FOOD ADDITIVES AND HYGIENIC STATUS OF FLAVOURED FRUIT JUICES MANUFACTURED IN DAR ES SALAAM, TANZANIA

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ABSTRACT

The purpose of this study was to assess the quality of juices manufactured and sold for immediate consumption in Dar es Salaam Municipalities, Tanzania. The assessments were based on identifying the levels of preservatives, hygienic practices in manufacturing industries and determine microbiological quality of the juices. High Performance Liquid Chromatographic (HPLC) method was modified and validated for determination of benzoic and sorbic acids, while direct titration method was used for quantitative estimation of total sulphur dioxide. Microbial quality was determined by counting viable micro-organisms using plate count method and coliform determination method. There were a total of 110 juice samples from five different manufacturers. A total of 22 different types of juices in replicates of five. Results show that more than 72% of samples tested did not comply with Tanzanian standards for soft drinks because of the presence of exceeded levels of allowable preservatives (> 350mg/ml for sorbic and benzoic acids) and only 40% complied with labelling standard. Samples which were found to have higher than specified levels of preservatives did not show any microbial growth. It was observed that 63 % of microbial growth (for total bacterial count) was from those samples without preservatives. The major micro-organisms found in juices were *Alicylobacillus* and *Lactobacillus spp.*

Therefore it is necessary for the Regulatory board to provide training on the effects of misuse of preservatives and education on hygienic procedures. It should also conduct inspections and increase efforts in enforcing laws to the manufactures.

DECLARATION

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

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DEDICATION

This work is dedicated to my family for their patience, tireless cooperation, encouragement and wonderful hospitality before, during and after this study.

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LIST OF ABBREVIATIONS

CFU……Colony Forming Unit

GHP……Good Hygienic Practices

GMP……Good Manufacturing Practices

HACCP……..Hazard Analysis Critical Control Point

HPLC …….High Performance Liquid Chromatography

SOPs……Standard Operating Procedures

SSOPs…….Standard Sanitation Operating Procedures

SUA…….Sokoine University of Agrculture

TFDA …….Tanzania Food and Drug Authority

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

A wide variety of fruit juices are found in Tanzania markets, ranging from pure juices, nectars, and flavored drinks. These products are manufactured either from direct blending of fruit juices, or reconstitution of fruit juice concentrates where water is the largest ingredient., corresponding to 80-85% of the final juice. The quality of reconstitution water significantly influences the final product. Water from municipal supplies or bore holes normally needs further on-site treatment to make it suitable for reconstitution of good quality juices (Bill *et al*., 2008).

The reconstitution is the step whereby concentrate is blended with water to make a ready-to-drink juice. The quantities of concentrate and water to be blended can be calculated approximately. After the correct ready-to-drink has been reached the product need to be packed into containers as soon as possible. For traceability, it is important that effective records are kept to document important information such as batch number of the concentrate, the final reading, colour, taste, fill height and volumes (Bill *et al*., 2008). Careful attention needs to be given to hygiene to produce juice of high microbial quality. Despite all precautions, it is impossible to exclude microorganisms from the process (Frazier and Westhoff, 1988). Preservatives are used to extend the shelf life of juices. These could be stored at room temperature. Pasteurized juices without preservatives have short-life and need refrigeration. Therefore, the cold chain needs to be maintained from receiving of the concentrate to blending, filling and storage (Bill *et al*., 2008). Sampling

and full laboratory analysis of juice properties produced from each batch of concentrate is recommended (Bill *et al*., 2008).

Food poisoning is usually caused by chemicals and/or micro-organisms. In order for micro-organisms to grow, they need a medium which provide all essential nutrients, ions, and moisture (Refai, 1979). Main factors that affect the microbial growth in a food product are water activity, pH of food, presence or absence of oxygen, availability of nutrients and the temperature (Wilson and Bahna 2005) ; James *et al.*, 2005). Hence preservatives are used to inhibit microbial growth and extend the shelf-life of a product and these have been used successfully for many years as direct food additives (Wilson and Bahna, 2005).

Preservation methods used to extend the shelf life of food include water removal, temperature control, drying, pH control, irradiation; vacuum packaging, modified atmosphere packaging, aseptic packaging, fermentation, heating (pasteurization and sterilization) and chemical preservatives (Riddervold and Ropeid, 1993*)*.

Effects of food additives may immediately or later be harmful if one has constant exposure or used above the recommended levels. Immediate effects may include headaches, change in energy level, and alterations in mental concentrations, behaviour, or immune response to diseases. Long-term effects may include increased risks of cancer, cardiovascular disease and other degenerative conditions (Wilson and Bahna, 2005).

1.2 Problem Statement

Mixing of additives commonly found in children's foods increases the mean level of hyperactivity (Ahmed *et al.*, 1991). The study that examined the effect of artificial colors and a sodium benzoate preservative has been found both to be problematic for some children (McCann *et al.*, 2007). With advanced technology, modern food industry's reliance on processing and food additives continues to increase (Taylor *et al.*, 2008). This seemingly abundance of food stuffs found in supermarkets of today is deceiving consumers by selling food products that are chemically altered and designed to appeal to people. For decades now, the food industry has continuously created new chemicals to manipulate, preserve, and transform natural food. Avoiding or minimizing toxins in our diets, is an important step towards enhancing our health and lowering consumer's risk of disease. Juices among other things, represents a source of these toxins (Wilson and Bahna, 2005).

Documented studies on the status of use of additives in fruit flavoured soft drinks and hygienic practices surrounding the manufacturing process in Tanzania are scarce. According to Tanzania Food and Drugs Authority (TFDA's), food production industries assessment and inspections indicates that more than 50% of the food factories are hygienically poor in food production procedures (TFDA, 2009). It is from the above grounds that, this study was conducted. It is anticipated that results from this study would form the basis for advising manufacturers of fruit flavoured drinks the best way to ensure that they meet the required safety criteria to safeguard consumers.

1.3 Justification

Tanzania does not have co-ordinated mechanisms to monitor the safety, quality and nutritional status of imported and locally produced/processed food products. National food legislation and regulations are inadequate to guide industries, importers, exporters, public regulatory officials and consumers in assessing the suitability of food for human consumption (Codex Stain, 2001).

Manufacturing of fruit flavoured juices requires addition of acceptable ingredients and food additives which are intended to achieve various technological purposes such as imparting flavour, stabilising, colouring and preservation of the final product. If such products are not manufactured in accordance to the principles of Good Manufacturing Practices, among the major risk that a consumer may face from consumption of such products is microbiological contamination and its associated health risks. On the other side, if the additives including preservatives used do not comply with the prescribed safe levels of their use may as well lead into health risks to the consumers (Codex Stain, 2001).

Demand for consumption of fruit flavoured juices in Tanzania as in other countries is on the increase (Rachel, 2010). This is associated with urbanisation and demand for new tastes. School children form the major group of population with high preference in the consumption of such products presumably due to their sweet taste and fruit colour(s) and flavour(s) (Rachel, 2010). In response to the demand, manufacturing industries for fruit flavoured soft drinks in the country is increasing, resulting into cheap products and therefore more affordable by many people compared to the processed identical natural

fruit juices. Thus demand for these products in Dar es Salaam City like in many other regions is very high.

Good manufacturing practices including proper use of food additives in line with the acceptable safe levels, combined with good hygienic practices contributes to a safe product of fruit flavoured juices. As documented, studies on the status of use of additives in fruit flavoured soft drinks and hygienic practices surrounding the manufacturing process in Tanzania are scarce. Therefore, this study was important to inform food control authority and juice industries on the status of fruit flavoured juices produced in Dar es Salaam for possible improvement to safeguard public health.

