ASSESSMENT OF QUALITY AND SAFETY OF STREET VENDED SUGAR CANE JUICE IN DAR ES SALAAM REGION, TANZANIA

STEPHEN PAUL RWABUNYWENGE

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ABSTRACT

Human infections and diseases outbreaks have been found in unpasteurized street vended juices. So, there is a need to analyse the quality and safety of street vended juices for human consumption. A study was conducted to assess the quality and safety of sugarcane juice vended along the streets and restaurants in all three districts of Dar es Salaam city, Tanzania. A total of 60 samples of sugarcane juice were collected and studied during the months of September, 2017 to August, 2018 and vendors were interviewed followed by physical-chemical and microbiological laboratory analysis. The results for unpasteurized sugarcane juices showed that the pH of the juices ranged between 3.6 to 5.9 while the acidity ranged from 0.083% to 0.084%. The total soluble solids (TSS) ranged between 2.4 to 22.1^{0}Brix. Most of unpasteurized sugarcane juice had TSS levels below Codex recommended minimum values and 72.1% (n=60) were classified as weak and watery. On microbial analysis the total plate counts (TPC) of the juices ranged between 3.9 to 6.7 log (cfu/ml). About 90.1% (n=60) of sugarcane juice samples had TPC above Codex recommended maximum levels of 3.5-4 logcfu/ml. The results of *E. coli* ranged from 0.61-4.6 log cfu/ml were above recommended by Tanzania Standards (TZS 585:2003) Codex general standards). Prevalence of 96.6% of all the samples were contaminated with *E. coli*. The results of pasteurized sample showed significantly different ($p<0.05$) in TPC and *E. coli* levels as compared to unpasteurized sugarcane juice. Generally, 81.6% of vending premises were unhygienic and encouraged contamination of the sugarcane juice. It is concluded that, the overall handling practices of unpasteurized sugarcane vended in Dar es Salaam city was poor. Based on the results there is an immediate need of monitoring and educating vendors to improve sugarcane juice quality and safety.

DECLARATION

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

20/11/2018

Stephen Paul Rwabunywenge **Date**

(MSc. Student)

The above declaration is confirmed by;

20/11/2018

Dr. A. Issa-Zachariah **Date**

(Supervisor)

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DEDICATION

I dedicate this work to my brother, Mr. Charles Paul Rwabunywenge for his support in all fields of my life including all my academic achievements and my Children Linus Stephen, Vivian Stephen and Violet Stephen for their encouragement.

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LIST OF ABBREVIATION AND ACRONYMS

%	Percent
AOAC	Association of Official Analytical Chemists
C.I	Confidence Interval
CAC	Codex alimenterius commission
CFU/ml	Colon forming unit per millitre
E. coli	*Escherichia coli*
FAO	Food and Agriculture Organization of the United Nations
g	Gram
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practices
h	hour
ISO	International Organization for Standardization
Kg	Kilogram
L	Litre
MAFSC	Ministry of Agriculture, Food Security and Cooperatives
mg	milligram
min	minutes
ml	millilitre
MPN	Most Probable Number
NA	Nutrient Agar
^{o}C	Degree Celsus
PCA	Plate Count Agar

pH	Hydrogen ion concentration
S.Dev	Standard Deviation
Sec	Seconds
SMEs	Small and medium Enterprises
spp.	species
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TNTC	Too Numerous To Count
TSS	Total Soluble Solids
US. Dollar	United State Dollar

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The street food industry offers a significant amount of employment, often to persons with little education and training (Latham, 1996). Fresh juices including sugarcane juice are among the street foods vended in urban areas mostly in developing countries. They are prepared by low income vendors who have poor premises and facilities (Muinde and Curia, 2005).

Sugarcane juice is a result of sugarcane crushing in electric or manual grinders by rollers and is characterized as drink of low acidity with pH range 5.0 - 5.5 and high-water activity (0.89-0.99) (Yusof *et al.*, 2000). Sugarcane juice provides 40 Kcal/100 ml of energy (Khare *et al.*, 2012). It also contains vitamins A, C, B and numerous other health-supportive compounds (Caffrey and Kumar, 2016).

Sugarcane juice can also help in prevention of cancer, aid in digestion, prevent heart diseases, good for treating diabetes and clear skin imperfections (Abbasi *et al.*, 2015). Being a nutritious product containing natural sugars, minerals and organic acids, it strengthens the stomach, kidneys, heart, eyes, brain and sex organs (Krishnakumar *et al.*, 2013). Sugarcane juice contains polyphenolic flavonoids (phytochemicals that reduce the risk of age-related chronic disease (Singh, 2015).

People are developing interest on freshly prepared juices due to their freshness and absence of preservatives compared to industrially processed juices. In Tanzania, specifically Dar es Salaam city, there has been an increase in consumption of fresh extracted sugarcane juices because of availability of sugar cane and the health benefits

(MAFSC, 2009) and due to increased urbanisation, availability of sugarcane, their affordability in terms of price and other nutritive and healthy benefits of the sugarcane juice (Kumar, 2016).

Despite the numerous advantages the sugarcane juice, the juice is consumed raw after being extracted. Literature demonstrates that many outbreaks of food borne diseases have been reported due to consumption of unpasteurized and contaminated juices (Aparna *et al.*, 2011).

1.2 Problem Statement and Study Justification

In 2015, report of WHO showed that there were an estimated 582 million cases of 22 different foodborne enteric diseases and 351 000 associated deaths with the African region recording the highest burden for the diseases (WHO, 2015).

Biological hazards are the most common sources of food safety problems (Winter, 2011). These are the result of poor hygiene, inadequate processing and temperature controls (Kim, 2008). The Codex principle of food hygiene on food and beverages insist proper use of Good Hygienic Practices and Good Manufacturing Practices.

Poor handling, lack of pasteurization and unhygienic conditions (Iqbal *et al.*, 2015) leads to potentially dangerous hazards that pose risks to consumers. There have been a big number of food-borne diseases in the country including diarrhoea and cholera (Hawking and Penrose, 2010). It is not well known how much of these diseases have been contributed by the consumption of unpasteurized sugarcane juices which is currently a popular product in Tanzania cities including Dar es Salaam.

Many outbreaks of food borne diseases have been reported due to consumption of unpasteurized and contaminated juice (Aparna *et al.*, 2011). Sugarcane juice is consumed fresh unpasteurized after extraction (Schmidt *et al.*, 1999).

Previous research shows that pathogenic organisms such as *E. coli*, coliforms, *enterococci*, *Salmonella spp* have been isolated from sugarcane juice worldwide (Aparna *et al.*, 2011). Salmonella outbreaks have been reported involving unpasteurized fruit juices worldwide (Poonam *et al.*, 2013)

A study on the quality and safety of street sugarcane juice in Noida city India reported that fresh sugarcane juice was found to be contaminated with pathogenic microorganisms (Verma and Gaur, 2017). The contamination was mainly due to improper washing by workers, improper personal hygiene, unhygienic surroundings, vehicular transmission, sewage and the absence of good manufacturing practices (Verma and Gaur, 2017).

In another study it was reported that sugarcane juice was contaminated with coliforms such as *E. aerogenes* and *E. coli* that can cause diseases like urinary tract infections, chronic broncho-pulmonary diseases, pneumonia, septicemia, meningitis etc (Souza *et al.*, 2003)

In majority of the area worldwide sugarcane juices is produced under unhygienic conditions either during transportation or extraction of the sugarcane juice (Aparna *et al.*, 2011). Food borne diseases are spread through consumption of contaminated food products such as juices that have shown to be potential sources of bacterial pathogens like *E. coli*, and *Vibrio cholerae* (Verma and Gaur, 2017). In the year 2013, a study conducted

in Morogoro on fruits juices revealed that 94% of juice samples were contaminated with *E. coli* (Tiisekwa, 2013).

In Tanzania study, it is not well known how much of these diseases have been contributed by the consumption of unpasteurized sugarcane juice. There is limited information on current status of contamination, and therefore Tiisekwa, (2013) recommended a periodic surveillance of pathogenic microorganisms from street vended foods.

A study to assess the quality and safety of sugarcane juice is necessary in order to know the current status of contamination, to provide clear information to the regulator body which will guide in protecting the public and will provide awareness about the contamination. The information obtained from this study will help Tanzania Bureau of Standards in formulating the standard/specifications of sugarcane juice. In this regards it necessitates to assess the quality and safety of sugarcane juice vended.

1.3 Objectives of the Study

1.3.1 Overall objective

The overall objective of this study was to assess the quality and safety of street vended sugarcane juice consumed in Dar es Salaam, Tanzania.

1.3.2 Specific objectives

(i) To assess the handling practices during preparation of street vended sugarcane juice.

(ii) To determine the physical-chemical status of raw sugar cane juice vended with focus to total soluble solids (TSS), acidity, and pH.

iii) To determine the level of microbial contamination of sugarcane juice with focus to Total Bacterial Count, *E. c*oli and *Salmonella spp*).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Street Vended Food and Sugarcane Juice Handling

According to World health organisation (WHO) Street-vended foods is defined as ready to eat foods and beverages prepared and/or sold by vendors in streets and other (similar conditions) public places for immediate consumption or consumption at a later time without further processing or preparation (WHO, 2014). This definition includes fresh fruits and vegetables and juices which are sold outside authorized market areas for immediate consumption (WHO, 2014).

The street food industry offers a significant amount of employment, often to persons with little education and training Latham, (1996) and Abdalla *et al.* (2009) reported that most of the food vendors were females. Street vended fresh juices are preferred by the consumers due to easy access, nutritional profile, natural pleasant taste, fresh flavour and low prices (Akhtar *et al.*, 2013). Clean water supply and hand washing or toilet facilities are not available to food street vendors (Abdalla *et al.*, 2009).

Fresh street vended sugarcane juice was found to be contaminated with pathogenic microorganisms that cause a major public health concern in Noida city India (Verma and Gaur (2017). In Tanzania, research showed that urbanisation coupled with low wages offered to employees and labourers has led to proliferation of street food vendors who offer commercial meals but of high microbial contaminants due to poor hygiene and handling methods (Kinabo, 2003).In growing cities such as Dar es Salaam, street foods including sugarcane juice are available at all places of work where they are required, such as factories, construction sites, offices, schools, transit points and market places (Kinabo, 2003).

