COMPLIANCE BY STREET SALAD AND JUICE VENDORS WITH NATIONAL FOOD SAFETY AND QUALITY REQUIREMENTS

A CASE OF MOROGORO MUNICIPALITY, TANZANIA

BY

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ABSTRACT

Compliance by street fruit salad and juice vendors with national food safety and quality requirements was evaluated. Evaluation was based on good manufacturing practices (GMP), good hygienic practices (GHP), selected heavy metals, physicochemical properties and microbiological quality parameters. Compliance by street vendors was also compared to vendors in university cafeterias and town restaurants. Among the fruit salad and fruit juice vendors, 71.4% and 54.3%, respectively, had no food safety knowledge. All vendors were not compliant with GHP and GMP. Fruit salad (94.1%) and 85.7% juice vendors were familiar with TBS and none, familiar with TFDA. A general compliance assessment using various indicators showed a range of 50-71% compliance for the right location and premises, 8-25% sanitation level, 0-50% processing and cleaning and ≤25% for personal hygiene. Samples were found free from heavy metals cadmium and lead, with one juice sample having 0.24mg/L copper. Ninety seven percent of juice and 85% of salad samples contained $>3\times10^6$CFU/ml or g of aerobic mesophilic count. Fifty one percent of the juice samples had fungi$>2.5\times10^6$CFU/ml with 41% having an average of 1.64×10^5 CFU/ml while for salads, 48% of the samples had an average fungi of 1.18×10^3 CFU/g. Moulds tentatively identified were *Aspergillus* spp and *Cladosporium* spp. Twelve percent of fruit juices had 2.8×10^3 MPN/ml of coliforms and 88% of the samples had $\geq1.6\times10^4$ MPN/ml of juice. Salads had an average load of 2.4×10^3MPN/g with 62% having $\geq1.6\times10^4$ MPN/g of coliforms. Ninety four percent of juice and 82% of salad samples were contaminated with *E. coli* with one sample containing *E. coli* O157:H7. Vendors on streets, at Universities and in restaurants were not significantly different in their compliance to hygiene requirements and microbiological quality criteria ($P>0.05$).Results of this study indicated the need for educating street vendors and other stakeholders in food hygiene to improve food safety.

DECLARATION

I, ADILI BENDANTUNGUKA TIISEKWA, 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.

_____ _____

Adili Bendantunguka Tiisekwa Date

The above declaration is confirmed

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Prof. J.K. Mugula Date

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DEDICATION

I dedicate this work to my father, Prof. B. P. M. Tiisekwa and mother, Dr. (Mrs.) J. B. Tiisekwa, for their hard work in raising me, and all their support in all fields of my life including all my academic achievements and my Beatha Mkojera and twin sons Leon and Luis for their encouragement.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

′	Coordinate minutes
″	Coordinate minutes
°	Degrees
°C	Degree Celsius
AAS	Atomic Absorption Spectrophotometer
AGA	Animal Production and Health Division (FAO department)
AGN	Nutrition and Consumer Protection Division (FAO department)
AGP	Plant Production and Protection Division (FAO department)
APC	Aerobic Plate Count
BAM	Bacteriological Analytical Manual
BGBB	Brilliant Green Bile Broth
BHMTF	Buguruni Healthy Market Task Force
CAC	Codex Alimentarius Commission
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
DDT	Dichloro Diphenyl Trichloroethane
DNA	Deoxyribonucleic Acid
E	East
E.C broth	*E. coli* broth
FAO	Food and Agriculture Organization
FII	Fisheries Industry Division (FAO department)
FOS	Department of Food Safety, Zoonoses and Foodborne Diseases (WHO department)
g	gramme
GAP	Good Agricultural Practices

GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
h	hour
HACCP	Hazard Analysis Critical Control Points
HITM	Hospitality Institute of Technology and Management
HIV/ AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
IMVC	Indole, Methyl Red, Voges-Proskauer and Citrate
JICA	Japan International Cooperation Agency
Kg	kilogram
L	Litre
LSTB	Lauryl sulfate tryptose broth
MAFSC	Ministry of Agriculture, Food Security and Cooperatives
MBW	Morogoro Ben's Winery
mg	milligram
min	minutes
ml	millilitre
MPN	Most Probable Number
MSFV	Mobile and Stationary Fruit vendors
NA	Nutrient Agar
NGO	Non-Governmental Organisation
NHD	Department of Nutrition for Health and Development (WHO department)
OMAFRA	Ontario Ministry of Agriculture and Food and Rural Affairs
ORP	Oxidation-Reduction Potential
PCR	Polymerase Chain Reaction

PDA	Potato Dextrose Agar
PHE	Department of Public Health and the Environment (WHO department)
POPs	Persistent Organic Pollutants
RBCA	Rose Bengal Chloramphenicol Agar
S	South
sec	seconds
SIDO	Small Industries Development Organsiation
SNV	Netherlands Development Organisation
SSFP	Small Scale Fruit Processors
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
TNTC	Too Numerous To Count
URT	United Republic of Tanzania
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Fresh foods are vulnerable to microbial contamination at all stages of production up to post harvest handling. Food-borne micro-organisms are widespread in the natural environment and can easily cross contaminate food surfaces, cooking utensils, food production systems and other food products which may result into food borne illnesses. Governments and the food industry today are more concerned with food-borne disease infection and intoxications than in the past (Todd, 1997). Different researches and technologies are innovated and applied in the food industry so as to ensure safe food at all stages of production (Institute of Medicine and National Research Council, 1998). Interventions are also carried out to educate producers and consumers on the importance of food safety so as to reduce the number of food borne outbreaks (Codex Alimentarius, 2007).

Unsanitary food handling is one of the major public health hazards. Food vendors should have a clear and direct responsibility to ensure that food products are safe for consumption. Poor practices can lead to potential dangerous hazards that can be physical, chemical or biological putting a risk to the handler as well as the consumer (HITM, 2006).Of these, biological hazards are the most common sources of food safety problems(Winter, 2011). Moulds, yeasts, viruses and bacteria are common microorganisms that present the greatest health risk. Poor hygiene, inadequate cooking and temperature controls are among the most common sources of biological hazards (Kim, 2008).

Bacteria are the most serious cause of foodborne illnesses and are usually the result of the presence of pathogens which can cause serious illness and even death (Teinaz, 2007).Should a foodborne illness be traced to food sold at a market, all those involved in the market from the vendors to the organizers, to the owners of the facilities could be open to legal liability and/or civil action (Winter, 2011).

Chemical hazards on the other hand come from additives, allergens, pesticides, drug residues, toxins, metals and cleaning agents (Tuomisto, 2009). Proper cleaning and management in the use of chemicals can usually eliminate chemical hazards from the product, while improper cleaning which should be corrected by occasionally changing chemicals may induce bacteria resistance (Nuneaton and Bedworth Borough Council, 2005). Most food processors in Tanzania use additives in high concentrations from the specified maximum requirement or ones that have been banned from use due to their toxicity effects (Xu and Ruteri, 2009).

At national level, Tanzania Food and Drugs Authority (TFDA), under the Ministry of Health and Social Welfare, is a regulatory body responsible for controlling the quality, safety and effectiveness of food, drugs, herbal drugs, cosmetics and medical devices (The Tanzania Food, Drugs and Cosmetics Act, 2003). TFDA works hand in hand with the Tanzania Bureau of Standards (TBS) whose mission is to develop and promote standardisation and quality assurance work in industry and commerce of Standards Act No. 2 of 2009 (TFDA, 2009). Municipal health departments at district levels are also responsible for ensuring safety of food in urban and rural areas.

TFDA is also responsible for monitoring vendor practices by initiating unannounced inspections. However, these inspections are sporadic and vendors without permits, food

safety training, and even chronic health violators continue to sell food on the streets and high way road sides of Morogoro. In contrast to restaurants with established locations, information regarding the past history of health violations of mobile vendors is much more difficult for the public to acquire.

Morogoro as one of the key fruit basket regions in Tanzania has many food vendors dealing with fruit juices at bus stands, highway sides and streets. Despite training and dissemination of food safety knowledge efforts by the government, NGOs and educational institutions and various achievements obtained, every year, prevalence of foodborne cases increase leading to illnesses and deaths (Batterman *et al*., 2009). Like in industrialized countries, the percentage of the population suffering from foodborne diseases each year has been reported to be up to 30% while less well documented, developing countries bear the brunt of the problem due to the presence of a wide range of foodborne diseases, including those caused by parasites (WHO, 2007). The high prevalence of diarrhoeal diseases in many developing countries suggests major underlying food safety problems (WHO, 2007). For example, the 1998 outbreak of cholera in Tanzania resulted in costs of US $36 million (Mosha and Magoma, 2002).

1.2 Problem Statement and Justification

Food trade, foreign travel and tourism are increasing each year in Tanzania contributing to the economic growth and social welfare (Kweka *et al*., 2001; Ministry of Foreign Affairs, 2013). Safe food is vital for public health which in turn improves the labour force productivity and hence contributes to economic development. Food consumed may also impose a high chance for foodborne illnesses and spoilage leading to economic loss and productivity (Codex Alimentarius, 2003).

WHO/ FAO joint committee (Codex Alimentarius) laid guidelines to GMP, GHP and Hazard Analysis and Critical Control Point system (HACCP) so as to assure food safety (Codex Alimentarius, 2003). As food regulatory and standards organs in Tanzania, TFDA and TBS work according to these guidelines to implement, formulate and enforce national laws, regulations and standards so as to deem food safe for consumption to the public (Ndabikunze *et al*., 2009).

In Tanzania, a large proportion of the food dealers and small scale processors are not aware of the food law, quality and hygienic practices and different hazards that may cause contamination of the food rendering it unsafe for human consumption and thus imposing a health risk to the public (Mashindano and Nyange, 2012). However, most of the research in food safety has not adequately addressed or published the level of compliance of food vendors to food safety regulations. As a result, there is a possibility of non-compliance to national, WHO/FAO food safety and quality requirements. Morogoro, where most of the foods, especially cereals, fruits and vegetables are abundantly grown acts as a gateway to other regions for mass movement and comprises of small scale food processors and vendors.

Do street-vendors conform to the codes of GMP, GHP, microbiological, physical and chemical criteria as stipulated by national and WHO/FAO food safety requirements? This question thus becomes important as a basis of this study with the aim of evaluating their compliance to these codes.

1.3 Objectives

1.3.1 Overall objective

To evaluate the compliance of street fruit salad and fruit juice vendors in Morogoro (Tanzania) with national food safety, quality legal requirements.

1.3.2 Specific objectives

i. To evaluate the compliance by street fruit salad and fruit juice vendors to GHP and GMP as per national standards (Tanzania).

ii. To determine occurrence of copper, lead and cadmium in street vended fruit salad and juice.

iii. To evaluate physic-chemical and microbiological quality of street vended fruit salad and juice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Agriculture in Tanzania

Tanzania is located on latitudes 1° – 12° south of Equator and longitudes 29° – 41° East of Greenwich having land area of 945 000 km^2, inland water of 62 000 km^2 and arable land of 44 million hectares out of which 10.1 million hectares are currently under cultivation (MAFSC, 2008). About 29.4 million hectares are suitable for irrigation; out of which 2.3 million hectares are of high development potential and 4.8 million hectares are of medium development potential (MAFSC, 2011). There are many lakes, permanent and seasonal rivers and underground water sources for irrigation and other uses. Agriculture accounts for about 80% of the employed population and contributing about 50% of GDP (Gross Domestic Product) and about 66% of merchandise exports (The World Bank, 2012).

The government through the agricultural sector lead ministries is responsible for services such as policy formulation, regulatory functions, research, extension, training, and provision of information aiming at creating a conducive environment for increased private sector participation in production, processing, marketing of agricultural commodities and inputs (MAFSC, 2008).

The government and stakeholders, vision the agricultural sector that by 2025 it will be modernized, commercialised, highly productive and profitable, utilizing natural resources in a sustainable manner and acting as an effective basis for inter-sector linkages (MAFSC, 2008).

Investment opportunities are available in production, processing, marketing and service provision in the crop sub sector such as, coffee, cotton, tobacco, sisal, spices, tea, pyrethrum, cashew nuts, sugarcane, floriculture, fruits and vegetables, maize, wheat, rice, oil seeds and other crops as cassava, Irish potatoes, sorghum, millets and various legumes like beans and peas (Tanzania Invest, 2008).

2.2 Fruit Production and Processing in Tanzania

Tanzania is richly endowed with a variety of fruits. Less than 10% of fruits and vegetables produced are processed (SNV, 2005). It is estimated that, due to limited processing capacity, poor storage facilities and poor infrastructure, about 30% of the fruits are destroyed either in the farm or on transit to the urban market or at the market level (SNV, 2005).

MAFSC (2008) reported that, room for large scale production of tropical as well as temperate fruits and investment in the fruit sector can be in:

(i) Processing, canning and packaging factories in regions with high potential for production of fruits such as Morogoro.

(ii) Open fruit and vegetables plantations for domestic and export markets in areas for horticultural crops such as Arusha, Kilimanjaro, Tanga, Morogoro, Dar es Salaam, Dodoma, Iringa, Mbeya, Mwanza and Kagera.

Despite the potential in the fruit sector where a demand in its products for both regional and international markets is high, fruit growing in Tanzania is less developed compared to other crops such as grain and plantation crops like coffee, tea, cashew, cotton and sisal (SNV, 2005). The production of major fruits in Tanzania in year 2009 and 2010 can be seen in Table 1.

Table 1: Annual production of fruits (in metric tonnes) in Tanzania

Fruit category	Year	
	2009	**2010**
Bananas	3 219 000	2 924 700
Citrus fruit	41 480	43 000
Fresh	195 527	200 000
Tropical fresh	48 643	50 000
Grapes	17 748	18 000
Lemons and limes	267	290
Mangoes, mangos teens, guavas	320 000	25 000
Oranges	939	1000
Pears	206	210
Pineapples	84 000	87 500
Plantains	653 890	660 000
Plums and sloes	209	240

Source: FAO Stat (2012)

2.3 Fruit Production and Processing in Morogoro

Morogoro produces about 8% of citrus, 9% for pineapples, 7% for mangoes, between 8-30% bananas and over 50% of pawpaw national production suggesting that Morogoro is probably one of the leading producers of fruit crops in Tanzania (SNV, 2005).

Potential fruits grown in Morogoro are mangos, pineapples, oranges, tangerines, passions, bananas and pawpaws including other indigenous fruits. A large number of postharvest losses are encountered each year due to poor handling and storage and negligible processing (Paavola, 2008). Fruit processing factories in Morogoro still remain at small

scale where most of the fruits are marketed fresh or processed by small scale food processors and food vendors in streets into dried forms, juices, jams, pickles and wines. Less than 10% of fruits and vegetables produced are processed (MAFSC, 2008). Notable challenges the small scale groups of producers face simply remain their inability to penetrate into the international market due to poor quality and quantity as well a failure to attain the required standard in the packaging of their agro products (Mashindano and Nyange, 2012).

The government and stakeholders have seen these setbacks and have taken some initiatives to improve the situation, particularly in quality and quantity, and modernise the packaging process so as to make the products meet the market demands (The Citizen, 2011).Facing similar problems, Morogoro Ben's Winery (MBW), an enterprising member of a fruit processing cluster in Morogoro for several years, has resorted to finding market solution to its processed juice, wine and vegetables of various kinds.

UNNAT Fruits Processing Limited was a medium scale factory in Morogoro with the potential to process oranges, tomatoes and pineapples and packaging mangoes, passion fruit and grapes to be exported. It had a capacity of contracting an estimate of 15 000 farmers who had already agreed to supply the factory with pineapples and oranges, and the company hoped to register an additional 60 000 farmers established in the Morogoro and Tanga regions by the year 2010. Eventually the factory was shut down and is not operational due to management problems. "If we process our products locally, their market value increases as well, and this will benefit farmers, processors and the government," said president Kikwete reported by Tanzania Invest (2008).

2.4 An Overview of Fruit Salad and Juice Safety

The human population is subjected to many life sustaining functions. Of these food is what we share in common and what makes us have life, thus it is very important to the human race. Food as it contains many nutrients it, also contains pathogenic agents which can lead to food borne illnesses, death and cause economic losses like in trade and employment (Codex Alimetarius, 2003). Safe food is therefore important. Food safety implies absence or acceptable and safe levels of contaminants, adulterants, naturally occurring toxins or any other substance that may make food injurious to health on an acute or chronic basis (FAO, 2003). Food safety is therefore an important aspect to attain good health as well as physical and mental stability of the body (FAO, 1998). Thus hygiene is very important to avoid human health and economic consequences related to food borne illness and spoilage. Farmers, processors and manufacturers, food handlers, governments and consumers have a responsibility to ensure that food is safe and suitable to consume (Codex Alimetarius, 2003).

