit / juice handling practices.
- Not good awareness on hygienic conditions required and sanitizing practices to be followed during process.
CHAPTER 06- RECOMMENDATIONS AND SUGGESTIONS

Based on the findings of this study and above mentioned identified hygienic problems associated with locally prepared fresh fruit juices, following recommendations are made:

1. Local Councils should continue to assess these premises during routine inspections and compliance checks and evaluate key food-handling practices including:
   - Washing all fruits thoroughly prior juicing regardless of whether they are to be peeled or skinned as the surface may contain pathogens such as *Listeria* and *Salmonella*. Cross contamination can occur in the process of peeling the fruits so it is also advisable to wash peeled or skinned fruits.
   - Cleaning and sanitizing utensils including chopping boards as often as possible throughout the day.
   - Ensuring the cleaning and sanitizing schedule identifies the dismantling of the juicing equipment.
   - Encouraging proprietors to keep prepared fruits under temperature control as growth of pathogenic organisms can occur on cut surfaces.
   - Encourage proprietors to remove badly damaged or bruised fruits.

2. The importance of personal hygiene, storage of fruit at cold temperature, using boiled water for diluting the juice/cleaning equipment should be informed to people involved in preparing and handling fruit juices.

3. Hygienic principles should be applied from production to consumption of fruits (agriculture, transport, manufacturing and processing, distribution, and preparation for consumption). Disinfection should be applied where appropriate. However, interventions to minimize contamination of fruits and vegetables at any point in the chain should be preferred to remedial or corrective action.

4. Since current study was conducted on small sample size, the researcher also recommends further study by increasing sample size in order:
   - To better understand modes of contamination of raw fruits before harvesting, when subjected to various agronomic practices, and during post-harvest handling.
   - To determine conditions that influence attachment, growth, survival and death of human pathogenic microorganisms on fruits.
To establish and validate disinfection methods, individually and in combination, that minimize risk of illness caused by microorganisms on fruits, vegetables and other plant materials eaten raw.

To develop highly effective treatments for removing pathogens from a wide range of raw produce (a single type of treatment will not be suitable for all fruits). Caution must be taken in the development of sanitizing or disinfecting treatments so that degradation of nutritional components of fruits is minimal and the formation of compounds with potential toxic or carcinogenic properties is avoided.

To develop or improve analytical techniques to detect low numbers of microorganisms, particularly viruses and parasites, on or in raw fruits, and plant materials before and after treatment with various disinfectants.

5. Further research is required to fully understand the risks, if any associated with mixed businesses producing freshly squeezed juices. The microbiological results from this survey indicated that the number of satisfactory samples dropped slightly in businesses that have a number of food preparations or when juice products were not the prime product for sale.

6. Serotyping and antimicrobial sensitivity should be carried out on identified pathogens in order to know resistance pattern to different antibiotics.

7. Control of pathogenic microorganisms should involve multidisciplinary teams with a wide range of technical, sociological, educational and administrative skills. Health education programmes should fully describe the impact of infections that can be associated with raw fruits and fresh fruit juices.

8. Epidemiological studies should be carried out to address the role of raw fruits as vehicles of microorganisms capable of causing human diseases.

9. Professional and domestic food handlers and consumers should be better educated about the principles of personal hygiene and decontamination of raw fruits, vegetables and plant materials. Changes in risk of illness related to ageing and health status of consumers should be more clearly defined.

10. There should be continual training of fruit growers and handlers at all levels in order to control microbiological hazards that may be influenced by current and changing agricultural, agronomic, processing, distribution and preparation practices.
11. Conferences, workshops and symposia specifically addressing the control of human infections associated with the consumption of contaminated raw fruits should be held in places where they will have greatest impact.

12. The establishment of effective GAP, GMP and HACCP programmes that would cover all aspects of growing, harvesting, packing, transport, processing, distribution, and preparation of raw fruits and fresh fruit juices is strongly encouraged. Since contamination of raw produce with pathogens can occur at any point in the system, all stages must be considered when devising HACCP programmes. Assistance from, and collaboration between, academic institutions, public health authorities, food control agencies, trade organizations and the private sector in developing HACCP programmes for the raw fruit and fresh fruit juices vendors will be necessary to minimize the risk of disease. An integral part of this process will involve identifying critical control points at which measures should be taken to prevent contamination and to decontaminate. In the interim, where lack of science-based information prevents identification of critical control points for specific segments of the fruit or vegetable growing and handling chain, this should be acknowledged and GAP and GMP established. The development of HACCP programmes will eventually follow. Several diseases caused by viruses, parasites and some types of pathogenic bacteria that are linked to the consumption of raw fruits and vegetables are transmitted via the fecal-oral route. It is imperative that individuals handling raw produce at every stage, from the field to the point of consumption, have an understanding of hygiene sufficient to prevent contamination. Application of improperly composted manure or water containing raw sewage to fields, or the use of water contaminated with feces during processing of fruits and fresh fruit juices, must be avoided. These responsibilities must be shared by the grower as well as the processor, and food handlers. Training of food handlers at all level of the food chain, as well as education of the consumer, are key elements in a total system approach to reducing the risk of produce-borne illnesses.

