KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI,

GHANA

MICROBIAL HAZARD ANALYSIS AND DEVELOPMENT OF CONTROL MEASURES FOR BISSAP DRINK (*SOBOLO*)

BY

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A thesis Submitted to the Department of Food Science and Technology,

College of Science, in partial fulfilment of the requirement for the degree of

MASTER OF PHILOSOPHY IN FOOD SCIENCE AND TECHNOLOGY

AUGUST, 2016

DECLARATION

I hereby declare that this submission is my own work towards the MPhil, and that, to the best of my of knowledge; it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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AKNOWLEDGEMENT

My utmost gratitude goes to the Almighty God for His strength and grace throughout my studies. I am most grateful to my parents, Dr. and Mrs. Yeboah- Awudzi, for their encouragement and especially for their financial support throughout the entire period.

My sincere gratitude goes to my supervisors Dr. Herman Erick Lutterodt (Department of Food Science and Technology, KNUST) and Dr. Felix Charles Robertson-Mills (Department of Biochemistry and Biotechnology) for the consistent guidance and immense input which made it possible for the successful completion of this thesis. I thank the entire staff of the Department of Food Science and Technology, KNUST for encouragement and advice.

My gratitude also goes to the Ghana Street Food Project for providing funds for me to conduct my research work and the contributions they made to make this work a success. The tremendous assistance provided by the staff of Microbiology Laboratory, CAnLab, Kwame Nkrumah University of Science and Technology is also much appreciated.

Big thanks to all my friends especially Ben Awaitey and Jeffery Kweku Dadson for their contributions in diverse ways to the success of this work.

ABSTRACT

In most developing countries street food vending remains a growing industry providing daily foods for most urban dwellers. Sorrel drink (*sobolo*) made from *Hibiscus sabdariffa* is now a widely patronized drink sold on the streets of Ghana. Despite its high patronage, microbial contamination from the preparation and packaging raises concerns. This study evaluated the microbial loads in *sobolo* drink sold in the Kumasi Metropolis using standard microbiological methods and proposing more hygienic production techniques for manufacturers. The *sobolo* drink was sampled from three widely used packaging materials, thus cups, used bottles and polythene baggies and assessed for total aerobic, enterobacteraceae and yeast counts. Qualitative coliform test was also performed together with pH and total soluble solids test. Observations made at both production and vending sites showed unhygienic conditions and practices such as use of unclean water, unwashed utensils, improper storage conditions and vending on bare floors. Total plate count revealed that all samples collected during late afternoon (between 4:00 and 4:30 PM) exceeded tolerable limits of 10^6 for aerobic microorganisms in ready to eat foods. However, 75 % of the samples collected early afternoon (between 1:00 and 1:30 PM) was below this limit. Enterobacteraceae counts showed that samples served in cups contained high levels of enterobacteraceae above the acceptable limit irrespective of the time of day. The manufacturing and vending processes observed showed possible microbial contamination due to unhygienic practices. High microbial counts were seen in total plate, enterobacteraceae and yeast. A flow diagram was developed to reduce the microbial contamination to acceptable levels.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Street food vending is a multimillion-dollar industry which provides affordable, convenient meals for the urban populations (FAO, 2009). The street food industry plays a very important role in meeting food requirements of urban dwellers in many cities and towns of developing countries by feeding most of its populace daily with a wide variety of foods that are relatively cheap and easily accessible (Tambekar *et al.,*2008). These foods are ready-to-eat foods and beverages prepared and or sold by vendors and hawkers especially in streets around markets and other public places (Umoh & Odoba, 1999).

In Ghana, foods sold by the road side do not only help meet the challenge of feeding the urban population but also it makes an important contribution to employment, household revenue and food security, hence significantly impacting national economy. Food production is very complex and involves several stages which may expose food to various forms of contamination. Therefore controlling contamination is always critical in the production process (Sargeant, Torrence, Rajić, O'Connor, & Williams, 2006).

Street-vended foods are diverse and include meat, fish, vegetables, cereals, frozen produce and beverages (World Health Organization, 1996). Several concerns have been raised over their safety and quality (von Holy & Makhoane, 2006).

Consumption of herbs and vegetables is believed to contribute to the improvement of human health (Biesalski *et al*., 2009). Plants for many years have served as a good source of therapeutic agents (Owulade *et al*., 2004).

Sobolo is the red coloured leaf of Roselle, which is scientifically known as *Hibiscus sabdariffa* from the family Malvaceae. It has several names depending on the country (see table 2.6 under literature review).

The growing popularity of the *sobolo* drink in Ghana has been rapid recently. The drink has not even been branded properly but the demand for it keeps rising. *Sobolo* drink is prepared by boiling the dry calyces in water for about 10 - 15 minutes to extract the pigment or embedded flavour. The raw extract has a sharp and sour taste and is usually sweetened with granulated sugar and fruits depending on choice. The *sobolo* drink has a short shelf life of 24 hours when kept under room temperature due to spoilage by microbial activities (Oboh & Elusiyan, 2004).

Commercial production of *sobolo* consists of a small number of operations, but this process can lead to the introduction of microorganisms or the proliferation of those already present. Food borne diseases are transmitted through consumption of contaminated food, drink or water. Improper washing of the Hibiscus leaves can lead to contamination of the product. Besides, use of unhygienic water for the preparation, dressing with contaminated ice, improper storage, the use of unclean harvesting equipment, production in unclean surroundings and places where dust and flies are not properly controlled may also act as sources of contamination (FDA, 2001).

Consequently, the drink so prepared could be a potential source of transmission of microbial contaminants, notably *Escherichia coli, Salmonella sp., Shigella sp., Staphylococcus aureus* and some filamentous fungi. Some of these contaminants cause serious disease conditions like typhoid, dysentery, enteric fever and peripheral as well as systemic mycoses, which represent threats to human health (Barro *et al*., 2006).

1.2 Rationale of Study

A large number of claims have been made of the therapeutic benefits of *sobolo*, from Ayurveda menstrual remedies to hangover cures and treatments for cancer. In addition to its popular appeal as a beverage, *sobolo* is widely regarded as a healthy drink in many cultures (Guardiola & Mach, 2014). As a result, it is massively patronized by Ghanaians making it a lucrative business for the rapidly increasing number of *sobolo* vendors.

However observations have shown that most *sobolo* vendors do not produce the drink under hygienic condition (Musah *et al*., 2014). The use of untreated water, unsterilized equipment and packaging materials by *sobolo* producers raises concerns about the safety of the drink on our streets. Contaminated *sobolo* poses health threat to most people especially pregnant women who usually take *sobolo* due to its spicy taste to prevent nausea.

Some *sobolo* vendors package the drink in small-sized polythene bags and sell to the populace including school-going-children. Such packaging is a potential source of microbial contamination due to the direct contact of the hands and other parts of the body with the drink.

1.3 JUSTIFICATION

In order to curb this alarming situation there is a need to outline a simple, economical and effective hygienic process that can be easily implemented by the small scale. Hence the focus of this study is to gather data on the levels of microbial contamination at the vending stage.

The documentation of the microbiological safety of *sobolo* will create awareness of the health risk associated with unhygienic preparation of *sobolo*. Such information will also inform Ghanaians whether street vended *sobolo* meet the required safety levels for consumption. The work will also

propose a simple and hygienic way for *sobolo* producers to use to reduce microbial contamination to safe levels.

1.4 OBJECTIVES

- To observe and document the manufacturing process and handling practices in the preparation and sale of *sobolo*.
- To determine the microbial quality of *sobolo*.
- To propose a process flow chat based on HACCP for the production of *sobolo*.

CHAPTER TWO

2.0 LITERATURE REVIEW 2.1 FOOD SAFETY ISSUES IN DEVELOPING COUNTRY

Several cases of foodborne diseases occur in developing countries annually as reported in several studies. Annually, over 1.8 million people lose their lives as a result of food borne diseases (Scallan *et al*., 2011).

There are cases where foodborne diseases occur in the homes of individuals which are not accounted for in reports related to foodborne diseases. An assumption is made that the incidence of foodborne diseases in developing countries is actually higher than reported. Children who suffer from foodborne diseases sometimes have negative effect on their growth and development (Adak *et al*., 2005). Bacterial contamination has been reported to be abundant in foods sold and prepared in Nigeria (Clarence *et al.,* 2009). The Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) have stated that the most prevalent health problem and an important cause of loss in economic productivity is as a result of diseases that arises from contaminated food (Käferstein & Moy, 2003).

An estimation of about 84,000 deaths occurred from diarrhoea outbreak as a result of consumption of foods that are contaminated in the years 2004 to 2008 this accounted for 25% of deaths among infants below the age of five while 297,104 out patients were reported with similar cases of foodborne illness (Omari *et al.,* 2014). Also in 2006, the Food and Drugs Authority reported that Ghana recorded 90,692 deaths in the area of food and personal hygiene-related illnesses alone but 297,104 patients were reported at the various Out-Patient Departments of clinics and hospitals with similar cases (Omari *et al*., 2014). An incidence due to cholera outbreak resulted in 922 cases being reported in various health facilities in the Cape Coast metropolis and the loss of four lives

(AsieduAddo, 2014). Large amounts of money from both government and developmental partners are channelled into solving food-borne diseases yearly (Omari *et al*., 2014). Tackling food and water safety together would serve as an essential tool to reduce the diseases, reducing the level of contamination associated with food would minimize unsafe food that are consumed and this would go a long way to ensure that individuals enjoy good health which turns to improve the economic growth in developing countries.