2.4.1 Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP)

The potential effects of primary production activities on the safety and suitability of food should be considered at all times. In particular, this includes identifying any specific points in such activities where a high probability of contamination may exist and taking specific measures to minimize that probability (Codex Alimentarius, 1997).

Good Manufacturing Practices (GMPs) (or Prerequisite Programs) are common-sense practices for creating the conditions required to prevent, minimize or control microbial, chemical and physical contamination in a food production environment (European Commission, 2002). There are four basic elements in a GMP food safety program. Each is

based on the principle of risk reduction through prevention (OMAFRA, 2006). They include:

(i) Control Programs: written operational and training policies and descriptions of the resulting operational and training procedures, which are applied during Training and through Operational Controls
(ii) Training: the training procedures
(iii) Operational Controls: the operational procedures in the food production facility
(iv) Environmental Controls: creation of a physical environment favourable for the production of safe food.

The joint FAO/WHO (Codex Alimentatrius) has established The Codex General Principles of Food Hygiene, which identify the essential principles of food hygiene applicable throughout the food chain (including primary production through to the final consumer) through good hygienic practices, to achieve the goal of ensuring that food is safe and suitable for human consumption by:

(i) Recommending a HACCP-based approach as a means to enhance food safety;
(ii) Indicating how to implement those principles; and
(iii) Providing guidance for specific codes which may be needed for sectors of the food chain; processes; or commodities; to amplify the hygiene requirements specific to those areas.

Effective hygiene control, through GHP along the food chain, therefore, is vital to avoid the adverse human health and economic consequences of foodborne illness, foodborne injury, and food spoilage. Everyone, including farmers and growers, manufacturers and

processors, food handlers and consumers, has a responsibility to assure that food is safe and suitable for consumption (Codex Alimentarius, 2003).

2.4.2 Status of compliance with GMP regulatory requirements

TFDA observed that, by their nature as small scale entrepreneurs, most applicants of food registration find it very difficult to obtain information on scientific data such as proportions of food additives used in manufacture of foods and or stability data that was used to establish shelf life of food products (TFDA, 2009).It was also observed that even large scale food importers are not able to access GMP data or health certificate that is required by TFDA as assurance that such foods are approved for human consumption in the exporting countries. Applying the HACCP based approach is difficult for food vendors and small scale processors in Tanzania, thus TFDA recommends applying GMP and GHP to produce safe products. A little has been published on the adherence and compliance of ready-to-eat foods especially fruit juice and fruit salads in Tanzania.

2.4.3 Management and activities related to provision of scientific advice by FAO/WHO

Management, coordination and supervision within FAO and WHO are engaged in providing advice on food safety and nutrition. FAO has the following bodies:

(i) Nutrition and Consumer Protection Division (AGN)

(ii) Animal Production and Health Division (AGA)

(iii) Plant Production and Protection Division (AGP)

(iv) Fisheries Industry Division (FII)

WHO in the provision has the following departments:

(i) Department of Food Safety, Zoonoses and Foodborne Diseases (FOS)

(ii) Department of Nutrition for Health and Development (NHD)

(iii) Department of Public Health and the Environment (PHE)

The coordination is facilitated by the Codex Alimentarius Commission (CAC) which brings together government institutions, non-governmental organizations, stakeholders along the different food value chains, professional and scientific organizations and academic institutions on different food safety aspects such as regulations for Food Additives and Contaminants (FAO/WHO, 2007).

The role of governments goes beyond adopting and monitoring compliance with national food legislation: they should actively promote food safety measure through the adoption of food safety management systems such as HACCP. Businesses such as small or less developed types bear the ultimate responsibility for assuring the quality and safety of the foods they produce. To add, they are required to interact and comply with government requirements regarding food safety management systems, inspection and auditing so as to meet basic requirements such as GHP and HACCP (FAO/WHO, 2006).

2.4.4 Microbiological Food Safety

Food provides a good environment for harboring microorganisms due to the nutrients and moisture it attains suitable for their growth. Bacteria are the most widespread microbes in the natural environment. They can easily be cross contaminated to food from pre to post harvest handling causing spoilage and food borne illness which ultimately affect the consumer's health (Greig *et al*., 2007).

Some ready-to-eat foods as such fruits and fruit products are also regarded as ‘potentially hazardous’. Such foods can support the growth of pathogenic (food poisoning) bacteria and must be kept at certain temperatures to minimise the growth of any pathogens that may be present in the food or to prevent the formation of toxins in the food (Mukhopadhyay and Basu, 2011; Stewart and Williams, 2009). Foods may contain a variety of microorganisms, many of which are harmless (NSW, 2009).

Non-spore-forming bacteria such as enterotoxigenic and enterohemorrhagic *Escherichia coli, Campylobacter jejuni, Listeria monocytogenes, Salmonella, Shigella* spp., *Staphylococcus aureus,* and *Vibrio* spp. could contaminate fresh produce by cross contact with humans or animals carrying these organisms with the fecal–oral route being possibly the main mechanism of transfer (James, 2006). All of these bacteria have been associated with publicized fresh produce foodborne outbreaks of public health significance (WHO, 2006). The transfer of these organisms could be controlled by practicing good personal hygiene, cleaning food contact surfaces, and always using potable water when water is required (James, 2006).

Data regarding foodborne diseases in the African Region are extremely scarce. However, limited studies have shown that the following pathogens are prevalent: *Campylobacter, Salmonella, Shigella, Hepatitis, Brucella, Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and rotavirus. Foodborne bacterial infections are particularly common: children in the African Region may experience five episodes of diarrhea per year and 800 000 children die each year from diarrhea and dehydration (WHO, 2007).

In addition, children’s exposure to pesticides in the African Region is suspected of causing immunological and endocrine defects, neurotoxic disorders, and sometimes

cancer. The number of consumers who are highly vulnerable to foodborne illness is growing in this region (WHO, 2006). In sub-Saharan Africa, where approximately 25 million adults and children live with HIV/AIDS, bacterial infections such as Salmonella can cause particularly serious complications, including death. Among the elderly, infections such as enterohemorrhagic *Escherichia coli* can be particularly fatal (De Waal *et al.*, 2005).

Maintaining low temperature throughout distribution is critical to maintaining quality of fresh-cut fruits and its fresh products like juices. Low temperatures reduce enzymatic reactions and greatly slow down the multiplication of spoilage organisms. Low temperatures also prevent the multiplication of most foodborne pathogens, with the exception of *Listeria monocytogenes* and a few others that are capable of growing, albeit slowly, at refrigerated temperatures (Gorny and Zagory, 2004).

2.4.4.1 Bacteria commonly associated with food poisoning in fruits

A wide range of bacteria are associated with fruits in bringing about spoilage or food poisoning which include e.g. *E. coli, L. monocytogenes, Salmonella* spp and *S. aureus*. Lactic acid bacteria are also predominan *L. agilis and L. plantarum. Other species identified included L. bifermentans, L. minor, L. divergens, L. confusus, L. hilgardii, L. fructosus, L. fermentum*and *Streptococcus* spp (Nyanga *et al*., 2007). *Staphylococcus aureus* is a facultative anaerobic, gram-positive *coccus*, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates (Wikipedia, 2012). Skin infections are the most common infections associated with *Staphylococcus*. Staphylococcal food poisoning is an illness of the bowels that causes nausea, vomiting, diarrhea, and dehydration. It is caused by eating foods contaminated with toxins produced

by *S. aureus*. Symptoms usually develop within one to six hours after eating contaminated food. The illness usually lasts for one to three days and resolves on its own (Stoppler, 2009). *S. aureus* food poisoning is often caused when a food handler contaminates food products that are served or stored at room or refrigerator temperature (Centre for Health Protection, 2012). Common examples of such foods are desserts (especially custards and cream filled- or topped desserts), fruits, salads (especially those containing mayonnaise), or baked goods (Medline Plus, 2007). A study on biofilm formation of both food-related and clinical *S. aureus* strains grown under different stress conditions (temperature, sodium chloride, glucose and ethanol) relevant for food processing was conducted were strong biofilm formers were identified, among food-related *S. aureus* strains, and biofilm formation was affected by environmental conditions relevant for the food industry (Rode *et al*, 2007). *E. coli* is a Gram negative, facultative anaerobic and non-sporulating. Most strains are harmless, but serotypes types like O157:H7 can cause serious food poisoning in humans (Soller *et al*., 2010). Most of the dangerous strains like the *E. coli* O157:H7 produce Shiga toxins which cause various symptoms to humans (CDC, 2008) Common routes of transmission include: unhygienic food preparation, farm contamination due to manure usage, irrigation using grey water or raw sewage, feral pigs on crop land. Food products associated with *E. coli* outbreaks include; raw ground beef, raw seed sprouts or spinach, raw milk, unpasteurized juice, and food contaminated by infected food workers via fecal-oral route (Wikipedia, 2012). *Salmonella* spp. on the other hand is closely related to *E. coli*. It is a rod shaped gram negative, non-spore forming and predominantly a non-motile bacterium. They cause illnesses to humans and animals such as, typhoid fever, paratyphoid fever and the food borne illness salmonellosis (Wikipedia, 2012). Salmonellosis can be caused by eating food contaminated during processing or handling; from an infected food handler. *Salmonella* spp are usually found in feces. A frequent cause is a food handler who does not wash his or her hands with soap after using the

bathroom. Salmonella infection usually stems from undercooked meat and poultry, raw eggs, unpasteurized milk, contaminated fruits or water containing Salmonella sp. Pet turtles and other animals can carry the bacteria (CDC, 2008; Health Square, 2009). Symptoms associated with Salmonella food poisoning include; diarrhea or constipation, headaches, stomach cramps, nausea and vomiting, fever and a possibility of blood in feces. Dehydration may also occur leading to weakening of the body, urine defects and increased thirst (Mahajan *et al*., 2003). It is therefore important to develop ways so as to ensure safe food from food related pathogens like *E. coli, Salmonella* spp *and S. aureus*.

2.4.4.2 Yeasts and Moulds commonly associated with food poisoning in fruit products

Yeasts and moulds are highly efficient at causing foods to spoil and are a problem for most food manufacturers (Scudder, 2011). There are several factors that enable these microorganisms to colonise a wide range of foodstuffs (Fisher Scientific, 2009):

(i) Some species can grow over a wide pH range, enabling them to survive in very acidic environments, such as fruit juices and pulps.

(ii) Some can tolerate extremes of temperatures. The temperature range for the growth of yeasts is 0 to 47°C. Many species are able to grow at low temperatures and at low pH, making them a particular problem for fermented milk products.

(iii) Many species are xerotolerant (able to grow in environments with very low water activity (a_w) as low as 0.65), and can grow in foods such as dried fruits, nuts, grains and spices. Other osmophilic and halophilic species are able to grow in environments with high osmotic pressure due to the presence of sugar or salt respectively. Such organisms can be a particular problem for bakery products and dried/cured meats.

Different fungi found in fruits include: *Penicillium, GeotrichuFusarium, Botrytis, Colletotrichum, Mucor, Monilia, Rhizopus* and *Phtyophthor*(UC Davis, 2013; Tournasa *et al*., 2006). The shelf life (i.e., the length of time that corresponds to a tolerable loss in quality of a processed food and other perishable items) of fresh-cut fruits and vegetables ranges from 1 to 35 days depending on types of shelf life (such as marketing shelf life, food safety shelf life, sensory shelf life, or microbiological shelf life), food safety concerns, marketing strategies of fresh-cut processors, produce commodities, raw materials, refrigerated storage temperatures, preparation methods, and packaging methods (Barth *et al.*, 2009).

Yeasts and some bacteria, including *Erwinia* and *Xanthomonas*, can also spoil some fruits and these may particularly be a problem for fresh cut packaged fruits (Ellin, 2007).They often colonize foods with high sugar, with low pH or salt content and contribute to spoilage of maple syrup, pickles, sauerkraut, fruits and juices. They have a diverse secondary metabolism producing a number of toxic and carcinogenic mycotoxins. Spoilage may be manifested as surface pellicles or fibrous mats of molds, cloudiness, and off-flavors (Ellin, 2007). Tanzania having a tropical climate becomes a potential harbor area for spoilage types.

2.4.5 Chemical contaminants in foods

Chemical contaminants may be naturally occurring or may be added during agricultural production, post-harvest handling and other unit operations. According to Codex Alimentarius (Codex standard 193-1995), a contaminant is any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine),

manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination. The term does not include insect fragments, rodent hairs and other extraneous matter (Codex Alimetarius, 1995). There are too many other insidious hazards which can affect food and consumers health. The main other insidious hazards in food are: heavy metals mainly in canned foods, natural toxins, irradiated food products, toxic chemicals, and so many others. Gloves contain harmful chemicals such as thiurams, and di-thio-carbamates (Sekheta *et al*., 2008). Chemical contaminants in foods arise from food additives if used in concentrations higher than set maximum limits, cleaning agents in food processing, processing equipment, handlers, and environment.

Codex Alimentarius (1995) has established the Codex general standard for food additives (CODEX STAN 192-1995). According to this standard, the use of food additives is justified only when such use has an advantage, does not present an appreciable health risk to consumers, does not mislead the consumer, and serves one or more of the technological functions set out by Codex and the needs set out from (a) through (d) below, and only where these objectives cannot be achieved by other means that are economically and technologically practicable:

a) To preserve the nutritional quality of the food; an intentional reduction in the nutritional quality of a food would be justified in the circumstances dealt with in sub-paragraph

(b) and also in other circumstances where the food does not constitute a significant item in a normal diet;

c) to provide necessary ingredients or constituents for foods manufactured for groups of consumers having special dietary needs;

d) to enhance the keeping quality or stability of a food or to improve its organoleptic properties, provided that this does not change the nature, substance or quality of the food so as to deceive the consumer;

e) to provide aids in the manufacture, processing, preparation, treatment, packing, transport or storage of food, provided that the additive is not used to disguise the effects of the use of faulty raw materials or of undesirable (including unhygienic) practices or techniques during the course of any of these activities.

The Global Chemicals Outlook (2012) reported that poisonings from industrial and agricultural chemicals are among the top five leading causes of death worldwide, contributing to over 1 million deaths annually. The agricultural chemicals including pesticide residues accumulate in the body and possibly lead to two types of serious health effects namely acute poisoning and chronic poisoning. The acute effects are almost immediate and have symptoms such as headache, skin rashes, nausea, vomiting, blurry vision, dizziness, sweating, diarrhoea, unusual weakness, loss of concentration, difficulty in breath, convulsion, coma and death. The chronic effects are usually felt or seen after repeated exposures. The effects take a long time to appear and normally difficult to cure and some of the symptoms include cancer, birth defects, miscarriage, still-births, sterility in men, liver and bone damage to the nervous system, asthma and allergies (Johnson and Yawson, 2009).

Pesticides are also called Persistent Organic Pollutants (POP's). Some commonly used pesticides, commonly called the dirty dozen are DDT, lindane, aldrin, dieldrin, endrin, chlordane, heptachlor, mirex, toxaphene, hexachlorobenzene, poly chlorinated biphenyles, dioxins and furanes. These pesticides poison the soil and water for many years and enter

our food chain through fruits, vegetables and grains as well as through meats and fish (Mallya, 2008).

Metals such as lead, cadmium, mercury and copper are cumulative poisons (HITM, 2006). They have been reported to be exceptionally toxic. Lead has been associated with intoxications leading to problems in the kidney and liver, the central nervous system, reproductive organs and anaemia. Although Copper is an essential trace element in the functions of the human body, chronic and excessive intake has been linked with digestive tract problems and cirrhosis of the liver (HITM, 2006; Tuomisto, 2009).

2.4.6 Physical contaminants in foods

These are hard foreign objects that can cause illness or injury that may be, inherent to the food or ingredient, contaminant during processing, metal, glass, wood splinters, rocks, insects, hair, dust and dirt (Keener, 2002; Mallya, 2008).Removal of these hazards is usually by filter or sieve (meat grinder), water bath (vegetables), metal detector (all foods), good handler practices (jewelry), good sanitation and quality control programs.

Illness and serious injuries can result from foreign material in produce; these physical hazards can result from poor practices during harvesting, washing, sorting and packaging operations (Goldman, 2002; Keener, 2002). Filth and foreign matter in fruits and vegetables are listed in many instances among the main barriers to international trade (HITM, 2006). FAO (2010) showed different physical hazards, risks and reasons for occurrence as shown in Table 2.