A study on street foods reported that, low and middle-income people who are the main customers of these locally vended juices are at risk of acquiring food-borne diseases (Edeltruds, 2013). In 2015, WHO warned that more than 200 diseases ranging from diarrhoea to cancer and announced a global awareness campaign on food safety (WHO, 2015). The Citizen (7/4/2015) reported that most people in Dar es Salaam city were risking their health by drinking fresh juices such as sugarcane juice sold by vendors at bus stand and roadsides as they believe that natural fruits juice is safer and healthier compared to the industrial processed juices. Sugarcane juice as street business has recent years grown in the country business capital city Dar es Salaam. (Citizen, 2015).

A study on street foods reported that, potential sources of entry of microorganisms are by environmental exposure, inappropriate washing of fruits, unhygienic surroundings often with flies and dust, use of unhygienic water, use of unhygienic ice and sustained preservation without refrigeration (Verma and Gaur, 2017).

2.2 Production and Distribution of Sugarcane

Sugarcane is one of the major industrial crops worldwide (Singh *et al.*, 2015). It is used both as cash crop as well as alternative energy source.

The distribution and production of sugarcane worldwide was presented by FAO as per figure below;

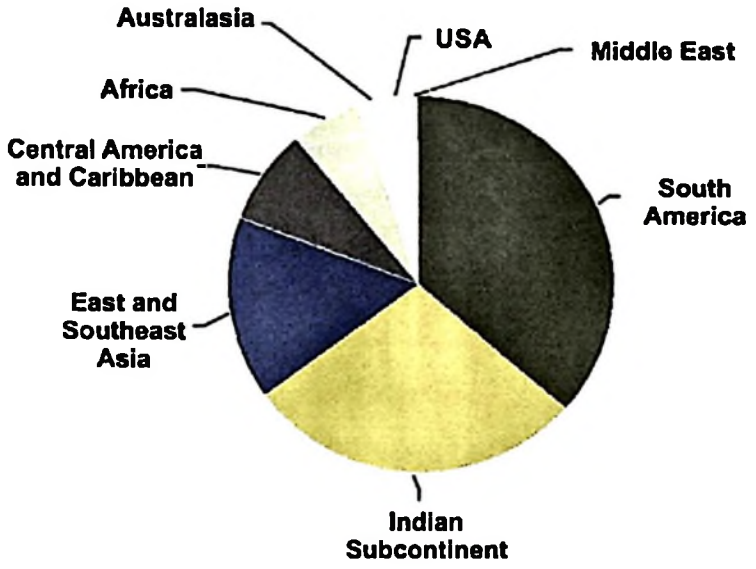

Figure 1: Distribution and production of sugarcane worldwide
Source: FAO (2013)

Tanzania is well situated for the production of sugarcane in East Africa and sugarcane is one of the important foods and commercial crops to the economy (Sambuo,2015). Its production is concentrated mainly in three regions, Morogoro, Kagera and Kilimanjaro (Tarimo and Takamura, 1998). Tremendous increase of the rate of sugar production in Tanzania has led to the raise of sugar consumption level the in the country (Sambuo, 2015). Sugar production and consumption by the year 2014 in Tanzania was at 320,000 metric tons and 480,000 metric tons respectively leaving a deficit of about 160,000 metric tons yearly demand (SBT, 2015).

2.3 Sugarcane Juice and Its Importance

Sugarcane juice is extracted from sugarcane by crushing sugarcane between roller crusher and consumed with (or) without ice. The earliest record came from a 9th century in which sugarcane juice was described as sweet drink called *Nalaka Rasa* which translate as sugarcane juice in South East Asia and India (Wikipedia, 2018).

According to Mesin (2017), sugarcane juice sold are always served cold with ice cubes. Traditionally, it is sold throughout the country especially among street vendors that set their stall on the street side. However, today sugarcane drink vendors with much improved hygiene, can also be found in food malls and shopping centres Mesin, 2017.

Sugarcane juice is also a refreshing drink in Africa. In Madagascar, sugarcane juice is fermented to make an inexpensive alcoholic beverage called *betsa-betsa*. The drink is popular with locals because it is cheaper than beer (Buah *et al.*, 2011). In Vietnam is commonly for sugarcane juice to be sold in small plastic bags filled with ice, with the open end attached around a drinking straw by a rubber band (Buah *et al.*, 2011).

2.4 Composition of Sugarcane Juice

Sugarcane juice contains water (75 to 85%), non-reducing sugars (sucrose, 10 to 21%), reducing sugars (glucose and fructose, 0.3 to 3%), organic substances (0.5 to 1), inorganic substances (0.2 to 0.6) and about (0.5 to 1) nitrogenous bodies (Swaminathan, 1995). Sugarcane juice of 100 ml provides 10 mg of iron and 6 µg of carotene (Parvathy, 1983).

According to Ravelo *et al.* (1991), sugarcane juice is composed of sugars, salts, organic non-sugars and insoluble mater as described in the (Table 1).

Table 1: Composition of sugarcane juice

		%brix
Sugars	Sucrose	81.00-87.00
	Reducing sugars	3.00-6.00
	Oligosaccharides	0.06-0.60
	Polysaccharides	0.20-0.80
Salts	Inorganic salts	1.50-3.70
Organic non-sugars	Organic acids	0.70-1.30
	Amino acids	0.50-2.50
	Dextrans	0.10-0.60
	Starch	0.11-0.50
	Gums	0.02-0.05
	Colourants	0.10
Insolubles	Sand, bagasse etc	0.15-1.00

Source: Ravelo *et al.* (1991)

2.5 Health and Nutritional Benefits of Sugarcane Juice

A health benefit of sugarcane juice includes numerous health-supportive compounds (Caffrey and Kumar, 2016). Shaikh (2014) reported that sugarcane juice can also help in prevention of cancer, aid in digestion; Liver functioning prevent heart diseases, good for treating diabetes and clear skin imperfections. Also, sugarcane juice aid in weight loss, eliminate toxins from the body, beneficial in treating Urinary tract Infection (UTI) and boosts immunity.

Due to significant amount of natural sugars, minerals and organic acids, it helps in strengthens the stomach, kidneys, heart, eyes, brain and sex organs (Krishnakumar *et al.*, 2013). The nutritional profile of sugarcane juice is shown in Table 2.

Table 2: Nutritional Profile of Sugarcane Juice

Quantity- 28.35 g	
Nutrients	**Amount**
Basic Components	
Proteins	0.20 g
Water	0.19 g
Ash	0.66 g
Fat	0.09 g
Calories	
Total Calories	111. 43
Calories From Carbohydrates	
Calories From Fats	0.03
Calories From Proteins	
Carbohydrates	
Total Carbohydrates	27.40 g
Sugar	25.71 g
Vitamins	
Riboflavin	0.16 mg
Niacin	0.20 mg
Pantothenic Acid	0.09 mg
Minerals	
Calcium	32.57 mg
Iron	0.57 mg
Magnesium	2.49 mg
Phosphorus	0.01 mg
Potassium	162.86 mg
Copper	0.09 mg
Manganese	0.09 mg

Source: Tanya (2018)

2.6 pH and Acidity of Sugarcane Juice

Sugarcane juice has low acidity, high water activity and high sugar content hence rapidly deteriorates even when refrigerated (Yusof *et al.*, 2000). Reduction of pH below 4.6 is an important action to inhibit growth of most pathogenic microorganisms.

According to Carlton *et al.* (2007), addition of an acid fruit pulp acts as a barrier, preserving the beverage though the development of filamentous molds, yeasts and aciduric bacteria, which can be present in natural sugarcane. The unpasteurized sugarcane juice had higher pH value range of 5.2 - 6.8 (Carazza *et al.*, 2001). The addition of citric acid to the sugarcane juice also gave good pleasant dull orange color to the juice.

Study by Chauhan *et al.* (2002) reported that addition of lemon and gingers followed by pasteurization reduce physical chemical changes during storage of ready to drink sugarcane juice. Low pH of the fruit and juices has inhibitory effects to microbial growth. Effects of pH against microbial contamination have also been reported by Mesfin (2011) and Sunday *et al.* (2011).

2.7 Total Soluble Solids (TSS) of Sugarcane Juice

Total soluble solids expressed in degrees °Brix is the sugar content of an aqueous solution. One-degree °Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass. Brix can be defined as the percentage in weight, or in volume, of soluble solids expressed as sucrose. According to Sabrinho *et al.* (2011) the brix variation in sugarcane juice was 14 to 22 depending on extraction process. Juice blends or beverages with less than 7°Brix are deemed weak and watery (Bates *et al.*, 2001).

2.8 Bacteria Commonly Associated with Unpasteurized Sugarcane Juice

Sugarcane juice is considered to be the best for breeding microorganisms. Sugarcane juice has low acidity, high water activity and high sugar content and therefore deteriorates rapidly even when refrigerated (Yusof *et al.*, 2000). It is liable to microbial contamination

mainly from the handlers, juice machines, raw materials, and unhygienic conditions (Iqbal *et al.*, 2015).

A study conducted by Nagalakshmi (1995) found raw sugarcane juice have $2.7x10^6$ bacterial colonies per ml. *E. coli* count was found to be $4.99x10^4$ cfu/ml. Similar results were reported by Subbannayya *et al.* (2007) stating that bacterial count in sugarcane juice ranged from 10^5 to 10^7 cfu/ml. Moreover, presence of *E. coli*, other coliforms indicated faecal contamination of juice (Subbannayya *et al.*, 2007).

In another study conducted by Aparna *et al.* (2011), (90%) of sugarcane juice sample were found to be contaminated with bacteria (Aparna *et al.*, 2011). The bacterial count for all the isolates was $<10^5$ cfu/ml. *Enterococci* (55.5%) and *E. coli* (48.1%) were the predominant isolates followed by *Citrobacter* spp. (18.5%), *Klebsiella* spp. (18.5%), and *Enterobacter spp.* (14.8%).Furthermore, the higher amount microorganisms have been found out in street vended juices in India and observed pathogenic *Pseudomonas aeruginosa* (25%), *Salmonella spp* (57.5%), *Klebsiella spp* (90%), *S. aureus* (82.5%) and *Escherichia coli* (72.5%) (Verma and Gaur, 2017).