2.2 FOOD SAFETY ISSUES IN GHANA

In a work done by (A. Omemu, Edema, Atayese, & Obadina, 2006), on the microflora of *Hibiscus sabdariffa* "*sobolo*" juice where dried calyces were bought and prepared into juice and this was compared to commercially sold *sobolo* on the streets. The comparison was done based on microbial load. The findings showed lower counts for laboratory prepared *sobolo* than the ones sold on the streets. Comparing the counts for commercially sold *sobolo* the counts of viable microorganisms were generally low compared with counts of microflora in related food materials stated in similar works done by (Frazier and Westhoff, 1986; Prescott *et al.,* 2005). Omemu *et al*., (2006) found no enteric organisms in the dried calyx but found *Serratia spp*, *Proteus mirabilis* and *Escherichia coli* were present in the juices. The work done by Duhain, (2011) showed the presence of 30 % faecal coliforms in the food samples which showed the high possibility of contamination from other pathogenic organisms too. *E. coli* was present in 22.5 % of the samples which was assumed it was possibly from the raw vegetables as well as bad hygienic practices. B. *cereus* was seen to pose a greater percentage risk among the pathogens. *S. aureus* was found in only one sample (3log CFU/g) (2.5 %) which suggested that there was low possibility of food being contaminated after it has been cooked.

2.3 STREET FOODS

Humans depends on food as his source of energy and strength but food consumed can also be source of food borne diseases and infections. Food is consumed mainly to build and support life but otherwise is seen and reported that food can pose detrimental effect to the health of consumers through chemical, biological or physical hazards. There is the need to research and bring out solutions that would ensure that food eaten only support and build the human system and not vice versa.

Street vended foods according to von Holy & Makhoane, (2006) are foods and beverages prepared and or sold for immediate consumption without the need for further processes. Globally, street food vending is a business practiced in many countries especially in Africa. Foods are seen being sold in the urban cities around places like work, schools, hospitals, universities, railway stations, bus terminals and taxi ranks in the urban areas (Kok & Balkaran, 2014) and this practice has become an acceptable lifestyle in many developing countries, especially in Africa. Street food vendors in most cities usually have varieties such as snacks, drinks and even full meals. The street food business is now a large food industry providing affordable food for majority of the population and income for the vendors (Ohiokpehai, 2003).

Street foods represent a significant part of urban food consumption for millions people on a daily basis especially for low-and-middle-income consumers. For many low income people, street foods maybe the least expensive and most accessible means of obtaining nutritionally balanced meal outside their homes (FAO, 2009). In developing countries, street food business provides a regular source of income for millions of men and women who have dropped out from schools, most people are able to engage in this business because the activity requires low initial investment. This activity

also promotes the work of local agricultural producers and food processors and contributes to local and national economic growth (Hanashiro *et al*., 2005).

Street food vending are small businesses are prepared from homes or pavements of the streets, they are mostly not registered and hence considered informal. They are relatively small in size and are usually operated from homes, street pavements with informal arrangements. Campbell (2011) states that "There is an assumption that by their nature, street food contamination is inevitable" but yet still a huge number of people depend on this source for nutrition and economic livelihood. Street food vendors normally benefit from a positive cash flow and evade taxes, and have the luxury to determine their own working hours (Oladipo & Adejumobi, 2010).

Workers, shoppers, travellers and people on low incomes are more often interested in the low price and convenience of the food than the safety, quality and hygiene of the food (Mensah *et al*., 2002).

2.3.1 FOOD SAFETY ISSUES RELATED TO STREET FOODS

Food safety is an issue of growing importance due to several worldwide trends in food systems. The growing movement of people, live animals in food handling, and the emergence of new pathogens or antibiotic resistance in pathogens all contribute to increasing food safety risks (Rheinländer *et al*., 2008). The burden of food borne diseases though preventable remains huge contributing to worldwide morbidity. The WHO estimates that each year, unsafe food makes at least two billion people, representing about one third of the global population ill worldwide. Simple prevention techniques could significantly reduce this burden (Yapp & Fairman, 2006).

The number of reported outbreaks of food-borne illnesses has been high, both in developed as well as developing countries (Afele, 2006). However, the problem is exacerbated in developing countries due to economic reasons, poverty, the lack of adequate health care facilities, and the dearth of data regarding food-borne diseases (Feglo & Sakyi, 2012). Food contamination in developing countries is caused by many factors including traditional food processing methods, inappropriate holding temperatures, and poor personal hygiene of food handlers which is really seen in the street food vending industry in Ghana.

2.4 HIBISCUS SABDARIFFA

There are over three hundred species of Hibiscus distributed in tropical regions around the world. Hibiscus species are normally used for ornamental purposes but are said to have other benefits such as medicinal properties of which Hibiscus *sabdariffa* forms part (Nivedita, 2007).

Roselle (*Hibiscus sabdariffa*) belongs to the family Malvacea. It is an annual herb cultivated for its leaves, stem, seed and calyces (Umechuruba & Biol, 1997). *H. sabdariffa* is an important annual or perennial erect, mostly branched, shrub that is grown successfully in tropical and sub-tropical climates. It takes about five months from planting to harvesting. The crop is native to India but was introduced to other parts of the world such as Central America, West Indies and Africa. It is best grown in tropical and sub-tropical regions. The plant is resistant to short periods of drought, and it can be cultivated throughout the tropics and subtropics during hot and rainy seasons (Eslaminejad & Zakaria, 2011). The plant thrives in a wide range of soil conditions. It grows satisfactorily in relatively infertile soils, but for commercial cultivation, a soil rich in organic materials and essential nutrients is essential (Eslaminejad & Zakaria, 2011). Hibiscus *sabdariffa's*

flowers are yellowish in color and has a dark red pigment at the the centre of the flower. It bears fruits that are 2.5 cm in length and are surrounded by calyces which are fleshy in nature. The seeds are dark brown with a weight of 0.025g and 4-6 cm long per seed. About 22-34 per capsule are found in the calyces sometimes occurring with dark red pigmentation at the center (Omemu *et al*., 2006).

The plant is widely cultivated for commercial purposes in the Tropical and Sub-tropical regions for its fibre and edible calyx; the most important part being the fleshy calyx (sepals) that surrounds the fruit (Ademiluyi & Oboh, 2013).

The part of the Hibiscus which is cooked and eaten as vegetables are the young shoots and leaves while the fleshy, the calyces which are red are used in colouring and seasoning foods, it is also used in preparing the drink called *zobo* in Nigeria and *sobolo* in Ghana. The physical and chemical characteristic of Roselle shows that it is very acidic fruit with the sugar content being low as well. The acids found in Roselle are succinic acid and oxalic acid and they have been quantified as the two predominant acids in Hibiscus. Comparing ascorbic acids in hibiscus to that found in orange and mango the vitamin C content of hibiscus is said to be higher. (Wong *et al*., 2002).

2.4.1 NAMES OF HIBISCUS

Hibiscus is known by different names in various parts of the world. The table below shows some of the various names of Hibiscus used in different parts of the world.

Table 2.1 Various Names for Hibiscus In Some Parts of The World

Country	Local name (s)
Ghana	Bissap, *sobolo*

Indonesia	Rosella, Rosella fruit
France, Senegal	Bissap
Mali	Dah, Dah bleni
Gambia	Wonjo
Caribbean, Latin America	Sorrel
Nigeria	*Zobo*
Iran	Chaye-Torosh
Sudan, Saudi Arabia, Egypt	Karkade

(Adanlawo & Ajibade, 2006)

2.4.2 CALYCES OF *HIBISCUS SABDARIFFA*

The calyces are the most used part of the hibiscus plants and they are acquired from the removal of the calyces from the capsules containing the seeds which serves a commercial interest. Akwa & State, (2009), reported the presence of vitamins such as D, B1, B2 and B complex. It has also been studied in other works that it helps to reduce hypertension (Adegunloye *et al*., 1996) (Onyenekwe *et al*., 1999). Fresh forms of the calyces from literature contain calcium, iron, icarotene ascorbic acid in 1.72mg, 57mg, 300mg and 14mg per 100 grams (Duke, 2001). The dried calyces contain a higher amount of vitamin C between the ranges of 260-280 mg per 100g and said to have a higher content of ascorbic acid than guava and orange (Tee *et al*., 1997).

According to Morton (1987), the dried calyces also contain the flavonoids gosypetine and sab-daretine. Hibiscin has been redefined as daphiniphylline and considered as a major pigment in the calyces.

Plate 2.1: Calyces of *Hibiscus sabdariffa*

2.5 TRADITIONAL USES OF CALYCES *HIBISCUS SABDARIFFA*

The calyces of *Hibiscus sabdariffa* has multi use in Africa countries especially and also in some tropical countries. The calyces are normally sold as fresh to cook soup, stew and sauces or dried. In western Nigeria, hibiscus calyces are used in cooking vegetable soup which is usually prepared by steeping it with wood ash overnight or parboiled with wood ash and washed thoroughly prior to it used for the preparation of soup. The calyces have been shown to contain anthocyanin which serves as food colorants in food products such as confectionery products, snacks, cake, pudding, ice cream and beverages (Abou-arab *et al*., 2011). After oil extraction the residue is fermented and used as soup or cake, the oils extracted serves as castor oil substitute (Fasoyiro *et al*., 2005). In India, hibiscus calyces are used in brewing beverages, for producing jams, jellies and also serves as food preservatives (Clydesdale *et al*., 1979). In Ghana, the dried calyces are prepared into a cold and refreshing drink called '*sobolo*'. The drink has gained popularity and acceptance because it is easily processed at home. It is mainly served and sold when is chilled by packaging in plastic

bottles or polythene films or cups or glasses when at home. It serves as income generation source for many women.