1.4 Objectives of the Study

1.4.1 General Objective

To assess the level of additives and hygiene in fruit flavoured juices manufactured in different industries in Dar es Salaam City, Tanzania.

1.4.2 Specific objectives

1. To determine hygiene practices in manufacturing sites for fruit flavoured drinks in Dar es Salaam municipality;
2. To determine microbiological quality in ready to sale and on sale fruit flavoured drinks in Dar es Salaam municipality;
3. To determine types of preservative used, amount and its comparison to the permissive values in Dar es Salaam municipality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Food Hygiene

The term food hygiene means the maintenance of good health and prevention of damages to health, and is an essential part of healthy living (Schothorst, 1999). Food hygiene can be defined in two main groups: Food safety and Food quality. Food safety is defined as the prevention of food borne diseases whereas food quality means acceptability of food referring to taste, aroma, palatability and appearance (Gardner, 1999). In the past, the term of food safety has been used for the hazards coming from chemical substances found in foods whereas food hygiene has been referred to microorganisms. It has been reported that consumers still tend to be more concerned about chemicals used during processing of foods (Eilers, 1990).

Recently quality assurance programs in food industry are designed to achieve good manufacturing practices (GMP) (Gardner, 1999). Besides GMP and Good Hygienic Practices (GHP), an important approach that can be applied at all stages in the production, processing and handling of food products is the Hazard Analysis Critical Control Point system (HACCP), an approved system as the best food safety management system all over the world (Codex Alimentarius, 1997). An effective HACCP plan cannot be implemented without prerequisite programs. The programs provide the basic environmental and process conditions for safe food production. GMP, Standard Operation Procedures (SOPs) and Standard Sanitation Operation Procedures (SSOPs) are the most common prerequisite programs (Sheridan, 2000).

GMPs are designed to ensure that the foods are produced under hygienic conditions and that microbiological, chemical and physical hazards are prevented (Gardner, 1999). SOPs are established in order to protect food products from microbial, chemical, and physical hazards; to control microbial growth that can result from temperature abuse; and to ensure procedures are in place for maintaining equipment, as food can transmit disease from person to person as well as serve as a growth medium for bacteria that can cause food poisoning (Satin, 2008).

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. Care should be taken to manage wastes, and store harmful substances appropriately (Codex Alimentarius, 2006). To achieve optimum consumer protection it is essential that safety be embodied in food products from production through consumption. Prevention, control at source, and identification of unsuitable products at an early stage make better scientific and economic sense that the traditional approach to food control which relied mainly on final product inspection and testing. A food control system must be developed and implemented in a transparent manner and the involvement of stakeholders is essential in this regard. Transparency based on stakeholder participation and will improve the efficiency of food control systems and contribute an increased rate of compliance with food safety requirements.

2.2 Laboratory Services

Laboratories play a vital role in the enforcement of regulatory food control measures and are an essential and highly technical component of the system. They are engaged in the physical, microbiological and chemical analysis of food samples sent to determine whether there is non-compliance with food standards. The utmost care is necessary to ensure the efficient and effective performance of the laboratory.

2.2.1 Microbiological control

Although micro-organisms are of great importance, but they use human foods as source of nutrient for their growth. This can result in a deterioration of food. They do this by increasing their numbers, utilizing nutrients producing enzymatic changes, contributing to flavour by breaking down products or synthesis of new components. To prevent this, micro-organisms must be minimized to a level that will cause no harm to the food products.

The juice extracted from fruits is mostly acidic, and is responsible for the growth of yeast and bacteria. The normal changes to be expected in raw fruit juices at room temperature are an alcoholic fermentation by yeast followed by the oxidation of alcohol and fruit acid by film yeast or mould growing on the surface, or the oxidation of alcohol acetic acid if acetic acid bacteria are present (Macrae *et al.*, 2010).

Yeast is by far the most common spoilage organism in juice. Yeast contamination is usually accompanied by extensive gas formation, especially when the oxygen supply is limited. Hence, spoilage by yeasts tends to result in blown packages. Some strains of acid-tolerant *Lactobacillus* grow faster under low oxygen pressure and usually result in a high

level of defects. Sampling to detect yeast spoilage is recommended throughout a production run.

Soft drinks bottled at low temperatures have high values of the water activity, which allow microbial growth. The pH, the sugar content and the addition of preservatives prevent the micro organisms' growth in soft drinks. Some species of moulds *Aspergillus Niger* and *Penicillum spinulosum* are resistant to chemical preservatives, as well as the sorbic and the benzoic acid and they can tolerate acid environments and low values of the water activity (Mihaela *et al.*, 2009).

The factors which influence the microbial alteration of the foods include the number and type of contaminating microorganisms, humidity (and water activity), the pH (the level of acidity and alkalinity), the presence or absence of oxygen, the type and availability of nutrients, the temperature and the food physical condition (Mihaela *et al.*, 2009).

2.3 Food additives

Food additives are substances added to food to preserve flavor or enhance its taste and appearance. With the advent of processed foods in the second half of the 20th century, many more additives have been introduced, both natural and of artificial origin (Codex Alimentarius, 2006). With the increasing use of processed foods since the 19th century, there has been a great increase in the use of food additives of varying levels of safety (Techkriengkrai and Surakarnkul, 2007). This has led to legislation in many countries regulating their use. For example, boric acid was widely used as a food preservative from the 1870s to the 1920s, but was banned after World War I due to its toxicity, as demonstrated in animal and human studies. During World War II the urgent need for

cheap, available food preservatives led to it being used again, but it was finally banned in the 1950s. Such cases led to a general mistrust of food additives, and an application of the precautionary principle led to the conclusion that only additives that are known to be safe should be used in foods (Wijk and Engelen , 2003).

The microorganisms growth inhibition is achieved through the interaction of the system of the two conjugated double bound in the aliphatic chain with cellular dehydrogenises which most of the yeasts and moulds cannot metabolize (Mihaela *et al.*, 2009).

2.3.1 Common juice preservatives and additives

Sulphur dioxide, benzoates, sorbates, carbon dioxide and ascorbic acid are the most common juice preservatives and additives. The sorbic acid and the sorbates are the preservatives mostly used in the soft drinks, which act efficiently against the yeasts and moulds growth, and of some bacteria, acting at a low pH. However, it continues to be efficient at a pH of 6.5 (Glevitzky *et al.*, 2009). Other preservatives such as benzoic acid, sorbic acid, co_2 can be used individually or synergistically. Sodium benzoate and potassium sorbate are the preferred forms given greater solubility of the salts (Branen *et al.*, 1989). Both function best below pH 4.0. Sorbic acid is effective over a broader range, up to pH 6.5. Benzoates and sorbates are often used together in combination with low temperatures to extend the shelf life of minimally processed juice drinks (Somogyi *et al.*, 1996).

2.3.2 Effects of food additives

There has been significant controversy associated with the risks and benefits of food additives. There were small but statistically significant differences of measured behaviors

in children who consumed the food additives compared with those who did not (Bateman *et al.,* 2004). In each case increased hyperactive behaviors were associated with consuming the additives (McCann *et al*, 2007). Also some artificial food additives have been linked with cancer, digestive problems, neurological conditions, and diseases like heart disease or obesity. Even "natural" additives may be harmful in certain quantities or because of allergic reactions in certain individuals (Wilson and Bahna, 2005).