In Tanzania study, Robert (2011) reported that the level of contamination of food and water by *E. coli* was significantly high, fun instance 75% of water supplies in Iringa region were contaminated with *E. coli.* Another study investigated water wells in Dar es Salaam found that 87% were contaminated by *E. coli* (Mushi *et al.*, 2012).

In another study, in fruit juice in Dar es Salaam the TPC ranged between 2.32 to 8.54 log(cfu/ml). About 72.2% juice samples had TPC above Codex recommended maximum

levels ($5x10^3$ - 10^4cfu/ml). The prevalence of *E. coli* in the juices was 80% with a range between -1.13 to 4.97 log MPN/ml) (Edeltruds, 2014).

2.9 Effects of Pasteurization on Physical Chemical Parameters of Sugarcane Juice

Pasteurization is used to reduce the bacterial load and hence prolong the shelf life of the drink (Lee *et al.*, 2006). Pasteurization at 90°C for 2 min led to a significant improvement in the organoleptic characteristics of the juice (Lee *et al.*, 2006) also reduced the ascorbic acid content during shelf life while fresh juice preserved this compound (Lee *et al.*, 2006). Sucrose level was decreased by 25% for fresh juice and 16% for pasteurized juice. After pasteurization, a 7% increase in viscosity and a 22% decrease in cloud were observed after 21 days of shelf life (Astolfi-Filho, 2018).

2.10 Production of Standardized and Pasteurized Sugarcane Juice

A combination of natural preservatives and low temperature storage was found as an effective way of preservation of sugarcane juice for more than a month with satisfactory sensory qualities (Ciência, 2014).

Lemon lowered the pH of sugarcane juice and inhibited the growth of microorganisms during storage. Combination of morning seed extract with lemon and ginger showed high antimicrobial activity when compared with sodium benzoate (as chemical preservative), at the permitted level (Ciência, 2014).

Good quality sugarcane juice (100 mL) with satisfactory storage stability at refrigeration could be prepared from heat-treated juice (72°C for 15 sec) before addition of lemon (3 ml) as a combination of flavor, color enhancer and source of citric acid (antioxidant); moringa (10 ml); ginger (0.6 ml) as flavor enhancer (Ciência, 2014) (Fig. 2).

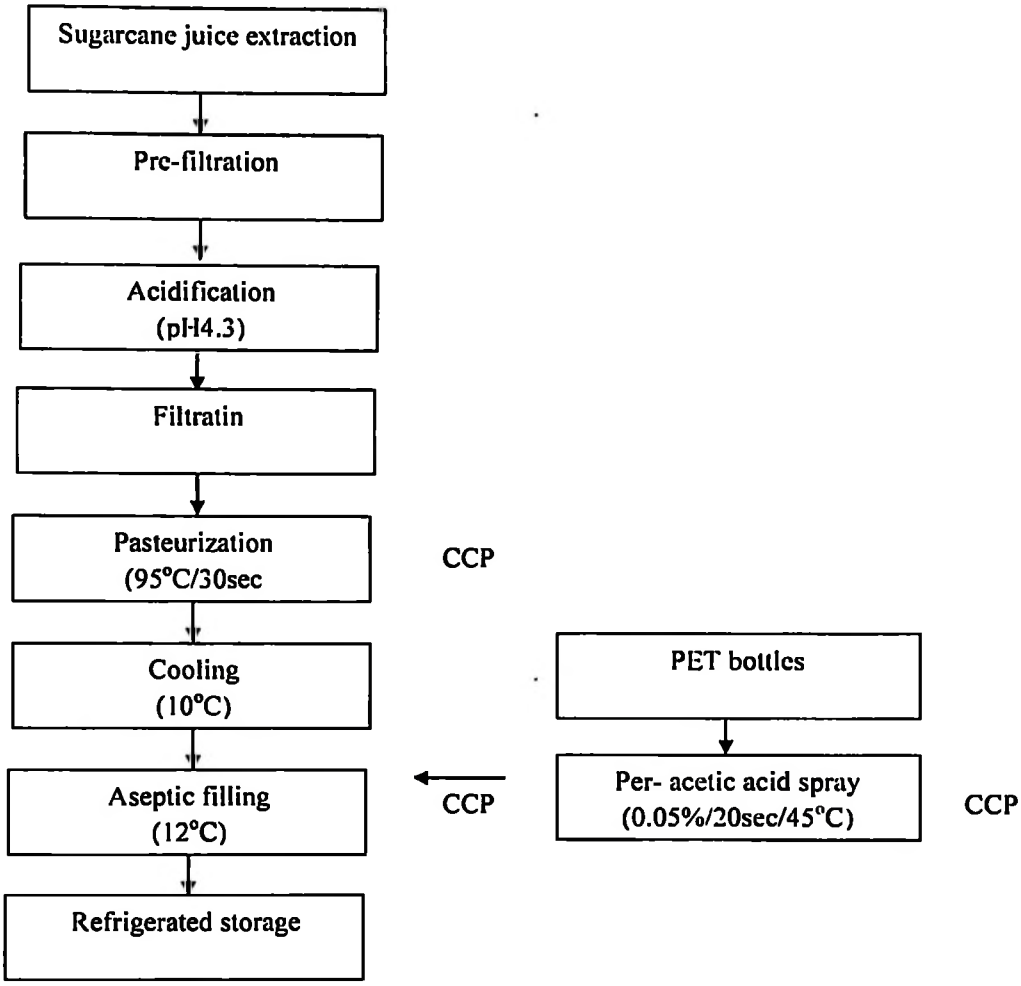

Figure 2: Flowchart shows stages taken in the processing of standardization and pasteurization of the sugarcane juice (Physicochemical and microbiological evaluation)

Source: Sabrinho *et al.* (2011)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Duration

This study was conducted in Dar es Salaam City, Tanzania from September 2017 to August, 2018. Dar es Salaam has been chosen for study because of its population density of 4,364,541 (Official Census of 2012) and rapid increase of sugarcane juices vending activities (Daily News, June 23, 2016). Dar Es Salaam is located between (60, 45' S - 390, 15E) and it borders in the north by Tanga Region, in the west and south by Coast Region and Lindi, and Indian ocean in the east (Fig. 3).

Figure 3: Map showing the location of Dar es Salaam city

3.2 Study Design

A cross sectional design was employed where observation (site examination) and sample for Laboratory were collected. Sugarcane juice vendors were interviewed with structured questionnaires (Appendix 1) followed by observation of the premises using observation checklist (Appendix 2) after which the samples were collected for Laboratory analysis. The method was used because of resource constraint and it saved time.

3.3 Materials

Chemicals, reagents and media used were provided by Tanzania Bureau of Standards and were pure manufactured by OXOID® Ltd and Basingstoke, U.K and Liofilchem (TE) Italy. Plate Count Agar(PCA), Triptone bile agar Medium (TBX), Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn), Rappaport-vassiliadis soya peptone broth (RVS) and Brilliant Green Agar(BGA) the brand used OXOID® Ltd., Basingstoke, U.K. Buffed peptone water (PBW), Xylose-Lysine-Desoxycholate Agar (X.L.D) and urea agar, brand used was Liofilchem (TE) Italy, Sampling containers were sterile autoclaving bottles

3.3.1 Study population

Sugarcane juice vendors were randomly selected based on their availability in different location in the city. They were identified from markets, along road sides/streets and Town restaurants in Dar es Salaam city. These includes; Mwenge, Sinza, Shekilango road, Kariakoo Lukoma street and Agrey streets, Mbezi Luis market, Ilala Bungoni, Buguruni, Kimara, Sinza, Ubungo Mawasiliano, Bamaga, Kinondoni, Urafiki, Temeke, Buguruni Malapa and Kigogo.

3.3.2 Sample size

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

$n = Z^2.SD^2/e^2$ ..... (1)

Where: n = size of sample, z = standard variant at 95% confidence level (1.96), SD = the standard deviation of population, and for the case of this study it will be taken at 19% and e = acceptable error which will be taken at 5% (0.05).

The sample $n = (1.96)^2 (0.19)^2/ (0.05)^2 x1 = 60$ ..... (2)

Therefore, a total of 60 samples were analysed in which 30 were iced and 30 were raw sugarcane juice vended from street and Restaurant vendors.

3.3.3 Sampling plan

The purposively sampling of sugarcane juice vendors was employed in which 60 vendors were purposively selected based on availability. Inclusion criteria was to those willing to participate in the study while exclusion criteria were to those vendors not willing to participate or not ready to give the required information. A total of 60 unpasteurized samples including 30 iced (with addition of ice blocks), and 30 raw (fresh without anything added) sugarcane juice from streets and restaurant were taken for Laboratory analysis.

The study also involved collection of pasteurized samples from one of SMEs (Small and medium Entrepreneur) in Goba village for comparison purposes in which 20 pasteurized sugar cane juice samples (at 80^0C for 10 min) and 20 pasteurized at (80^0C for 10 minutes) plus addition of 40mg Citric acid per litre of sugar cane juice sample were taken for Laboratory analysis of physical chemical and Microbiological analysis.

Table 3: Categories of samples collected for analysis

Juice Categories	Sample size	Sugarcane Juice types
60 Unpasteurized	30	Iced (with ice blocks)
	30	Raw (fresh without ice)
40 Pasteurized	20	Pasteurized
	20	Pasteurized+Citric acid

3.3.4 Structured questionnaires and observation checklist

This study collected only primary data. Respondents were interviewed on and respond on structured questions (Appendix 1) as data collection tool. In structured questionnaires, (both closed-ended questions were used in most of the questions and open-ended questions) were used. Observation checklist (Appendix 2) was used to collect information from the respondents' site (premises).