As final objective it was required to identify juice types those are more susceptible to contamination. According to the results, Papaya juice was shown higher counts rather than other juice types. Therefore it was identified Papaya juice was more susceptible for contamination. Since papaya juice has the pH value which is favorable for bacterial growth, it was more susceptible for contamination rather than other juice types which have more acidic pH values.
The following limitations were identified during this study.

- As an expected limitation of this study, it was identified human errors had huge impact on bacterial analysis results.

- These study findings were interpreted with standard introduced for ready to serve fruit drinks than locally prepared fresh fruit juices.

- As it is known, not all *Staphylococcus aureus* are associated with staphylococcal food poisoning. 50% *Staphylococcus aureus* strain produce heat stable enterotoxins. Therefore current study cannot address strains that secrete toxin as well as preformed toxin.

Similarly present study identified total coliforms. However, strains that associated with food poisoning *E.coli (EHEC O157: H7)* and preformed toxin were not determined.
CHAPTER 06 – REFERENCES


8. Centers for Disease Control and Prevention: Outbreak of *Escherichia coli* 0157:H7 infections associated with drinking unpasteurized commercial apple juice - British


26. PHLS. Microbiological guidelines for some ready-to-eat foods sampled at the point of sale: an expert opinion from the PHLS. *PHLS Microbiology Digest* 1996; 13: 41-43


APPENDICES

APPENDIX A – Survey Questionnaire

Locally Prepared Unpasteurized Fruit Juice Survey Questionnaire

Date -

Answer to all questions. Underline correct answer.

1. What type of premises is this?
   a) Restaurant
   b) Cafe
   c) Juice Bar

2. Is safety program available at your premises for training purpose?
   a) Yes
   b) No

3. Is the cleaning and sanitizing process for juicers covered in the premises Food Safety Program?
   a) Yes
   b) No

4. How often are juicers/blenders cleaned?
   a) After each use
   b) Between different juice types
   c) Once per day
   d) 2-3 times per day
   e) >3 times per day
   f) 2-3 times per week
   g) Other (please specify) ____________________________

5. How often are juicers/blenders sanitized?
a) After each use
b) Between different juice types
c) Once per day
d) 2-3 times per day
e) >3 times per day
f) 2-3 times per week
Other (please specify) ....................................................................................

6. How often are the juicers dismantled for cleaning and sanitizing purposes?

a) After each use
b) Between different juice types
c) Once per day
d) 2-3 times per day
e) >3 times per day
f) 2-3 times per week
Other (please specify) ....................................................................................
If not dismantled please briefly describe any alternative cleaning/sanitizing procedure (eg juicer flushed with cleaning/sanitizing solution)
....................................................................................................................
....................................................................................................................
....................................................................................................................
....................................................................................................................

7. Please underline the processes that best describes the usual cleaning and sanitizing Procedure for dismountable parts of the juicer: (you may underline more than one for
Example water and scrub surface plus soak in water with sanitizer added)

a) Cleaned with water and detergent in container (e.g. Sink)
b) Cleaned with running water
c) Soak in a container of water and detergent
d) Cleaned with water and scrub surface
e) Through the dishwasher
f) Cleaned in container of water with sanitizer added

g) Cleaned then soak in container of water with sanitizer added

8. Answer following questions with short answers
   a) Name of sanitizer used and active ingredient (eg chlorine, quats)

   b) Concentration of sanitizer

   c) How long is soaking process (minutes)

   d) (If another method not mentioned above please describe)

9. How often are utensils/chopping boards used in fruit juice preparation cleaned?
   a) After each use
   b) Between different juice types
   c) Once per day
   d) 2-3 times per day
   e) >3 times per day
   f) Once a month
   g) Other (please specify)

10. How often are utensils/chopping boards used in fruit juice preparation sanitized?
    a) After each use
    b) Between different juice types
    c) Once per day
    d) 2-3 times per day
    e) >3 times per day
    f) Once a month
    g) Other (please specify)
11. Please underline the processes that best describes the cleaning and sanitizing procedure for utensils/chopping boards: (you may tick more than one for example rinse and scrub surface plus soak in water with sanitizer added)
   a) Cleaned with water and detergent in container (e.g. Sink)
   b) Cleaned with running water
   c) Soak in a container of water and detergent
   d) Cleaned with water and scrub surface
   e) Through the dishwasher
   f) Cleaned in container of water with sanitizer added
   g) Cleaned then soak in container of water with sanitiser added

12. Answer following questions with short answers
   a) Name of sanitizer used and active ingredient (eg chlorine, quats)
      ..........................................................
   b) Concentration of sanitizer
      ..................................................................
   c) How long is soaking process (minutes)
      ..................................................................
   d) (If another method not mentioned above please describe)
      ..................................................................
      ..................................................................