Daily consumption of juices, candy, and soft drinks with additives is fuelling disruptive behaviour in child. Nearly every kid's food is "decorated" or "tainted" with artificial food colour and additives. While they make food look pretty, there may be a downside, and when children's daily consumption of juices, candy, and soft drinks with these additives be fuelling disruptive behaviour, restlessness and lack of concentration (Bateman *et al.,* 2004).

Over the past 40 years, the United States Food and Drug Administration (FDA), British authorities and researchers have insisted that there was little or no link between hyperactivity and food preservatives like sodium benzoate or artificial colouring like sunset yellow food dyes. Yet, a study published in the British journal Lancet has brought this under question (Holley and Hill, 2007). Stevenson (2007) a psychologist at the University of Southampton, recruited 300 children, age 3, 8 and 9, and spiked their juices with differing quantities of additives and food colour. The first group gulped down the usual amount of food with dye that a British child drinks, the second group had half as much, while the third group received no food additives or dye in their juices. The parents, teachers and a computer were used to measure the children's

level of hyperactivity. To the surprise of the researchers, food industry and government officials, the children consuming the drinks with preservatives and dye exhibited higher levels of hyperactivity. Some symptoms were seen within one hour of taking the drink (Holley and Hill, 2007).

The effect of additives was more pronounced in 3-year-olds and a selected group of children (Dangour *et al.*, 2010). Scientists believe there may be a genetic link between food and release of histamines causing hyperactivity. Some kids with the genetic predisposition may be super-sensitive to food additives (McCann *et al.*, 2007). The sulphites, metabisulphites and sulphur dioxide which found in cold drinks, fruit juice concentrates and wine, dried fruits, can trigger asthma attacks due to their irritant effect on the airways (Doughari *et al.*, 2009).

Consumption of aspartame probably increases the risk of cancer. (Wilson and Bahna, 2005) reported that, people especially young children should not consume foods and beverages sweetened with aspartame. Caffeine and quinine is the drug that is present naturally or added to widely consumed foods and it is mildly addictive, one possible reason that makers of soft drinks add it to their products. Many coffee drinkers experience withdrawal symptoms, such as headaches, irritability, sleepiness, and lethargy, when they stop drinking coffee. Because caffeine increases the risk of miscarriages (and possibly birth defects) and inhibits fetal growth, it should be avoided by women who are pregnant or considering becoming pregnant. It also may make it harder to get pregnant (Wilson and Bahna, 2005).

2.3.3 Flavours enhancers

The most important and widely used flavour enhancer is monosodium glutamate, or MSG. Well known for producing 'Chinese Restaurant Syndrome', the symptoms of which range from tightness in the chest and palpitations to faintness, flushing, sweating, headache and low blood pressure, MSG is now believed to be an endocrine disrupter. This means that it can upset the body's endocrine (hormonal) system. The long term effects on all aspects of health that this could have are potentially far worse than 'Chinese Restaurant Syndrome' and for this reason particularly disturbing. MSG is also reported to trigger attacks in some asthmatics. Unfortunately, the additive-labeling system is not infallible. Certain categories of products are exempt, such as alcoholic drinks, food and drink served in catering establishments, and medicines. Food sold without wrapping such as cheese, delicatessen items and bread may also be exempt, even though they are likely to contain additives. Even with labeled food, manufacturers may not be required to list all the substances that came already added to the ingredients. Some categories of additives such as flavorings do not have to be listed on labels either (Macrae *et al.*, 2010). They have never been tested for safety, and the assumption that they are safe relies upon the fact that they are used in very small quantities.

2.4 Importance of avoiding or minimizing toxins in diet

Avoiding or minimizing toxins in diet is an important step toward enhancing health and lowering the risk of disease. Foods, amongst other things, represent a source of these toxins. Effects of food additives may be immediate or may be harmful in the long run if one has a constant exposure. Immediate effects may include headaches, change in energy level, and alterations in mental concentration, behavior, or immune

response. Long-term effects may increase the risk of cancer, cardiovascular disease and other degenerative conditions (Food and Drug Administration's (FDA's) **International, 2001).**

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the study

The research was conducted in Dar es Salaam which is the largest city in Tanzania located in the coast of Tanzania. It is also the country's richest city and a regionally important economic centre (United Nations Human Settlements Programme, 2009). Dar es Salaam is actually an administrative province within Tanzania, and consists of three municipalities; Kinondoni to the north, Ilala in the center of the region, and Temeke to the south. The Region has a population of more than 2.5 million as of the official 2002 census, (United Nations Human Settlements Programme, 2009). The analysis of the samples was conducted at the laboratory of the Tanzania Food and Drugs Authority (TFDA) headquarters located in Dar es Salaam.

3.2 Samples collected

Twenty two different types of juices were collected from five different registered industries in Dar es salaam by collecting five units of every type of juices from each manufacturer at each visit; making a total of 110 samples. The industries and juices in the brackets were: Chemi and cotex; (Chemicola orange drink and Chemicola black currant), A-One products and bottlers Ltd; (Pride orange drink, Pride orange flav.drink, Pride cocktail drink, Pride passion drink, Pride passion drink and Pride cocktail), Sayona Drinks Ltd; (Sayona orange drink, Sayona mango drink, Sayona orange drink and Sayona pineapple drink), Salim said Bakhressa (Azam apple and blackcurrant beverage, Azam pineapple, Azam apple ready to drink, Azam orange ready to drink,

Azam mango/orange ready to drink, Azam mapera juice and Azam mango juice), and Noble foods Ltd (Tropicana orange squash, Tropicana blackcurrant flavoured, Tropicana mango flavoured and Tropicana orange flavoured drink).

Determination of hygienic practices in manufacturing sites was done by using structured questionnaire to collect data where location, building construction, water supply, raw materials, processing and equipment, sanitation and staff hygienic and records were the main factors.

3.3 Study design and sampling procedure

A longitudinal study design was used with repeated observations of the same industries over three different phases of time from January 2011 to March 2011. Random sampling technique was used to select the 5 units of each type juice from each industry under the study.

3.4 Determination of hygienic practices in manufacturing sites

A structured questionnaire was used for data collection. Questions were based on the following factors; location, building/construction, water supply, raw materials, processing, equipment, sanitation and staff hygienic records.

3.5 Laboratory sample analysis

3.5.1 Preparation of samples for chemical analysis

Benzoic and sorbic acids in juices were determined using High Performance Liquid Chromatography (HPLC) method. Ten millilitres of sample were transferred into 50 ml volumetric flask then volume made up with 50% methanol in water. Turbid samples were

filtered using 0.45 µm pore diameter membrane filter to remove particulate matter. The filtrate samples were ready for HPLC analysis. Sodium metabisulphate were determined by using titration method (0.05 N iodine solution and starch as indicator) as explained by Ranganna (2001).

3.5.2 Preparation of chemicals for HPLC (Mobile phase)

Ammonia acetate buffer and methanol (HPLC grade) at ratio of 60:40 was freshly prepared. Ammonia acetate (0.77 g) was weighed and dispensed in 1000 ml volumetric flask with 250 ml distilled water. The pH was adjusted with acetic acid to pH 4.4 while stirring. The volume were made by adding distilled water, mixed well, and filtered through membrane filter made under vacuum. About 600 ml of buffer and 400 ml of methanol were mixed well. The mobile phase was degassed by magnetic stirring for 20 minutes.

3.5.3 Preparation of stock standard solution

Benzoic and sorbic acids 0.1 g each were weighed into separate 200 ml volumetric flasks and firstly dissolves in 70 ml of 50% methanol in water, and after 30 min sonication volume was made to 200 ml with the same solvent.