3.3.5 Laboratory sample collection for analysis

The laboratory samples for microbiological and physicochemical analysis were collected. The assumption in collecting the samples was that the population was unknown that a preliminary survey was made in October, 2017 to identify the vendor locations and sites, sugarcane juice samples were collected immediately after the questionnaire in the morning. One sugarcane juice sample was collected from their buckets with ice block of which 25ml was collected aseptically in sterile autoclaving bottles this was termed iced sample. Another sample was collected direct at the point of extraction from the roller machine outlet without mixed with ice blocks (this was termed raw). This sample was collected in the sterile autoclaving bottle. Another sample was collected from one factory sugarcane juice producer (SMEs) who was involved in the study for comparison purposes in which pasteurized sugarcane juice sample and pasteurized plus addition of Citric acid sugarcane juice sample were also taken.

All samples were marked for identification, immediately put into a sterile glass bottle, stored in a cool box with ice parks and taken directly for analysis at TBS Food Laboratory in Ubungo, Dar es Salaam.

Plate 1: Collected samples for Laboratory analysis

3.4 General Assessment on Handling Practices of Sugarcane Juice

General assessment of observed juice handling practices of the sugarcane juice vendors was done by selecting parameters and ranked them in score sheet assigning different levels of quality/labels example, effective, moderately and poor.

3.5 Physicochemical Analysis of Sugarcane Juice

3.5.1 Total soluble solids (TSS) determination

Total soluble solids TSS (°Brix) were determined using refractometer model Rf m860 BS (Bellingham and Stanley Ltd., London, U.K) at 20^0C. The reflectometer was standardized by using distilled water at 0 °Brix. The prism was cleaned with distilled water and dried with soft tissue after each reading. By using sterile pipette approximately 3 drops were drawn and applied to the reflectometer prism. The prism was covered and the start button was pressed to initiate the measuring. After 3 seconds the reading of °Brix and temperature appeared on the screen of the refractometer. The reading was recorded accordingly. The procedure was repeated for all samples.

3.5.2 Determination of pH

The pH of the sugarcane juice samples was determined by using digital meter analyser Mettler tolledo 10LF00, London, (U.K) which was first calibrated using standard buffer solutions of pH 7.0 and pH 4.0 (ISO 7218:2013). Ten (10 ml) of the sugarcane juice sample in a Teflon tube were shaken vigorously for 45 sec and used for pH determination.

3.5.3 Determination of total titratable Acidity (TA)

The titratable acidity of the sugarcane juice samples (expressed as % citric acid) was determined by the recommended method by AOAC, (1999). Ten 10 ml of the sample was against 0.1NaoH using phenolphthalein indicator. About 10 ml of the sample was pipette into a 250 ml conical flask and few drops of phenolphthalein indicator into the conical flask. It was titrated against 0.1 NaOH up to light pink end point with the solution sample. Titratable acidity of the sugarcane juice samples (expressed as % citric acid) was then calculated by the formula;

$$Titratable\ \%\ acidity\left(\frac{w}{w}\right) = \frac{Titratable\ volume(ml) \quad \times \quad (0.064)}{Samle\ weight} x100 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where: 0.064 = the acid factor for citric acid

3.6 Media preparation for Microbiological Analysis

3.6.1 Plate count agar (PCA)

Plate count agar (PCA) was prepared by mixing 17.5g of dehydrated powder in 1 litre of purified water in glass bottle, heated with frequent agitation and boil for 1 min. to complete dissolve powder, this is in according to manufacturer's instructions of the brand used OXOID® Ltd., Basingstoke, U.K. It was distributed to final containers and sterilized by Autoclaved at 121°C for 15 min. The media was left to cool in a water bath around 45 °C after being autoclaved before used for plating.

3.6.2 Buffed peptone water-(BPW)

Buffed peptone water-(BPW) is pre-enrichment medium prior to selective enrichment in the isolation of *Salmonella spp.* The media was prepared according to manufacturer's an instruction (Liofilchem (TE) Italy) in which 20g of the powdered medium was added into 1000 ml of distilled water. It was mixed well in final containers and sterilized/autoclaved at 121^{0}C for 15 minutes. The medium was cooled to about 20^{0}C before used.

3.6.3 Triptone bile agar Medium (TBX)

Triptone bile agar Medium (TBX) is the selective Chromogenic medium for detection and enumeration of *Escherichia coli.* Based on Tryptone Bile agar. Addition of a chromogenic agent X glucuronide which defects glucuronidase activity. The brand used was OXOID® Ltd (Basingstoke, U.K) 30g of the powder was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. This was followed by sterilization by autoclaving at 121^{0}C for 15 minutes. It was allowed to cool in the water bath at 50^{0} C to 44-47^{0}C (ISO 16649-2:2001) (E) before used for plating.

3.6.4 Xylose-Lysine-Desoxycholate Agar (X.L.D)

Xylose-Lysine-Desoxycholate Agar (X.L.D) is the selective enrichment for growth of *Salmonella spp.* The brand used was Liofilchem (TE) Italy. The medium was prepared as per manufacturer's instruction in which 55.4 g of powdered medium was suspended in 1 litre of distilled water. The medium was heated with frequent agitation to boiling point taking care to avoid overheating. Immediately the medium was transferred into water bath at 50°C for cooling. As the medium temperature reached around 45 – 47 °C it was poured into sterile Petri dishes placed on the lamina floor and it was left to solidify.

3.6.5 Urea Agar base

Urea Agar base is the growth medium used for confirmation of *Salmonella spp.* The brand used was Liofilchem (TE) (Italy). The medium was prepared according to manufacturer's instructions by suspending 24 g in 950 ml of distilled water and boiled to dissolve completely, followed by sterilization by autoclaving at 115°C for 20 min. The medium was cooled to 50°C and aseptically 5 ml of sterile 40% Urea solution was introduced. It was mixed well and then 10 ml were distributed into sterile containers and allowed to settle in slope position.

3.6.6 Triple Sugar Iron Agar (TSI)

Triple sugar Iron Agar (TSI) is the growth medium used for confirmation of *Salmonella spp. The* brand used was Liofilchem (TE) (Italy). The medium was prepared by suspending 65.5g of powdered medium in 1000ml of distilled water. It was boiled to dissolve completely, mixed well and distributed into final containers followed by sterilization of the medium by autoclaving at 121°C for 15 min. After sterilization, the medium was allowed to set in sloped form with a butt about 1inch deep.

3.6.7 Rappaport-vassiliadis soya peptone broth (RVS)

Rappaport-vassiliadis soya peptone broth (RVS) Rappaport is the selective liquid medium for isolation of *Salmonella spp.* The brand used was from OXOID® Ltd., Basingstoke, (U.K). The medium was prepared according to manufacturer's instructions by suspending 30 g in 1 litre of distilled water and heated gently to dissolve. Then, 10 ml was dispensed into screw-chopped bottles and were sterilised by autoclaving at 115°C for 15 min. Glass bottles with medium were placed on the lamina floor to cool about 20°C prior to use.

3.6.8 Brilliant Green Agar (BGA)

Brilliant Green Agar is recommended for the selective enrichment of *Salmonella* spp. Brilliant Green Agar - used brand was Liofilchem (TE) (Italy). The medium was prepared according to manufacturer's instructions by suspending 52 g in 1 litre of distilled water and gently heated to boil. It was allowed to cool to 45-50 ^{0} C and mixed gently and poured disposable sterile Petri dish plates. And left to dry for about 2 hrs with covers partially open.

3.6.9 Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn)

Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn) is the selective liquid medium for isolation of *Salmonella spp.* It was prepared according to manufacturer's instructions, the brand used was OXOID® Ltd. Basingstoke, (U.K). 89.5g of powder was suspended in 1000 ml of distilled water, mixed well and brought to boiling and then cooled to below 45°C. Immediately before use 20 ml of iodine were added followed by adding the contents of four vials of Novobiocin selective supplement. The medium was mixed well and aseptically dispensed into sterile screw-capped bottles.

3.6.10 Tryptone water medium

The brand used was OXOID® Ltd., (Basingstoke, U.K.). It was prepared according to manufacturer's instructions. The medium was prepared by dissolving 15 g of the powder in 1000 ml of distilled water and distributed in final containers. It was then sterilized by autoclaving the medium at 121°C for 15 min.

3.7 Laboratory Procedure for Microbiological Analysis

3.7.1 Determination of total plate count (TPC)

Total plate count (TPC) as per ISO 7218:2007(E)). Twenty-five (25) ml of the sugarcane juice sample was pippeted in a glass bottle containing 225 ml of the sterilized pre-enrichment BPW and mixed with frequency agitation for 1 min. Serial dilutions were carried out in tenfold from 10^{-1} to 10^{-6} in tubes containing 9 ml of the diluent (0.1% BPW) using sterile micro pipettes. One (1 ml) of the sample was added to 9 ml of diluent to make (10^{-1} dilution), then 1ml from this dilution was transferred to the second tube containing 9 ml to make (10^{-2} dilution). The procedure was repeated up to 10^{-6} dilution. Sterile disposable petri dishes size (15x100 mm) were used for plating, in which 1 ml from each dilution was pour plated in duplicate and two replicates were prepared for each dilution. About 12-15 ml of PCA which was cooled in the water bath to (44-47)^{0}C was poured onto each petri dish. The inoculum was allowed to mix with the media by carefully rotating the petri dish. The plates were left to cool and solidify on the horizontal surface of the lamina floor. The petri dishes were inverted and placed in the incubator at 30±1^0 C for 72±3 hrs (ISO 4833-1:2013) Two controls were also involved and the procedure was done parallel as per sample. Positive control was *Salmonella typhimurium* (ATCC13311) to assess the effectiveness of the media and the blank media was PCA agar media to assess sterility of the media and quality of preparation environment.