13. Specify preparation procedure used for fruits prior to juice preparation (eg. Peeling and washing, peeling and not washing, cut into pieces).
    ..................................................................
    ..................................................................
    ..................................................................
14. what is the type of water used for juice preparation and utensils washing and cleaning.

Juice preparation ..............................................................................................................
Washing utensils and cleaning .........................................................................................

15. Please specify about the Source of fruit, used for juice preparation? (eg. Direct from market, from farm).

........................................................................................................................................

16. Where do you store Fruits as a temporary storage site in premises, underline correct answer.
   a) In refrigerator
   b) On shelf
   c) In basket

17. Where do you store fruit pulp (If applicable only) storage site in premises, used for juice preparation?
   a) In refrigerator
   b) On shelf
   c) In basket

18. How long do you store fruit pulp (If applicable only) in storage site in premises, before used for juice preparation?
   a) One day
   b) Two days
   c) More than two days
APPENDIX – B – Part 01,

**Total Viable Count (Plate count method) Ref. ISO 4833:2003**

(10g sample + 90ml BPW)

Liquid sample ($10^0$) → ($10^1$) → ($10^2$) → ($10^3$)

(1ml+9ml BPW) → (1ml+9ml BPW) → (1ml+9ml BPW)

1ml each into 2 sterile petri-dishes

Pour 12-15ml PCA at 44-47°C

Mix contents & allow to solidify

(If surface overgrowing colonies are suspected,
Pour ~4ml overlay medium at 44-47°C onto the surface of inoculated medium & allow to solidify)

Incubate Inverted plates at 30±1°C for 72±3h

Count colonies using colony counter considering following;
• Count pinpoint colonies as well
• Avoid mistaking particles of undissolved or precipitated/foreign matter
• Count spreaders as single colonies
• If $<1/4^\text{th}$ of the dish is overgrown by spreading, count the colonies on the unaffected part of the dish & calculate the corresponding No. of the entire dish
• If $>1/4^\text{th}$ is overgrown by spreading, discard the count

### Calculation & Expression of results

1. At least one dish containing at least 10 colonies
   a. Use following equation to calculate result:
   b. Equation applicable only for,
      i. Inoculation of one 90mm-diameter Petri dish per dilution
      ii. Maximum No. of counts for the total colonies present: 300 per dish
      \[
      N = \frac{\Sigma C}{V \times 1.1 \times d}
      \]
      \[
      N = \text{No. of microorganisms (CFU/g) or (CFU/ml)}
      \]
      \[
      \Sigma C = \text{Sum of the colonies counted on 2 dishes retained from 2 successive dilutions, at least one of which contains a minimum of 10 colonies}
      \]
      \[
      v = \text{Volume of inoculum placed in each dish, in mm}
      \]
      \[
      d = \text{Dilution corresponding to the first dilution retained}
      \]
      • Round off the calculated result to 2 significant figures.
        > If the $3^{\text{rd}}$ figure $< 5$, do not modify the preceding figure
        > If the $3^{\text{rd}}$ figure $\geq 5$, increase the preceding figure by 1 unit
      • Express the result as a No. between 1.0 & 9.9 multiplied by the appropriate power of 10, or a No. with 2 significant figures.

2. Dishes contain $<10$ and $\geq 4$ colonies
   a. Calculate as equation (1)
   b. Report result as an estimated (CFU/g) or (CFU/ml)
3. Dishes contain 3 to 1 colonies
   a. Report the result as;
      \( <4 \times 1/d \text{ (CFU/g)} \) or (CFU/ml)

4. Dishes contain no colonies
   a. Report the result as;
      \( <1/d \text{ (CFU/g)} \) or (CFU/ml)
APPENDIX B – Part 02

Total Coliform Count (3-tube MPN method) Ref. ISO 4831: 2006

(10g sample + 90ml BP)

Liquid sample (10^6) → (10^1) → (10^2) → (10^3) →

(10ml+90ml BPW) (10ml+90ml BPW) (10ml+90ml BPW)

10ml each into 3x10ml

<-----double-strength LT tubes----->

<----- 1ml each into 3x10ml single-strength LT tubes -- -->

<--------Incubate at 30±1°C or 37±1°C for 24±2h--------->

Examine for gas formation

Re-incubate gas (-)ve tubes for additional 24±2h

Examine for gas formation
gas (+)ve tubes

\[ \rightarrow \]

Transfer into BGB broth

\[ \rightarrow \]

Incubate at 37±1°C for 24±2h

\[ \rightarrow \]

Examine for gas formation

\[ \rightarrow \]