Table 2: Physical hazards, risks and reasons for occurrence

Physical hazards	Risks	Reason
Soil	Presence in finished products:	Harvested with the crop
Machinery	(i) Soil and stones	(i) Dirty packaging materials
Equipment and containers	(ii) Metal	(ii) Inadequate inspection of field equipment and packing facilities
	(iii) Wood	(iii) Inadequate maintenance of containers and machinery
	(iv) Glass and plastic	(iv) Discarded rubbish, e.g. bottles, cigarette butts
	(v) Knives	(v) Inadequate cleaning schedule
	(vi) Plasters	(vi) Staff untrained in personal hygiene
	(vii) End product contains: jewelry and pieces of clothing	(viii) Inappropriate working clothes

Source: FAO, (2010)

2.4.7 Food safety control in Tanzania

The food regulatory organ in Tanzania is TFDA and the standards body is TBS. While a number of related problems keep foodborne diseases at high levels within the African region, the root cause is poverty, which disproportionately affects women and children. Poverty exacerbates food safety problems in many ways and contributes to:

(i) Unsanitary conditions in rapidly growing urban centers

(ii) Lack of access to clean water

(iii) Unhygienic transportation and storage of foods

(iv) Low education levels among consumers and food-handlers, leading to reduced information on food safety

In 1997 WHO Food Safety Programme conducted a case study In Buguruni market (WHO, 2006). The Buguruni Healthy Market Task Force (BHMTF) Plan of Action resulted in a number of successful outcomes, including:

(i) Improvement in road access (Plan International);

(ii) Construction of a solid waste storage bay (JICA);

(iii) Construction of toilet and hand washing facilities (WHO); and

(iv) Development of a system for the collection and sorting of solid waste for subsequent disposal.

As evidenced by improved handling of solid waste, activities within and outside the market have produced a synergism which has contributed significantly to the hygienic conditions in the market. Local resources were mobilized for an education program for market participants, consumers and other stakeholders to promote awareness that safe and nutritionally adequate food is the foundation of good health. The Healthy Food Market concept has also been introduced into several other markets in Dar es Salaam and other cities in Tanzania.

Foodborne diseases have many adverse economic consequences within the African region. For example, the 1998 outbreak of cholera in Tanzania cost US $36 million for medication. WHO has documented numerous food safety and quality problems that have affected food exports and imports in African countries including Tanzania (WHO, 2007). Those include: spoilage, substandard/fake products, failure to provide production dates, improper or deceitful labeling of food imports, poor product quality and packaging of

food exports, expired food, exceeding levels for preservatives/additives, lack of harmonization of food safety regulations and fraud (De Waal and Robert, 2005). Currently there is no existing food safety policy in Tanzania. This makes the population more susceptible to health hazards each year.

2.5 Street Vended Foods

Street vendors are an important source of affordable food. But street foods often do not meet proper hygiene standards, in large part because of weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food-handlers (De Waal and Robert, 2005).

There has been a continuing growth in urbanisation in developing countries, and governments face a major challenge in ensuring that street dwellers are able to procure sufficient food and safe food (WHO, 1996). According to WHO, Street-vended foods or its equivalent "street foods" are defined as foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation. This definition includes fresh fruits and vegetables which are sold outside authorized market areas for immediate consumption (WHO, 1996). Food handlers have shown potentially contaminating actions, including spitting on the sidewalk, smoking, or touching the mucous membranes of one's mouth or nose, were also noted(Burt *et al*., 2003). These actions including unsanitary areas may contribute to contamination of the different foods sold including fruit products. Abdalla *et al*. (2009) reported that socio-economic and demographic data showed that most of the food vendors were females, although, it is not certain whether the predominance of women in the street food vending trade is advantageous to food safety. The vendors studied, agreed that the hand must be washed (74.0%), because the

organisms such as *Salmonella typhi*, non-typhi *Salmonella*, *Compylobacter* spp. and *E. coli* can survive on fingers tips and other surfaces for varying periods of time and some cases after hand washing. Also clean water supply and hand washing or toilet facilities are not available to food street vendors (Abdalla *et al*., 2009).

Many foodborne illnesses are transmitted by foods sold on streets, making sure that food is handled and prepared in a safe and sanitary manner can reduce the chance of people contracting foodborne illnesses (James, 2006).The most effective food safety behaviour include keeping foods at safe temperatures, using a thermometer to determine the adequate cooking of foods, avoiding cross-contamination, washing hands, and avoiding high-risk foods (Simonne *et al*., 2008).

Street vended foods contribute significantly to the economic enhancement of those involved in its production, particularly suppliers of raw produce, food processors and vendors. In Morogoro it was observed that the water used to prepare street vended foods (like stiff porridge, and rice) is not safe due to large total counts of *E. coli* contained including the foods and drinking water sold indicating low and non-compliance to GMP and GHP as laid by WHO/ FAO, 1997 guidelines(FAO, 2005). It is therefore important to evaluate the safety of fruit products such as juices and salads which are consumed by many people.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location and Duration

The study was carried out in Morogoro Municipality of Morogoro region for 8 months starting in August 2012 to March 2013. Morogoro produces about 8% of citrus, 9% for pineapples, 7% for mangoes, between 8-30% bananas and over 50% of pawpaw national production suggesting that Morogoro is probably one of the leading producers of fruit crops in Tanzania (SNV, 2005). Morogoro is located between 6° 49′ 0.12″ S and 37° 40′ 0.12″ E and it is bordered to the north by the Tanga Region, to the east by the Pwani and Lindi Regions, to the south by the Ruvuma Region and to the west by the Iringa and Dodoma regions. Fig.1 shows the map location.

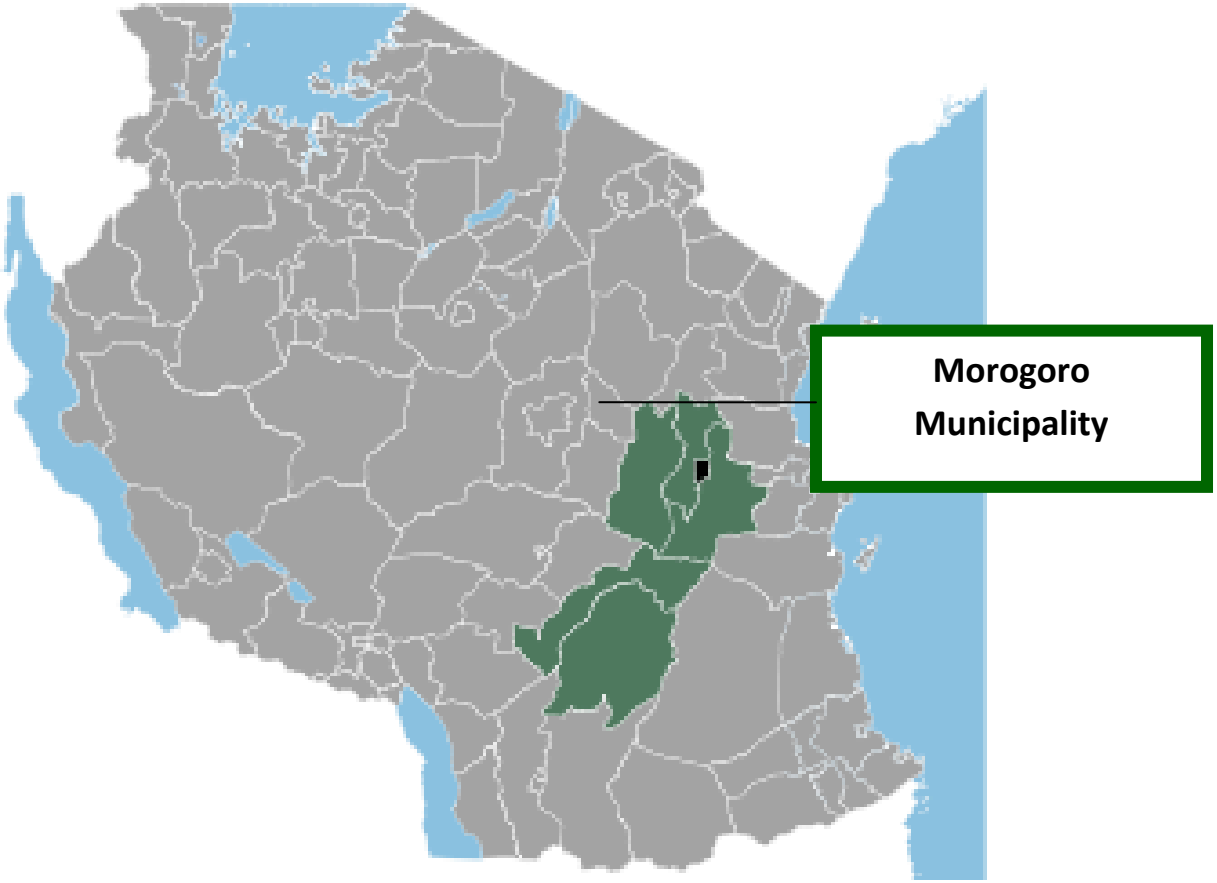


Figure 1: Morogoro location

3.2 Study Population

The population evaluated during the current study was categorised into three groups: (i) Street Vendors (ii) University Cafeterias and (iii) Town Restaurants. These included mobile and stationary street fruit salad and juice vendors. They were identified along road sides/streets in the Morogoro municipality centre, in municipality market areas (Morogoro central market, Mawenzi market), transportation terminals and bus stops (Morogoro bus terminal, Msamvu bus terminal), restaurants (Morogoro municipal centre), universities and colleges (Sokoine University of Agriculture, Mzumbe University, Jordan University) and secondary and primary schools (Uhuru, Mwere, Bungo, Morogoro secondary school). From the preliminary survey it showed that some places were more congested with vendors compared to others e.g. schools thus the respondents were randomly selected based on the preliminary survey carried out prior to start of the research. The survey also showed that some respondents were not willing to be interviewed due to fear of the government overtaking their businesses. Some also requested to make a follow up so as to improve their practices through knowledge dissemination and trainings.

3.3 Study design

This study consisted of two parts. The first part was a survey of street vendors (located along road sides, bus terminals, market areas, town centre, primary and secondary schools), University cafeteria and restaurants to collect data on knowledge and practices related to GMP and GHP by using a questionnaire. In the second part, 35 fruit juice and 35 fruit salad samples were collected from interviewed vendors for laboratory analysis of microbiological and physico-chemical quality parameters.

3.4 Sample Collection

Samples were randomly collected from the vendors in the afternoon (1300 to 1400h) in February 2013, during rainy season. The samples were put in aseptic containers (liquid and solid 500ml and 400g, respectively) and stored in a cool box containing sterile ice packs and taken for immediate analysis. The assumption in collecting the samples was that the population was unknown, thus a preliminary survey was made to identify locations where they are mostly found (Section 3.2). A total of 70 samples were collected, 35 from fruit salad vendors and 35 samples juice vendors. The samples were taken to laboratories at the Department of Food Science and Technology, Department of Crop Science and Production and Department of Soil Science, Sokoine University of Agriculture for analysis.

3.5 Survey: Evaluation of Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP)

Evaluation of GMP and GHP was carried out as per Codex Alimentarius Codes of practice. The codes were compiled in the questionnaire (Appendix I) which was modified from WHO and TFDA codes on essential safety requirements for street vended foods and guidelines to good manufacturing practices, respectively (WHO, 1996; TFDA, 2007). Main aspects evaluate included: demographic characteristics, business details, product and preparation practice, food safety knowledge, types of infrastructure and a general assessment of location and premises, sanitation status, maintenance and cleaning, processing and personal hygiene.

3.6 General Assessment of Location, Sanitation level, Processing and Cleaning and Personal Hygiene of Fruit Salad and Juice Vendors

These parameters were ranked in score sheet assigning different levels of quality. The scale was from very good = 2; good = 1 to poor = 0. The sums and percentages were then

computed from different criteria for each category giving a general picture of the premises and sanitation status (Appendix 1).

3.7 Physico-chemical Analysis

3.7.1 pH

The pH of samples was determined using a standard pH meter (Benchtop pH HI-207/208, HANNA instruments). Fruit juice samples (100ml) were agitated by vigorously shaking for 45sec and then used for pH determination. Fruit salads were homogenized using sterilized motor and pestle and 100g of each sample was weighed using an electronic balance (Denver Instruments TP-3002, Germany) where pH measurement was taken from slurry/ pulp.

3.7.2 Oxidation-Reduction Potential (ORP)

The oxidation-reduction potential was determined using an ORP/ pH combined meter (pH 510-cyberscan, EUTECH Instruments, USA). Fruit juice samples were agitated by vigorously shaking for 45sec were approximately 100 ml was used for ORP determination in millivolts (mV) while for fruit salads, samples were homogenized using sterilized motor and pestle to obtain the slurry which was less viscous. Approximately 100g was used from each respective sample for ORP (mV) determination.

3.7.3 Heavy metals

Lead, cadmium and copper were determined as described by Jeng and Bergseth (1992). Both fruit salad and fruit juice samples were agitated and homoginised of which, each sample was filtered using a whatman filter paper No. 1 (Whatman, England) overnight get 20 ml of filtrate. The filtrates of juice and salad samples were then put in the atomic absorption spectrophotometer (AAS Pye UNICAM 919, England) and concentrations of

the metals were determined from their absorbance using their respective lamps (Pb, Cd and Cu).

3.7.4 Colourings and Flavourings

Food colours and flavourings as additives were assessed by using the questionnaire (Appendix 1) administered to the respondents so as to evaluate compliance with recommended standards as described by Codex general standard for food additives-CODEX STAN 192-1995 (Codex Alimentarius, 1995).

3.8 Microbiological Analysis

The microbiological quality of the products was determined as described in Bacteriological Analytical Manual (1998). Samples were collected and carried in a cool box containing ice of potable water quality for immediate analysis to prevent proliferation of microorganisms.

3.8.1 Total Mesophilic Aerobic Count

Enumeration of samples for total mesophilic aerobic microorganisms was done according to procedures described by Maturin and Peeler (2001) in BAM. Methylated spirit and 70% ethanol were used to sterilize working surfaces while a lamina flow unit (Telstar PV 100, Spain) was used for culturing and plating. For fruit salad, samples were weighed to 250g and homogenized by motor and pestle that were pre-sterilised at 150°C for 120 min in an oven (Memert UM 400, Germany) while fruit juice samples were homoginised by shaking. Standard nutrient agar (NA) (Himedia laboratory, Pvt, India) was used as media. NA was prepared in glass bottles containing magnetic stirrers by dissolving 28g in 1000 ml of distilled water. The bottles were then heated in an electric water bath to achieve geletinisation temperature (Precision Scientific 180 series. Chicago, USA) and then sterilised using an autoclave (SHi- AVX 90.E electronic, France) at 121°C for 15 min. The

temperature of the NA for plating (around 45ºC) was regulated using the water-bath (Precision Scientific 180 series. Chicago, USA).The media was left to cool to around 45 ºC after being autoclaved before used for plating.

Glass petri dishes for plating were cleaned and also sterilized at 150°C for 120 min using the oven (Memert UM 400, Germany).An 8.5% buffered polypeptone physiological solution (PPS) was used for dilution and serial dilution of the samples. It was prepared by taking 1g of polypeptone (Becton, Dickinson and Company, Sparks, USA) together with 8.5g of Sodium chloride (common salt) and dissolving them in 1000 ml of distilled water. The PPS was then dispensed in test tubes each containing 9 ml (for serial dilution) and glass bottles containing 225ml with magnetic stirrers (for first dilution of 25ml of juice) and then autoclaved at 121ºC for 15 min. A heat-magnetic stirrer (Yellow line-MSHC: MSC basic, Germany) was used to agitate 25 ml of juice or 25g of fruit salad samples in 225 ml of 8.5% buffered polypeptone water. Twenty five grams of the homogenized fruit salad sample or 25 ml of fruit juice sample was the dispensed in a glass bottle containing 225 ml of PPS and mixed using a magnetic stirrer for 1min. Serial dilutions were then carried out in a series of tubes containing 9 ml of PPS to a 10^{-4} dilution. After the sample was serially diluted, 1 ml from each respective dilution was pour plated in duplicate using nutrient agar. The plates were left to cool and solidify then incubated(Binder 960285, Germany) upside down at 37°C for 24-48 hrs, after which colonies were counted in plates containing the range of 30-300 colonies and recorded. The CFU/ml (fruit juice) and CFU/g (fruit salad) were calculated as shown in equation 1.

$$\boldsymbol{X = {}^{A \times V}/_{I}} \quad \ldots\ldots(1)$$

Where,

X= CFU/ ml or CFU/g.