2.6 MEDICINAL USES OF CALYCES OF *HIBISCUS SABDARIFFA*

The human body is made up of systems comprising of natural enzymatic and non-enzymatic antioxidants that defend and protect it from free radicals which are harmful to the body. Consuming food containing antioxidants found in food help to enhance protection from free radicals by scavenging (Vertuani, Angusti, & Manfredini, 2004). Work done by many researchers show that extract from the red calyces of *H. sabdariffa* contain/have antioxidant properties ((Ologundudu *et al*., 2009)

The calyces of roselle (green) is very rich in vitamin C and riboflavin with some major mineral present (Babalola *et al*., 2001). Hibiscus calyces are used as a digestive and purgative agent and a folk remedy for abscesses, billows, cancer, hypertension (Y. A. Duke, 1985). In Chinese traditional medicine and also in Senegal it is used in the management of hypertension, as well as pyrexia and liver diseases (Odigie *et al*., 2003).

Its sepal extract has been used as a valuable treatment option against leukemia. Infusions of the leaves and calyces are employed as diuretic, choleretic and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis. It has antispasmodic, anthelmintic and antibacterial activities as well. Roselle extract is claimed to decrease the rate of absorption of alcohol and so lessen its effect on the system (Tseng *et al*., 2000) thus in Guatemala, roselle is a favourite remedy for the after-effects of drunkenness. H. *sabdariffa* is rich in anthocyanin and protocatechuic acid. The dried calyces contain the flavonoids gossypetin, hibiscine and

sabdaretine. The major pigment, formerly reported as hibiscine, has been identified as daphniphylline. Small amounts of myrtillin (delphinidin-3-monoglucoside), chrysanthenin (cyanidin-3-monoglucoside), and delphinidin are also present. Roselle seeds are a good source of lipid- soluble antioxidants, particularly γ-tocopherol (Mohamed *et al*., 2007). The calyces are good sources of antioxidant like anthocyanin and ascorbic acid (Jung, Kim and Joo, 2013). It has also showed hypocholesterolemic and antihypertensive properties in animal models (Allison L. Hopkins, Marnie G. Lamm, 2014).

2.7 NUTRITIONAL AND BENEFITS OF *SOBOLO*

Hibiscus *sabdariffa* is highly rich in vitamins, minerals and bioactive compounds such as organic acids, phytosterols, and polyphenols. The phenolic content consists mainly of anthocyanins, flavonoids and glycosides (Oguntona, 1995, Ekwunzi, 1995). Table 2.2, 2.3, 2.4 show the nutritional and anti-nutritional composition of *Hibiscus sabdariffa* calyx.

Table 2.2: Proximate of Calyces of Hibiscus sabdariffa

Constituent	Composition (g/100g)
Moisture	7.6
Protein	4.71
Fat	2.01
Crude Fibre	4.69
Carbohydrate	68.75
Ash	12.24

Source: Adanlawo & Ajibade 2006

Table 2.3: Mineral Composition of Hibiscus *sabdariffa* calyces

Constituent	Composition (g/100g)
Sodium	96.66
Potassium	49.35
Calcium	12.65
Manganese	2.39
Iron	3.22
Zinc	12.22
Magnessium	38.65
Nickel	1.78
Phosphorus	36.0

Source: Adanlawo & Ajibade, 2006

Table 2.4: Anti-nutritional content and ascorbic acid composition

Constituents	Composition (%)
Phytic acid	0.32
Oxalate	6.15
Tannic	2.00
Hydrocyanic	0.16
Ascorbic	16.67

Source: Adanlawo & Ajibade 2006

2. 8 PREPARATION OF *SOBOLO* BEVERAGE

The name *Sobolo* originates from the Northern part of Nigeria from the local Hausa name for the Roselle plant which is, 'Zoborodo'. *Sobolo* is a made from extracting the anthocyanin in the calyces, it is indigenous and said to be non-alcoholic. The calyces are either boiled with water or

left in water over night. It is sieved with a colander to remove the calyces. The extract is sweetened with sugar, fruits, ginger, spices and other flavours are added to enhance its taste, the drink is stored refrigerated (Akwa & State, 2009).

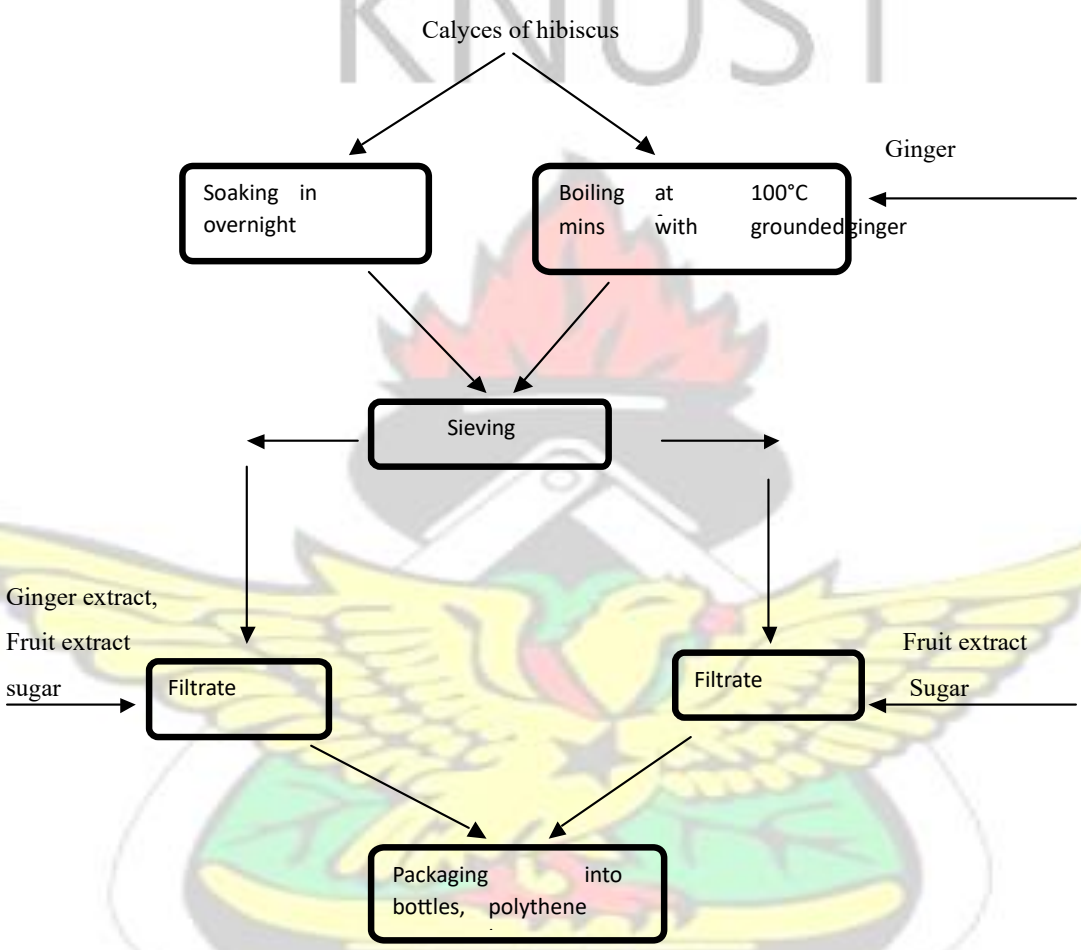


Fig 2.1: TRADITIONAL PROCESS FLOW OF SOBOLO DRINK

2.9 FOOD SPOILAGE BY MICROORGANISMS

Spoilage is a consequence of yeast growth in the product. Food components are utilized for the growth of microbes. Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending

operations because of their diversity, mobility and temporary nature (Estrada-Garcia *et al*., 2004). People who patronize street food, have been reported to suffer from food borne diseases like diarrhoea, cholera, typhoid fever and food poisoning (Rane, 2011).

2.10 MICROBIAL CONTAMINATION OF SOBOLO BEVERAGE

Food borne diseases are transmitted through consumption of contaminated food, drink or water. Improper washing of the Hibiscus leaves may introduce microbes into extracts leading to contamination of the product. Besides, use of unhygienic water for preparation, dressing with contaminated ice, improper storage, the use of unclean harvesting equipment, production in unclean surroundings and places where dust and flies are not properly controlled may also act as sources of contamination (Musah *et al*., 2014). The drink has a very short life span, lasting approximately twenty-four hours following production if not refrigerated. A study by Iloh, (2007) found *Aspergillus sp.* to be the microbial contaminant with the highest occurrence (21%) in both used and unused bottles. This is of concern, in that the presence of fungi is associated with the production of Aflatoxin, which is heat stable and as such boiling the beverage does not destroy the toxin (Frazier & Westhoff 1998; Adebayo-Tayo & Samuel, 2009). Furthermore, the toxin is carcinogenic and therefore the need to investigate the long term effect of continuous consumption of the locally prepared Hibiscus tea on human health. However, the fungal contamination of the drink and hence propensity for aflatoxicosis can be reduced or even averted by proper handling of the calyces and proper hygienic practices during preparation of the drinks. The need for proper handling and hygienic practices are underscored by the finding of various microbial contaminants which indicate human contact *(S. aureus* and *Klebsiella sp*.), water-borne (*E. coli* and *Pseudomonas sp*.), or use of sludge as manure (*E. coli*) and environmental sources of

contamination (*Bacillus sp.).* In view of the possible contamination and species heat stability, good manufacturing practices (GMPs), appropriate hygienic measures as well as brewing by boiling instead of cold water brewing should be preferred for enhanced consumption safety of locally prepared Hibiscus tea (Musah *et al*., 2014).