Re-incubate gas (-)ve tubes for additional 24±2h

\[ \rightarrow \]

Examine for gas formation

\[ \rightarrow \]

gas (+)ve tubes

\[ \rightarrow \]

[Coliform (+)ve]

\[ \rightarrow \]

Calculate MPN of Coliform based on confirmed LT tubes

\[ \rightarrow \]

Express the results as;

Coliforms (MPN/g) or (MPN/ml)
APPENDIX B – Part 03

Total Staphylococcal Count (Direct plate count method) Ref. ISO 6888-1: 1999

(10g sample + 90ml BPW)

Liquid sample ($10^6$) → ($10^1$) → ($10^2$) → ($10^3$) →

(10ml+90ml BPW) (10ml+90ml BPW) (10ml+90ml BPW)

0.1ml into 2 BPA plates or 0.4, 0.3, 0.3ml into 3 BPA plates
(if high counts are expected) (if low counts are expected)

Spread inocula & let stand 15min

Incubate at 35 or 37°C for 24±2h

Examine plates & mark typical colonies

Re-incubate for further 24±2h

Count & record each type of colonies
S. aureus ---- Typical colonies → Black or grey, shining, convex & surrounded by a clear zone. Opalescent ring contacts with the colony.

Atypical colonies → a) Shining black colonies with or without a narrow white edge; the clear zone is absent or barely visible & the opalescent ring is absent or hardly visible.

b) Grey colonies free of clear zones.

Confirmation

1. Coagulase test
   a. Transfer suspect colonies into 5ml BHI broth & emulsify.
   b. Incubate at 35°C or 37°C for 24±2h.
   c. Add 0.1ml BHI culture to 0.3ml rabbit plasma & mix.
   d. Incubate at 35°C or 37°C
   e. Examine after 4h to 6h for clot formation. If (-)ve re-examine after 24h.
      - S. aureus → Volume of clot occupies more than half of the original volume of the liquid.
   f. As a (-)ve control, add 0.1ml sterile BHI broth to 0.3ml rabbit plasma & incubate.

Calculation & Expression of results

1. Calculate No. of S. aureus for each plate selected
   a. Use following equation:

      \[ a = b_c \times c_c + b_{nc} \times c_{nc} \]

      \[ \frac{A_c}{A_{nc}} \]

      \( a \) = No. S. aureus per plate

      \( A_c \) = No. of typical colonies submitted to the coagulase test

      \( A_{nc} \) = No. of atypical colonies submitted to the coagulase test
b_c = No. of typical colonies which are coagulase (+)ve

b_{nc} = No. of atypical colonies which are coagulase (+)ve

c_c = Total No. of typical colonies on the plate

c_{nc} = Total No. of atypical colonies on the plate

2. Calculate No. of *S. aureus* in the test portion
   a. Use following equation for dishes containing maximum 300 colonies, with
      150 typical and/or atypical colonies,

      \[
      N = \frac{\Sigma a}{V (n_1 + 0.1 n_2)d}
      \]

      \(\Sigma a\) = Sum of *S. aureus* colonies on all the plates

      \(V\) = Volume of inoculum on each dish, in ml

      \(n_1\) = No. of plates selected at the 1\textsuperscript{st} dilution

      \(n_2\) = No. of plates selected at the 2\textsuperscript{nd} dilution

      \(d\) = Dilution of the 1\textsuperscript{st} dilution selected

3. Estimation of low numbers
   a. If the two dishes contain less than 15 identified colonies;
      I. For liquid products,

      \[
      N_e = \frac{\Sigma a}{V x 2}
      \]

      \(\Sigma a\) = Sum of *S. aureus* colonies on the two dishes selected

      \(V\) = Volume of inoculum on each dish, in ml
II. For other products,

\[ N_e = \frac{\Sigma a}{V \times 2 \times d} \]

\[ \Sigma a = \text{Sum of } S. \text{ aureus colonies on the two dishes selected} \]

\[ V = \text{Volume of inoculum on each dish, in ml} \]

\[ d = \text{Dilution rate of the initial suspension} \]

b. If the two dishes do not contain any colonies of coagulase-positive staphylococci

I. If the inoculation has been performed with 0.1 ml of sample;
   a) For liquid products, report the results as;
      Less than 10 \( S. \text{ aureus} \) per ml
   b) For other products, report the results as;
      Less than \( 10/d S. \text{ aureus} \) per g,

      where \( d = \text{dilution rate of the initial suspension} \)

II. If the inoculation has been performed with 1 ml of sample;
   a) For liquid products, report the results as;
      Less than 1 \( S. \text{ aureus} \) per ml
   b) For other products, report the results as;
      Less than \( 1/d S. \text{ aureus} \) per g,

      Where \( d = \text{dilution rate of the initial suspension} \)
APPENDIX B – Part 04