V= number of colonies in a plate.

A= dilution factor for accepted plate and I= volume of inoculum (1 ml for pour plate)

3.8.2 Enumeration of coliforms and *E. coli*

3.8.2.1 Enumeration and presumptive confirmation of coliforms

The detection and enumeration of coliforms and *E. coli* (indicator organisms) was carried out according to BAM procedures described by Feng *et al*. (2002). A presumptive test for coliforms was first carried out followed by a confirmatory test of coliforms and finally *E. coli*. Lauryl sulfate Tryptose Broth (LSTB) single and double strength, Brilliant Green Bile Broth (BGBB) and E.C broth (*E. coli* broth) and Kovacks reagent-Indole (all products of Himedia laboratory, Pvt India) were used for enumeration and confirmatory tests. Forty grams and 15g of BGBB and LSTB respectively, were weighed and dissolved in 1000 ml glass bottles, where 10 ml of BGBB and LSTB were dispensed in a series of test tubes containing Durham tubes, then autoclaved (SHi- AVX 90.E electronic, France) at 121ºC for 15 min. All media were adjusted to suitable pH using 0.1N $HCL_{(aq)}$ and 0.1N $NaOH_{(aq)}$. The tubes were also left to cool to around 45ºC in a clean chamber with lamina flow (Telstar PV 100, Spain) for use for coliform evaluation. Fruit salad, samples were weighed to 250g and homogenized by motor and pestle that were pre-sterilised in an oven at 150°C for 120 minutes (Memert UM 400, Germany). Fruit juice samples were homogenized by shaking. Twenty five grams of the homogenized fruit salad or 25 ml of fruit juice sample was dispensed in a glass bottle containing 225 ml of PPS and mixed using a magnetic stirrer for 1min. One millilitre of diluted sample was serially diluted in 5 consecutive dilutions (10^{-1} to 10^{-5}) of 5 tubes containing Durham tubes and 9 ml each of LSTB with each respective dilution containing a set of 5 tubes for a 5 tube MPN. The tubes were then incubated at 35°C± 0.5°C for 48h. The tubes were recorded for reactions displacing the medium through gas production in Durham tubes from fermentation. From each gassing

LSTB tube, a loopful of suspension was transferred to a tube of BGBB. The BGLB tubes were incubated at 35°C± 0.5°C and examined for gas production after 48h. Positive tubes were recorded and the most probable number (MPN) of coliforms based on number of confirmed gassing LSTB tubes for 3 consecutive dilutions was determined (close to dilution to extinction tube showing no growth).

3.8.2.2 Confirmatory Test for Fecal coliforms and *E. coli*

From double strength LSTB tubes from the Presumptive test, a loopful of each suspension was transferred to a tube of E.C broth (the loop-stick was sterilized before transfers). The EC broth tubes were incubated at 45.5°C for 24hrs and examined for gas production for presence of fecal coliforms and enumerated by using MPN tables. *E. coli* confirmation was carried out by testing for indole production IMVC pattern. Suspensions were inoculated in tubes of tryptone broth and incubated for 24hrs at 35°C. Kovacs' reagent (0.2-0.3 ml) was added to test for indole production. The appearance of a distinct red color in the upper layer of the suspensions was positive indication of presence of *E. coli*. The enumeration of coliforms and *E. coli* is summarized in Fig. 2.

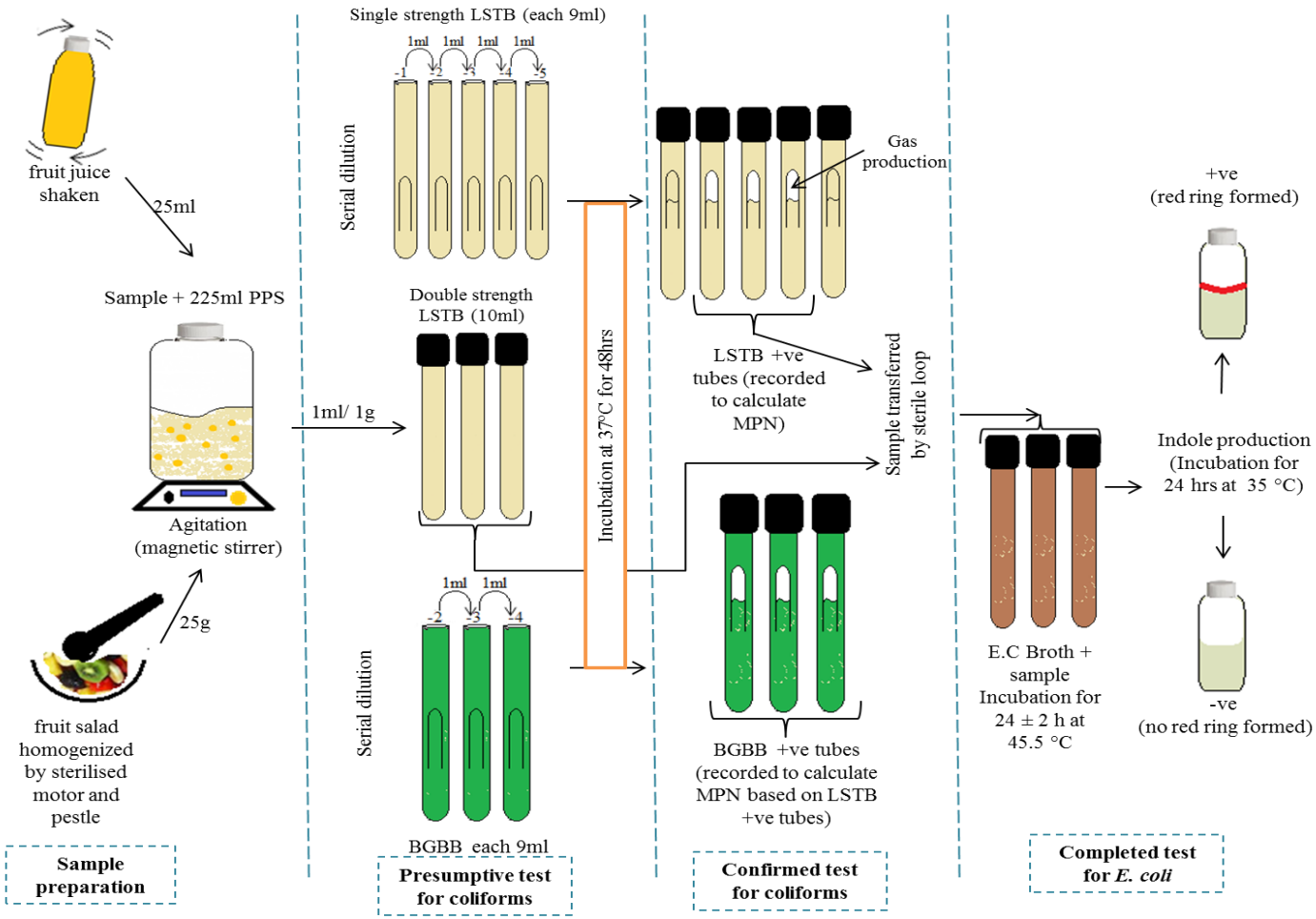


Figure 2: Illustration of Coliforms and *E. coli* enumeration (Source: Feng *et al*., 2002).

3.8.2.3 Polymerase Chain Reaction (PCR) Analysis

The molecular standard technique was used to identify pathogenic *E. coli* 0157:H7 in a street fruit juice sample using their specific primers as described by Wang *et al*. (1997). DNA from the *E. coli* culture was extracted using the "QIAamp® Viral RNA Mini Kit-Qiagen" (QIAGEN Sciences, Maryland 20874, USA) following the manufacture's protocol. A known positive isolate for *E. coli* O157:H7 was obtained and DNA was extracted by boiling at 80ºC for 30 min. PCR amplification for *E. coli* was then carried out. The PCR mixture consisted of 25 µl containing0.5 µl of Taq DNA polymerase, 12.5 µl of 2X reaction buffer, 8 µl of RNase free water, 10 pmol of each primer and 2 µl of DNA template. The PCR reaction was performed in a thermo cycler at a denaturation temperature of 72ºC for 10 min. A total of 35 cycles at 95ºC, 55ºC and 72ºC each for 30 sec followed denaturation. The PCR products were analyzed using 1.5% gel electrophoresis which was run at 100 voltages for 30 min and viewed under ultra violet light where a photograph was taken.

3.9 Moulds and Yeasts

Moulds and yeasts were enumerated as described by Tournas *et al*. (2001) in BAM. For fruit salad, samples (250g) were weighed and homogenized by motor and pestle that were pre-sterilised at 150°C for 120 min in an oven (Memert UM 400, Germany) while fruit juice samples were homoginised by shaking. Rosebengal chloramphenicol agar (RBCA) (Himedia laboratory, Pvt India) was used for enumeration. RBCA was prepared in glass bottles containing magnetic stirrers by dissolving 32.15g in 1 000 ml of distilled water. The bottles were then heated in an electric water bath to achieve gelatinization (Precision Scientific 180 series. Chicago, USA) and then sterilised using an autoclave (SHi- AVX 90.E electronic, France) at 121°C for 15 min. The media was left to cool to around 45ºC, poured in sterile petri dishes and left to solidify for surface spreading. Twenty five grams

of the homogenized fruit salad sample or 25 ml of fruit juice sample was dispensed in a glass bottle containing 225 ml of 8.5% buffered polypetone water (PPS) and agitated using a magnetic stirrer for 1min. Serial dilution was then carried out in a series of sterile test tubes containing 9ml of PPS to a 10^{-4} dilution. After the sample was serially diluted, 0.1 ml from each respective dilution was plated in duplicate by surface spreading on solidified RBCA in 15 x 100 mm sterile glass petri plates. The plates were then incubated (Binder 960285, Germany) upside down at 25°C for 5 days. The number of colonies counted in plates containing a range of 25-250 colonies. The CFU/ml of fruit juice or fungi/g of fruit salad where then computed. Isolates from plates with growth were sub-cultured and incubated PDA at 25°C for 3-5 days for morphological analysis. The isolates were assessed for morphological characteristics for identification using an electric light microscope (Brunel microscope B25, UK) under a ×1 000 magnification. A loop sterilized by flame and ethanol was used to swab a fungi colony. The colony was spread on a microscope slide and wet mounted using distilled water. The colony was then stained using lacto phenol cotton blue where a cover slip was placed on top. The edge of a dry paper was touched on the cover-slip to withdraw excess water. The slide was then spread with an oil immersion for higher magnification view (for ×100 objective lens). The morphology of colony, size, shape, budding, mycelia and fruiting bodies were studied for specie identification according to Mathur and Kongsdal (2003).

3.10 Data Analysis

In combination with Excel software for Windows, data were subjected to one way analysis of variance (ANOVA) and Tukey HSD multiple range test was used to determine the differences at $p \leq 0.05$ using SPSS 21.0 (SPSS software for Windows, release 21.0, SPSS, Inc., USA).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Production of Street Fruit Salad and Fruit Juice

4.1.1 Production of fruit juice

This parameter addressed the first specific objective using the questionnaire (Appendix 1). Fruit salad and fruit juice vending in streets, University cafeterias and restaurants is a common practice and the majority of the population consumes the products. Fruit juice was produced using simple equipment which included, aluminum pots; knives; plastic buckets; cool boxes; plastic cups and re-used plastic bottles. The preparation after purchasing fruits from local markets (Morogoro central market and Mawenzi market) included washing, peeling, extraction of juice (using a home electrical blender) and blending with water. The mixture was mechanically blended using an electrical blender. The juice was then transferred into a cool box containing frozen ice blocks in plastic packets. The juice was either sold as pre-packed in 250 ml or 500 ml re-usable plastic bottles or poured using a plastic cup into the re-usable plastic bottles according to consumer preference (Plate 1). The juice was transported by using mobile carts for street vendors and sold on site at University cafeterias and town restaurants.

Plate 1: Street vended pre-packed juices for sale (Note: The names Mountain Dew, Kilimanjaro and Azam Cola are names of drinks previously packed in the respective bottles. These bottles have been reused by vendors)

4.1.2 Production of fruit salad

The production of fruit salad was not much different from production of fruit juice. This addressed the first specific objective using the questionnaire (Appendix 1). The equipment used by vendors were; knives; plastic buckets; fridge; plastic plates and polyethylene bags. Fruits were bought from local markets (Morogoro central market and Mawenzi market). From own observations, some vendors washed the fruits before peeling and chopping while others did not. After the fruits were peeled using knives they were then chopped on a plate and served or stored in a refrigerator uncovered waiting to be sold. Sometimes fruit salad was packed into polyethylene bags and sold to the consumer on site at universities and restaurants or sold by mobile vendors on streets (Plate 2).

Plate 2: Fruits salad preparation for sale

4.2 Status of Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) of Street Fruit Salad and Fruit Juice

The characteristics to follow addressed the first specific objective using the questionnaire (Appendix 1).

4.2.1 Demographic characteristics

The demographic features of fruit salad and fruit juice vendors on streets, at University cafeterias and restaurants are shown in Table 3. The results indicated that the majority (88.6%) of the fruit juice vendors were men while women involved were only 11.4%. Similarly, a majority (74.3%) of the fruit salad vendors were men while women were only 25.7.4%. Findings also showed that vendors had a relatively low level of education. The

majority (94.3%) of the fruit juice vendors had primary level education while 5.7% had none. Similarly all fruit salad vendors had primary level education. Similar to other studies, illiteracy is presumed to characterize the street food vendors (Dinda, 2010; Campell, 2011). The low education level may be associated with poor manufacturing and hygiene practices during handling, storage and preparations of the salads and juices which can increase the risk of street food contamination (Muyanja *et al*., 2011). Most fruit juice vendors (62.9%) were mobile businesses while 37.1% were stationary (sold on site). On the other hand, the majority (85.7%) of fruit salad vendors were stationary businesses while 14.3% were mobile businesses. This implies a diverse accessibility by consumers in different locations around the municipality for these products.

Table 3: Demographic and business characteristics of food street vendors in Morogoro municipality

Characteristic	Fruit juice (n=35)	Fruit salad (n=35)
Gender		
Male	31 (88.6)	26 (74.3)
Female	4 (11.4)	9 (25.7)
Vendor type		
Street vendor	25 (71.4)	25 (57.1)
University	5 (14.3)	5 (14.3)
Restaurant	5 (14.3)	10 (28.6)
Marital status		
Single	33 (94.3)	29 (82.9)
Married	2 (5.7)	6 (17.1)
Education level		
Primary	33 (94.3)	35 (100)
None	2 (5.7)	0 (0)
Type of business		
Mobile	22 (62.9)	5 (14.3)
Stationary	13 (37.1)	30 (85.7)
Type of preparation		
Prepacked	35 (100)	0 (0)
Prepacked or prepared on site	0 (0)	35 (100)
Business location		
Along road sides	7 (20)	0 (0)
Market area	0 (0)	7 (20)
Transport terminal	13 (37.1)	0 (0)
University	5 (14.3)	5 (14.3)
Primary school	5 (14.3)	0 (0)
Town centre	5 (14.3)	23 (65.7)

*Figures in parenthesis indicate percentage of total population (n)

4.2.2 Composition of the street vended fruit juice and fruit salads

The composition of fruit juice and fruit salad is shown in Table 4. For fruit salad a majority (80%) of the vendors used mangos, pineapples and avocados for making the mix while only 20% used mangoes, bananas, pineapples, avocados, cucumbers and oranges to make the chopped mix. A majority of fruit juice vended (82.9%) was sold as mango juice followed with a mix of mango and orange fruits sold as only 8.6% of vendors identified. The use of mangos and pineapples in most formulations might have been attributed to the maturity of the fruits and lower price at the fruit markets. Most of these citrus fruits are locally grown in large quantities in Morogoro by small scale farmers (FAO, 2012; SNV 2005).However, juice and fruit salad vendors did not use any food colour, flavouring or preservative.