The numbers of colonies were counted on plates after incubation and the number of cfu/ml were counted at least two critical dilution, where two consecutive plates with 15 to 300 colonies were considered for record. The countable colonies from two conservative plates were converted into cfu/ml of sugarcane juice (ISO 7218:2007(E)). and the results were expressed using equation 4

$$N = \frac{\Sigma C}{V(n1 + 0.1n2)\, d} \quad \ldots (4)$$

Where N = Colony forming unit (cfu/mL)

ΣC = sum of colony counted in two successful dilutions,

N1= number of dishes retained in the first dilution

N2 = number of dishes retained in the second dilution

d=dilution factor corresponds to the first dilution

V=volume of inoculum, in millilitres, applied to each case, (in this case 1 ml was used) for pour plate count

3.7.2 *E. coli.* determination in sugarcane juice

E. coli. in sugarcane juice was determined as per ISO 16649-2:2001(The colony-count technique at 44^0 C on solid medium (TBX) containing a chromogenic ingredient for detection of the enzyme beta – glucuronidase). Twenty-five (25) ml of the sugarcane juice sample was pippeted in a glass bottle containing 225 ml of the sterilized pre-enrichment BPW and mixed with frequency agitation for 1 min. Serial dilutions were carried out in tenfold from 10^{-1} to 10^{-6} in tubes containing 9 ml of the diluent (0.1% BPW) using sterile micro pipettes. One 1 ml of the sample was added to 9 ml of diluent to make (10^{-1} dilution), then 1ml from this dilution was transferred to the second tube containing 9 ml to make (10^{-2} dilution). The procedure was repeated for further dilution up to 10^{-4} dilution. Sterile petri dishes size (15x100 mm) were used for plating in which 1 ml from each dilution was pour plated in duplicate and two replicates were prepared for each dilution. About 12-15 ml of the sterilized TBX agar at 44-47 ^{0} C was mixed gently and poured to the disposable sterile Petri dish plates. The plates were left to cool and solidify on the horizontal surface of the lamina floor. The petri dishes were inverted and placed in the incubator. In order to capture both stressed cells and active cells, the plates were incubated

for initial period of 4 h at 37 ^{0}C and then raised the incubation temperature to 44^0 C for 24 h (ISO 16649-2:2001). Three controls were involved and the procedure was done parallel as per sample (positive, negative and blank). Positive *E. coli* (ATCC8739) was employed to assess the effective of the media, negative control was (*Staphylococcus aureus* ATCC 6538) to assess selectiveness of the media and blank control was (TBX media) to assess sterility of the media and preparation environment.

The numbers of colonies were counted on plates after incubation by aid of colony counter (Schutt count plus and the number of cfu/ml were counted at least two critical dilution, where two consecutive plates with 15 to 300 colonies were considered for record. The TBX critical dilution of 10^{-1} to 10^{-3} were best for countable range 15-30 colonies. The countable colonies from two conservative plates were converted into cfu/ml of sugarcane juice and the results were expressed using equation 4.

3.7.3 Detection of *Salmonella spp.* in sugarcane juice

Salmonella spp. was detected as per by ISO 6579:2002 and the following steps were involved.

Step 1: Pre-enrichment in non-selective medium (BPW)

Twenty-five (25 ml) of the sample was prepared in culture bottle containing 225 ml of the sterilized pre-enrichment BPW and mixed with frequency agitation for 1 min followed by incubation at 37 ^{0}C for 18±2 hrs.

Step 2: Enrichment in selective liquid medium (RVS) broth

The volume of 0.1ml of the prepared culture in pre-enrichment medium step 1 was inoculated in 10 ml (RVS) broth and incubated at 41.5 ^{0}C for 24 hrs, at the same time 1ml of the same culture was inoculated in 10ml of (MKTTn) broth and incubated at 37 ^{0}C for 24 hrs.

Step 3; Plating and identification of *Salmonella spp.*

The RVS and MKTTn obtained in step 2 was shifted to XLD agar by streaking where the surface of the plates with XLD was divided into two halves, one half was streaked the culture from RV broth and the other half by the culture from MKTTn broth. The same process was repeated for BGA agar where 4 plates where prepared in duplicate. The dishes were inverted and incubated at 37 ^{0}C for another one day (24hrs). After one day incubation the culture were examine for typical colonies of *Salmonella spp.* and atypical colonies that may be *Salmonella spp.* Typical colonies of *Salmonella spp* grown on XLD agar have a black centre and light transparent zone of redish color due to change of phenol red indicator.

3.7.4 Conformation test for salmonella spp.

3.7.4.1 Detection of salmonella spp. in sugarcane juice

Selected plates were identified for confirmation and suspected colonies from each plate were picked by means of sterile inoculating loops and streaked on surface of nutrient agar plates to allow well development of isolated colonies then the inoculated plates were incubated at 37 ^{0}C for 24 hrs. After one day the resulting pure culture was used in biochemical test which were confirmation in TSI and Confirmation in urea agar as follows;

With confirmation in TSI, each of the pure culture was inoculated to the agar slant surface and stubbed the butt of the agar by means of sterile inoculating loop and incubated at 37 ^{0}C for 24 hrs, after one day incubation the changes in the medium were interpreted as; Typical salmonella culture shows alkaline (red) slants and yellow butts with gas formation (bubbles) and in 90% of the cases produce hydrogen sulphide detected by blackening of the agar. When lactose is positive *Salmonella spp.* is isolated the TSI slant is yellow.

With Confirmation in Urea agar, each of the pure culture was incubated on the agar slant surface by means of a sterile inoculating loop, then incubated at 37 ^{0}C for 24 hrs. The incubated agar was examined at interval. The positive reaction (which is apparent after 2-4hrs) indicating the splitting of urea to reberate ammonia, which changes the color of phenol red to rose-pink and later to deep cerise.

3.8 Statistical Data Analysis

Data were analysed by using Statistical Packages for Social Science (IDM SPSS Version 20). Descriptive statistics was used to compute the frequencies and percentages of questionnaires data. Bacteria counts were normalized by Log transformation. Analysis of Variance (One -way ANOVA) was used to compute the mean, Standard deviation and range of Laboratory data and compared the significance at ($P<0.05$): (95% C. I) of variation between main factors. Results were expressed as mean±SD and presented in tabular and graphic forms.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic Characteristics of Sugarcane Juice Vendors

The assessed demographic parameters of sugarcane juice vendors are presented in Table 4. The study assessed a total of 60 vendors. The results of gender indicate that the majority of sugarcane juice vendor were males (96.7%) as compared to female vendors (3.3%). Furthermore, education level of the sugarcane juice vendors showed that the majority (51.7%) had primary school education while (48.3%) had secondary school education. There was no sugarcane juice vendor with college education. The majority of the Sugarcane juice vendor (63.3%) had less experience of vending duration of less than 1 year. However, fewer vendors (10%) had long experience of more than 3 years in sugarcane juice vending.

Table 4: Demographic characteristics of sugarcane juice vendors

Parameter	Category	Frequency	(%)
Gender	Male	58	96.7
	Female	2	3.3
	Total	60	100.0
Educational level	Primary	31	51.7
	Secondary	29	48.3
	Total	60	100.0
Duration of vending sugarcane juice	Less than 1 year	38	63.3
	1-2 year	16	26.7
	3-5 years	6	10.0
	Total	60	100.0
Vending type	Street	40	66.7
	Restaurant	20	33.3
	Total	60	100.0

Values are expressed as frequencies and percent distribution of vendors in respect to gender, education level, duration of vending and type of vending.

4.2 Handling Practices and Methods of Production of Sugarcane Juice

4.2.1 Production of sugarcane juice

Sugarcane juice is produced by using the roller machinery and other simple equipment including knives, plastic buckets, cool boxes, plastic cups, glass cups, re-used plastic bottles, mags, plastic funnel. Cold facilities are commonly bucket with ice. The raw material is sugarcane which was purchased by vendors from Dar Es Salaam markets including (Tandale, Mabibo and Buguruni). The ice blocks and lemon added to the sugarcane juice were obtained from the local supplies in Dar es Salaam market. Water used for washing utensils was tap water.

The production steps included chopping, cutting, squeezing to obtain sugar cane juice. Sugarcane stems were chopped and cut into pieces of about 100 cm, followed by squeezing by a roller machine with stainless steel cylinders, operated either mechanically or by an electric motor. Ice blocks were introduced into the buckets, which received the sugarcane juice and allowed to mix with ice blocks. Lemon was added in the juice in small proportions to improve the sensory quality of the sugarcane juice. During preparation bare hands were used for handling the ice and sieving of sugarcane juice. Sugarcane juice was poured into about 250ml and 500ml glass cup and sold at Tanzania shillings between 500/= and 1000/=respectively. A glass or cups of 250 ml of cold sugarcane juice with iced was sold at 500/= while fresh (raw) sugar cane juice was sold at 1000/=. Depending on customer preference the juice was consumed in glass cups or sold as take away in re-usable plastic bottle.

Plate 2: Steps in production of sugarcane juice in Dar es Salaam city
Key: (a) Chopped stems at 100 cm (b) inserting stems to the roller machine (c) pressing to obtain juice (d) filtration to obtain fresh sugarcane juice.

4.2.2 Sugarcane juice handling practices

The results of sugarcane juice handling practices showed that, all interviewed sugarcane juice vendors (100%), declared that sugarcane juice pasteurization was not done by street and restaurant vendors. It was also noted that all interviewed vendors (100%) were not adding water to the sugarcane juice instead ice blocks were commonly added. Lemon added to the sugarcane juice by all interviewed vendors (100%) to improve the sensory property of the sugarcane juice. Majority of the vendors (75%) used buckets with ice as the cold storage which is not effective for intended use. Majority of the vendors (55%) used cold water to clean the utencils and only (5%) use hot water and soap indicate poor washing methods.

The majority of the vendors (76.7%) did not know the quality of the ice used, as they are getting from their local suppliers and only 23.4% declared to treat by boiling the water used for ice at home.