Total Salmonella Count - Ref. ISO 6579:2002

25g sample + 225ml BPW

↓

Incubate at 37°C for 18±2h

↓

Inoculate 0.1ml into 10ml RVS broth
Inoculate 1ml into 10ml MKTTn broth

↓

Incubate at 41.5±1°C for 24±3h
Incubate at 37°C for 24±3h

↓

Streak onto XLD
Streak onto BGA

↓

Streak onto XLD
Streak onto BGA

↓

Incubate at 37°C for 24±3h

↓

• XLD → Typical colonies – Black centered; lightly transparent zone of reddish colour
  → H₂S (-)ve variants – Pink with a darker pink centre
  → Lactose (+)ve Salmonella – Yellow with or without blackening

• BGA → Typical colonies – Pink with bright red surrounding medium
Selection of colonies for confirmation

Streak onto Nutrient Agar

↓

Incubate at 37°C for 24±3h

↓

Biochemical confirmation

1. Reaction on TSI
   a. Streak TSI slant & stab butt.
   b. Incubate at 37°C for 24±3h.
   c. Observe colour change.
      - Typical *Salmonella* ➔ red slant; yellow butt; form gas; form H₂S
      - Lactose-(+)ve *Salmonella* ➔ Yellow slant

2. Reaction on Urea
   a. Streak Urea agar slant.
   b. Incubate at 37°C for 24±3h. Examine at intervals.
   c. Observe colour change.
      - Typical *Salmonella* ➔ (-)ve reaction (No colour change)

3. Reaction in L-Lysine decarboxylation medium
   a. Inoculate the medium.
   b. Incubate at 37°C for 24±3h.
   c. Observe colour change.
      - Typical *Salmonella* ➔ (+)ve reaction (Turbidity & purple colour)

4. Detection of β-galactosidase
   a. Inoculate 0.25ml saline solution.
   b. Add 1 drop of toluene & shake.
c. Put in a water bath at 37°C for about 5min.
d. Add 0.25ml of β-galactosidase reagent and mix.
e. Put again in the water bath at 37°C for 24±3h. Examine at intervals.
   - Typical *Salmonella* → (-)ve reaction (No yellow colour)

5. Voges-Proskauer (VP) reaction
   a. Inoculate into 3ml VP medium.
   b. Incubate at 37°C for 24±3h.
   c. Add 2 drops of creatine solution, 3 drops of ethanolic 1-naphthol, & 2 drops of KOH solution; Shake.
      - Typical *Salmonella* → (-)ve reaction (No pink colour)

6. Indole reaction
   a. Inoculate 5ml tryptone medium.
   b. Incubate at 37°C for 24±3h.
   c. Add 1ml Kovacs reagent.
      - Typical *Salmonella* → (-)ve reaction (yellow-brown ring)

Serological confirmation

1. Elimination of auto-agglutinable strains
   a. Disperse part of the colony in small amount of water to obtain a homogeneous solution.
   b. Place a drop of this solution on a clean slide.
   c. Add a drop of saline solution & mix.
   d. Rock the slide gently for 30–60s.
   e. Observe against a dark background with the aid of a magnifying glass.
      - If the bacteria have clumped, the strain is auto-agglutinable & shall not be submitted for serology tests.
2. Examination for O-antigens
   a. Proceed as above replacing saline solution by anti-O serum.
      - *Salmonella* agglutinate.

3. Examination for Vi-antigens
   a. Proceed as above replacing saline solution by anti-Vi serum.
      - *Salmonella* agglutinate.

4. Examination for H-antigens
   a. Inoculate semi-solid NA with the culture.
   b. Incubate at 37°C for 24±3h.
   c. Use this culture for the test.
   d. Proceed as above replacing saline solution by anti-H serum.
      - *Salmonella* agglutinate.

<table>
<thead>
<tr>
<th>Biochemical reactions</th>
<th>Auto-agglutination</th>
<th>Serological reactions</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical</td>
<td>No</td>
<td>O-, Vi- or H-antigen (+)ve</td>
<td><em>Salmonella</em> (+)ve</td>
</tr>
<tr>
<td>Typical</td>
<td>No</td>
<td>All reactions (-)ve</td>
<td>May be <em>Salmonella</em></td>
</tr>
<tr>
<td>Typical</td>
<td>Yes</td>
<td>Not tested</td>
<td></td>
</tr>
<tr>
<td>No typical reactions</td>
<td>No/Yes</td>
<td>O-, Vi- or H-antigen (+)ve</td>
<td></td>
</tr>
<tr>
<td>No typical reactions</td>
<td>No/Yes</td>
<td>All reactions (-)ve</td>
<td><em>Salmonella</em> (-)ve</td>
</tr>
</tbody>
</table>

Table 06: Interpretation of confirmatory tests
Appendix B – Part 05

Water Analysis for Thermotolerant (fecal) coliforms

Refer - AFNOR validated method 3M 01/2-09/89C (all food types): 3MTM PetrifilmTM

Coliform Count Plates

Place Petrifilm plate on level surface. Lift top film.