Table 4: Composition and use of additives in fruit juice and fruit salads

Ingredients	Fruit juice (n=35)	Fruit salad (n=35)
Fruits		
Mango	29 (82.9)	0 (0)
Mango and avocado	2 (5.7)	0 (0)
Mango and orange	3 (8.6)	0 (0)
Mango and baobab	1 (2.9)	0 (0)
Mango, banana, pineapple, avocado, cucumber and orange	0 (0)	7 (20)
Mango, pineapples and avocado	0 (0)	28 (80)
Food colours		
Use	0 (0)	0 (0)
Do not use	35 (100)	35 (100)
Preservatives		
Use	0 (0)	0 (0)
Do not use	35 (100)	35 (100)
Flavourings		
Use	0 (0)	0 (0)
Do not use	35 (100)	35 (100)

*Figures in parenthesis indicate percentage of total population (n)

4.2.3 Food safety knowledge and health of vendors

4.2.3.1 Fruit juice vendors food safety perception and health

The perception of basic food safety knowledge and the health status of fruit juice vendors is summarized in Table 5. Of the vendors, 54.3% were not familiar with the term “food safety” while 45.7% were familiar with it. In other countries including developed ones such as Ireland, it was observed that some of vendors did not know food safety concepts (McCarthy *et al*., 2007).In terms of awareness, all vendors had never heard of TFDA. However, 85.7% of vendors were aware and only 14.3% unaware of TBS. This could be attributed to the public sensitization by TBS and the importance of the mark that usually appears in TBS certified food products. All juice was sold pre-packed in reusable plastic bottles or paper cups. All vendors were not familiar with food safety programs including GMP and GHP, and implemented neither. The majority of the vendors (94.3%) went for check-up only when sick, and only 5.7% went for medical check-up twice a year. However, all of them did not remember the last time they went for medical check-up. WHO (2006) reported that most countries had insufficient inspection personnel, insufficient application of the HACCP concept and noted that registration, training and medical examinations were not amongst selected management strategies.

Table 5: Food safety and health knowledge

Characteristic	Fruit juice (n=35)	Fruit salad (n=35)
Familiarisation with food safety terminology		
Familiar	16 (45.7)	10 (28.6)
Not familiar	19 (54.3)	25 (71.4)
Heard about TFDA		
Have heard	0 (0)	0 (0)
Have not heard	35 (100)	35 (100)
Heard about TBS		
Have heard	30 (85.7)	33 (91.4)
Have not heard	5 (14.3)	2 (8.6)
Quality registration certificate		
Posses	0 (0)	0 (0)
Do not posses	35 (100)	35 (100)
Type of preparation		
Prepacked	35 (100)	0 (0)
Prepacked or prepared on site	0 (0)	35 (100)
Familiarisation with food safety program and implementation		
Familiar and implement	0 (0)	0 (0)
Not familiar and do not implement	35 (100)	35 (100)
Heard of GMP		
Have heard	0 (0)	0 (0)
Have not heard	35 (100)	35 (100)
Heard of GHP		
Have heard	0 (0)	0 (0)
Have not heard	35 (100)	35 (100)
Do medical check-up		
Yes	35 (100)	35 (100)
No	0 (0)	0 (0)
Frequency of medical check-up		
Once per year	2 (5.7)	0 (0)
Twice per year	0 (0)	0 (0)
At least once every month	0 (0)	0 (0)
When sick	33 (94.3)	35 (100)
Last time for medical check-up		
Remembered month/ year	0 (0)	0 (0)
Do not remember	35 (100)	35 (100)

*Figures in parenthesis indicate percentage of total population (n)

4.2.3.2 Fruit salad vendors food safety perception and health

The food safety knowledge and health characteristics of fruit salad vendors are shown in Table 5. The majority of the vendors (71.4%) were not familiar with the term food safety while 28.6% were familiar. Similarly, with fruit juice vendors none had heard about TFDA. Ninety four percent were aware of TBSwhile8.6%were not. This may also be attributed to the sensitization by TBS and the logo/ mark that usually appears in TBS certified food products. Fruit salad was sold as either pre-packed or prepared on site on plates or packaged in polyethylene bags. All vendors were also not familiar with food safety programs including GMP and GHP and implemented neither. All vendors went for medical check-up only when sick. However, as in the case of fruit juice vendors, all of them did not remember the last time they went for medical check-up. Food safety sensitization/ education may be effective and additional strategies are necessary to develop specific food safety messages targeted to vendor mind-sets and measure the effectiveness of the delivered food safety messages (Saulo and Moskowitz, 2011). Basic training in food hygiene is recommended in order to ensure that food vendors follow the required rules for proper hygiene and sanitation (Chukuezi, 2010).

4.2.4 Types of infrastructure and gear used

Table 6 shows the type of infrastructure used by vendors. Most of the vendors (80% for juice and 42% for salad) indicated that they had access to potable water but relied on the safety measures taken by suppliers, with only 40% of juice vendors boiling the water. This is similar with observations made in Nigeria on use of poor quality water by vendors (Chukuezi, 2010).The major source of water used was municipality supply tap water. A majority of juice and salad vendors, 57.1% and 71.4%, respectively, had no access to toilets. Fruit salad vendors used waste bins while juice vendors (57.1%) used Pit/earth holes for disposal of waste. A large proportion of juice vendors (71.4%) had no processing

outfit gear while 46.7% of fruit salad vendors wore hair nets/caps together with aprons. Multiple comparisons showed no statistical significance difference among vendors on streets, university cafeterias and restaurants ($P>0.05$). This indicated presence of poor infrastructure facilities used by vendors which might lead to more cross contamination. All vendors did not wear gloves, although gloves could be hazardous if not properly used. Street vendors in Uganda also were observed to have variable poor infrastructure for preparation of foods (Muyanja *et al*., 2011)

Table 6: Type of infrastructure and gear used in processing

Type	Characteristic	Fruit juice (n=35)	Fruit salad (n=35)
SV[a]	*Potables water availability*		
U[a]	Yes	35 (100)	35 (100)
T[a]	No	0 (0)	0 (0)
	Source of water for processing		
SV[a]	Wel	0 (0)	0 (0)
U[a]	Municipal tap	28 (80)	15 (42.9)
T[a]	Hawked in plastic containers	7 (20)	20 (57.1)
SV[a]	*How is water ensured safe*		
U[a]	Boil	14 (40)	0 (0)
T[a]	Rely from supplier	21 (60)	35 (100)
SV[a]	*Are toilets available*		
U[a]	Yes	10 (28.6)	15 (42.9)
T[a]	No	25 (71.4)	20 (57.1)
SV[a]	*Hand washing facilities*		
U[a]	Yes	35 (100)	15 (42.9)
T[a]	No	0 (0)	20 (57.1)
	Cooling facilities		
SV[a]	Refrigeration	6 (17.1)	15 (42.9)
U[a]	Cool box	25 (71.4)	0 (0)
T[a]	Cooling dispenser	4 (11.4)	0 (0)
	None	0 (0)	20 (57.1)
SV[a]	*Waste disposal available*		
U[a]	Yes	35 (100)	35 (100)
T[a]	No	0 (0)	0 (0)
	Type of waste disposal		
SV[a]	Waste bin	8 (22.9)	35 (100)
U[a]	Pit/ earth hole	20 (57.1)	0 (0)
T[a]	Plastic bag	7 (20)	0 (0)
	Plastic bucket	0 (0)	0 (0)
	Processing gear		
	Hair net/ cap	3 (8.6)	6 (17.1)
SV[a]	Gloves	0 (0)	0 (0)
U[a]	Apron/ Processing coat	0 (0)	0 (0)
T[a]	Boots	0 (0)	0 (0)
	Hair net / cap and apron	7 (20)	16 (46.7)
	None	25 (71.4)	8 (22.9)

*Figures in parenthesis indicate percentage of total population (n): U= University cafeterias, SV= Street vendors and T= Town restaurants. Same lowercase letters denotes no difference amount the street vendors (P>0.05).

4.2.5 General assessment of location, sanitation level, processing and cleaning and personal hygiene of fruit salad and juice vendors

4.2.5.1 Location and premises

The parameters assessed include: location and surroundings, waste/garbage around that could harbor animals, pests or germs, dusts and bad smell, toxic substances, pets roaming around, water logged areas and lighting and ventilation. As indicated in Fig. 3, most of the fruit juice vendors in restaurants assessed for sanitation, location, processing, cleaning and personal hygiene were better, scoring a higher level (above 57%) as compared to universities and street vendors and the three vendors differed significantly (P<0.05).Fruit salad vendors were observed to have compliance levels below 50% (Fig. 4) with restaurants being better-off than universities and street vendor's premises and environment (Plate 3).

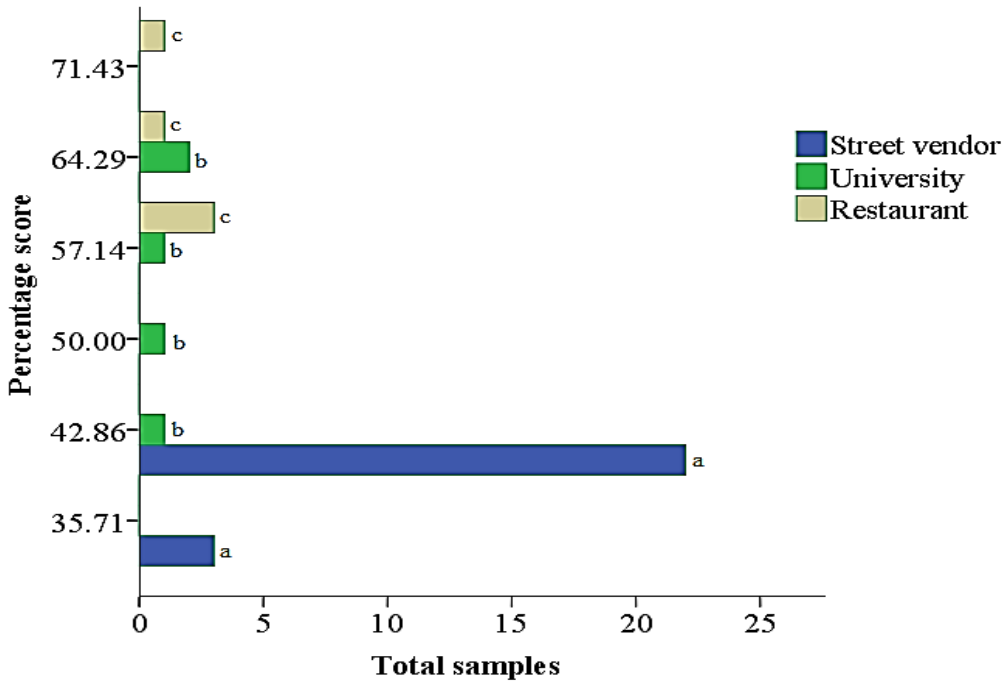


Figure 3: Status of location and premises of fruit juice vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

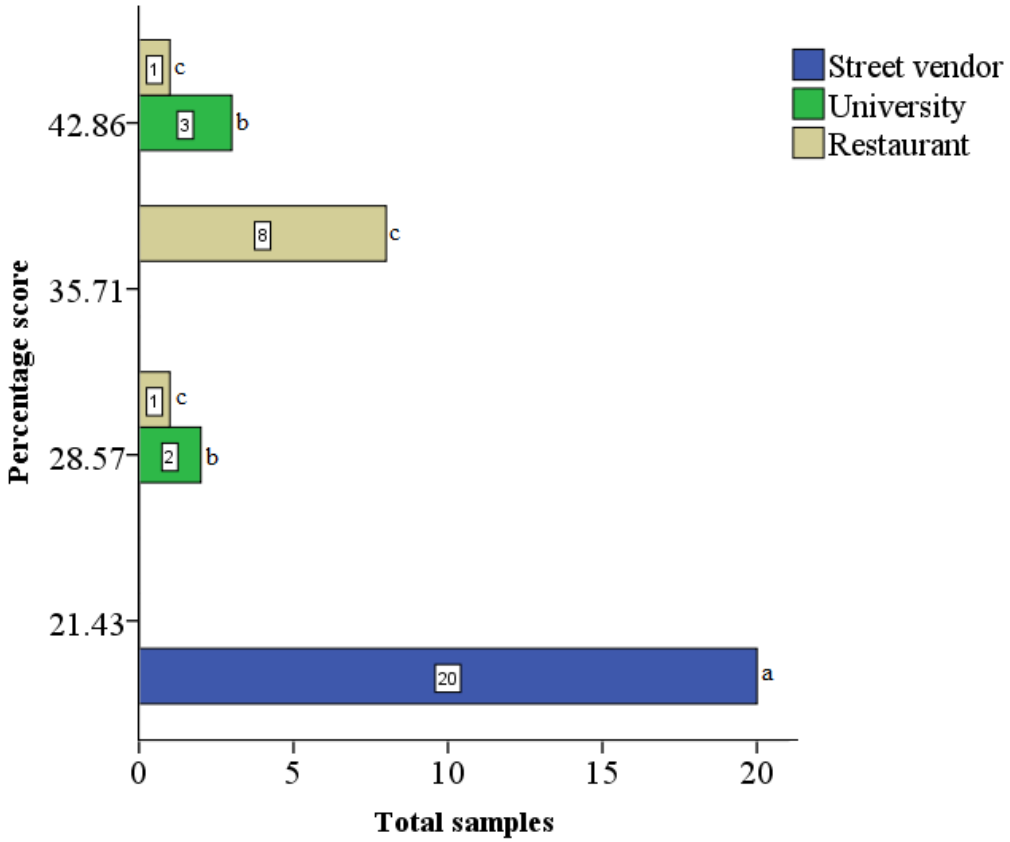


Figure 4: Status of location and premises of fruit salad vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

Plate 3: Fruit juice and fruit salad vending premises and environment (a) Bus terminal (b) University cafeteria

4.2.5.2 Sanitation level

The parameters that assessed were water availability, water quality being clean/potable; lid type containers for disposal if sufficient, proper disposal system, detergent/disinfectant, presence and preventive structures for animal/insect infestation. Water used for processing was physically dirty and of poor quality including poor infrastructure. Based on national and Codex Alimentarius guidelines, the sanitation level of the fruit salad vendors ranged from 8-25% showing a very low compliance (Fig. 5). However, there was no significance difference between universities and street vendors (P<0.05%).As for fruit juice vendors the sanitation level ranged from 16-58% (Fig. 6).Restaurants and universities showed a moderate sanitation with street vendors having poor sanitation levels with all vendors were significantly different (P<0.05%). Key findings showed that infrastructure developments were relatively limited with restricted access to potable water (47%), toilets (15%), refrigeration (43%) and washing and waste disposal facilities (WHO, 2006). In Nigeria, it

was reported that water used by street vendors was dirty (Abdalla *et al*., 2009; Chukuezi, 2010). Studies carried out in Morogoro municipality (Jiwa *et al*., 1991) and in the suburb Kingolwira (Shayo *et al*., 2007) indicated that water used was of poor quality and highly contaminated and needed proper treatment before consumption.