Food borne diseases are transmitted through consumption of contaminated food, drink or water. Improper washing of the Hibiscus leaves may introduce microbes into extracts leading to contamination of the product. Besides, use of unhygienic water for preparation, dressing with contaminated ice, improper storage, the use of unclean harvesting equipment, production in unclean surroundings and places where dust and flies are not properly controlled may also act as sources of contamination (Musah *et al*., 2014). Consequently, the drink so prepared could be a potential source of transmission of microbial contaminants, notably *Escherichia coli, Salmonella* sp., *Shigella* sp., *Staphylococcus* aureus and some filamentous fungi. Some of these contaminants cause serious disease conditions like typhoid, dysentery, enteric fever and peripheral as well as systemic mycoses, which represent threats to human health (Salle, 1943). However, although dried, the calyxes like any other raw material or food is prone to deterioration and spoilage caused by food microbes which lead to already contaminated drink and reduced quality of the drink in terms of colour, taste and nutrition. Most of the fungal contaminant can cause spoilage and they are known to produce mycotoxins which are detrimental to human health (Adebayo-Tayo & Samuel, 2009).

2.11 MICROORGANISMS FOUND IN SOFT DRINKS

Soft drinks are mostly spoilt by yeasts and a few acid-tolerant bacteria and fungi. Drinks that have low levels of fruit juice like *sobolo* tend to exhibit similar spoilage flora to fruit juices (Stratford *et al*., 2000). A number of microorganisms are found in soft drinks as environmental or raw material contaminants but a few can grow in the acidic region (Aneja *et al.*, 2014; Doyle et al., 2009).

Sobolo which a street beverage is also sometimes considered as a fruit juice as fruits like pineapples, watermelon and bananas are added to it. Unpasteurized fruit juice is defined as the product produced by pressing or squeezing of the fruits (Raybaudi-Massilia *et al*., 2009). Fruit juices contain a microflora which is normally present on the surface of fruits during harvest and postharvest processing which include transport, storage, and processing (Tournas *et al*., 2006). Many microorganisms such as acid tolerant bacteria and fungi (moulds, yeasts) use them as substrate for their growth. Yeasts form the main flora of fruits before processing because of acidic pH. The major genera include *Candida, Saccharomyces, Aspergillus* and many others are filamentous fungi most frequently isolated from fresh fruits and juices (Baird-Parker & Kooiman, 1980).

The other factors that contribute to the spoilage of juices include juice pH, oxidation reduction potential, water activity, availability of nutrients, presence of antimicrobial compounds, and competing microflora. Among these factors, pH and water activity are the two most influential factors affecting the spoilage of juices (Sperber & Doyle, 2009). Beverages like *sobolo* have pH in the acidic range below 4.5, and this serves as important barrier for microbial growth. However food borne pathogens such as *E. coli* and *Salmonella* survive in acidic environment of fruit juices due to acid stress response.

2.11.1 Bacteria

The most common reported bacterial genera include *Acebacter, Alicyclobacillus, Bacillus, Gluconobacter, Lactobacillus, Leuconostoc, Zymomonasand Zymobacte*r. Among yeasts, *Pichia Candida, Saccharomyces,* and *Rhodotorula* are commonly encountered genera responsible for spoilage of juices (Bevilacqua & Corbo, 2011). Total plate count TPC (aerobic, mesophilic organisms defines how many aerobic (oxygen-loving), mesophilic (moderate- temperatureloving), micro-organism colonies such as bacteria, yeast and mould fungi will grow in 72 hours on an agar plate that was normed for microbiological testing at a controlled temperature of 30 ^{0}C.

2.11.2 Yeast

Yeast are widely found in nature and are said to cause spoilage of foods such as wines, cheese, bakery goods, jams, fruit products, beverages, juices, salads and many others (Souza *et al.*, 2007). Food spoilage caused by yeast is mostly detected by the alteration of organoleptic and sensorial properties of food, which mostly occur in acid drinks like *sobolo*. There is gas production which turns to deform or blow up the packages (Doyle *et al*., 2009).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 STUDY AREA

Kumasi Metropolis has a population of 2, 035,064, representing 42.6 % of the total Ashanti region, with a land area of 254 km^2 and 250-300 meters' elevation range above sea level. It is located 270km north of the national capitol Accra, within a transitional forest zone. The Metropolis is bounded to the north by Afigya Kwabre and Kwabre East Districts, to the west by Atwima Nwabiagya District to the east by Ejisu Juabeng and Bosomtwe-Atwima Kwanwoma Districts, and to the south by Atwima Kwanwoma District (Darimani, Akabzaa, & Attuquayefio, 2013; Ghana Statistical Service, 2012).

3.2 OBSERVATION OF PRODUCTION AND VENDING SITE

Sobolo samples were collected from Twelve (12) different vendors from KNUST campus and its environs. Their vending as well as production sites were visited twice (2) in a week for three (3) conservative weeks to observe the conditions under which *sobolo* was prepared and sold. The observational tool used is shown in table 3.1, and was adapted from Rheinländer *et al.*, (2008).

TABLE 3.1 OBSERVATION GUIDE FOR *SOBOLO* BEVERAGE

VENDORS OBSERVATION TOOL	
Observations	
Observations were made at each session and the following data were obtained	
Producing and vending sites	Observations were made on source of water supply (availability, distance) Availability and the distance to toilet facilities, hand washing facility and use of soap. Presence of dirt, dust, ants, flies, animals, garbage and interruption of children Condition and presentation of point of sale in terms of flooring, cleanliness, refuse, flies, ants. Presentation of beverage whether they are covered properly or left open
Vicinity facilities	A general inspection of the immediate surroundings streets, social standards, public or private sanitation facilities.
Food hygiene practices	Preparation of beverage (how, when, by who) Treatment of *bissap* leaves and other raw materials. Storage conditions of *bissap* beverage The use of utensils, bare hands, plastic cover when handling the drink Separation of raw and finished product Handling of leftover beverage (what is kept and disposed) Quality of water and raw materials used (source of water used for cooking/washing, quality of leaves and other raw materials used
Vendor's personal hygiene	Hand washing (when, how often, in which water, soap) Cleanliness of clothes, hands, fingernails, open wounds Use of apron, head cover, plastic gloves Unhygienic behaviors while vending food such as nose blowing, child care, coughing, touching food, cross contamination

Source: Rheinländer *et al.,* 2008).

3.3 STERILIZATION OF EQUIPMENT AND MATERIALS

The materials used in this study were sterilized under laboratory conditions (Obire *et al*., 2008). Aseptic techniques were ensured in the preparation of media to prevent contamination. The use of ultra violet light in sterilizing the hood before the use of the inoculating hood was done to prevent or reduce contamination in the hood.

3.4 SAMPLE COLLECTION

Three types of *sobolo* packaging materials frequently used in selling and distributing the beverage in the Kumasi Metropolis were considered for this study; cups, used bottles, and polythene bags. *Sobolo* were sampled during late morning, early afternoon and late afternoon on different days from the 12 vendors, two days per vendor making a total of 72 samples. Samples purchased were kept in an ice chest and sent to the laboratory for analysis.

3.5 MICROBIAL SAMPLE PREPARATION

Ten milliliters (10 ml) of each sample collected was pipetted into sterile sample bottles containing 90 ml sterile peptone water and subjected to vortexing for about 30 seconds at moderate speed. Serially dilutions of five were prepared (10^{-1} - 10^{-5}) and aliquots of 0.1 mL these dilutions were used for the analysis.

3.5.1 ENUMERATION OF YEAST

Yeast enumeration was carried out with Sabroud Dextrose Agar (SDA) incorporated with 100mg/L of chloramphenicol and 50 mg/L Oxytetracycline, to ensure no growth of bacteria.

Spread plate method of inoculation was used (Egbuta et al., 2014). The plates were incubated at 25 °C for 5 to 7 days and plates with counts between the range of 30-150 were selected and manually counted. The number of colony-forming units per millilitres (cfu/ml) of samples was calculated by multiplying the number of organisms by the dilution factor.

3.5.2 ENUMERATION OF ENTEROBACTERACIE

The media Violet Red Bile Agar was used in enumerating enterobacteraceae from the five serial dilutions of samples collected using spread plate technique. The plates were incubated at 37 °C for 2 days, dilutions with 30-300 colonies after incubation were selected and manually counted. The number of colony-forming units per millilitres (cfu/ml) of samples was calculated by multiplying the number of organisms by the dilution factor.

3.5.3 TOTAL PLATE COUNT

From the prepared serial dilutions, enumeration was carried out using the spread plate method on Plate Count Agar. The plates were incubated at 37 °C for 48 h. After the appropriate incubation, plates with 30-300 colonies were selected and manually counted. The number of colony- forming units per millilitres (cfu/ml) of samples was calculated by multiplying the number of organisms by the dilution factor.

3.6 PHYSICOCHEMICAL ANALYSIS

3.6.1 pH

All pH measurements were done after microbial analysis. The pH meter was calibrated and used according to the manual's guide. it was calibrated to the same figure. The electrode was removed and rinsed with distilled water. It was then placed into the samples to be tested. Readings were taken thrice for each sample. The electrode was made not to touch the sides of the beaker (Elia, 2016).