With pipette perpendicular to Petrifilm plate, place 1 mL of sample onto center of bottom film.

Carefully roll top film down to avoid entrapping air bubbles. Do not let top film drop.

With flat side down, place spreader on top film over inoculums. Gently apply pressure on spreader.

to distribute inoculum over circular area before gel is formed. Do not twist or slide the spreader.

Lift spreader. Wait a minimum of one minute for gel to solidify. Incubate plates in stacks of up to 20 for 24h ± 2h at 44°C ± 1°C. Incubator humidification is required at this elevated temperature.

Count all the colonies.

Results record as cfu/ml
APPENDI B – Part 06

Swab Analysis for Food contacting surfaces - ISO 18593:2004(E)

Sawb wrap and sterilize at 121°C for 15minutes.

↓

Serilize individually wrapped templates enclosing in area 25cm²

↓

Remove swab from wrapping.

↓

Moisten with immersing it in a bottle containing 100ml of sterile buffered peptone water

↓

Press the tip of the swab against the wall of the bottle to remove excess water.

↓

Unwrap a template and place on the surface to be investigated.

↓

Strak inside the area of template while rotating the swab between thumb and forefinger

↓

Put the swab in the bottle containing dilution liquid and aseptically break off the stick

↓

Repeat four times to cover an area 100cm²

↓

Mis thoroughly

↓

Inoculate duplicate plates of plate count agar with 1ml of initial suspension using pour plate technique
Incubate inverted plates at 35°C for 48 ±2h.

Count colonies

Calculation

\[ N_s = \frac{N \times F \times D}{A} \]

Ns – No of cfu in 1 ml of dilution liquid

N – No of colonies

F – Amount in ml of dilution fluid in the bottle

D – Reciprocal of the dilution used

A – Surface area investigated
APPENDIX C – TEST RESULTS

1. Questionnaire Results

1.1. Cleaning and Sanitizing frequencies of Juicers/Blenders

Table 01: Cleaning and sanitizing frequencies of Juicers/Blenders results

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Cafes</th>
<th></th>
<th>Restaurants</th>
<th></th>
<th>Juice Bars</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>After each use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between different juice types</td>
<td>3</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once per day</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2-3 times per day</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>&gt;3 times per day</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2 or 3 times a week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

1.2. Cleaning and Sanitizing frequencies of utensils

Table 02: Cleaning and sanitizing frequencies of utensils results

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Cafes</th>
<th></th>
<th>Restaurants</th>
<th></th>
<th>Juice Bars</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>After each use</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between different juice types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once per day</td>
<td>9</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3 times per day</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3 times per day</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 or 3 times a week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Once a month</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
### 1.3. Method used for cleaning & sanitizing

**Table 03: Method used for cleaning & sanitizing results**

<table>
<thead>
<tr>
<th>Method</th>
<th>Cafes</th>
<th>Restaurants</th>
<th>Juice Bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using sanitizers</td>
<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Using dishwashers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using water with detergent/soap</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Using only water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 1.4. Methods used prior to preparation

**Table 04: Methods used prior to preparation results**

<table>
<thead>
<tr>
<th>Method</th>
<th>Cafes</th>
<th>Restaurants</th>
<th>Juice Bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinsed with running tap water</td>
<td>4</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Rinsed and scrubbed the fruits in water</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Rinsed with sanitizers</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Not wash prior to preparation</td>
<td>5</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

### 1.5. Fruits storage condition

**Table 05: Fruits storage condition results**

<table>
<thead>
<tr>
<th>Method</th>
<th>Cafes</th>
<th>Restaurants</th>
<th>Juice Bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf</td>
<td>7</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Bucket</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
2. Analytical Results

2.1. pH Measurement

Table 06: pH Measurement results

<table>
<thead>
<tr>
<th>Juice Type</th>
<th>No of samples Analyzed</th>
<th>pH Range</th>
<th>pH Mean</th>
<th>pH Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange</td>
<td>30</td>
<td>3.11 – 4.48</td>
<td>3.80</td>
<td>3.79</td>
</tr>
<tr>
<td>Papaya</td>
<td>30</td>
<td>5.14 – 5.76</td>
<td>5.50</td>
<td>5.47</td>
</tr>
<tr>
<td>Pine Apple</td>
<td>30</td>
<td>3.35 – 4.03</td>
<td>3.72</td>
<td>3.73</td>
</tr>
<tr>
<td>Mango</td>
<td>30</td>
<td>3.62 – 4.90</td>
<td>4.10</td>
<td>3.94</td>
</tr>
</tbody>
</table>