Table 5: Sugarcane juice handling practices

Assessed parameter	Category	Frequency	Percent
Cold storage containers	Ice box	12	20
	Deep freezer	3	5
	Bucket with ice	45	75
	Total	60	100.0
Cleaning service utensils	Cold water and soap	33	55
	Cold water alone	23	38.3
	Hot water and soap	3	5
	Hot water alone	1	1.7
	Total	60	100.0
Roller machine cleanliness	washing with cold water	27	45
	Washing with cold water and soap	22	36.7
	Washing with hot water	8	13.3
	Washing with hot water and soap	3	5
	Total		100.0
Is ice used in sugarcane juice	Yes	60	100.0
Is roller machine covered after squeezing	Yes	39	65
	No	21	35
	Total	60	100.0
Is water used to make ice treated/boiled?	Yes	14	23.3
	No	46	76.7
	Total	60	100.0
Is water added to sugarcane juice?	No	60	100.0
Addition of lemon or ginger in sugarcane juice	Yes	60	100.0
Sugarcane juice pasteurization is done	No	60	100.0
Serving utencils	Glass	39	65
	plastic cups	15	25
	disposable plates	6	10
	Total	60	100.0
Waste disposal	in plastic bags	49	81.7
	dust bin	11	18.3
	Total	60	100.0
Medical check up	Yes	24	40
	No	36	60
	Total	60	100.0

Values are expressed as frequencies and percent distribution of vendors in respect to the assessed parameters.

4.2.3 Observation on preparation practices of sugarcane juice vendors

The general observation from the preparation practices of sugarcane juice vendor showed that, the sugarcane juice was prepared in unhygienic environment (Table 6). Poor washing methods were observed in which 27 vendors (45%) observed washing utensils using cold water without detergents. Hand washing equipment and disinfectant were not observed in majority of the vendors (81.7%) (Table 6) which indicate poor hygiene practices. Most vendors (78.3%) had pests in their premises which encourage the presence of flies and bees which are source of microorganisms. It was also observed that the majority (71.7%) of the sugarcane juice assessed was not protected from source of contamination, the buckets with juice, roller machine was left uncovered.

Table 6: Observed preparation practices of sugarcane juice vendors

Assessed parameter	Category	Frequency	Percent (%)
Does preparation set min contamination?	Moderately	11	18.4
	Poorly	49	81.6
	Total	60	100.0
Does washing process min contamination?	Moderately	33	55
	Poorly	27	45
	Total	60	100.0
General cleanliness of handler	Good	42	70
	Poorly	18	30
	Total	60	100.0
Vendor has working uniform	Fully	5	8.3
	Partial	23	38.3
	No uniform	32	53.3
	Total	60	100.0
Hand washing equipment and disinfectant present	Yes	11	18.3
	No	49	81.7
	Total	60	100.0
Waste receiving receptacle present	Yes	31	51.7
	No	29	48.3
	Total	60	100.0
Presence of pests	Yes	47	78.3
	No	13	21.7
	Total	60	100.0
Is the juice protected from source of contamination	Yes	17	28.3
	No	43	71.7
	Total	60	100.0
Is the storage facility effective for the intended purpose?	Yes	15	25
	No	45	75
	Total	60	100.0

Values are expressed as frequencies and %age distribution of vendors in respect to preparation setting, washing process, cleanliness of handlers and uniforms, disinfectants, waste disposal, pest control and effectiveness of storage facilities

4.3 Physical-chemical Properties of Sugarcane Juice

4.3.1 TSS of sugarcane juice (^{O}Brix)

The physical chemical characteristics of the sugarcane juice were investigated as presented in (Table 7). There was statistically significance difference ($P<0.05$) in TSS ^{o}brix between all sugarcane juice types. The TSS of iced sugarcane juice ranges from (2.4-22.1) ^{o}brix. The results showed a big range of variation in TSS for iced sugarcane juice. The TSS of raw sugarcane juice range from 12.2-22.1 ^{o}brix. Majority of the TSS of raw sugarcane were where higher and within recommendation Fig. 4. Raw sugarcane juice showed higher values of TSS than pasteurized and iced (Table 7) sugarcane juice vended.

Table 7: Physical-chemical characteristics of Sugarcane juice

Physical parameter	Unpasteurized		Pasteurized	
	Iced	Raw	Pasteurized	Pasteurized+Citric acid
^{o}Brix (20^{0} C)	9.73±3.5^{a}	18.22±2.2^{b}	14.90±0.7^{c}	15.20±0.7 ^{d}
pH	4.80±0.2^{a}	4.90±0.2^{a}	4.30±0.3^{b}	3.10±0.2^{c}
Acidity	0.08±0.04^{a}	0.08±0.04^{a}	0.11±0.03^{a}	0.57±0.03^{b}

The mean difference is significant at the 0.05 level. Values are Mean+ standard deviation of duplicate samples with sample size n=20. Means with different (superscript) on the same row are significantly different at $P<0.05$. Raw=Fresh sample without any treatment, Iced=sugarcane juice with added ice blocks, Pasteurized+Citric acid =Pasteurized sample with addition of Citric acid.

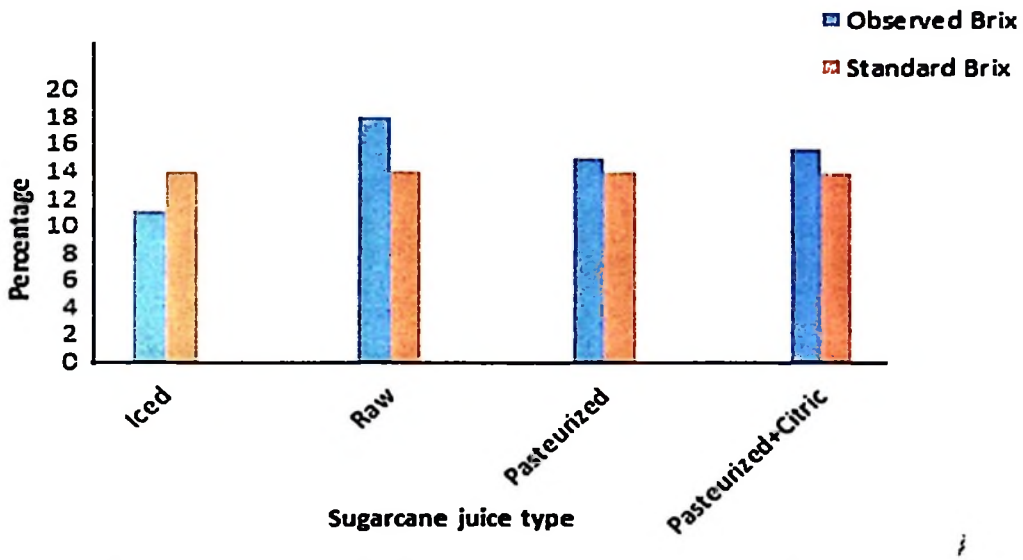

Figure 4: Bar chart showing TSS (°brix) of different types of sugarcane juice

4.3.2 The pH and acidity of sugarcane juice

The pH of unpasteurized juice was determined and found to range from 3.68-4.80 for iced sugarcane juice (Table 7). The pH of raw sugarcane ranges from 3.6-5.9. There was no statistical significance difference ($P<0.05$) in pH between Iced and raw sugarcane juice sugarcane juice vended.

Pasteurized sugarcane juice had low mean pH (Table 7). There was statistical significance difference ($P<0.05$) in pH between Iced and pasteurized sugarcane juice and also there was a statistical significance difference ($P<0.05$) in pH between raw and pasteurized sugarcane juice. Furthermore, the pasteurised sugarcane juice with addition of 40 mg of citric acid per litre had the lowest mean pH values (Table 7).

The acidity of unpasteurized sugarcane juice ranges from (0.083-0.084). There was no statistical significance difference ($P>0.05$) (Table 7) between acidity of two types of unpasteurised sugarcane juices (raw and iced), However, the results showed that there were higher values of acidity in iced sugar cane juice than in raw sugar cane juice vended.

Furthermore, higher values in acidity was observed in pasteurized sugarcane juice than unpasteurized (Table 7)

4.4 Microbial Status of Sugarcane Juices Vended in Dar es Salaam

4.4.1 Total Plate count (TPC)

The results of microbiological levels of sugarcane juice were determined and presented in Table 8. The results of total plate count of all unpasteurized ranges from 3.6 – 6.7 log cfu/ml depending on juice type.

There was no significance ($p>0.05$) between raw and iced but higher TPC levels in sugarcane juice with ice blocks (iced) than in raw Fig. 5. The results of pasteurized sample showed significantly different ($p<0.05$), compared to unpasteurized (iced and raw) in TPC levels (Table 8).

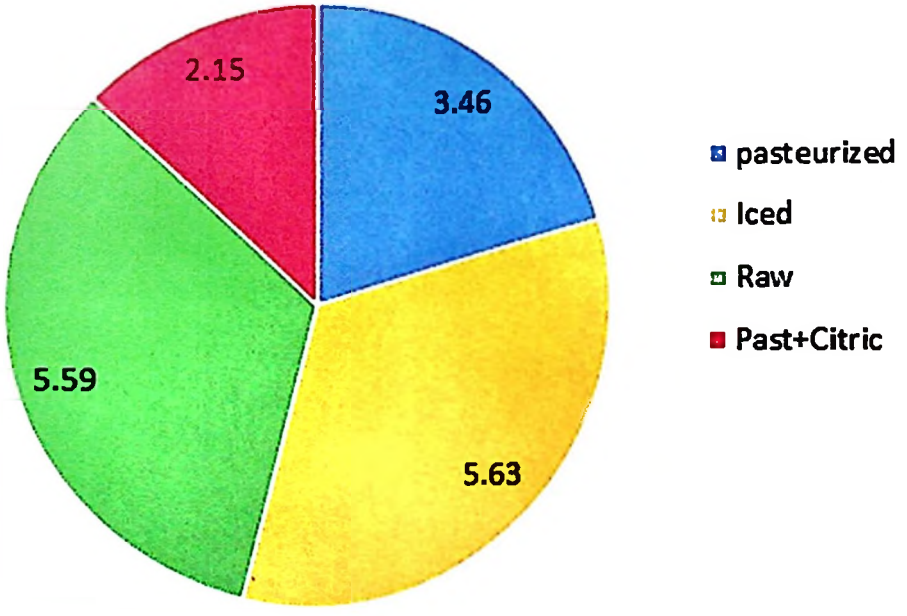

Figure 5: TPC levels against sugarcane juice types in log cfu/ml.