3.6.2 TOTAL SOLUBLE SOLIDS

A hand held refractometer (Atago's Master Series, Japan) was to determine the °Brix of the samples. The refractometer was first calibrated with deionized water. One to two drops of drink was placed on the prism and covered. The brix level in degrees was recorded from the blue line when viewed. One °brix (1°Bx) is equivalent to 1%. The prism surface and cover were cleaned after every sample. Triplicate measurements were taken for each sample (Elia, 2016).

3.7 Statistical Analysis

Microsoft Excel 2010 was used in the tabulation of the results obtained, the data was exported to Statistical Package for Social Sciences (SPSS version 20) and one-way analysis of variance ANOVA was for easy interpretation. Microbial counts were expressed as means and the significant differences among them were also compared using Tukey's post hoc test. P-values which showed values ≥ 0.05 were considered as statistically not significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Observations at Vending and Production Site

Observations made at each step of the production process and vending sites showed various hazards at the different stages of the production site. Stationed vending sites also showed a number of unhygienic conditions and practices. Microbial hazards and the associated risks observed at both sites are summarized in table 4.1.

TABLE 4.1: Observations made at Vending Sites

NO	Source	Observations Made
1	Stand and production facilities	Drinks are normally kept in containers with ice and kept on the floor during point of sale or when vendor is tired. No designated utensils are kept for this production Surfaces of utensils have remains of food prepared earlier Improper waste disposal Production is done in the open and on the bare ground. Beverage exposed to flies and other insects
2	Raw Materials	The raw materials were stored with other food products. Fruits used in the production are mostly deteriorating
3	Food hygiene practices	Beverage are uncovered Beverage is not properly stored until time of sale and are not kept at the right conditions during sales Left over beverages and fruits are left at room temperature and not stored in the fridge. Used bottles are normally rinsed with just water
4	Vendor's personal hygiene	No washing of hands before production and before start of sales Unclean cloth during production hair not covered Excessive talking during production and selling Feeding of children during production

All the 12 vendors worked with were females with the exception of one. Majority of street food vendors are women as reported by Donkor *et al.,* (2009). The overall food vendors sampled in their work comprised of 98.4 % female and 1.6 % men.

Street foods are basically produced either at home or at stalls or selling site (Rane, 2011). Observations made in this work showed 8.4 % of the vendors prepare the beverage at the point of sale and 91.6 % prepare their beverage at home and transport them to the site of sales. Most times the production floors and sites are not cleaned before production is done. Production is normally done in the open space of the premises. From the production sites visited, 25 % of the floors were cemented whiles the remaining 75 % were bare grounds. The drink is exposed to flies and duct throughout production and packaging. It has been reported that 70-90 % cases showed contamination at preparation areas by animals, insects and liquid waste (FAO, 2009).

The two main sources of contamination in the production area are improper food handling and waste disposal. Handling of foods in an unsanitary manner has been found to be a common source of contaminants. The hands of food handlers are the main transfer of organisms from faeces, nose, skin to the food being prepared (Omemu and Aderoju, 2008). During the observation, all the vendors observed improper washing of hands before and during production. When the calyces are left on fire for it to boil, producers use this time to attend to household chores like sweeping, washing dishes, bathing and feeding little ones and others, at best they rinse their hands with water without soap. Organisms such as *Campylobacter* and *E. coli* can be introduced into the drink. Studies have shown that that *Salmonella*, non-typhi *salmonella*, *Campylobacter* can survive on finger tips for varying periods of time and shows traces after washing (Rane, 2011b).

The quality of raw materials used in the production of the drink is very critical as contamination from them can be consistent throughout preparation. The calyces were kept in store with other

household food stuffs, back tracing the raw material from purchasing site; the calyces are packed in sacks and stored in a very moist storage house with other materials such as maize, *ayoyo* leaves and many others. The quality of water used in the preparation of *sobolo* drink is very paramount since contaminated water can contain pathogens such as *E. coli, Salmonella spp.* and *Campylobacter spp*. and others (Angulo *et al*., 1997). Most vendors use well water for their production. Apart from the water added to the calyces during boiling the addition of water to the drink at further stages of production does not go through any form of treatment. One vendor soaks the calyces in water overnight in order to conserve energy.

Cooking utensils used for general household cooking were used for production. The utensils were mostly not washed properly causing build-up of residues of food which facilitates microbial growth. During production little water is used to wash all items due to proximity of water source. These observations were similar to a study conducted by (Barro *et al*., 2006). Stationed vendors wash their hands and dishes in one bucket without soap. The water used for washing the utensils gets dirty overtime and it's still used for the washing of all chores (Kaul and Agarwal,1998). Cardinale *et al.,*(2005) stated that serving utensils used at the vending sites are often contaminated with *Micrococcus spp* and *Staphylococcus* which may originate from the water used in dish washing and food preparation surfaces.

Preparation of food and storing before consumption influences food contamination. *Sobolo* prepared has to be kept refrigerated after packaging but this wasn't observed for most vendors due to lack of refrigeration, the drink was held at ambient temperature for long period of time contributing to food contamination. The drink is usually held for several hours after cooking and this includes overnight holding temperatures, until sold, thus can harbour high microbial populations which was evident in some of the sampled drinks.

Production was done under unhygienic conditions given that garbage and waste were conspicuously close to the point of production. The lack of sanitary facilities, garbage disposal and waste water results in garbage being dumped near production and vending site serving as breeding grounds for pest like flies and it also serves as media for growth of microorganisms (Muinde & Kuria, 2005). This exposed the beverage to contamination from flies and other insects as well as dust and dirt from the ground. In most cases, running water was not available at both vending and production sites, particularly vending sites which resulted in vendors not practicing hand washing throughout the time of selling for non-stationed vendors especially.

4.2 GUIDELINE LEVELS FOR READY-TO- EAT FOODS

The Food Standards Code for determining the microbial quality of ready-to-eat foods, which categorizes food as acceptable, good and unsatisfactory based on levels of microbial load in foods shown in Table 4.2 below was employed in this study to determine the safety of level of microbial contamination of the samples (Safety *et al.,* 2015).

TABLE 4.2: GUIDELINE LEVELS FOR DETERMINING THE MICROBIAL QUALITY OF READY-TO-EAT

Standard plate count test		Microbial Quality l (CFU/m)/	
Food	Good	Acceptable	Unsatisfactory
Category A	$< 10^4$	$<10^5$	$\measuredangle 10^5$
Category B	$<10^6$	$<10^7$	$\measuredangle 10^7$
Category C	N/A	N/A	N/A

Source: (New South Wales (NSW Food Authority 2009).

N/A – not applicable, Category A-applies to ready to eat food in which all components are fully cooked for immediate sale consumption (example meat pie), Category B-applies to ready to eat foods that are fully cooked with further handling or processing before consumption (example custard filled pastry), Category C- food that contain uncooked fermented ingredients or fresh fruit and vegetables (e.g. takeaway fruit and yoghurt mix).

4.3 Total Plate Counts

To determine microbial loads in each sample, total plate count was performed to assess the total aerobic microorganisms present. Samples were analysed in triplicates and the mean TPCs for each sample are summarized in the table below. The *sobolo* sampled for total plate count showed viable microbial count notwithstanding the type of packaging material used.

Table 4.3 Total plate counts (log cfu/ml)

Sample Code	Morning	Early Afternoon	Late Afternoon
MSP	3.861 ± 0.041 [a]	5.578 ± 0.655 [c d]	5.706 ± 0.015 [d]
ASP	3.256 ± 0.754 [a]	4.236 ± 0.650 [a]	4.284 ± 4.764 [a]
HSP	2.834 ± 0.360 [a]	3.400± 0.189 [a]	4.293 ± 4.738 [a]
LSP	3.627 ± 0.070 [a]	4.514 ± 0.200 [ab]	4.752± 0.204 [a b]
HSB	3.422 ± 0.019 [a]	3.466 ± 0.021 [a]	3.498 ± 0.115 [a]
ASB	2.943 ± 0.208 [a]	4.241 ± 0.862 [a]	4.538 ± 0.185 [a]

ASSB	3.130 ± 0.045 [a]	4.016 ± 0.150 [a]	4.640 ± 0.818 [a]
MSB	3.716 ± 0.264 [a]	5.998 ±1.307 [b c]	6.257 ± 4.282 [c d]
SSC	3.011 ± 0.215 [a]	3.207 ± 0.070 [a]	4.074 ± 0.120 [a]
RSC	3.275 ± 0.064 [a]	4.840 ± 0.650 [a]	5.727 ± 0.217 [a b]
ISC	4.520 ± 0.400 [a]	4.696 ± 0.125 [a b]	4.733 ± 0.015 [a b]
QSC	3.651 ± 1.014 [a]	4.269 ± 0.300 [a]	4.317 ± 0.264 [a]

Key: Sample codes ending in P are samples collected from polythene baggies, sample codes with B are from used bottles and samples codes with C are samples obtained from cups. Significant differences ($P<0.05$) are seen for means in the same row that do not share the same letter (superscript). Morning (10:30 – 11:30) Early Afternoon (13-13:30) Late Afternoon (16:00-16:30 GMT)

Results obtained from the experiment indicated that *sobolo* packaged in cups, used bottles and polythene bags sold on the streets of Kumasi were contaminated with microorganisms. This was confirmed by the different microbial analysis done. Although some samples showed significant difference with respect to time of sampling, this wasn't so for all samples. *Sobolo* sampled from polythene baggies showed no significant differences with the exception of samples from MSP. The MSP sample recorded a high viable mean count value of 3.8611 log cfu/ml for late morning, which indicates that the sample was already contaminated before selling began. This could be due to unhygienic working conditions and practices during production. *Sobolo* from MSP vendor was prepared at her residence in Ayigya-KNUST. The house compound is shared among 6 tenants and every household work such as washing, cooking and bathing of young children by each tenant is

done within this space, creating a high possibility of already contaminated drink before selling start. Mishandling and disregard of hygienic measures such as blowing air into the polythene bags to open them enable pathogens to come into contact with the food which can be passed on to the consumer (Mensah *et al*., 2002). During selling, hands are used in so many activities and not washed at all before serving a customer. All the samples from the polythene bags irrespective of the times and location were highly contaminated with mean viable counts above 8.97 x 10^3 comparing this to counts 1.1 to 2.2 x 10^4 from retail *sobolo* juice shows that vended *sobolo* is indeed contaminated (Omemu *et al*., 2006).