3.5.4 Preparation of working standards for calibration curve

Fifty millilitre of stock solution was transferred into 100 ml volumetric flask and diluted with 50% methanol/water to volume. The procedure was repeated by using 25 ml, 15 ml, 10 ml, and 5 ml, to make calibration solutions with concentrations of 50 ml, 100 ml, 150 ml and 200 ml. The solutions were transferred to HPLC special vials for injection into HPLC.

3.5.5 Liquid chromatography conditions

Detector with wavelength at 235nm was used with column (Column Hypersil, 250 x 4.6 mm C_{18}, 5µm.) at low pressure gradient. Buffer/methanol (40/60) was pumped at flow rate of 1.00 ml per minute. Injection volumes were 20 µl which flow for 12 minutes. Retention times were expected from 6.7-10.5min.

3.5.6 Calculation of Benzoic and sorbic acids in juice sample

$$\text{Benzoic or sorbic acids in mg/kg} = \frac{PAsa}{PAst} x \frac{Vst}{Vsa} x1000 \quad \text{..... (i)}$$

Where;

PAsa = Peak Area of same compound in sample.

PAst = peak Area of same compound in standard.

Vst = volume of standard injection.

Vsa = volume of sample injection.

1000 = factor to convert from mg to µg.

3.6 Determination of sodium metabisulphate

The titratable sodium metabisulphate was determined by following the standard procedure.

Fifty millilitre of juice were mixed with 25 ml of 1 N NaOH and allowed to stand for 10 min, and ten millilitre of diluted H_2SO_4 (1:3) added and titrated with 0.05 N iodine solution using starch as indicator. Bluish colour (Blue colour persisted for 2 min.). One millilitre of 0.05N iodine is equal to 0.0016 g of SO_2 (Ranganna, 2001).
SO_2 was calculated by using the expression below:

$$SO_2 \text{ mg/l} = \frac{\text{Titre value x } 0.0016 \text{x } 10^6}{} \text{ .....(ii)}$$

$$\frac{50}{}$$

Where;
50 ml = volume of sample taken
10^6 = ppm (parts per million)
0.0016 = grams of 1ml of 0.05N iodine

3.7 Microbial Analysis

Microbial analyses of the sample to estimate the viable bacterial number and coliform were done by plate count method for East Africa standards (EAS, 2008a). Ten-fold dilutions of each sample were made by diluting 1:9 with alkaline peptone water (adding 10 ml of sample to 90 ml of peptone water). After two hours, pre-enriched samples were diluted serially by using phosphate buffer saline at a ratio of 1:9 (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). One ml of each of the above dilutions for each sample was inoculated into plate count agar and incubated at 37^0C for 48 hours. The plates with the countable number of colonies were selected for counting. Colonies were counted from plates containing between 30 and 300 colonies, and number of bacteria were calculated as bacteria per ml by the following formula (EAS, 2008a):

$$N = \frac{C}{(n_1 + 0.1n_2)\, d} \quad \text{..... (iii)}$$

Where;

C is the sum of colonies counted on all the dishes retained.

n_1 is the number of petri dishes retained in the first dilution

n_2 is the number of petri dishes retained in the second dilution

d, is the dilution rate corresponding to the first dilution selected.

For coliform test, 10 ml of the sample were taken and lactose broth medium added to make 100 ml, and incubated for 48 hours at 30^0C. Few tubes show growth, after slightly

shaken and a portion of the fluid taken by using an inoculating loop and streaked on MacConkey agar medium, and incubated for 24 hours at 45^{0}C (EAS, 2008b).

3.8 Statistical data analysis

Data obtained were analysed by using statistical software STATA (Elise and Jonathan, 2002) where the t-test examines how far the estimated mean values of different samples from different products lies from national standard and was computed to determined significant deference between single mean with a hypothesized value at P<0.05.

The t statistic, from which a P value is derived, is as follows.

$$t = \frac{\text{Sample mean – hypothezed mean}}{\text{SE of sample mean}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{(iv)}$$

The results were expressed as means ± SD. Data were also analyzed for analysis of variance at 95% confidence level to determine which explanation is most likely to use calculated confidence intervals for the mean and to perform a hypothesis test.

CHAPTER FOUR

4.0 RESULTS

4.1 Comparison of hygiene status in manufacturing sites for juices between Industries in Dar es Salaam municipalities.

4.1.1 Location

Four factories have scored above 70% in the category “location requirement”, which means, located within industrial area and easily accessible by road. Noble Food industry is located very near to the main road. This increases the risk of contamination from heavy dust pollution from the roads and carbon monoxide from vehicles. Due to this situation, this factory scored poor in “location requirement” category (Fig. 1).

4.1.2 Building / construction

Among the five manufacturers, Salim S. Bakhressa scored the highest (100%) in sections under building and construction. All buildings are of permanent material and good state of repair. The building have proper adequate artificial lighting and air ventilation and the floor are hard, smooth, non-absorbent with no accumulation of grime or dirt with good floor drainage; no stagnant water and smooth to invert. Walls are internally plastered, smooth easy to clean, while the roof was in good state, clean not gapped and provides adequate area to accommodate activities carried out (Fig. 1). Sayona Drinks followed Salim Bakhressa with (70%) score in other aspect except floor, which was poor with a lot of cracks, not smooth, with poor drainage and a lot of stagnant water. A-One Products score (30%) poor in all sections except walls that are of permanent material. Chemi & Cotex scored poor in floor as the finish was not smooth, with accumulation of grime or dirt, and poor floor drainage. Noble Foods was the poorest and scored zero to almost all sections (Fig. 1)

4.1.3 Water supply

Chemi & Cotex, A-One Product, and Salim S. Bakhressa scored (100%) very good in water supply aspect, they have both source of water (Municipal or private) and available all the time, adequate in volume and pressure, have reservoir with capacity to supply adequate water in case of shortage with available additional water treatment. Sayona Drinks and Noble Foods score (25%) poor because of depending on municipal water with no water treatment process, no good quality of reservoir in case of shortage
(Fig.1).

4.1.4 Raw materials

All the factories except Salim S. Bakhressa scored poor (less than 40%) in raw material handling and storage especially additives and concentrates, which are stored in not recommended temperature (cool dry place) (Figure 1). It was observed that same materials were expired, and there were no details of quality assurance methods that would enable workers to produce safe juices. There were no reporting procedures and records keeping of the activities taking place.

4.1.5 Processing and equipment

A-one and Noble Food factories scored (less than 40%) poorly in equipment arrangement according to flow of operations especially in processing and packaging. bottles were not properly washed, sanitized and rinsed. There are no cleaning procedures in place, no good production planning, and no technical requirements for protection of the products against contamination. The cleaner and rinsers are not constructed in good manner, staffs are not

aware for GHP and GMP procedures. Chemi & Cotex score poor only in proper inspection of cleaned bottles, and there were also no manuals to guide the cleaners on proper cleaning of the facilities. Sayona Drinks and Salim S. Bakhressa scored very well in all sections of this aspect because all equipment arrangement and processes follow GHPs and GMPs. For Salim S. Bakhressa factory, more than 80% of production processes are done automatically by machines, that is why number and type of workers are mainly those needed to operate machines, and properly trained to use machines safely (Fig. 1). No chance of personal contamination throughout the whole process even in packaging of finished product.