Table 8: Microbiological level of sugarcane juice log cfu/ml

Parameter	Unpasteurized		Pasteurized		Recommend
	Iced	Raw	Pasteurized	Pasteurized+Citric acid	
TPC	5.64±0.2[a]	5.59±0.2[a]	3.45±0.3[b]	2.18±0.1[c]	3.50-4.00
E. coli	2.10±0.7[a]	1.79±0.8[a]	X	X	Absent
Salmonella	X	X	X	X	Absent

The mean difference is significant at the 0.05 level. Values are mean±standard deviation of duplicate samples, n=sample size. Means with different superscript on the same row are significantly different at P<0.05. TPC=Total bacterial count, S. D=Standard deviation, X=Not detected, Raw=Fresh sample without any treatment, Iced=sugarcane juice with added ice blocks, Pasteurized sample with addition of Citric acid

4.4.2 *E. coli* contamination in sugar cane juice vended

The results of *E. coli* contaminations in unpasteurized sugarcane juice were determined and are presented in Table 8. The Prevalence was that 96.6% of the samples were contaminated with *E. coli*. The results between *E. coli* contamination in Raw and Iced sugar cane juice were not statistically significant (p>0.05), moreover the results showed higher *E. coli* contamination levels in iced sugarcane juice (52%) than in raw sugar cane juice (48%)

The *E. coli* contamination in Street vended and Restaurant vended sugarcane juice were not statistically significant (p>0.05). The results showed street vended sugarcane juice was more unhygienic poor as indicated by high load of *E. coli*.

4.4.3 *Salmonella spp.* contamination in Sugarcane juice

Salmonella was absent in all samples.

CHAPTER FIVE

5.0 DISCUSSION

The present study was conducted to determine quality and safety and handling practices of sugarcane juices vended along street and restaurants in Dar es Salaam city. The results of the findings indicated that sugarcane juice was contaminated and was prepared in unhygienic conditions. The findings were supported by the laboratory results on TPC and *E. coli* (Table 8) which were significantly high in sugarcane juice above the recommended limits.

5.1 Sugarcane Juice Handling and Preparation Practices

Sugarcane are transported from upcountry and are delivered into Dar es Salaam markets by trucks with soil deposited on the stems. The production of the quality and safe sugarcane juice depends on the Good Hygienic Practices of the vendor and environment for handling the production of the sugarcane juice.

In the present study, the socio-economic and demographic data showed that majority (96.2%) (Table 4) of the sugarcane juice vendors were young males. However, Abdalla *et al.* (2009) reported that most of the street food vendors were females. From the current study observations, the reason could the business itself is mechanical by nature and is different from production of fruits juice by using simple blenders. The results of the sugarcane juice handling examined by structured questionnaire showed that, the general practices of the vendors towards, assessed parameters were poor (Table 5). The majority of the vendors did not know the quality of the ice used, as they are buying them locally with no information (quality certificate) about the quality of ice used. Fewer of the vendors (23.3%) treat water used to make ice by boiling at home (Table 5). In this study the iced sugarcane juice was found to be more contaminated than fresh (raw) one (Table

8). At the same time, sugarcane was not washed before squeezing these could be the factors contributed for higher microbial load found in sugarcane juice. Furthermore, the roller machine (press) were left uncovered before and after squeezing, this could contribute to contamination by dust, flies. According to recommendation a place of food preparation should be kept clean at all times and should be far from any source of contamination (FAO, 1995). Majority of the vendors used plain cold water for washing utensils and the roller machine which could contribute to contamination. Poor cooling facilities were observed as majority of the vendors use buckets with ice cubes which could not provide adequate and long-lasting cooling (Table 5).

The observed practices of sugarcane juice vended (Table 6), showed that; the general practices of the vendors towards, preparation and vending practices were observed to be poor. The preparation of street sugarcane juice showed to predispose the sugarcane juice to contamination (Table 6). Poor hygiene includes; poor preparation methods, poor washing process, poor handling of the equipment and disinfectants, presence of pests, poor storage facilities and furthermore sugarcane juice was not protected from source of contamination (Table 6), Furthermore, the current study results suggested that the factors observed contributing to poor hygienic environment includes on observed parameters includes; inadequate sanitary conditions of the sugarcane itself (sugarcane are coming with soil deposits), they are not washed before squeezing are just chopped(plate 2), the water used for ice blocks making are not treated (the quality of the ice block used), the roller machine (press) left uncovered, the equipment and utensils are washed with plain cold water and the water used to wash the roller machine (press) is not treated.

The general results show that the general principle of food hygiene Codex Alimentarius food safety standards are not followed by the sugarcane juice vendors. A study on the

quality and safety of street sugarcane juice in Noida city India reported that contamination was mainly due to improper washing by workers, improper personal hygiene, unhygienic surroundings, vehicular transmission, sewage and the absence of good manufacturing practices (Verma and Gaur, 2017). Similar agreements were reported by Edeltruds, (2013).

5.2 Physical-chemical Characteristics of Sugarcane Juice

The current study determined the °Brix, pH and Acidity in order to know the quality status of the sugarcane juice vended and to relate with the microbial status.

The TSS of raw sugarcane juice was 18.2±2.2 °brix (Table 7). Similar findings were reported by (Sabrinho *et al.*, 2011) in which the TSS was found to range between 14 to 22 °brix (Sabrinho *et al.*, 2011). Iced sugarcane juice had average TSS of 9.7 °brix (Table 7) which is very low compared to recommended value (14 to 22) °brix (Sabrinho *et al.*, 2011). However, according to FAO, juice blends or beverages with less than 7°Brix are deemed weak and watery (Bates *et al.*, 2001). Low values of °Brix of iced sugarcane juice observed in this study may be due to over dilution of the sugarcane juice by addition of large amount of ice blocks. The reason might be due to lack of standardized amount of ice block added, the amount of ice added is diluting the juice and reducing the TSS.

The pH of sugarcane juice was presented in (Table 7). There was no statistical significance difference($p>0.05$) in pH between raw and iced sugarcane juice. The results of pH obtained in this study for unpasteurized showed similar findings to that reported by Carazza *et al.* (2001); Sobrinho *et al.* (2011). The results showed a statistical significance difference ($P<0.05$) in pH between unpasteurized and pasteurized (Table 7). However, the lowered pH of pasteurized sugarcane juice showed to inhibit microbial contamination of

the juice compared to unpasteurized, while on the other hand, higher pH of unpasteurized sugarcane juice showed to support growth of microbes as majority of the juices were contaminated with *E. coli* and had high TPC counts (Table 8) supported by Zahra (2010). The suppress of microorganism in pasteurised sugarcane juice might be attributed by lower level of pH. Lacks of pasteurization contribute to microbial load (Speck, 1976). Low pH such as 4.27 might still allow some TPC and cause deterioration (Tiiskwa, 2013). The pasteurised sugarcane juice with addition of 40mg of citric acid per litre has dropped the pH of sugar cane juice to average of 3.1, this is lower compared to recommended pH near 3.9 (Ivan *et al.*, 2014) the reason could be unstandardized or lack of standards for levels of citric acid added/used by the SMEs. According to Ciência (2014), good quality sugarcane juice (100 ml) with satisfactory storage stability at refrigeration could be prepared from heat-treated juice at (72°C for 15 sec) before addition of lemon (3 ml) as a combination of flavor, color enhancer and source of citric acid (antioxidant); moringa (10ml; ginger (0.6 mL) as flavor enhancer (Ciência, 2014). The effects of pH against microbial contamination have also been reported by Mesfin (2011) and Sunday *et al.* (2011).

Acidity of unpasteurized sugarcane juice was found to have mean value of 0.084±0.04 for iced while that of raw was 0.083±0.04. The results agree with Yusof *et al.* (2000) that Sugarcane juice had low acidity, (Yusof et *al.*, 2000). Juices with more than ~1.2% acid are sour, (Bates *et al.*, 2001). Fortunately, the acidity of majority of the sugarcane juices in this study was within recommended level (Yusof *et al.*,2000)

5.3 Microbial Quality of Sugarcane Juice

The microbiological quality of sugarcane juice was assessed in terms of total plate count (TPC), *E. coli* and *Salmonella spp.* The sugarcane juice samples of iced and raw analysed were found to be contaminated with higher levels of TPC and *E. coli* (Table 8)

The results of total plate count of all unpasteurized ranges from 3.6 – 6.7 log cfu/ml depending on juice type (Table 8). The results showed higher prevalence of contamination in which 90% of all samples were above recommended maximum limits 3.5-4 log cfu/ml (Kader *et al.*, 2014). Similar findings were reported by (Hameed *et al.*, 2016) in Pakistan but this is higher than that reported by Nagalakshmin (1995) in Brazil in which 30% of the sugarcane juice sample were above recommended (Nagalakshmin, 1995).

There was no significant difference in TPC ($p>0.05$) between iced and raw sugarcane juice but the results tended to show higher contamination levels in sugarcane juice with ice blocks (iced) than in raw sugarcane juice vended (Table 8). The variation in TPC of both types may be due to the quality of the ice block added to the sugarcane juice. A study by (Raheem, 2015) reported that the microbiology quality of ice was not good which could be due to use of bare hands, not cleaning of utensils and quality of water used to prepare ice. From the current study the juice handling practices showed that 76.7% of the sugarcane juice vendors were not treating water used to make ice, or another reason may be due to unhygienic maintenance during preparation (Ankond *et al.*, 2011). There was no significant difference in TPC between the street vendors, and restaurants on microbial quality ($P>0.05$). However, the results showed increased TPC contamination levels in street sugarcane juice vended (51%) than in restaurant sugar cane juice vended (49%). The high microbial load in sugarcane juice elucidates poor hygienic status of street vended sugarcane juice (Hemed *et al.*, 2016). There was statistically significant difference

($p<0.05$) between unpasteurised and pasteurised sugarcane juice (Table 8). The TPC results of the pasteurized sample analysed was found to be within the recommended level of 3.7-4.7 log cfu/ml This implied that low level of pH due to pasteurization inhibit the growth of bacteria, where the high pH of unpasteurized sugarcane juice supports the bacterial growth (Lee *et al.*, 2006). The overall results of TPC reflect the inadequate sanitary conditions of the sugarcane itself, and the results suggested that sugarcane juice could be contaminated due to the factors such as; use of unboiled water for making ice, the roller machine press left uncovered, no washing of the sugarcane stem before squeezing instead chopping only was done. Use of plain/cold water for washing utensils instead of using hot water and detergents. The major factors contributing to foodborne disease in majority of countries reported as contamination of food from raw food, infected handlers, inadequately cleaned equipment and time and temperature abuse to be (WHO, 2006).