Samples from used bottles showed no significant difference between samples HSB and ASSB and the various times of sampling. There was significant difference between samples ASB and MSB as well as the various times samples were purchased. Samples from MSB recorded very high count right from morning. MSB had a stationed vending site and preparation of the *sobolo* was done at the vending site, where other food and non-food products are sold. The product was kept at ambient temperature and sold under this condition, which turns to create favourable conditions for growth of foodborne pathogens. The utensils used during production were also used for other purposes. Secondly, the used bottles were not washed or cleaned in any way before used for repackaging, and this will also contribute to the high contamination of the samples from MSB.

The total viable mean count of samples packaged in cups obtained from KNUST campus showed significant differences for samples SSC and QSC in comparison to RSC and ISC. Samples from QSC and SSC showed no significant difference between them and with respect to time of sampling, also their contamination were minimal compared to the RSC and ISC. Samples from vendor ISC recorded the highest viable mean count of 8.20 x10^6 (4.520 log cfu/ml) 12.3 x 10^6 (4.696 log cfu/ml) 38.0 x10^6 (4.733 log cfu/ml) for the various times of sampling. The high count recorded

for this vendor was related to unhygienic working conditions and cleaning practices. The cold dispenser used for dispensing the drink into cups is not regularly cleaned. The dispenser is emptied after the day's sales and left unwashed; the unwashed dispenser is then filled with either left over drink or freshly made drink. The unwashed dispenser could be the main cause of the high contamination of the drink even at early start sales. The contamination increased significantly from mid-afternoon sample to late afternoon.

However, comparing the means of viable count obtained in this study and the reference range for microbial contamination of ready-to-eat foods in table 4.2. By this standard, *sobolo* drink fell under category B due to further processing of mixing and sieving after cooking. Looking at *sobolo* packaged in polythene baggies sampled during morning, samples ASP and HSP of 8.97x103 and 3.40 $x10^3$ CFU/ ml were below < 10^6. Comparing this with standard in table 4.2 the microbial quality is considered to be good no food safety concern is needed. Whilst MSP-and LSP of $1.18x10^6$ (3.861 log cfu/ml) and 1.05×10^6 (3.627 log cfu/ml) respectively exceeded 10^6 showing microbial load that were really high at the initial stage of sale and fell within the acceptable range of $<10^7$ CFU/ml for microbial quality of ready-to-eat foods, implying that good manufacturing practices (GMP's) must be enforced and applied. Early afternoon samples ASP-02 and HSP-02 recorded values of 8.75×10^4 (4.236 log cfu/ml) and 1.25×10^4 (3.400 log cfu/ml) showing increase in the microbial load with no significant difference between morning and early afternoon samples but was still categorized within the region of good. Samples from vendors LSP and MSP also showed an increase in microbial load from morning to early afternoon of 8.10×10^6 (5.578 log cfu/ml) and 9.37×10^7 (4.514 log cfu/ml) respectively. LSP-02 showed no significant difference and was still categorized within the acceptable region but significant increase was seen in MSP02, the microbial counts recorded for this sample fell under unsatisfactory category. By late afternoon

the microbial load for samples ASP and HSP of 9.57 x 10^4 (4.284 log cfu/ml) and 9.7 x10^4 (4.293 log cfu/ml) respectively had higher microbial load compared to samples taken from early afternoon but the results obtained was still within the acceptable range of <10^7 from the standard. This is within expected microbiological levels for this type of product (upper range) and presents no food safety concern and no action needs to be taken.

However comparing these results to work done by Musah *et al.*, (2014), mean viable counts of 5.27 x 10^2 and 4.39 x 10^2 CFU/ml were recorded for used and unused bottles respectively for standard plate count. Both used and unused samples showed counts lower than counts obtained in this work for used bottles and the other forms of packaging materials. This could be due to the different approaches used in sampling and analysis of microbial counts. It was way lower compared to microbial limits for ready- to- eat foods in category B. Their work showed significant difference between the two forms of packaging material with the used bottles recording highest viable count, which may be due to inefficient washing of the bottles or by use of non-potable water in washing and or inadvertently exposure of the washed bottles to contaminants prior to packaging which was similar in this study.

Sobolo sampled from packaged cups showed mean viable count less than <10^6 for samples taken from vendors SSC and QSC irrespective of the time of sampling. Samples from SSC recorded the lowest count among all four. ISC and RSC on the other hand recorded higher count of 8.20 x 10^6 (3.275 log cfu/ml) and 1.27 x 10^5 (4.520 log cfu/ml) for morning respectively. ISC was within the acceptable region whilst RSC was considered as good compared to the standard for ready-to-eat foods. Samples taken during early afternoon for both samples ISC and RSC increase in microbial load but only showed significant difference for sample ISC-01 and ISC-02. By late afternoon the

results obtained were 3.59 x 10^7(4.733 log cfu/ml) and 1.34 x 10^7 (5.727 log cfu/ml) for ISC and RSC respectively were significantly different from samples taken during early afternoon. Both samples exceeded the acceptable category compared to the reference. This means that *sobolo* beverage from cups were more contaminated than *sobolo* sampled from polythene bags and used bottles. The higher counts seen in cups could be due to the continuous exposure of the drink during refilling the dispenser, dispensing in the cups, and the exposure of the empty cups to the environment. The dispenser can take a certain quantity at a time; the drink is left at ambient temperatures and sometimes in the sun creating a favourable environment for already present microbes to thrive. The longer the sample stayed the higher the rate of contamination which was evident in all forms of packaging.

The pH of the *sobolo* was recorded to be very low in the range of 2.5 - 3.4. Most bacteria cannot survive under this condition with the exception of aciduric bacteria such as lactobacillus species. reported that Bacillus and Lactobacillus species were readily and mostly found in low pH foods. *Sobolo* is a beverage with low pH and has been reported in this studies and other studies (Azeredo *et al.,* 2016; Fasoyiro, Babalola, & Owosibo, 2005; Mbaeyi-Nwaoha & Egbuche, 2012; Mohamed *et al*., 2007). The total plate and enterobacteracie counts could be due to the presence of lactobacillus and other bacteria that survive and multiply under very low pH, this could possibly count for the increase in bacteria count irrespective of the time of sampling. Similar results was seen in Omemu *et al*., (2006), they found lactobacillus and other enteric microorganisms like *Serratia spp, Proteus mirabilis* and *E. coli* in retailed *sobolo* drink sampled from the streets of Nigeria.

4.4 Enterobacteraceae Counts

To determine post processing contamination of the samples, enterobacteraceae determination was analysed. Below are means of the samples analysed in triplicates.

Table 4.3: Counts of Enterobacteraceae (log cfu/ml)

SAMPLE CODE	Morning	Early Afternoon	Late Afternoon
MSP	2.771 ± .0030 [a]	2.941± .0380 [a]	2.950 ± .0305 [a]
ASP	1.824 ± 0.577 [a]	2.301± 1.732 [a]	3.356 ± 0.208 [a]
HSP	2.647 ± 2.645 [a]	3.147 ± 0.200 [a]	3.326 ± 0.251 [a]
LSP	2.891 ± 0.25 [a]	4.389 ± 0.055 [b]	5.276 ± 0.062 [c]
HSB	2.171 ± 0.017 [a]	2.716± 0.043 [a]	2.922 ± 0.568 [a]
ASB	1.869 ± 3.605 [a]	2.374 ± 0.500 [a]	3.724 ± 0.902 [a b]
ASSB	2.804 ± 0.404 [a]	3.352 ± 0.070 [a b c]	5.035 ± 1.044 [h]
MSB	3.281± 0.264 [a]	4.672 ± 0.655 [b]	4.721 ± 4.853 [bc]
SSC	2.790 ± 0.136 [a b c]	3.723 ± 4.842 [d e f]	4.981 ± 1.802 [a b]
RSC	2.779± 0.117 [e f g]	5.390 ± 13.308 [h i]	6.221 ±1.695 [i]
ISC	3.221± .096 [a b c d]	3.687± 4.148 [c d e f]	4.014 ± 0.378 [g]
QSC	3.211± .109 [a b c d]	3.916± 0.133 [f g]	3.922± 0.165 [f g]

Key: Sample codes ending in P are samples collected from polythene baggies, sample codes with B are from used bottles and samples codes with C are samples obtained from cups. Significant

differences (P<0.05) are seen for means in the same row that do not share the same letter (superscript) Morning (10:30 – 11:30) Early Afternoon (13-13:30) Late Afternoon (16:00 -16:30 GMT)

In assessing hygiene and post-processing contamination enterobacteraceae becomes a useful tool, the presence of enterobacteraceae in levels > 10^4 /millilitres in ready-to-eat foods implies poor hygiene resulting from contamination or undercooking. This renders a food unsatisfactory for consumption (Gilbert *et al.*, 2000).