4.1.6 Sanitation

There was a big problem with Noble Foods, Chemi & Cotex, and A-One Products in sanitation aspects where by Noble Foods scored zero. They employ staff with no ability to create a barrier between themselves and the product, and the company do not provide enough coats/smocks, plastic aprons or plastic sleeves, hairnets and snoods, and gloves, to operators during operations. Staffs wear protective clothing outside the plant premises and not regularly changed. No controlled laundering, jewellery and body piercing. Pre-shift checks of the condition of clothing or footwear for frayed edges or loose items, such as buttons or snaps, are not done. Footwear is also not controlled to minimise microbial contamination from humans. No policy that guides employees to leave their footwear at the facility in order to mitigate contaminants carried into the plant from home. Not all personnel in the manufacturing environment regularly and adequately wash hands to prevent microbial contamination. No rules that apply to everyone when entering the production facility. Hand washing policies are not properly followed, as there are no

instructions that require employees to wash their hands after any type of activity that could contaminate the hands with pathogens, such as after using the washroom, blowing the nose or touching body parts, handling raw food, waste or non juice-contact surfaces such as light switches or pipes. There are no appropriate and enough facilities for hand washes to accommodate all employees and in other place hand washing basins are not placed in convenient locations, that results to employees skipping washing hands. Sayona Drinks and Salim S. Bakhressa staff are aware for GHP and GMP procedures and equipment arrangement and processes follow GHP and GMP procedures. Sayona score 78%, while Bakhressa score 100%

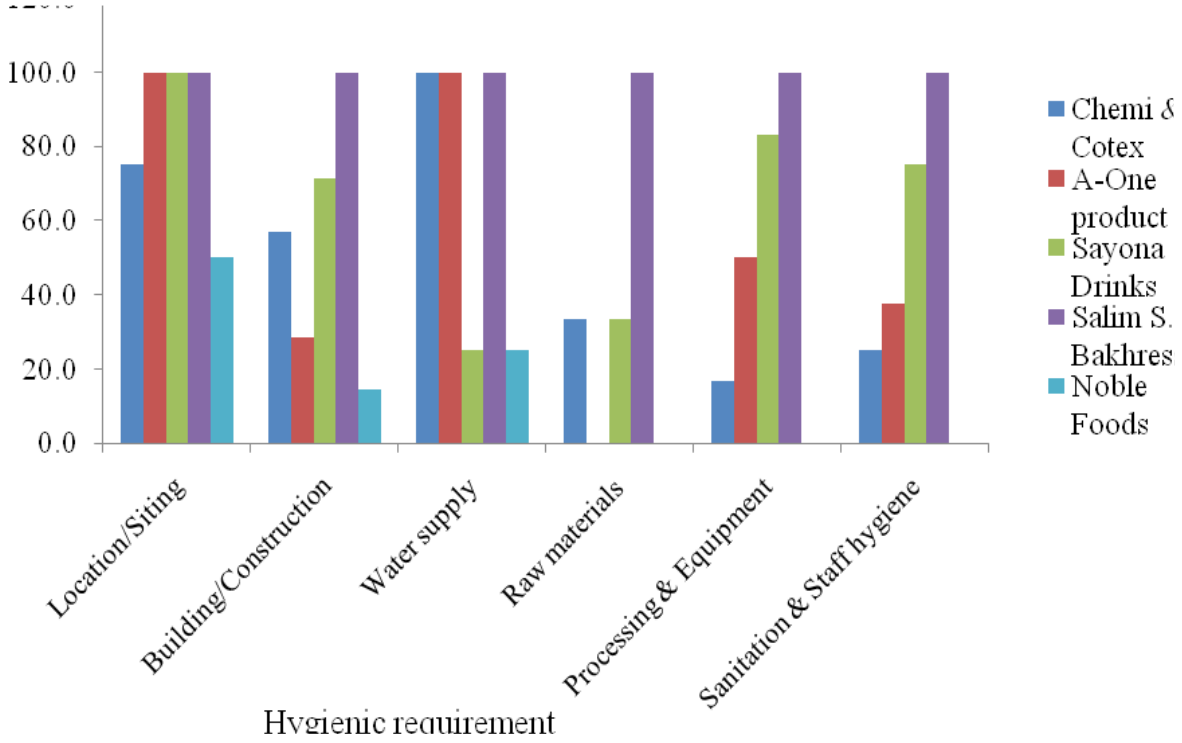

Figure 1: **Comparison in percentage scores on hygienic status of juice manufacturing sites in Dar es Salaam, Tanzania, 2011.**

4.2. Levels of additive used and their conformity to standard

(i) The result show that, Chemicola orange and Chemicola blackcurrant (Chemicola & Cotex Ltd), and Pride orange (A-one product Ltd) were found to contain benzoic acid which were significantly ($p< 0.001$) higher than Tanzanian standards of 350mmg/L.

(ii) It was also found out that, Pride orange, pride cocktail, (A-one Product Ltd.), tropicana orange, tropicana blackcurrant and Tropicana mango (Noble foods Ltd) had significantly ($p<0.001$) higher levels of both Benzoic and Sorbic acids than Tanzanian standards of 350mmg/L.

(iii) Moreover, Pride passion (A-one product Ltd), and all products from Sayona had lower values of Benzoic acid than maximum allowable units.

(iv) The results also show that Chemicola orange, chemicola blackcurrant and all Sayona products had no Benzoic acid at all whereas all Bakhressa products had no both Benzoic and sorbic acids (preservatives at all).

Table 1: **Comparison of mean values of amounts of Benzoic and Sorbic acids used for various purposes in fruit juice from different manufactures with the National Standard (350 mg/l).**

Manufacturer	Product name	Mean value Benzoic Acid (mg/L)	Mean value Sorbic Acid (mg/L)
Chemi & Cotex Ltd.	Chemicola Orange	701.8 ± 19.0*	0.00
	Chemicola B/currant	706.8 ± 39.7*	0.00
A- One products Ltd.	Pride Orange	730.7 ± 34.5*	639.7 ± 44.8*
	Pride C/tail	727.9 ± 21.1*	568.3 ± 15.3*
	Pride Passion	173.8 ± 6.7	128.9 ± 5.4
	Pride Mango	763.3 ± 30.3*	0.00
Salim S. Bakhressa	Azam Apple	0.00	0.00
	Azam Pineapple	0.00	0.00
	Azam Orange	0.00	0.00
	Azam Guava	0.00	0.00
	Azam Mango	0.00	0.00
	Azam apple &B/currant	0.00	0.00
	Azamto	0.00	0.00
	Azam Mango/Orange/Guava	0.00	0.00
Sayona Drinks Ltd.	Sayona Orange	77.0 ± 2.0	0.00
	Sayona Mango	69.7 ± 4.0	0.00
	Sayona Nanasi	100.3 ± 4.6	0.00
	Sayona B/currant	92.3 ± 20.5	0.00
	Sayona Pineapple	76.9 ± 4.4	0.00
Noble Foods Ltd.	Tropicana Orange	712.5 ± 17.5*	609.3± 12.5*
	Tropicana B/currant	719.9 ± 3.8*	634.1± 9.0*
	Tropicana Mango	688.2 ±34.2*	609.7± 4.5*

*Values are higher than standard value required (350 mg /L)

4.3 Microbiological quality of the juices

Results for total plate microbial analysis have been presented in Table 2

The results show that eight samples yielded microbial growth in total plate count method where two samples from Noble Foods had growth above Tanzanian Standards ($> 10^3$ CFU/L), five samples from Salim S. Bakhressa and one sample from Sayona Drinks yielded microbial growth within Tanzanian Standards ($<10^3$ CFU/L). Most of the growth was spore forming soil micro-organism resembling *Alicylobacillus* and *Lactobacillus*.