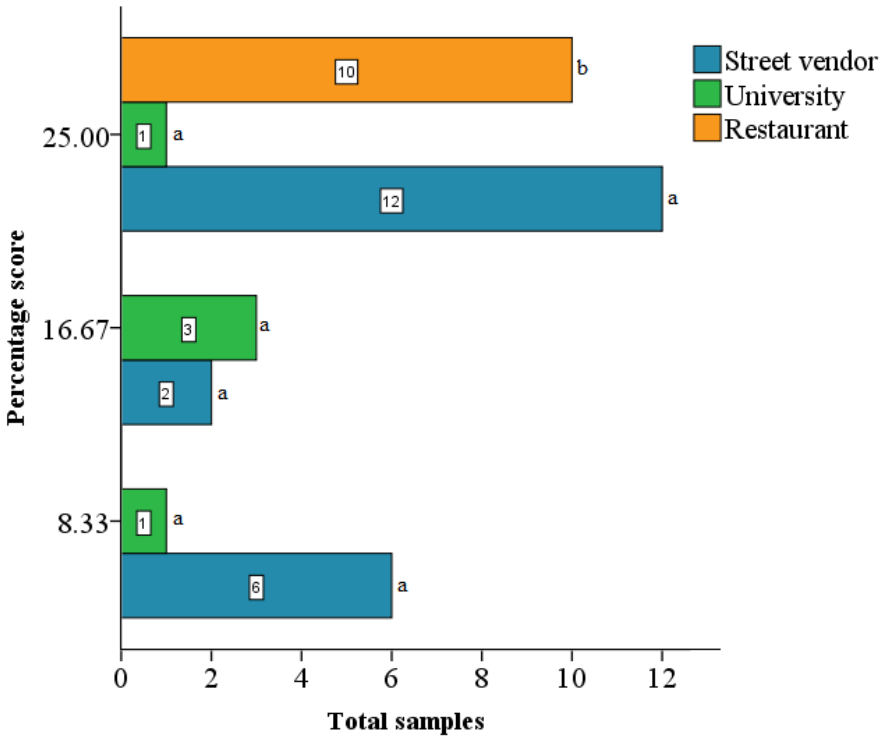


Figure 5: Sanitation level of fruit salad vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

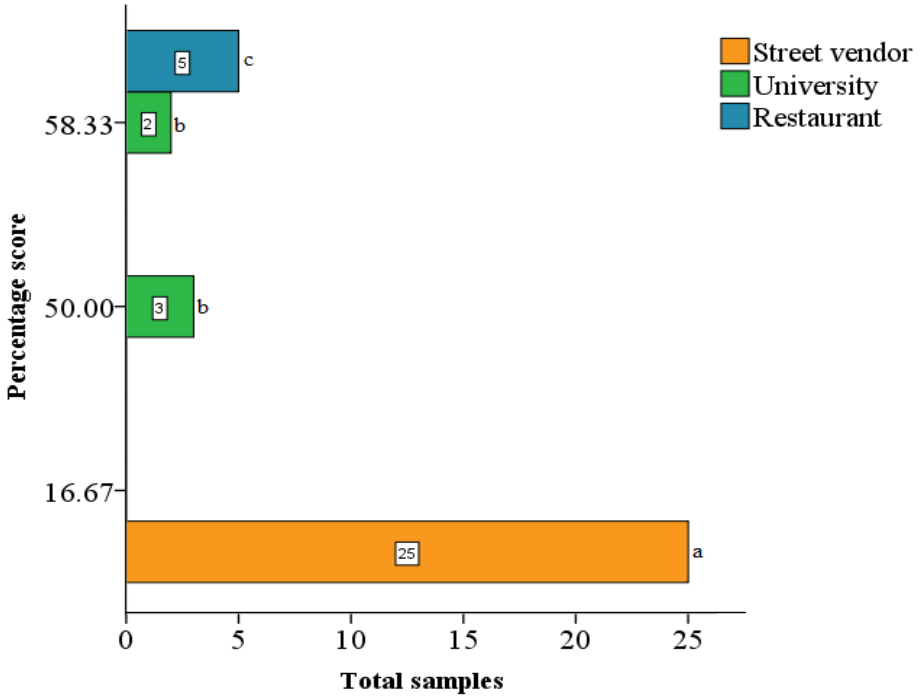


Figure 6: Sanitation level of fruit juice vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

4.2.5.3 Processing and cleaning

The parameters investigated included cleanliness and hygienic status of premises, and appropriateness of tools used for food processing and packaging. The level of processing and cleanliness of fruit juice vendors ranged from 0-50% (Fig. 7 and plate 4). Street vendors scored 0% compared with university cafeterias and restaurants scoring an average of 50%. There was no significant difference between universities and restaurants (P>0.05), but on the other hand, street vendors differed significantly with latter two categories (P<0.05%).This could be attributed to poor equipment used for processing, especially by street vendors using cool boxes as sole means for juice storage. Juice vendors also packed the juice in used plastic bottles that were just rinsed with the same water (batch), being used repeatedly for washing consecutive bottles that were used for packing and serving juice to consumers. The same water that was used for rinsing could contain high microbial

loads and dirt which could impose a risk by increasing the microbial load in the packed juice.

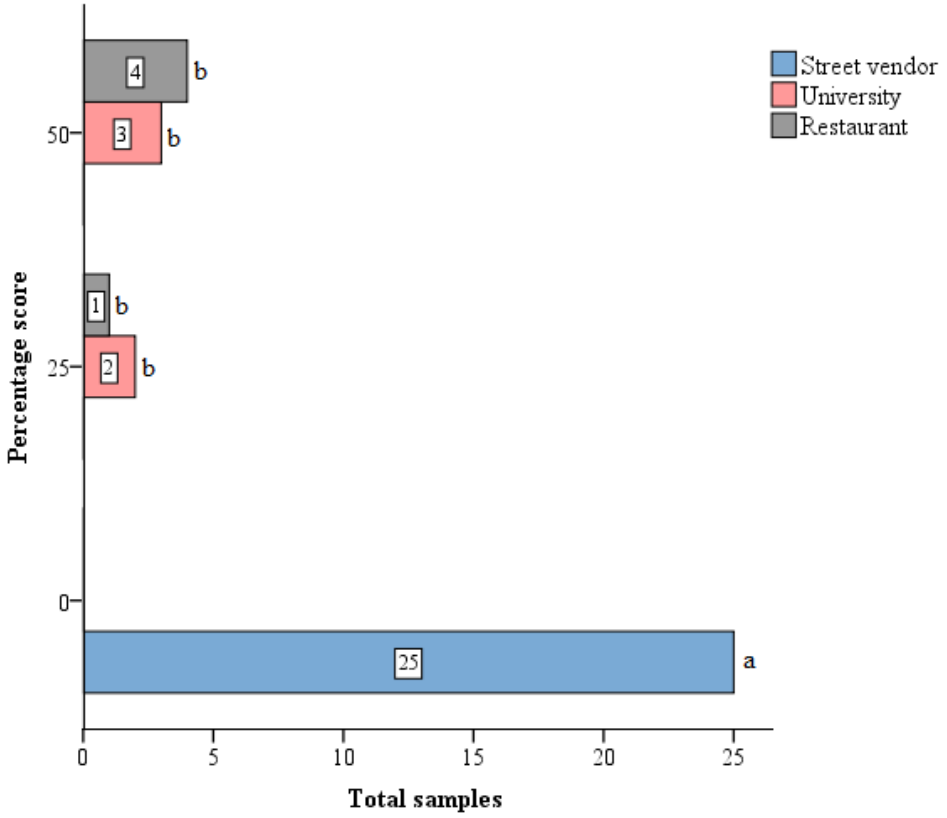


Figure 7: Processing and cleaning level of fruit juice vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

Plate 4: Fruit juice vending equipment. (a) along road side and (b) in bus terminal.

There was no statistical significant difference among fruit salad vendors compliance on the same parameters (P>0.05) where they generally scored low (25%) (Fig.8 and Plate 5).

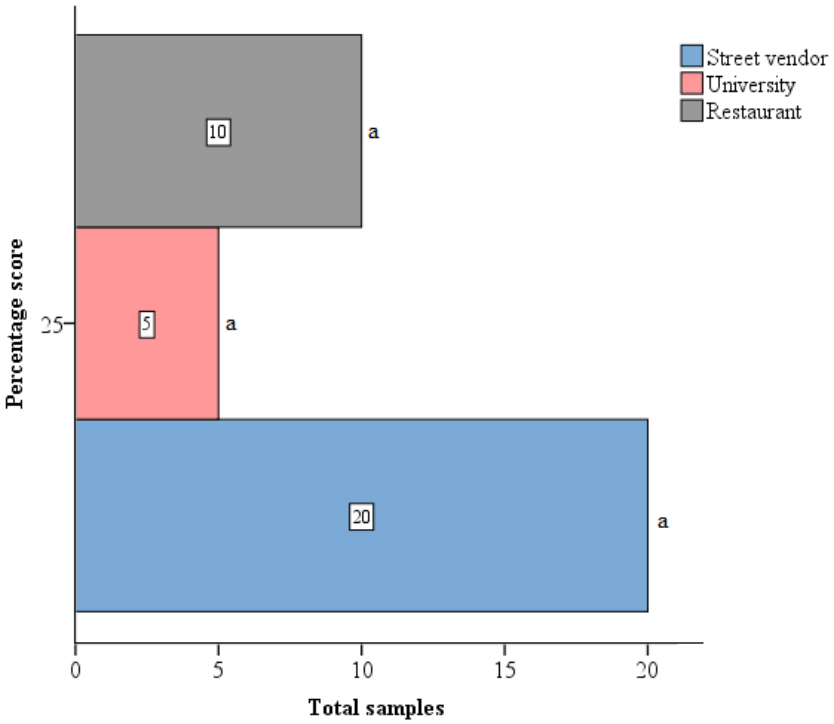


Figure 8: Processing and cleaning level of fruit salad vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

Plate 5: Fruit salad preparation equipment, (a) and (c): University and (b) restaurant

4.2.5.4 Personal hygiene

Parameters assessed for personal hygiene were if a worker was healthy not suffering from any communicable diseases and attending medical examination after every 6 months, suitable clean clothes for processing, no decorative articles, cleanliness of hands and nails, washing of hands before and after work, usage of gloves, hair net/ hat and presence of first aid kit. It was observed that personal hygiene compliance by fruit juice vendors was poor (below 19%) (Fig. 9) and there was a significant difference among vendors ($P<0.05\%$). Similarly, fruit salad vendors showed low levels of personal hygiene (≤25%) (Figure 10), with no significant difference between restaurants and universities ($P>0.05$), but significantly different from street vendors ($P<0.05$).The poor hygiene was attributed to vendors having dirty hands, lack of gloves while handling food and unsuitable and dirty clothes that might cause contamination. A similar study carried out in Sudan urged that routine medical examination of food handlers should be carried out by health officials in order to regulate safe street food handling, preparation and vending (Abdalla *et al*., 2009).

The majority of street vendors did not comply with the general codes of hygiene as laid by national and Codex Alimentarius requirements. This emphasizes the importance of training

street vendors on importance of personal hygiene in order to avoid contamination of food (James, 2006).

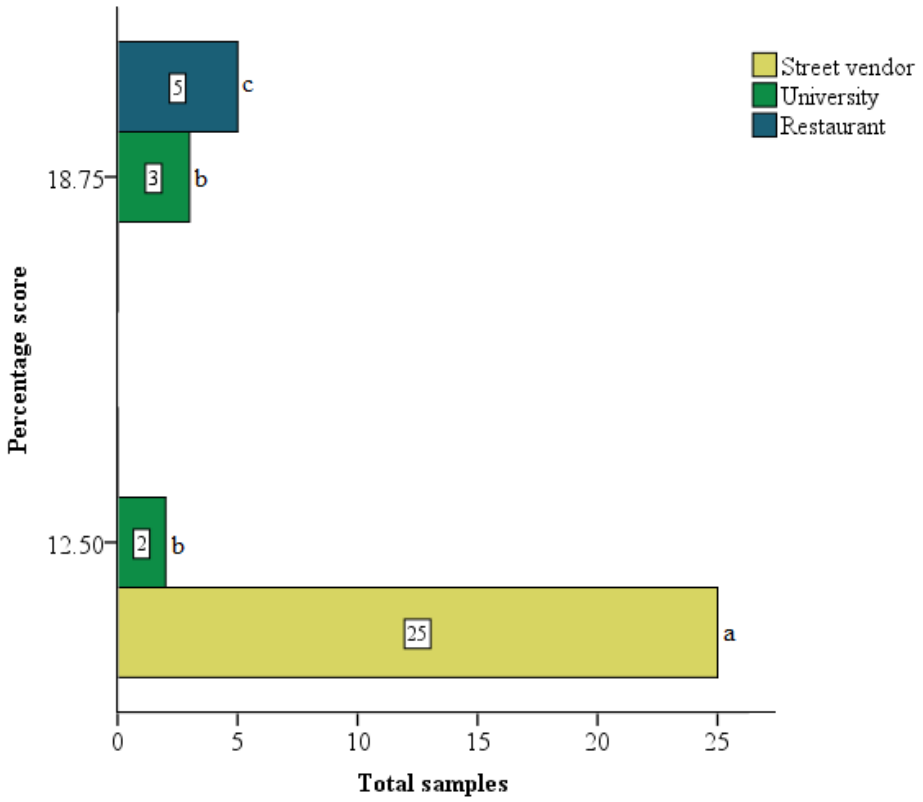


Figure 9: Personal hygiene level of fruit juice vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

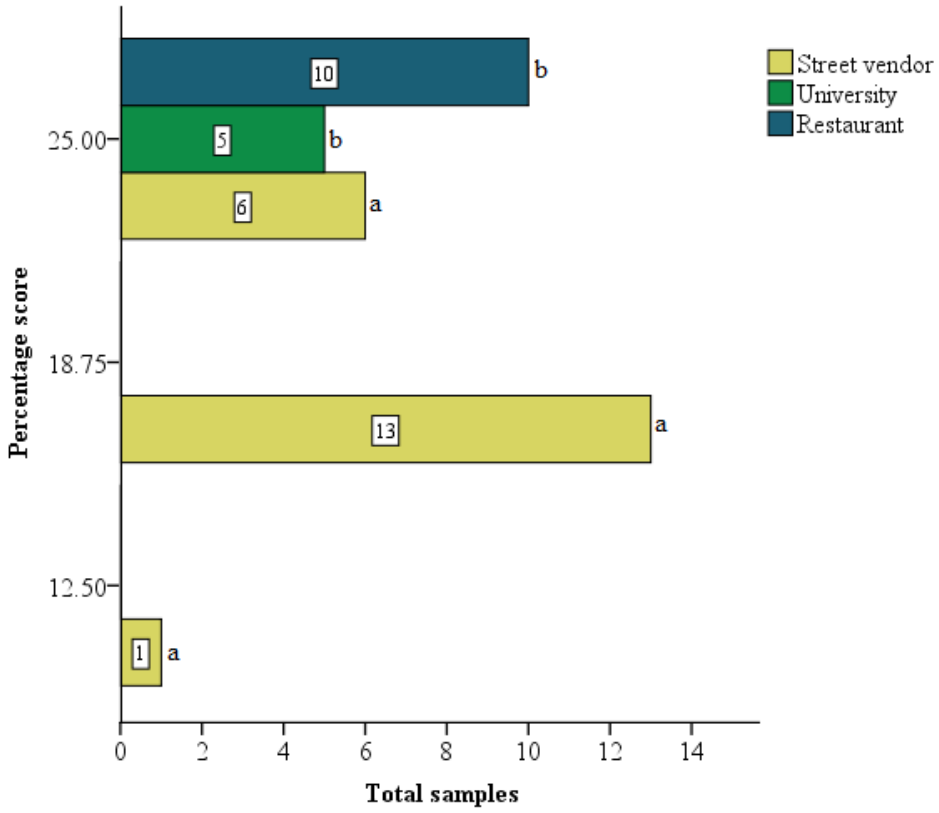


Figure 10: Personal hygiene level of fruit salad vendors. Bars with same lowercase letters means there is no significant difference of vendors for different percentage scores (p>0.05).

4.3 Physico-Chemical Properties of Fruit Juice and Fruit Salad

The physico-chemical parameters (pH and ORP) of vended fruit juice and fruit salad were investigated. The average pH and ORP for fruit juices were 4.27 and 136.8 mV, respectively, while the average pH and ORP for fruit salads were 4.43 and +130 mV respectively (Table 7). Most aerobic bacteria grow at an ORP range of +200 to 800mV while anaerobic bacteria grow well at -700 to +200 mV (McPherson, 1993). Despite the low pH, the samples contained high microbial counts. This might be attributed to contamination, presence of acidophilic microorganisms and microbial growth parameters e.g. nutrient composition of the fruit juices and fruit salads such as sugar, vitamins and minerals; high a_w; and lack of pasteurization (Speck, 1976). However, the presence of moulds in commercial fresh fruit juices has been reported. Heat-resistant moulds e.g.

*Chrysoniliasitophila, Aspergillus*spp may survive if heat treatment is not sufficient (De Donno *et al*., 1998). It has been reported that high ORP (> +1000mV) may lead to the oxidation of membranes and disruption of metabolic fluxes in the bacterial cells (Liao *et al*., 2007; Marriott and Gravani, 2006).

The ORP may be conducive for proliferation of aerobic microorganisms including moulds and film yeasts which tend to grow on surface of juices and cause spoilage. The low pH (4.27) might still allow some of the bacteria to proliferate and cause food spoilage or poisoning (especially the lactic acid bacteria) (Charlier *et al*., 2009). Elaboration of mycotoxins e.g. patilin, byssotoxin, byssochlymic acid A, etc. in fruit products by e.g. *B. fulva*, *B. ruvea* etc. has been reported.