According to the standard there is an indication of possible contamination which could have resulted from poor temperature and time control of the cooking and storing of the drink, from surfaces of equipment used, personal handling the food. Sample MSP recorded mean viable counts of 1.08×10^4 (2.771 log cfu/ml) which exceeded the limit of 10^4 for enterobacteraceae of ready-toeat foods. This could be as a result of poor hygiene during preparing and selling of the drink. Poor temperature and time control during the boiling of the calyces could be a contributing factor as well as blowing air into polythene baggies to open it, residues from improper cleaning of utensils.

Samples collected from ASP for early afternoon and late afternoon all recorded a higher count from morning. The counts increased with respect to time but showed no significant differences among them. Counts from early afternoon and late afternoon were in the borderline margin and possible contamination may have resulted during vending as hands were not washed, ice blocks from contaminated sources used in keeping the drinks cold, exposure of the drinks to the environment for advertisement and many others.

Sample HSP also recorded a higher count for early afternoon samples compared to morning and further increased by late afternoon. No significant difference was seen among them and they were considered to be in the borderline, also indicating poor hygienic conditions. Samples collected from LSP and MSP for early afternoon and late afternoon showed counts higher than 10^4 that rendered the products unsatisfactory. Samples from used bottles HSB, ASB and ASSB which were collected in the morning recorded high counts of 2. 01 x 10^2 (2.171 log cfu/ml), 1.00 x 10^2 (1.869 log cfu/ml) and 3.17 x 10^3 (2.804 log cfu/ml) at the initial stage of sampling thus morning, all three samples were between 10^2-10^4 which was considered to be at the border when compared to the standards for ready to eat foods. Sample MSB was above the unsatisfactory level even for morning samples, there was increase in the microbial load with respect to time. Samples collected during early afternoon and late afternoon for HSB and ASB increased with time but showed no significant difference among the three times sampling was done and considered good for consumption. Although the food is still considered safe for consumption at these levels further checks should be put in measure to regulate the poor sanitary condition at both production and vending site to reduce the levels to the barest minimum. On the other hand, samples collected during early afternoon and late afternoon for samples ASSB and MSB recorded counts which rendered the samples unsatisfactory for consumption. Samples collected for ASSB during early afternoon and late afternoon showed no significant difference between them whilst there was a significant difference between the times of sampling for MSB. The high levels of enterobacteraceae counts from even the first sample analysed shows that processing of the beverage was done under unhygienic conditions. Also since the packaging material is used bottles of other products, improper washing of the bottles could cause contamination and cross contamination of the drink. These bottles are picked from rubbish dams and on the streets.

Work done by Musah *et al*., (2014) on unused and used bottles showed that the used bottles used for packaging *sobolo* recorded high counts of contamination compared to the unused bottles. The increase in enterobacteraceae contamination with respect to time could be due to the fact that *sobolo* does not go through any form of post-production treatment such as pasteurization that could have eliminated or reduce the microbial load to an appreciable amount hence the load increases with further exposure to the environment.

Samples from cups had enterobacteraceae counts greater than 10^4 for all samples analysed from the vendors irrespective of time. There were significant differences in counts among the various time of sampling for all vendors, as contamination increased significantly with respect to time.

For *sobolo* packaged in cups, most of the vendors prepare the *sobolo* a day before and the drink in kept at room temperature till it's poured in the cooling dispenser for sales. The dispenser used is normally not washed after used and it's reused each day. The cups and straws for serving are not covered and our exposed to the environment. Refilling of the dispenser also continually exposes the drink to dust, flies and the environment.

4.4 Yeast Counts

Table 4.4: Yeast counts of *sobolo* (log Cfu/mll)

SAMPLE CODE	Morning	Early Afternoon	Late Afternoon
MSP	3.885 ± 0.173 [a b]	3.900 ± 0.194 [a b]	5.083 ± 0.137 [f]
ASP	2.597 ± 1.060 [a]	3.059 ± 0.400 [b]	3.776 ± 0.208 [c]
HSP	2.407 ±1.266 [a]	1.533 ± 0.650 [a]	2.565 ± 0.731 [a]
LSP	2.639 ± 0.050 [a b]	3.494 ± 0.650 [c d]	4.674 ± 0.100 [e]
HSB	1.079 ± 0.113 [a]	1.230 ± 0.513 [a]	1.420 ± 0.167 [a]
ASB	1.13 ± 0.000 [a]	2.382 ± 4.423 [a]	2.894 ± 0.360 [a]
ASSB	3.571 ± 0.042 [a]	3.716 ± 0.351 [a b]	4.254 ± 0.026 [a b]
MSB	3.634 ± 0.315 [a]	3.681 ± 0.451 [a]	4.152± 4.743 [d e]
SSC	2.817 ± 0.832 [a]	3.140 ± 0.351 [a]	4.448± 0.500 [a b]
RSC	4.034 ± 0.556 [a b]	5.103± 0.521 [b]	5.278± 0.234 [c]
ISC	4.417 ±1.123 [c]	4.157 ± 0.125 [e]	4.620± 4.676 [d e]
QSC	3.550 ± 0.264 [a]	4.087 ± 0.404 [b]	3.844 ± 0.264 [a b]

Key: Sample codes ending in P are samples collected from polythene baggies, sample codes with B are from used bottles and samples codes with C are samples obtained from cups. Significant

differences (P<0.05) are seen for means in the same row that do not share the same letter (superscript) Morning (10:30 – 11:30) Early Afternoon (13-13:30) Late Afternoon (16:00 -16:30 GMT)

Yeasts are known to be the main contaminants of soft drinks and fruit juices compared to bacteria and because of the physical and chemical properties of the fruit juices (Obire *et al.,*2008). They are found in these products due to the rich nutrient composition of the juice and their ability to grow in acidic and also in carbonated environment (Azeredo *et al*., 2016). Pineapples and watermelon fruits which are used in production of *sobolo* are sources from which spoilage organisms originate from them especially when they are deteriorating. The calyces are bought from the markets, where the hibiscus calyces are stored in very moist places with other raw materials, which cause cross contamination and growth of yeasts.

Lactic acid bacteria, coliforms, moulds and yeast have been reported to use the carbohydrate content of the foods for undesirable fermentation processes (Amusa *et al*., 2005). Sample ASP and HSP had viable mean counts within the range of 10^3during late morning and early afternoon. The load of yeast increased by early afternoon but showed no significant difference between late morning and early afternoon for samples. By late afternoon, samples ASP and HSP had both increased but still showed no significant difference from the earlier times.

Sample MSP and LSP had high counts of 1.90 x 10^6 (3.885 log cfu/ml) and 1.08 x 10^6 (2.639 cfu/ml) respectively for mean viable counts of yeast during early morning. The counts increased by early afternoon for both samples but there was a significant difference for only LSP with counts of 7.73 x 10^6 (3.494 log cfu/ml). By late afternoon, both samples MSP and LSP showed significant difference in viable mean counts of 3.00 x 10^7 (5.083 log cfu/ml) and 1.17 x 10^7 (4.674 log cfu/ml)

respectively for their late afternoon samples. Contamination of the *sobolo* drink by yeast could be from the raw materials used for the production, the handling of the food and air vectors (Lachance, Bowles, Chavarría Díaz, & Janzen, 2001). Poor hygiene and lack of cleaning and sanitizing practices during production process causes the infestation of yeast. Poor hygienic conditions were seen at all production sites, although some production sites were generally worse than others. There was no standard procedure for the production of the beverage. No regulated form of cleaning or CIPs to prevent contamination.

Sample HSB and ASB recorded the lowest count for yeast contamination among the samples from used bottles. The mean viable count increased with respect to time. The various times of sampling showed no significant difference for both vendors. HSB had the lowest counts of 10^1. Samples ASSB and MSB recorded high counts of 2.51×10^5 (3.571 log cfu/ml) and 2.90×10^5 (3.634 log cfu/ml) for early morning samples. The counts increased by early afternoon but showed no significant difference. By late sample which is the last sample collection for the day, the viable counts had increased significantly by 1.21×10^6 (4.254 log cfu/ml) and 9.57×10^6 (4.152 log cfu/ml) for ASSB and MSB, respectively. The high level of yeast contamination of both samples could be due quite a number of factors such as poor working conditions, from the used bottles, poor quality of raw materials.

Samples SSC showed the lowest counts for viable yeast counts among *sobolo* packaged in cups. Sample SSC recorded counts of 8.87×10^2 (2.817 log cfu/ml), 1.87×10^3 (3.140 log cfu/ml) and 3.80×10^4 (4.448 log cfu/ml) for morning, early afternoon and late afternoon respectively. The counts increased with time as seen in both aerobic count and enterobacteracie. Sample QSC had

6.50 x 10^4 (3.550 log cfu/ml) for morning and showed significant difference with time for early afternoon and late afternoon samples. RSC showed high counts of 2.60x 10^6 (4.034 log cfu/ml for early morning sample and increased to 3.14 x 10^7 (5.103 log cfu/ml) and 4.73 x10^7 (5.278 log cfu/ml) for early afternoon and late afternoon respectively. Sample ISC recorded the highest contamination for yeast too with counts of 6.47 x 10^6 (4.417 log cfu/ml, 1.31 x 10^7 (4.157 log cfu/ml) and 3.80 x 10^7 (4.620 log cfu/ml) for late morning, early afternoon and late afternoon samples. There was a significant difference among the times of sampling, implying increase in contamination with prolong exposure of the drink to the environment.