2.2. Microbiological Test Results - Orange – Restaurants

Table 07: Microbiological Test Results - Orange – Restaurants

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>pH</th>
<th>TVC cfu/ml</th>
<th>TVC log cfu/ml</th>
<th>TCC MPN/g</th>
<th>Log salmonella</th>
<th>staphylococcus Log cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.41</td>
<td>4 x 10^4</td>
<td>4.60</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>4.03</td>
<td>2.7 x 10^3</td>
<td>5.43</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>3.11</td>
<td>1.8 x 10^4</td>
<td>4.25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>4.39</td>
<td>2 x 10^4</td>
<td>5.30</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>4.09</td>
<td>3 x 10^4</td>
<td>4.48</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>3.72</td>
<td>1.9 x 10^4</td>
<td>4.28</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>3.56</td>
<td>3.1 x 10^4</td>
<td>4.49</td>
<td>ND</td>
<td>ND</td>
<td>4.2 x 10^2</td>
</tr>
<tr>
<td>8</td>
<td>3.54</td>
<td>2.9 x 10^3</td>
<td>3.46</td>
<td>ND</td>
<td>ND</td>
<td>8.1 x 10^2</td>
</tr>
<tr>
<td>9</td>
<td>3.28</td>
<td>1.8 x 10^5</td>
<td>5.25</td>
<td>1.12</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>3.98</td>
<td>3.8 x 10^5</td>
<td>5.58</td>
<td>2.11</td>
<td>0.32</td>
<td>ND</td>
</tr>
</tbody>
</table>

2.3. Microbiological Test Results - Orange – Cafes

Table 08: Microbiological Test Results - Orange – Cafes

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>pH</th>
<th>TVC cfu/ml</th>
<th>TVC log cfu/ml</th>
<th>TCC MPN/g</th>
<th>Log salmonella</th>
<th>staphylococcus Log cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.35</td>
<td>1.1 x 10^5</td>
<td>5.04</td>
<td>46.22</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>3.29</td>
<td>3.6 x 10^3</td>
<td>3.56</td>
<td>9.33</td>
<td>0.97</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>4.43</td>
<td>1.4 x 10^5</td>
<td>5.15</td>
<td>109.89</td>
<td>2.04</td>
<td>9.3 x 10^5</td>
</tr>
</tbody>
</table>

xxiv
### 2.4. Microbiological Test Results - Orange – Juice Bars

**Table 09: Microbiological Test Results - Orange – Juice Bars**

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>pH</th>
<th>TVC Cfu/ml</th>
<th>TVC log Cfu/ml</th>
<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
<th>Staphylococcus Cfu/ml</th>
<th>Log cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.38</td>
<td>1.5x10^4</td>
<td>4.18</td>
<td>1.12</td>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>3.39</td>
<td>3.2x10^6</td>
<td>6.50</td>
<td>2.76</td>
<td>0.44</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>3.59</td>
<td>5.4x10^5</td>
<td>5.73</td>
<td>1.12</td>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>4.29</td>
<td>1.5x10^5</td>
<td>5.18</td>
<td>1.12</td>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>4.32</td>
<td>4.1x10^4</td>
<td>4.61</td>
<td>2.05</td>
<td>0.31</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>3.37</td>
<td>3.6x10^4</td>
<td>4.56</td>
<td>1.47</td>
<td>0.17</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>3.27</td>
<td>2.4x10^4</td>
<td>4.38</td>
<td>3.48</td>
<td>0.54</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>4.33</td>
<td>1.4x10^5</td>
<td>5.14</td>
<td>2.05</td>
<td>0.31</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>3.88</td>
<td>8x10^3</td>
<td>3.90</td>
<td>9.33</td>
<td>0.97</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>4.25</td>
<td>1.9x10^6</td>
<td>6.28</td>
<td>2.05</td>
<td>0.31</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

### 2.5. Microbiological Test Results - Papaya- Restaurants

**Table 10: Microbiological Test Results - Papaya- Restaurants**

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>pH</th>
<th>TVC Cfu/ml</th>
<th>TVC log Cfu/ml</th>
<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
<th>Staphylococcus Cfu/ml</th>
<th>Log cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.22</td>
<td>6.8x10^5</td>
<td>7.83</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>5.63</td>
<td>1.9x10^5</td>
<td>5.28</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>5.34</td>
<td>7.6x10^5</td>
<td>5.88</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>5.28</td>
<td>1.2x10^6</td>
<td>6.08</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>5.33</td>
<td>3.7x10^6</td>
<td>6.57</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>5.61</td>
<td>4.6x10^6</td>
<td>6.66</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>5.39</td>
<td>4.9x10^5</td>
<td>5.69</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>5.53</td>
<td>3.8x10^7</td>
<td>7.58</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>5.53</td>
</tr>
<tr>
<td>9</td>
<td>5.45</td>
<td>2.3x10^6</td>
<td>6.36</td>
<td>2.31</td>
<td>0.36</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>5.58</td>
<td>1.1x10^7</td>
<td>7.04</td>
<td>1.12</td>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