Microbial analyses for coliforms at 45^0C did not show any coliform growth in all samples. There was an indication of microbial growth inhibition related to presence of high level of preservatives in most samples as those samples with high levels of preservatives did not show any growth except two samples from Noble Food factory which was the poorest factory in hygienic status (score 12%).

Table 2: Total plate count (CFU/ml) following microbiological analysis of deferent juices products from different manufacturer in Dar es Salaam municipalities, Tanzania 2011.

Manufacture	**Sample name**	**Results (cfu/ml)**
Sayona Drinks	Sayona mango juice	1.9×10^1
Salim S. Bakhressa	Azam guava juice	6.4×10^2
Salim S. Bakhressa	Azam guava juice	4.6×10^2
Salim S. Bakhressa	Azam mango juice	4.5×10^2
Salim S. Bakhressa	Azamto juice	2.7×10^1
Salim S. Bakhressa	Azam mango guava juice	1.9×10^2
Noble Foods (Tropicana)	Tropicana black currant juice	1.64×10^3
Noble Foods (Tropicana)	Tropicana black currant juice	1.46×10^3

CHAPTER FIVE

5.0 DISCUSSION

This is the first study that has assessed the level of additives and hygiene in fruit flavoured juices manufactured in Tanzania. Hence this study will be important to inform food control authority and juice manufacturing industries on the status of fruit flavoured juices produced for possible improvement to safeguard public health. Because Tanzania does not have co-ordinated mechanisms to monitor the safety, quality and nutritional status of imported and locally produced/processed food products, and also because national food legislation and regulations are inadequate to guide industries, importers, exporters, public regulatory officials and consumers in assessing the suitability of food for human consumption, while demand for consumption of fruit flavoured juices in Tanzania is on increase. Thus this was the original idea for doing this research. Also the results of this study will stimulate necessary actions to be implemented by relevant stakeholders.

The results of hygienic practice analysis show that, there were no good personal hygiene policies and practices as the foundation for successful food safety and quality assurance in manufacturing facilities. Although there are no official standards for hygienic practices, Sanitation Standard Operating Procedures (SSOPs) that have been developed by the facility to ensure consistent hygienic practices must be followed. Plant personnel are among the most significant reservoirs and vectors of micro-organisms, chemical residues and foreign materials in the finishing products, therefore, their hygienic status should be controlled.

Salim S. Bakhressa factory scored the highest points because they have in place a system of the top-down approach are there to ensure that personal hygiene. Hygienic policies and procedures from the top management are implemented by all personnel including

management members, visitors, and production, sanitation and maintenance staff in each section in the facility. Noble Foods factory scored the lowest in overall because of poor or no control of contaminants such as those from the staff bodies, skin, mouth, hands or hair to the product, and indirectly via their personal equipments, such as clothing, footwear, utensils and other tools used in their daily tasks. People in the production environment do not have knowledge that, anything that travels through or is mobile in the facility is a potential source of contamination and must be tracked and controlled. Also there was no good system to institute designated and dedicated personnel in particular operation especially those requiring hygienic practices as a way to reduce contaminations; there were no way to distinguish those who are non-product handlers and those who are product handlers. Other hygienic problem sources apart from staff hygiene were in the handling of raw materials in storage and packages. Where by some manufacturers such as Chemi & Cotex, A-one products and Sayona Drinks had middle scores in overall. However, the Noble Foods factory had poor raw materials handling in terms of storage and proper package. Reasons for low scores in hygienic practises observed may be attributed to luck of knowledge in HACCP and GMP, luck of adequate number of qualified personnel and possibly low capital. Inadequate regulatory monitoring may also have an effect in these observations.

It is not known whether the higher levels of preservatives found to be used in the product from manufacturers were deliberately formulated to counteract the unhygienic practises observed or it was due to lack of knowledge on preservative use in food.

Use of large amount of Benzoic and or Sorbic Acids in juices products may have be toxic to human. The findings indicate that most factories are using lager amount of preservatives possibly to cover the problem of poor hygienic practices.

But according to other researchers use of high amount of preservatives more than maximum limits specified in national standards in a long-term will have health effects on children because of links to cancer or other problems, (Dangour *et al.*, 2010).

Microbiological analysis was performed to observe the total plate count and presence of coliforms. None of fruit juices was found to contain coliform bacteria. This is contrary to the observed hygienic practices in some factories where, with exception of Salim S. Bakhressa other manufactures performed poorly. A possible explanation to this lack of growth of microorganisms could be the higher level of preservatives added, and this is supported by the fact that products from these poor hygienic practising manufactures showed no growth of any microorganism.

It was found only two samples had microbial growth above allowed limits. These samples were Tropicana Black Currant Juice from Noble Foods, shows overgrowth of microorganisms which correlates to low score in hygienic practices though, surprisingly this product was also found to contain preservative at higher level than requirements. The only possible cause here could be that the preservative used was ineffective due to expiry or may be either because the antimicrobial effectiveness of the preservatives depends on the physical and chemical

properties of the soft drink, including pH (it works best between pH levels of 2 – 4), also storage temperature and storage length can be the reason (Branen *et al*., 1990).

Labelling was another problem observed in many products, either because of an effect in inadequate regulatory monitoring to strength inspections, and educate manufactures the importance of labelling. Correct information on the container labelling is very necessary mostly on nutritional levels and types of various additives because some part of the population may be sensitive to some of the added approved substances, for example people with asthma appear to be especially sensitive to some preservatives.

CHAPTER SIX

6. 0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study have shown that hygienic practises are generally inadequate in most manufacturing facilities studied. Compliance to the required levels of preservatives is poor as most factories are using lager amount of preservatives possibly to cover the problem of poor hygienic practices. The results have also shown that, microbial load of some fruit flavoured juices in these companies is high above permissible limits.

6.2 Recommendations

It is therefore important for the regulatory authority to enforce systems that ensures continued production of safe food under good hygienic and manufacturing practices (GHPs/GMPs). Under such practices special attention should be directed to important areas especially;

(i). Protective outer clothing:

- Juice processors should train employees on how to protect food by creating a barrier between themselves and the product. The employee should wear protective gears which include coats/smocks, plastic aprons or plastic sleeves, hairnets and snoods, and gloves, when appropriate. Essentially, protective clothing provided by the company should never be worn outside of the plant premises, should always be worn in the plant production areas and should be regularly changed.

● Factory clothing should be hygienically designed to prevent foreign bodies from shedding directly (i.e., lint, buttons) or indirectly (i.e., outside pockets from which objects can fall out into product). Whenever possible, smocks should not have outside pockets. Many aprons, gloves and smocks used in juice production are constructed and designed to prevent microbial cross-contamination of the product from the employee.

● Laundering has to be controlled by the company in order to achieve a greater level of confidence that these items have been cleaned and sanitized adequately before being worn in an area where it may come into contact with finished product.

● Jewellery do not allowed in the production area. In the era of tongue rings and body piercing, it is good to have a completely restrictive policy to prevent foreign material inclusions from personnel to the line. In addition, a pre-shift check of the condition of clothing or footwear for frayed edges or loose items, such as buttons or snaps, can help control inadvertent foreign material contamination of product during the shift.

(ii) Footwear:

● Footwear can be the good goal and effective barrier against microbial contamination from humans and/or equipment that travels through juice-contact areas.

● Foot dips need to be monitored not only for adequate concentration but also for appropriate volume of sanitizing solution. Often, by the end of the first break in a

given shift, there is so much organic material built up in the container that there is no longer any sanitizer effectiveness. In fact, the foot dip is a pool of bacteria that everyone walks through. Thus, routine monitoring of the volume and concentration and regularly changing the dip solution is best.