The results of *E. coli* contamination in unpasteurized sugarcane juice iced and raw were presented in (Table 8). The results of *E.coli* for unpasteurized both iced and raw were above recommended by Tanzania Standards (TZS 585:2003) Codex general standards). The results of *E.coli* of unpasteurized were higher with a prevalence of 96.6% of all the samples were contaminated with *E. coli.* Similar results were reported by Mahale *et al.* (2008) in which almost all sample were found contaminated by *E. coli.* Several foodborne diseases are associated with the consumption of the foods that are previously exposed to the pathogenic microbes (Akhtar *et al.*, 2013). The results in this study was higher than that found in unpasteurized sugarcane juice reported by (Nagalakshmi,1995) and (Edeltruds, 2013) in which the prevalence was 80%. higher prevalence compared to those obtained by Abid in Pakistan in which 75% of the sugarcane juice sample were contaminated with *Escherichia coli* (Abid, 2008). *Escherichia coli* must be absent in ready to drink beverages (TBS, 2003). The presence of *E. coli* is not allowed by safe food

consumption standards (Andres *et al.*, 2004). Higher levels of *Escherichia coli* contamination suggesting of direct faecal contamination, or contamination from environment (Rashed *et al.*, 2012). There was no significant difference ($p>0.05$) between iced and raw sugarcane juice in *E,coli* contamination levels but the results tended to show higher contamination levels in sugarcane juice with ice blocks (iced) than in raw sugar cane juice vended (Table 8).

All samples showed presence of *E. coli* which could be explained by poor hygienic condition of the premises and the handling practices of the sugarcane juice vendors. Factors contributing to high contamination could be inadequate or no washing of the sugarcane stem; observation from the current study showed that sugarcane are brought with soil deposit and no washing of the sugarcane stem is done to remove the soil. Another reason could be the use of unhygienic water to prepare the ice blocks as the water used to make the ice blocks was not treated by boiling it, majority receive the ice blocks from their local supplier but the quality of water used is not known. A study by Raheen, (2015) reported that ice was contaminated because of quality of water used to prepare it, hence there is need of set proper standard on the quality of ice. The high pH of sugarcane juice due to lack of pasteurization showed to support bacterial growth. *E. coli* was found to be predominant in street food due high pH (Subbannya *et al.*, 2007). According to Tambeker *et al.* (2009) presence of *E. coli* could be due to inadequate hand washing by vendor or absence of GMP. The main source of *E. coli* contamination might be due to unlashing of the sugarcane stem to remove the soil deposit on the sugarcane before squeezing. Another reason could be the use contaminated water for making ice blocks. All this contributes to the absence of good manufacturing practices (Tambekar *et al.*, 2009). According to Tanzanian Specifications (TZS 585: 2003), *E. coli* must not be present in ready to drink beverages (TBS, 2003).

In Tanzania study, Robert (2011) reported that the level of contamination of food and water by *E. coli* was significantly high that 75% of water supplies in Iringa region were contaminated with *E. coli.* Another study investigated water wells in Dar es Salaam and reported that 87% were contaminated by *E. coli* (Mushi *et al.*, 2012).

The results of *Salmonella* spp detection indicated that No typical *Salmonella* spp was detected in all 60 samples. Similar results were reported by Aline *et al.* (2006) in which *Salmonella* spp was absent in all samples, likewise reported by Edeltruds (2013) and Souza *et al.* (2008), in other juices. However, a number of *Salmonella* outbreaks have been reported involving fruit juices especially unpasteurized ones in several places around the world (Vojdani *et al.*, 2008 and Poonam *et al.*, 2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The Present study exhibited the quality and safety of street sugarcane juice vended in Dar es Salaam city, Tanzania to ensure consumption of safe food and control over public health risk (disease outbreak). From the data presented in the current study, it can be concluded that:

Juice handling practices, preparation, premises and person hygiene were poor. The preparation practices and vending environments were unsatisfactory and unhygienic. These factors might have contributed to the higher microbial load. The pH of most of unpasteurized sugarcane juice samples was higher and support bacterial growth. The pH of pasteurised sugarcane juice was lower and inhibits microbial growth. Acidity of most of fresh (raw) sugarcane juice samples were low and within the recommended level. Majority of iced sugarcane juice samples had low TSS (°Brix) beyond recommended limits. The majority of unpasteurized sugarcane juice samples showed higher levels of TPC above recommended maximum level by Codex. *E. coli* were detected in all samples of unpasteurized sugarcane juice and in most cases were above the recommended limit for consumption. Iced sugarcane juice was more contaminated with respect to TPC and *E. coli* than fresh (raw) sugarcane juice but *Salmonella* spp was not detected in all samples. Both streets vended and restaurant vended sugarcane showed high contamination than codex recommended limit.

The prevalence observed in the current study indicates that most of the unpasteurized sugarcane juices vended in Dare es Salaam are not fit for human consumption. Such

situation is likely to be contributed by limited education on hygiene and inadequate food safety measures as the majority of the vendors had primary level of education and are had no training on food safety.

6.2 Recommendations

The prevalence observed in the current study indicates that most of the raw unpasteurized sugarcane juices vended in Dare es Salaam are not fit for human consumption. It is therefore recommended that there should be;

i. Training of sugarcane juice vendors on Good Hygienic Practices (GHP) by the Government quality and safety authorities, TBS, TFDA; training to maintain hygiene, to use quality ice, to use good quality water, washing of the sugarcane stem before squeezing, proper covering of the roller machine before and after use, properly washed utensils, maintain personal hygiene, and a proper business cites to be enclosed from dust environment.

ii. Establishment of sugarcane juice standard by TBS and other stakeholders.

iii. Establish specifications for Ice blocks. The use of such ice in food items also questions their quality so there is need of set proper standard on the quality of ice so that the consumers could be protected from bad quality food and pathogenic microorganisms.

iv. Registration of sugarcane juice premises and Certification of all sugarcane juice products including ice block makers.

v. Regular monitoring of street vended foods including sugarcane on the quality and safety for safe human consumption to avoid any future contamination and disease outbreak, is recommended.

vi. Further research on how to establish simple pasteurization technique to juice vendors.

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APPENDICES

Appendix 1: Structured Questionnaires

A. Personal particulars of the respondents

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

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

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

A. General Information

1. Sex ---------- 1. Male 2. Female

2. Level of education ----------Age.....

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

e) Vocational training

3. How long have you been vending raw sugarcane juice? ----------

4. Where do you get sugarcane used to prepare the juice from? ----------

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

c) From farmers d) From hawkers d) Others: Specify ----------

5. Methods of preservation prior juice processing

a) In a refrigerator b) On the floor c) On an open space d) In a bucket e) Others: Specify --

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6. If the juice is diluted where do you get water used for dilution of the juice?

a) From tap b) From wells c) Rain water d) Bottled water e) Others: Specify ----------

7. Do you wash the sugarcane before you start preparation of the juice? 1. YES 2. NO

If YES;

8. How do you treat the water before use in washing the sugarcane

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

C. Sugarcane handling before extraction of juices

9. What is the first procedure done on the sugarcane?

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

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

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

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

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

D. Methods of juice extraction

12. What do use to extract the juice?

a) I use squeezing machine b) Others: Specify ----------

13. How do you ensure cleanliness of the roller/parts of the machine before extraction

a) clean with cold water b) clean with hot water c) clean with hot water and soap

14 What do you do after cleaning the machine

a) leave it openly b) cover it with clothing c) cover it with a plastic material

15. Treatment of water before use for dilution of the juice

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

16. Do you add ice in juice? 1.YES 2.NO If yes where do you get ice from?.....

a) I prepare myself b) I buy from streets c) I buy from my supplier

17.Is water used to make Ice a) boiled before used b) Not boiled c) I don't know d) Others: Specify--

18.What are you adding apart from ICE?

a) Lemon a) leaves c) Not adding anything d) other: specify----------

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

19. How do you pasteurize?

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

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

E. Sugar cane Juices handling and storage after preparation

20. Where do you keep the juice after preparation

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

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

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

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

22. Vending place/site

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

23. Serving equipment

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

24. How do you wash the equipment after serving and before serving to another customer?

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

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

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

If YES How often?

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

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

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

d) I dispose it

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

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

f) In a covered bin

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

If YES

29. How often do you do medical check-up??

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

Appendix 2: Observational Checklist

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

YES NO

1 Does preparation setting minimize cross-contamination? Effectively, Moderately, poorly

2 Washing process; Does it minimize contamination? Effectively, Moderately, poorly

3 General cleanliness of the handler Very good, Good, Poor

4 Vendor has working uniform Fully, Partial

3 Hand washing equipments and disinfectant present

4 Waste receiving receptacle present Covered, Uncovered

5 Presence of pests (flies, cockroaches) Specify ----------

6 Is the juice protected from sources of contamination?

6 Is the storage facility effective for the intended purpose?

Appendix 3: *Salmonella spp.* contamination in sugarcane juice vended in Dar es Salaam

Type of sugar cane juice	Source	Media	No growth	Atypical growth	Growth
Iced sample	Restaurant	XLD	11	0	0
		BGA	7	4	0
	Street	XLD	18	2	0
		BGA	11	9	0
Raw sample	Restaurant	XLD	11	0	0
		BGA	6	5	0
	Street	XLD	15	1	0
	Factory	XLD			
		BGA	0	0	0
Pasteurized sample			0	0	0

XLD= Xylose-Lysine Desoxycholate Agar BGA=Brillint Green Agar

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