Table 7: Physico-chemical characteristics of vended fruit juice and fruit salad

Source	Fruit Juice			Fruit salad		
	pH	ORP (mV)	Temperature (°C)	pH	ORP (mV)	Temperature (°C)
S.V	5.61	65.2	23.3	5.95	70.2	23.5
S.V	5.14	91.5	22.7	5.48	96.5	22.9
S.V	4.62	101.2	23	4.96	106.2	23.2
S.V	3.34	175.6	22.6	3.68	180.6	22.8
S.V	3.30	175.8	23.6	3.64	180.8	23.8
S.V	3.28	178.5	23.6	3.62	183.5	23.8
S.V	5.42	76.7	24	5.76	81.7	24.2
S.V	3.92	144.7	23.5	4.26	149.7	23.7
S.V	4.60	106.6	24.1	4.94	111.6	24.3
S.V	3.80	143.2	24.2	4.14	148.2	24.4
U	3.79	179.5	23.5	5.49	67.7	23.4
U	4.47	179.7	24	5.02	94	22.8
U	3.67	182.4	23.2	4.50	103.7	23.1
U	3.67	80.6	23.7	3.22	178.1	22.7
U	5.71	148.6	23.6	3.18	178.3	23.7
R	5.24	110.5	23.5	3.53	153.2	24.4
R	4.72	147.1	23.5	3.51	115.1	24.5
R	3.44	90.6	23.5	5.65	151.7	24.1
R	3.40	100.3	23.7	4.15	116	23.6
R	3.43	174.7	23.6	4.83	152.6	24.2
S.V	3.41	174.9	23	3.16	181	23.7
S.V	5.55	177.6	23.2	5.30	79.2	24.1
S.V	4.70	75.8	23.8	3.80	147.2	23.6
S.V	3.90	143.8	23.6	4.48	109.1	24.2
S.V	3.95	105.7	23.5	3.68	145.7	24.3
S.V	4.63	142.3	23.6	5.42	117.6	24.0
S.V	3.83	178.6	23.6	3.92	154.2	23.5
S.V	3.82	178.8	24	4.60	73.7	24.1
S.V	4.50	181.5	24.3	3.80	100	24.2
S.V	3.70	79.7	23	3.80	109.7	22.9
S.V	3.70	147.7	24.03	5.84	184.1	23.9
S.V	5.74	109.6	23.53	5.37	184.3	23.9
S.V	5.27	146.2	24.13	4.85	187	24.3
S.V	4.75	156.3	24.23	3.57	85.2	23.8

Source of samples: S.V= Street vendor, U= University and R= Restaurant

4.4 Heavy metals quality

Metals contaminants investigated were copper, lead and cadmium. All samples analysed did not contain any Pb and Cd. This may also be attributed by the limit of detection/ sensitivity of the ASS used (Pb: 0.10 mg/L and Cd: 0.032 mg/L). Only one sample out of 75 samples contained 0.24mg/L of copper. Consumption surveys indicated that the mean dietary copper intake by adults in Europe is between 1.0 and 2.0 mg/day. An MRL for essential micro-nutrients like copper in foods, has mostly been studied in products of animal origin e.g. milk: 0.2 mg/kg; eggs (fresh or cooked): 1.5 mg/kg., unless there is a clear consumer safety issue to do so then other products will be evaluated (EFSA, 2012).The fruit juices and fruit salads investigated were deemed safe in terms of these metals according to Codex Standard 193-1995 (Codex Alimentarius, 1995). Lead and cadmium in fruit juice should not exceed a level of 0.05mg/L while in fruit salads not more than 0.1mg/Kg.

4.5 Microbiological Quality

4.5.1 Microbial quality of fruit juice

The microbial quality of juice and salads was assessed in terms of total mesophilic aerobic plate (APC), fungi and coliforms counts.

A large proportion of the samples were heavily contaminated. A majority of the samples (97%) had an aerobic mesophilic microbial concentration exceeding>3×10^6 CFU/ml of juice with one sample having 3.6×10^5CFU/ ml of juice aerobic mesophiles (Table 8).

Table 8: Microbial quality of fruit juice

Vendor	APC (CFU/ml)	Fungi (CFU/ml)	Coliforms (MPN/ml)	Indole test (+ or -)	Intensity of red ring (Indole test)
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	3.6×10^5	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	5.9×10^4	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	3.5×10^3	+	S
S.V[a]	$>3\times10^6$	1×10^5	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	4.16×10^5	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	8.7×10^4	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	1.06×10^5	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	2.8×10^3	+	S
U[a]	$>3\times10^6$	1.53×10^5	$\geq1.6\times10^4$	+	S
U[a]	$>3\times10^6$	1.81×10^5	$\geq1.6\times10^4$	+	S
U[a]	$>3\times10^6$	1.68×10^5	2.8×10^3	+	S
U[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
U[a]	$>3\times10^6$	ND	$\geq1.6\times10^4$	+	L
R[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	8×10^3	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	2.55×10^5	$\geq1.6\times10^4$	-	-
S.V[a]	$>3\times10^6$	7.4×10^4	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	3.05×10^5	$\geq1.6\times10^4$	+	L
S.V[a]	$>3\times10^6$	1.17×10^5	2.8×10^3	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	L
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	L
S.V[a]	$>3\times10^6$	2.95×10^5	$\geq1.6\times10^4$	-	-
S.V[a]	$>3\times10^6$	8.7×10^4	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	2.34×10^5	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	1.46×10^5	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S

Key: Intensity of ring formed from indole test: S= Strong and L= Low. Source of samples: S.V= Street vendor, U= University and R= Restaurant. ND=not detected, (+) = positive and (-) = negative. APC= Aerobic mesophilic plate count. Same lowercase superscripts for vendors are not significantly different ($p>0.05$).

About 51% of the samples had fungal concentration exceeding 2.5×10^6CFU/ml of juice with 8% of the samples without fungal contamination, and 41% of the samples had a range of 8×10^3 to 4.16×10^5 CFU/ml of juice. The concentration of coliforms ranged from 2.8×10^3 (12% of samples) to $\geq1.6\times10^4$ MPN/ml of juice. However, most of the samples (88%) had coliforms $\geq1.6\times10^4$ MPN/ ml indicating poor hygiene and feacal contamination. The detection of *E. coli* through the production of indole from tryptone was indicated by formation of a red layer (Plate 6). Indole is a by-product of tryptophan metabolism by *E. coli* and the intensity of the ring correlates with its concentration. A majority (94%) of the samples presumptively contained *E. coli* of which 82% of the samples had a strong intensity of the redring formed with only 11% having low intensity. This indicated a high level of contamination by *E. coli*. Results on a study conducted on street vended foods in Sudan and South Africa also indicated high counts of coliforms (Abdalla *et al*., 2009; Mosupye and von Holy, 2000).There were no significant difference between the street vendors, universities and restaurants on microbial quality, implying the same poor compliance to hygiene ($P>0.05$). The majority of countries reported contamination of food (from raw food, infected handlers and inadequately cleaned equipment) and time and temperature abuse to be the major factors contributing to foodborne disease (WHO, 2006). The Tanzania Standards have no defined acceptable limits on microbial quality of unpasteurized-street vended fruit juice or fruit salads.

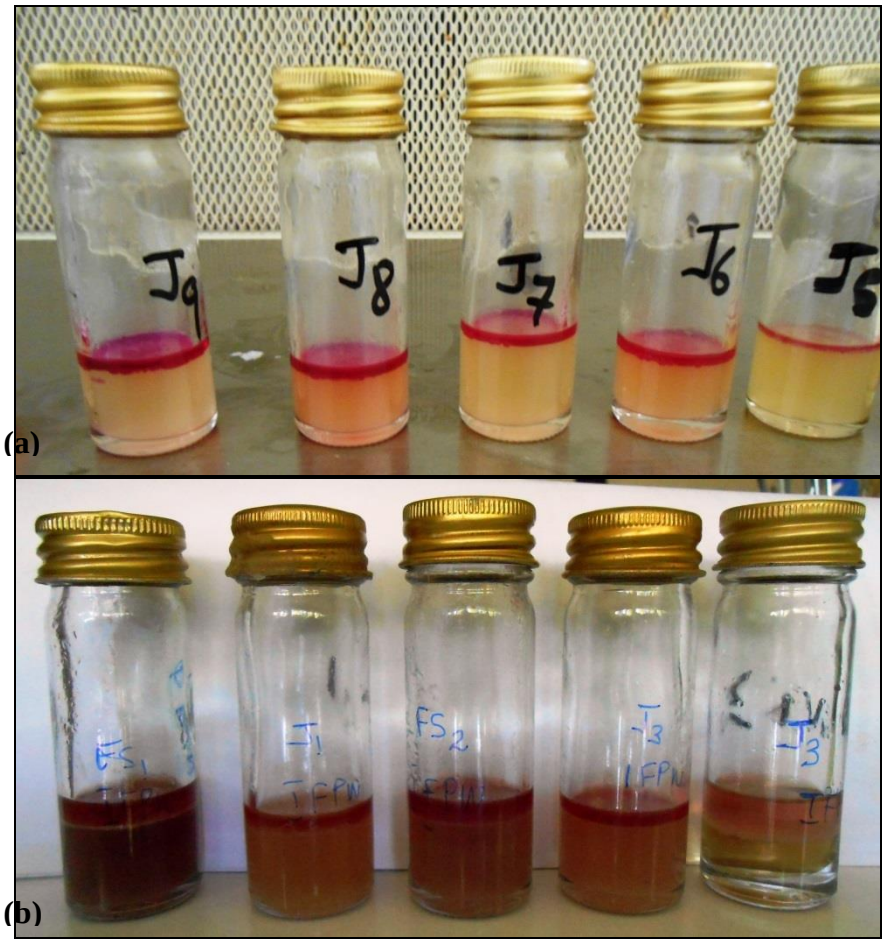


Plate 6: Samples showing red rings formedusing Kovack's reagent. (a) fruit juice samples and (b) fruit salad samples.

4.5.2 Microbial quality of fruit salad

The microbial quality of salads was also assessed in terms of APC, fungi and coliform counts (Table 9).

Table 9: Microbial parameters of fruit salad

Vendor	APC (CFU/g)	Fungi (CFU/g)	Coliforms (MPN/g)	Indole test (+ or -)	Intensity of red ring (Indole test)
S.V[a]	$>3\times10^6$	1.53×10^3	3.5×10^3	+	S
S.V[a]	$>3\times10^6$	3.55×10^2	1.6×10^3	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	1.60×10^3	2.2×10^3	+	L
S.V[a]	$>3\times10^6$	2.11×10^3	3.5×10^3	+	L
S.V[a]	$>3\times10^6$	4.10×10^2	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	7.95×10^2	340	+	L
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
U[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	-	-
U[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
U[a]	$>3\times10^6$	$>2.5\times10^6$	1.6×10^3	+	S
U[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	-	-
U[a]	2.70×10^4	$>2.5\times10^6$	$\geq1.6\times10^4$	-	-
S.V[a]	$>3\times10^6$	5.35×10^2	$\geq1.6\times10^4$	-	-
S.V[a]	9.55×10^4	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	2.8×10^3	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	3.5×10^3	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	3.5×10^3	-	-
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	1.3×10^3	+	S
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
S.V[a]	$>3\times10^6$	1.01×10^3	2.8×10^3	+	S
S.V[a]	$>3\times10^6$	4.30×10^2	2.2×10^3	+	S
S.V[a]	$>3\times10^6$	1.16×10^3	2.8×10^3	+	L
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	-	-
S.V[a]	$>3\times10^6$	7.05×10^2	$\geq1.6\times10^4$	+	L
S.V[a]	$>3\times10^6$	1.9×10^3	$\geq1.6\times10^4$	+	L
S.V[a]	5.8×10^4	1.01×10^3	$\geq1.6\times10^4$	+	L
S.V[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	L
R[a]	9.2×10^4	1.58×10^3	$\geq1.6\times10^4$	+	L
R[a]	9.25×10^4	1.29×10^3	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	$>2.5\times10^6$	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	1.89×10^3	$\geq1.6\times10^4$	+	S
R[a]	$>3\times10^6$	1.79×10^3	$\geq1.6\times10^4$	+	S

Key: Intensity of ring formed from indole test: S= Strong and L= Low. Source of samples: S.V= Street vendor, U= University and R= Restaurant. ND=not detected, (+) = positive and (-) = negative. APC= Aerobic mesophilic plate count. Same lowercase superscripts for vendors are not significantly different ($p>0.05$).

A large proportion of the samples (85%) had an aerobic mesophile concentration exceeding 3×10^6CFU/g of salad with 5 samples having an average of 7.3×10^4 CFU/g of salad. In India it was reported that fruits had APC up to 10^{10} CFU/g (Viswana than and Kaur, 2000). About 51% of the samples had fungi concentrations exceeding 2.5×10^6 CFU/g of salad and 48% of the samples had an average of 1.18×10^3 CFU/g of salad. The samples had coliforms (Plate 7) with an average of 2.4×10^3MPN/g(12% of samples) and most samples had$\geq1.6\times10^4$ MPN/g of salad(62%) also indicating poor hygiene and feacal cross contamination. A majority (82%) of the samples contained *E. coli*. About 57% of the samples in this study had a strong intensity of the red ring formed with only 25% having low intensity. This also indicates a high level of contamination. Similarly, as for fruit juice vendors, there were no significant differences ($p<0.05$) among the fruit salad street vendors, universities and restaurants on the microbial quality implying the same poor hygiene i.e. non-compliant. Street vended foods in South Africa were observed to be contaminated with *E. coli* (Mosupye and von Holy, 2000). Codex Alimetarius on the safety of fresh juices or salads recommends taking precedent and strict limits on production, harvest, transportation, storage, manufacture, processing, labeling and distribution. These are incorporated into Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) with Hazard Analysis and Critical Control Point (HACCP) procedures being applied throughout the food chain (FAO, 2001; CAC, 1997).

Plate 7: Gas production in Durham tubes by coliforms in fruit juice and fruit salad (a) BGBB tubes and (b) LSTB tubes

4.5.3 Polymerase Chain Reaction (PCR) Analysis

A PCR analysis was carried out to identify the presence of pathogenic strains of *E. coli*. A culture of *E. coli* from a positive street vended juice sample was sero-typed using PCR techniques and compared to the pathogenic serotype of *E. coli* O157:H7 (Wang *et al*., 1997).The results indicated presence of *E. coli* O157:H7 in the juice sample from streets (Plate 8). Similarly, this pathogenic strain of *E. coli* was detected in street vended foods in South Africa (Mosupye and von Holy, 2000).It has also been reported to survive in full-strength apple juice and apple juice diluted to 50 and 25% with pH of 3.7, 3.62, and 3.55, respectively (Janisiewicz *et al*., 1999; Wua *et al*., 2011).