● Footwear should be constructed of material that is cleanable. It should not be made of leather or cloth that will get and stay wet.

● Ideally, it is important to have a policy that employees leave their footwear at the facility in order to mitigate contaminants carried into the plant from home. Also, it is important to provide appropriate cleaning resources such as cleaners and brushes for all employees at end of the production shift, as well as appropriate storage conditions.

(iii) Hand hygiene:

● All personnel in the juice manufacturing environment must regularly and adequately wash their hands to prevent microbial contamination of juices and juice-contact surfaces. It needs to be stressed that this applies to everyone that enters the production facility. At bare minimum, everyone prior to going to work or coming into a production environment should have to stop and wash with soap and water, and when appropriate sanitize, their hands. Hand washing policies should require employees to wash after any type of activity that could contaminate the hands with pathogens, including using the restroom, blowing the nose or touching body parts, handling raw food, waste or non juice-contact surfaces such

as light switches or pipes. Employees also should wash before entering juice handling areas, changing clothing and putting on gloves.

● Providing appropriate hand washing resources has to be enough stations to accommodate that flux of employees. If hand washing stations are not placed in convenient locations, employees may skip washing.

(iv) Storage and package:

● Do provide a control plan for storage and package.

● Packaging which cannot withstand high temperatures must be filled in a sterile environment. The juice should be dispensed into containers which are new, non-porous, and non-corrosive, made of food grade materials and should be cleaned and inverted prior to use. New caps must be used. Glass containers may be reused if they have been properly cleaned, sanitized and rinsed prior to reuse.

● The Company must give its core group of sanitarians the resources to do their job properly. This means ensuring that their equipment is being maintained in a condition that can be cleaned and in sanitary condition.

● Colour-coding of sanitation tools is a tremendous tool because it works in all languages and makes these tools easily distinguishable from other production tools for better accountability. Sanitation tools and equipment should be designated and dedicated by three categories: juice contact, non-juice contact and drain.

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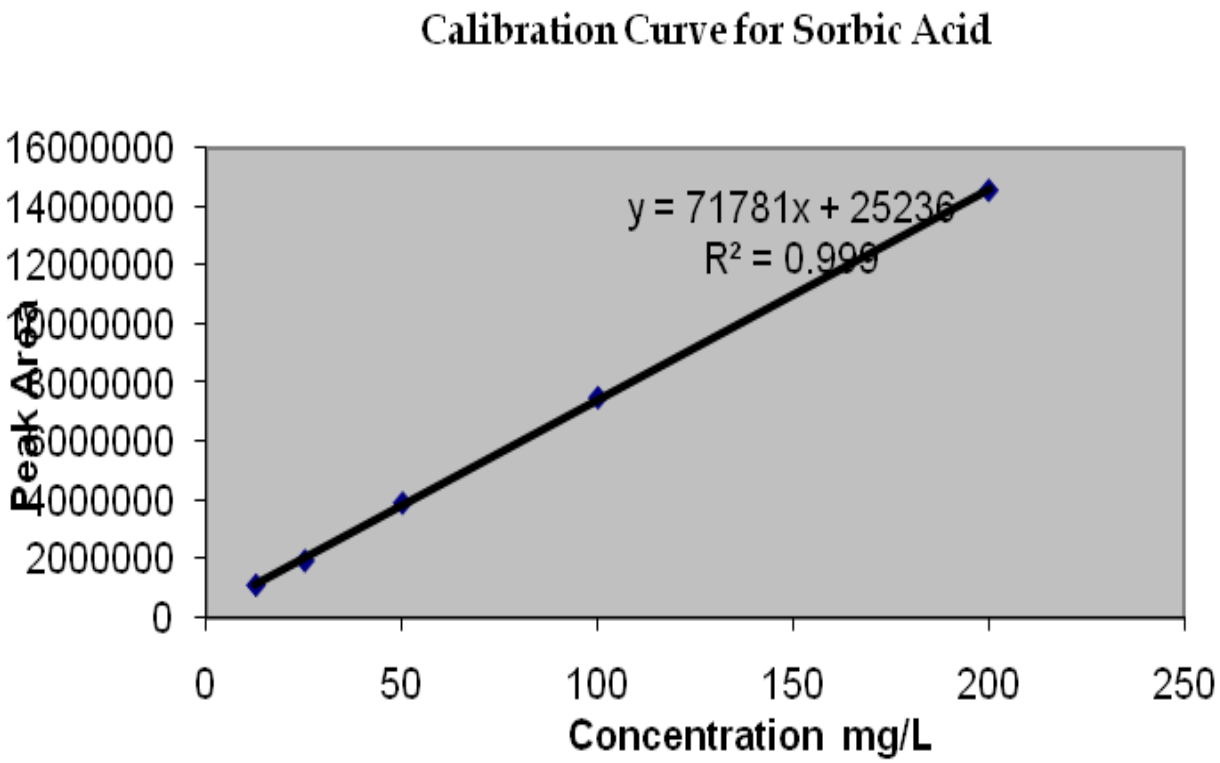
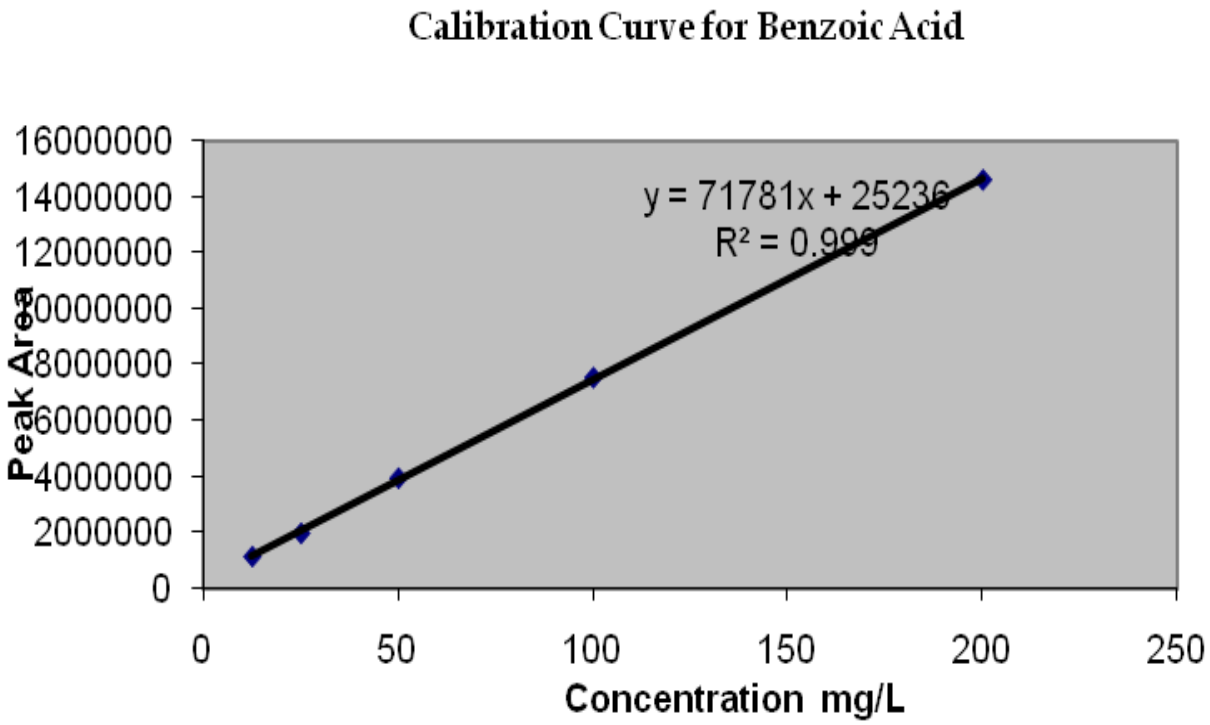
APPENDICES