xxv
2.6. Microbiological Test Results - Papaya - Cafes

Table 11: Microbiological Test Results - Papaya - Cafes

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>pH</th>
<th>TVC Cfu/ml</th>
<th>TVC log cfu/ml</th>
<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
<th>Staphylococcus Cfu/ml</th>
<th>Log cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.44</td>
<td>6.1 x10⁶</td>
<td>6.78</td>
<td>2.76</td>
<td>0.44</td>
<td>ND</td>
<td>ND</td>
<td>3.74</td>
</tr>
<tr>
<td>2</td>
<td>5.75</td>
<td>5.1 x10⁶</td>
<td>6.71</td>
<td>4.27</td>
<td>0.63</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.29</td>
<td>1.8 x10⁷</td>
<td>7.25</td>
<td>9.33</td>
<td>0.97</td>
<td>ND</td>
<td>ND</td>
<td>2.61</td>
</tr>
<tr>
<td>4</td>
<td>5.36</td>
<td>3.8 x10⁵</td>
<td>5.58</td>
<td>21.46</td>
<td>1.33</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.69</td>
<td>3.5 x10⁷</td>
<td>7.54</td>
<td>1.47</td>
<td>0.17</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.71</td>
<td>1.6 x10⁷</td>
<td>7.20</td>
<td>2.11</td>
<td>0.32</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5.38</td>
<td>8.1 x10⁶</td>
<td>7.91</td>
<td>1.12</td>
<td>0.05</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.46</td>
<td>7.5 x10⁷</td>
<td>7.87</td>
<td>14.94</td>
<td>1.17</td>
<td>ND</td>
<td>ND</td>
<td>2.78</td>
</tr>
<tr>
<td>9</td>
<td>5.72</td>
<td>2.9 x10⁵</td>
<td>5.46</td>
<td>7.49</td>
<td>0.87</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.47</td>
<td>4 x10⁷</td>
<td>7.60</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>2.75</td>
</tr>
</tbody>
</table>

2.7. Microbiological Test Results - Papaya - Juice Bars

Table 12: Microbiological Test Results - Papaya - Juice Bars

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>pH</th>
<th>TVC Cfu/ml</th>
<th>TVC log cfu/ml</th>
<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
<th>Staphylococcus Cfu/ml</th>
<th>Log cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.34</td>
<td>1.4 x10⁷</td>
<td>7.15</td>
<td>1.47</td>
<td>0.16</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.69</td>
<td>2.3 x10⁷</td>
<td>7.36</td>
<td>2.11</td>
<td>0.32</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.48</td>
<td>5.6 x10⁷</td>
<td>7.75</td>
<td>4.27</td>
<td>0.63</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.76</td>
<td>8.2 x10⁵</td>
<td>5.91</td>
<td>ND</td>
<td></td>
<td>ND</td>
<td>2.5x10⁵</td>
<td>3.40</td>
</tr>
<tr>
<td>5</td>
<td>5.74</td>
<td>6.2 x10⁶</td>
<td>6.79</td>
<td>9.33</td>
<td>0.97</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.71</td>
<td>1.6 x10⁷</td>
<td>7.20</td>
<td>9.33</td>
<td>0.97</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5.62</td>
<td>8.3 x10⁶</td>
<td>6.92</td>
<td>46.22</td>
<td>1.66</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
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Table 13: Microbiological Test Results - Pineapple – Restaurants

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<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
<th>Staphylococcus Cfu/ml</th>
<th>Log CFU/ml</th>
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Table 14: Microbiological Test Results - Pineapple – Cafes

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<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
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<th>Log CFU/ml</th>
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Table 15: Microbiological Test Results - Pineapple – Juice Bars

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<th>TCC MPN/g</th>
<th>TCC log MPN/g</th>
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<th>Staphylococcus Cfu/ml</th>
<th>Log CFU/ml</th>
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### Microbiological Test Results - Mango - Restaurants

#### Table 16: Microbiological Test Results - Mango - Restaurants

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<th>TVC MPN/g</th>
<th>TCC log MPN/g</th>
<th>Salmonella Cfu/ml</th>
<th>Staphylococcus Cfu/ml</th>
<th>Log cfu/ml</th>
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### Microbiological Test Results - Orange – Mango – Cafes

#### Table 17: Microbiological Test Results - Orange – Mango – Cafes

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<th>TVC MPN/g</th>
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<th>Log cfu/ml</th>
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2.13. Microbiological Test Results - Mango – Juice Bars

Table 18: Microbiological Test Results - Mango – Juice Bars

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Table 19: Water Analysis – Fecal Coliform presence/absence

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xxix
### 2.15. Swab Analysis of Juicer / Blender & utensils

#### Table 20: Swab Analysis results of Juicer / Blender & utensils

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