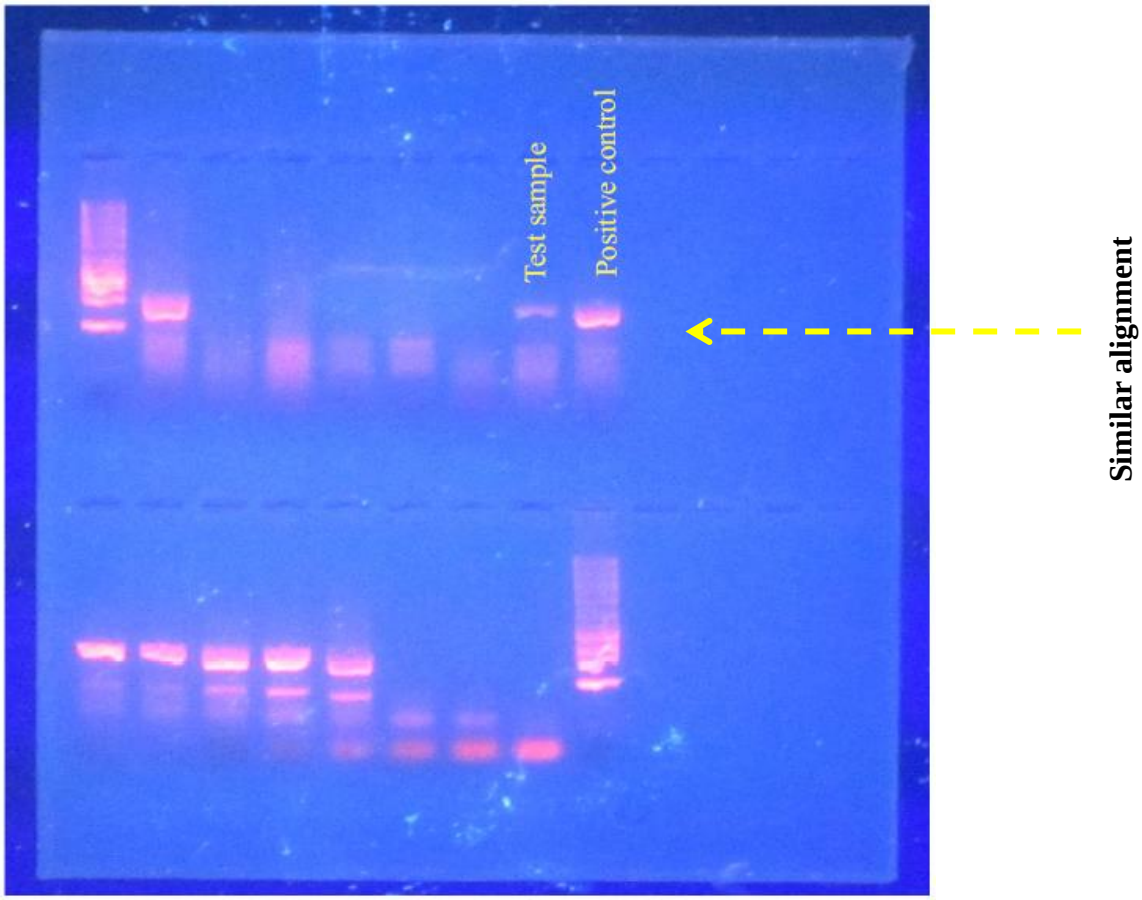
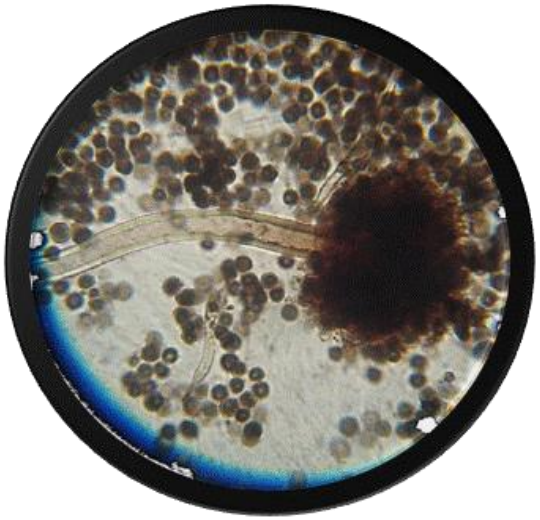


Plate 8: PCR output for *E. coli* O157:H7 showing test sample and positive control

4.5.4 Identification of Fungi

Four isolates were tentatively identified which included *A. niger*, *A. flavus*, *Saccharomyces* spp and *Cladosporium* spp. (Plates 9 and 10). *Aspergillus* species have potential to produce mycotoxins under stressful conditions, it is therefore important for further analysis to be done in fruit juice and fruit salad to determine presence of mycotoxins (Ellin, 2007). On the other hand *Cladosporium* is associated with allergens when inhaled and asthma attacks and causes spoilage in fruit juice forming cloudiness and bad odorous smells. It is also associated with most of spoilages in fruits (Kačániová and Fikselová, 2007; Tournas and Katsoudas, 2005). Al-Hindi *et al*. (2011) reported similar results that *Aspergillus* species are predominantly found in fruits. Moulds and yeasts are usually acid tolerant and are

therefore associated with the spoilage of acidic foods e.g. yeast may cause spoilage of juice through fermentation (FAO, 1998).

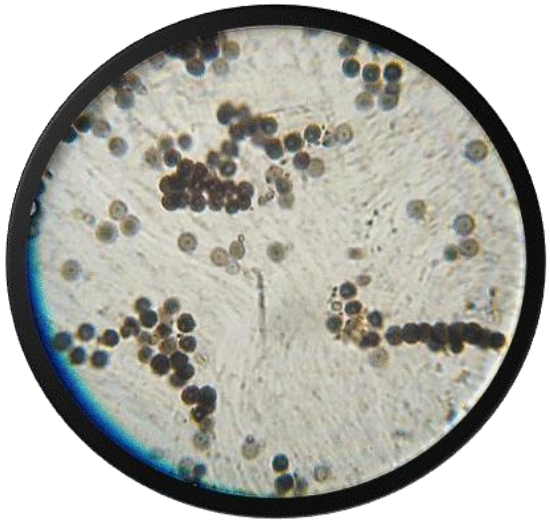


(b) *A. flavus*

(c) *A. niger*

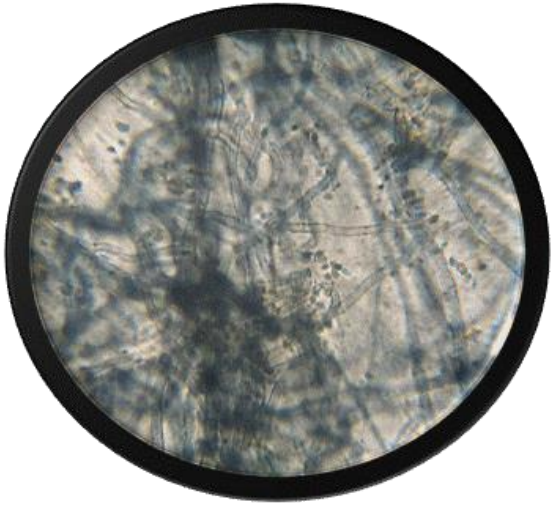
(d) *Saccharomyces spp* (Yeast cells)

(e) *Cladosporiumspp*

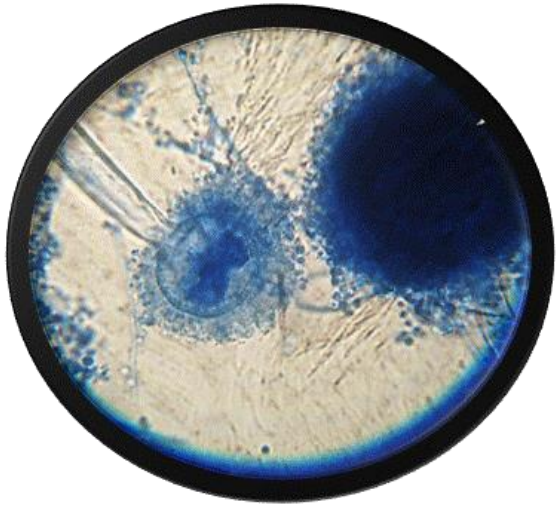


(f) *A. flavus* fruiting bodies

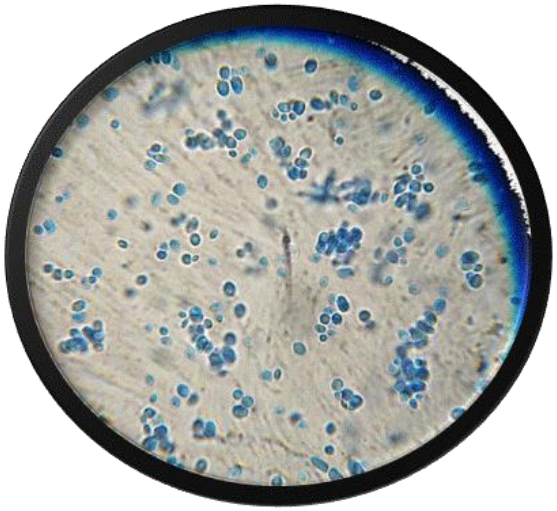
Plate 9: Fungi species identified as seen under a microscope (×1000). The morphology of colony, size, shape, budding, mycelia and fruiting bodies were studied for specie identification according to Mathur and Kongsdal (2003).



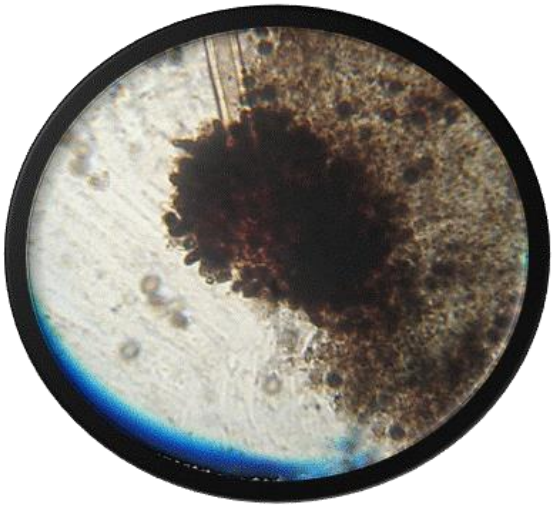
(g) *Cladosporiumspp*



(h) *A. niger*



(i) *Saccharomyces spp* (Yeast cells)



(j) *A. flavus*

Plate 10: Fungi species identified as seen under a microscope (×1000). The morphology of colony, size, shape, budding, mycelia and fruiting bodies were studied for specie identification according to Mathur and Kongsdal (2003).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

A large population is exposed to street vended juices and salads. This study indicated that fruit juice and fruit salad sold on streets, universities and restaurants had high levels of microbial contamination, thus not meeting national/ Codex Alimentarius food safety standards. This was mainly due to the poor processing and hygienic practices, causing cross contamination and deeming the products unsafe for consumption. The results have also shown that vendors on streets, at universities and in restaurants selling fresh fruit juice and fruit salads were not statistically significantly different in their compliance to general hygiene requirements and microbiological quality. The preparation practices and selling environments were unsatisfactory and unhygienic. These factors might have contributed to the higher microbial load and presence of pathogens. On the other hand, the products were chemically safe from contamination of heavy metals, lead and cadmium. The water used in processing should also be safe in order to prevent food poisoning and spoilage. The samples contained high concentrations of coliforms, indicating poor microbiological quality that could lead to food poisoning. The fungi tentatively identified included *Aspergillus* species, which have a potential of mycotoxins production in these products. Also, the *E. coli* O17:H7 was confirmed in one of the street vended juice samples and indicate possibility of a potential hazard to consumers from food poisoning. From this case study, it could be concluded that most food vendors did not comply with national/Codex Alimentarius food safety requirements.

5.2 Recommendations

(i) Poor hygiene and high level of microbial contamination observed calls for intervention. It is therefore recommended that, training of vendors on streets, at universities and in restaurants should be conducted on food safety emphasizing on good manufacturing practices and good hygienic practices. The knowledge on selection and quality of fruits used in production of juices and salads should also be disseminated to vendors. Boiling of water and use of hypochlorite tablets commercially available used for processing should also be stressed so as to minimize the microbial load. Storage of ready to eat/ drink salads or juice should be done in an appropriately such as covering of salads and avoiding temperature abuse of juices left open in the sun as it favors microbial growth.

(ii) The government should educate the society (ultimate consumers) on importance of food safety and advice not to purchase foods in unclean sources or areas. The importance of susceptible population; the young, old pregnant and immune-compromised, should also be taken into account as consumption to these foods may induce poisoning and cause ill-health effects or economic loss.

(iii) Further research on identification of pathogenic strains should be done. A quantitative microbial risk assessment evaluation should also be done so as to establish the population exposed to food safety hazard.

(iv) Local government authorities should intervene and enforce regulations in this sector for compliance with food safety standards. Countrywide research is recommended to map food safety and establish a periodic surveillance of pathogenic microorganisms from street vended foods.

(v) Simple disinfection technologies for preparation equipment and fruits should also be looked into like application of electrolyzed water and other sanitisers.

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APPENDICES

Appendix 1: Questionnaire

Date: …../…../…….

Product (circle one): **Fruit Juice** **Fruit Salad**

Vendor type (circle one): **Street V** **Restaurant** **University**

A. Personal details

1. Location (Physical):……
2. Sex: M / F
3. Marital status (Circle)
   - Married
   - Not married
4. Educational status (Circle)
   - No school
   - Primary
   - Secondary
   - University
   - Vocational training
   - Other (specify)……

B. Business details

5. Type of food business (Circle)
   - Mobile vendor
   - Stationary vendor
6. Type of preparation (Circle):
   - Ready to eat
   - Prepacked
   - Prepared on site
   - Sold as prepacked/ prepared on site
7. Location of business (Circle)
   - Along road sides/ streets
   - Market area
   - Transport terminal
   - University
   - Secondary school
   - Primary school
   - Town centre

Product details

A. Ingredients (list)	B. Food colour(s). If YES list	C. Preservatives and Concentration. If YES list	D. Flavouring material. If YES list	E. List equipment used in preparation	F. List detergents and disinfectants used
•	•	•	•	•	•
•	•	•	•		
•	•	•	•		
•	•	•	•		
•	•	•	•		

Preparation method

……
……
……
……
……
……
……
……

D. Knowledge on Food Safety

8. Are you familiar with the term food safety? (Circle) YES NO
9. Have you heard about TFDA? (Circle)
   YES NO
10. Do you know TFDA functions (If answer in 13 is YES)
    - Circle: YES NO
    - What are they
      - ……..
      - ……..
      - ……..
      - ……..
11. Have you heard about TBS? (Circle)
    YES NO
12. Do you know TBS functions (If answer in 15 is YES)
    - Circle: YES NO
    - What are they
      - ……..
      - ……..
      - ……..
      - ……..

13. Do you have a quality registration certificate? (Circle) YES NO
    Who issued the certificate? (If answer is YES) (Circle)
    - TFDA
    - TBS
    - Municipal Health Authority

14. Do you know anything about food safety programme? (Circle) YES NO
    What do you know?
    ……
    ……
15. Do you implement food safety programe? (Circle) YES NO
    How do you implement food safety programe?
    ……
    ……
16. Have you heard about GMP? (Circle): YES NO
17. Have you heard about GHP? (Circle): YES NO
18. Do you and your staff regularly go for medical check-up? (Circle) YES NO
19. (If YES) How often do u do check-up?
    - One a year
    - Twice a year
    - At least once every month
    - When sick
20. When was the last time you went for check-up?
    - Say Month and Year:…….....
    - Don't remember

E. Type of infrastructure

21. Is Potable water available? (Circle) YES NO
22. How do you ensure that water is safe?
    - Boil
    - Rely from supplier

23. Are Toilets available? (Circle) YES NO
24. Hand washing facilities? (Circle) YES NO
25. Cooling facility available?
    - Refrigeration
    - Cool box
    - Cooling dispenser
    - None
26. Is Waste disposal available? (Circle) YES NO
27. What type of waste facility?
    - Waste bin
    - Pit/earth hole
    - Plastic bag
    - Plastic bucket

28. Processing gear used
    - Hair net/ cap
    - Gloves
    - Apron/ processing coat
    - Hair net/cap and apron
    - Hair net/ cap and apron
    - None
29. Do you cover food? (Circle) YES NO

F. General assessment (Tick in each box and indicate total score)

30. **Location and premises**

Sn	Item	Very good 2	Good 1	Poor 0	Total Score
1	Location and surroundings				
2	Waste/ garbage around that could harbor animals, insects or germs				
3	Dusts or bad smell				
4	No toxic substances				
5	No pets or nearby farm				
6	No waterlogged areas				
7	Enough lighting and ventilation				
Total					

31. **Sanitation status**

Sn	Item	Very good 2	Good 1	Poor 0	Total Score
1	Water is available				
2	Water is clean and portable				
3	Lid type containers for waste disposal and are sufficient				
4	Proper disposal system				
5	Disinfectant and detergent presence and acceptable and safe for food and handler				
6	Preventive structures for animal/ and insects infestation				
Total					

32. **Maintenance and cleaning**

Sn	Item	Very good 2	Good 1	Poor 0	Total Score
1	Manufacturing building is clean and in good hygienic conditions				
2	Tools are clean and in good hygiene before and after production				
3	Tools used are proper for food processing				
4	Transportation tools are clean and proper				
5	Regular maintenance of equipment				
Total (10)					

33. **Processing and cleaning**

Sn	Item	Very good 2	Good 1	Poor 0	Total Score
1	Premise is clean and in good hygienic conditions				
2	Tools used are appropriate for food processing				
Total					

34. **Personnel hygiene**

Sn	Item	Very good 2	Good 1	Poor 0	Total Score
1	Workers are healthy and not suffering from any combinable diseases must be examined after every 6 months				
2	Suitable and clean clothes for processing				
3	No decorative articles				
4	Hands and nails clean				
5	Hand washing before and after work				
6	Gloves usage incase not measures for keeping hands clean are followed				
7	Hair net/ hat				
8	First aid kit for workers				
Total					

G. Other comments

……
……
……
……

Appendix 2: Sampling and analytical summary

Samples collected on random basis

Product	Vendor source	N
Fruit Juice	Streets	25
	University	5
	Restaurants	5
Total		**35**
Fruit Salad	Streets	20
	University	5
	Restaurants	10
Total		**35**
GROUP TOTAL		**75**

Analysis

Analysis	Parameters	Tools
GMP and GHP	GMP and GHP	Structured questionnaire
Metals	Pb, Cd and Cu	AAS
Physico-chemical properties	pH and ORP	pH/ ORP meter
Microbial analysis	APC	Microbial Media
	Fungi	Microbial Media
	Coliforms and *E. coli*	Microbial Media
		Microscopy
		PCR