Appendix I: Caliberation Curve for Sorbic acid



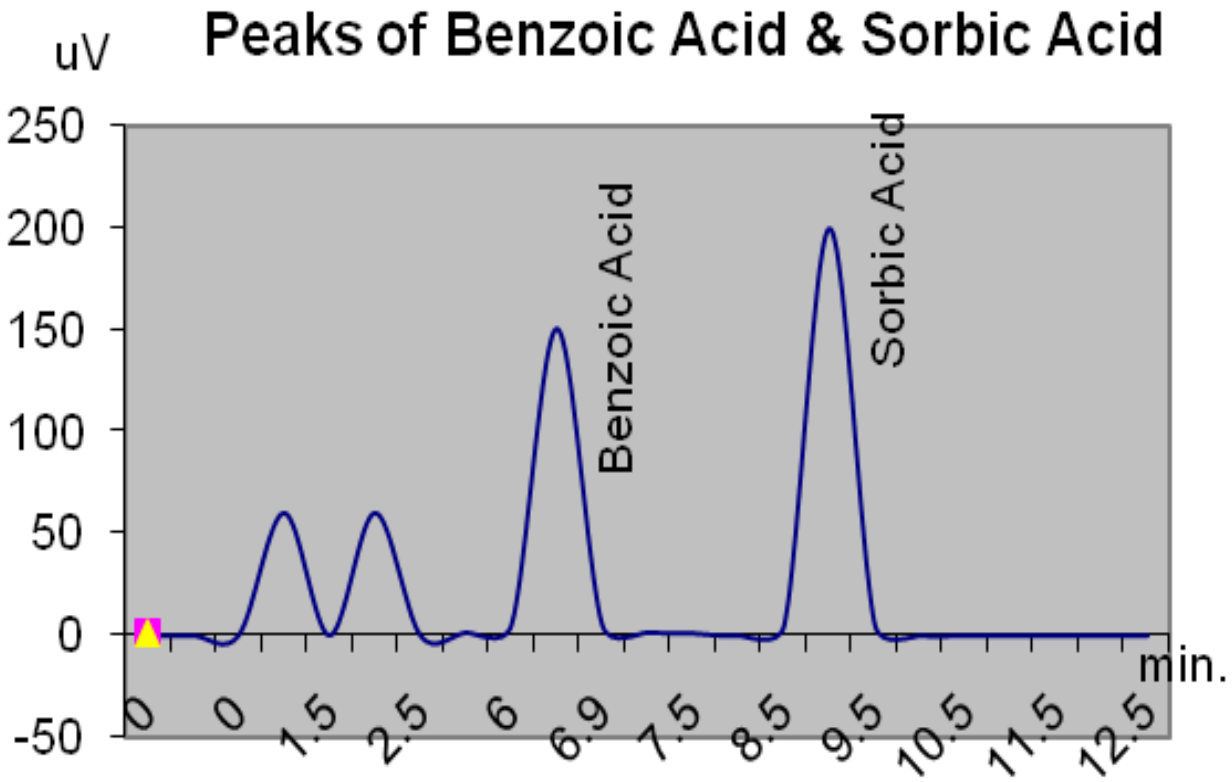
Appendix II: Caliberation Curve for Benzoic acid



Appendix III: The mean percentage recoveries of spiking and standard deviations

Concentration(mg/L)	Mean percentage recoveries		
	Benzoic Acid	**Sorbic Acid**	**Sodium Metabisulphate**
25	26.50 (105.8%)	27.0 (108%)	24.73 (98.92%)
50	45.86 (91.72%)	46.34 (92.68%)	47.12 (94.24%)
100	98.07 (98.72%)	87.97 (87.97%)	89.44(89.44%)
200	202.22 (101.11%)	181.83 (90.91%)	193.63 (96.82%)
Mean	**99.34%**	**94.89%**	**94.85%**

Appendix IV: The retention times for Benzoic and sorbic acids are about 6.8 and 9.0 min respectively



Appendix V: Determination of hygiene practices in manufacturing sites for fruit flavoured drinks in Dar es Salaam

ITEM /ASPECT/REQUIREMENT						
	Chemi & Cotex	**A-One product**	**Sayona Drinks**	**Salim S. Bakhressa**	**Noble Foods**	**Percentage**
1. **LOCATION / SITING**						
Within Industrial Area	1	1	1	1	1	100%
Free from sources of contamination	0	1	1	1	0	60%
Accessible by road	1	1	1	1	1	100%
Sound surface water drainage in place	1	1	1	1	0	80%
2. **BUILDING / CONSTRUCTION**						
Of Permanent material and good state of repair	1	1	1	1	1	100%
Proper adequate artificial lighting and air ventilation provided	1	0	1	1	0	60%
Floor finish hard, smooth, non-absorbent no accumulation of grime or dirt	0	0	0	1	0	20%
Good floor drainage, no stagnant water and smooth to invert	0	0	0	1	0	20%
Walls internally plastered, smooth hard cleanable and kept clean	1	0	1	1	0	60%
Roof in good state and repair, clean and not gapped	0	1	1	1	0	60%
Provides adequate area to accommodate activities carried on	1	0	1	1	0	60%
3. **WATER SUPPLY**						
Source is (Municipal or private) potable and available all the time	1	1	1	1	0	80%
Water supply is adequate in volume and Pressure	1	1	0	1	0	60%
Reservoir with capacity to supply adequate water in case of shortage	1	1	0	1	1	80%
Additional water treatment available	1	1	0	1	0	60%
4. **RAW MATERIALS**						
Food additives and concentrates properly packed and labelled held and not expired	0	0	1	1	0	50%
Raw materials stored at recommended temperatures	0	0	0	1	0	20%
Food additives approved by law & not expired	1	0	0	1	0	50%
5. **PROCESSING & EQUIPMENT**						
Equipment arranged according to flow of Operations	0	1	1	1	0	60%
Bottles properly washed, sanitized	0	1	1	1	0	60%

and rinsed						
Proper inspection of cleaned bottles carried Out	0	0	1	1	0	50%
Proper inspection of bottled / canned drink	0	0	1	1	0	50%
Glass fragments contamination control avalable	0	0	0	1	0	20%
Crown / Caps are of good quality and free from rust	1	1	1	1	0	80%
6. **SANITATION & STAFF HYGIENE**						
- Employees provided with protective clothing	0	1	1	1	0	60%
- Hand washing facilities provided	0	1	1	1	0	60%
- Workers are medically examined every six months.	0	0	1	1	0	50%
- Sufficient toilet/ cloak room facilities provided.	0	0	1	1	0	50%
- Cleaning schedules and methods recorded and followed	0	0	0	1	0	20%
- Employees do not smoke, pick nose, scratch their skin, sneeze during working	1	1	1	1	0	80%
- Proper sewage and drainage facilities available	0	0	1	1	0	50%
- Proper waste disposal in place	1	0	0	1	0	40%
7**. RECORDS**						
Quality control records physical, chemical and microbiological.	0	0	1	0	0	20%
Cleaning and disinfection/ disinfestations	1	0	1	1	0	60%
Total	**16**	**16**	**24**	**33**	**4**	
Percentage	**47%**	**47%**	**70%**	**97%**	**12%**	