4.5. pH

The pH of each samples were determined. All samples showed pH values within the acidic range (< 7). Below is a table showing the mean pH values of the samples collected.

Table 4.5 pH of *sobolo* sampled at different times of the day

Sample Code	Morning	Early Afternoon	Late Afternoon
MSP	2.67 ± 0.01 $^{a\,b\,c}$	2.503 ± 0.44 ^{a}	2.403 ± 0.35 ^{a}
ASP	3.14 ± 0.01 $^{d\,e}$	2.92 ± 0.01 $^{c\,d}$	2.89 ±0.01 $^{c\,d}$
HSP	2.85 ± 0.01 ^{cd}	2.79 ±0.01 ^{b}	2.82 ± 0.01 ^{c}
LSP	3.61 ± 0.01 ^{f}	3.58 ± 0.04 ^{f}	3.24 ± 0.01 ^{e}
HSB	3.79 ± 0.01 ^{f}	3.64 ± 0.01 ^{f}	3.62 ± 0.01 ^{f}
ASB	3.86 ± 0.01 ^{f}	3.67 ± 0.01 ^{f}	3.64 ± 0.01 ^{f}
ASSB	2.91 ± 0.01 ^{c}	2.88 ± 0.01 ^{c}	2.96 ± 0.01 ^{c}
MSB	3.63 ± 0.01 ^{f}	3.62 ± 0.01 ^{f}	3.57 ± 0.02 ^{f}
SSC	2.94 ±0.01 ^{e}	2.89 ± 0.01 $^{c\,d}$	2.88 ± 0.02 $^{c\,d}$
RSC	2.97 ± 0.00 ^{e}	2.92 ± 0.01 $^{c\,d}$	2.87 ± 0.00 $^{c\,d}$

ISC	2.72 ± 0.02^{b}	2.78 ± 0.06^{b}	2.71 ± 0.02^{b}
QSC	2.76 ± 0.02^{b}	2.77 ± 0.02^{b}	2.79 ± 0.02^{b}

Key: Significant differences ($P<0.05$) are seen for means in the same row that do not share the same letter (superscript) but those that share the same letter (superscript) do not differ significantly ($P>0.05$). Morning (10:30 – 11:30) Early Afternoon (13-13:30) Late Afternoon (16:00 -16:30 GMT)

The low pH of the *sobolo* drink is due to the low pH of the calyces used in the production of the drink. The drink produced from hibiscus has been said to be highly acidic from literature with succinic acid and oxalic acid being the two predominant organic acids (Wong *et al*., 2002). Sample MSP had mean pH values within the range of 2.4 to 2.7 for the three different times sampling was done. Sample ASP showed mean pH values of 2.89 for late morning, 3.14 for early afternoon and 2.92 for late afternoon. HSP also had pH value with the range of 2.5 to 2.9 for all the different times of sampling. LSP on the other hand, had pH values greater than 3.0 for all the three different times of sampling. Samples analysed from polythene baggies showed significant differences between early afternoon samples and late afternoon samples.

Samples from used bottles had pH values higher than 3.5 with the exception of ASSB samples. Sample HSB recorded 3.64, 3.79 and 3.86 for late morning, early morning and late afternoon respectively. Sample ASB recorded 3.64, 3.67 and 3.86 for late morning, early afternoon and late afternoon respectively. Sample MSB recorded higher values of 3.64, 3.67 and 3.86 for late morning, early afternoon and late afternoon respectively. All the samples showed no significant difference among the various times of sampling. On the other hand, ASSB samples recorded values of 2.91, 2.88 and 2.96 for the various times of sampling.

Samples analysed from cups had pH values reading between the ranges of 2.7 to 2.9 for all samples analysed at the various times of sampling. The samples at the different times showed no significant differences among them. A work done by (Fasoyiro *et al*., 2005) showed that pH of *sobolo* drink stored at ambient temperature and refrigeration temperature decreased with time which is attributed it to the decomposition of fermentable substrate like the carbohydrates in the fruits and sugar added that are decomposable thus reducing the pH. This was evident in this study as the pH of most samples decreased with time. The pH of the *sobolo* sampled from used bottles were in the range 3.57 to 3.86, comparing this to the pH of a work done on fruit flavoured *sobolo* mainly apple, pineapple and orange by Fasoyiro *et al.,* (2005), the study showed that pineapple flavoured *sobolo* had a pH of 3.14 recording the lowest among the three flavoured *sobolo*.

4.6 Total Soluble Solids (Brix)

The sugars present in the samples were determined and the mean brix value were recorded and tabulated below. The sugars present in each vendor's sample varied.

Table 4.6: Total soluble solids (°Brix)

Sample code	Morning	Early Afternoon	Late Afternoon
MSP	8.00 ± 1.00 [a]	7.00 ± 1.00 [b]	7.30 ± 0.2 [c]
ASP	9.10 ± 0.10 [a]	6.100 ± 0.10 [b]	8.10 ± 0.10 [c]
HSP	12.00 ± 1.00 [a]	11.00 ± 1.00 [b]	10.5 ± 0.10 [c]
LSP	9.30 ± 0.10 [a]	8.90 ± 0.10 [b]	7.9 ± 0.10 [c]
HSB	8.90 ± 0.01 [a]	8.2 ± 0.01[b]	8.60 ± 0.01[c]
ASB	7.70 ± 0.10 [a]	6.80 ± 0.10 [b]	5.80 ± 0.2 [c]
ASSB	9.50 ± 0.04 [a]	10.30 ± 0.01[b]	9.90 ± 0.03 [c]
MSB	7.60 ± 0.01[a]	6.30 ± 0.10[b]	6.80 ± 0.02 [c]

SSC	9.30 ± 0.10 [a]	8.40 ± 0.20 [b]	9.00 ± 0.10 [c]
RSC	6.40 ± 0.20 [a]	5.30 ± 0.10 [b]	5.90 ± 0.20 [c]
ISC	14.7 ± 0.20 [a]	12.20 ± 0.00 [b]	12.90 ± 0.40 [c]
QSC	7.20 ± 0.00 [a]	8.90 ± 0.10 [b]	8.40 ± 0.10 [c]

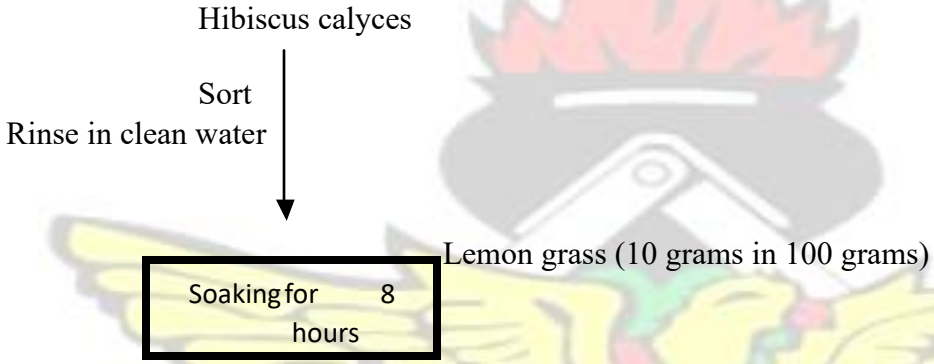
Key: Significant differences ($P<0.05$) are seen for means in the same row that do not share the same letter (superscript) but those that share the same letter (superscript) do not differ significantly ($P>0.05$). Morning (10:30 – 11:30) Early Afternoon (13-13:30) Late Afternoon (16:00 -16:30 GMT)

The lowest value was recorded for samples RSC of 6.40, 5.30 and 5.90 from morning to late afternoon. Sample HSP from polythene recorded the highest brix value of 12.00, 11.00 and 10.50 from morning to late afternoon. All the samples recorded different values for brix, because of the amount of sugar added to reduce the sour taste of the hibiscus calyces. The addition of the sucrose sugar depended on the vendor's preference as there is no stated and controlled recipe for the production of the sobolo drink. The total soluble sugars increased with time for most samples which is comparable to values reported by Ogiehor & Nwafor (2008) for *sobolo*. For each vendor there were significant differences among the various times of sampling in a day. The stated total sugar content (soluble sugars, °Brix) of roselle extract is between the range of 3.00 to 4.90 (Fasoyiro *et al*., 2005).

4.7 Proposed Flow Diagram for *Sobolo* preparation and GMP's

The preparation of the *sobolo* should be done according to the flow diagram proposed in this work which has been modified from a work done Fasoyiro *et al*. (2005). The leaves should be soaked overnight to ensure complete extraction of the colour anthocyanin during boiling and reduce the time boiling is done. The boiling serving as a critical control point in the production step, hence

any other raw material used in the preparation of the drink needs to be incorporated during the boiling stage. The blended ginger spices and lemon grass is added at this point. Boiling is done for 15 minutes at a temperature of 100 °C. Antimicrobials are used in foods to control natural spoilage processes and control/ prevent the growth of microorganisms' especially pathogenic microorganisms. Natural antimicrobials can be obtained from animals or plants and the use of natural antimicrobials into packaging materials to protect the food surface rather than protecting the food have also been developed (Davidson, 2006; Gaysinsky & Weiss, 2007; Gracia-Valenzuela, Orozco-Medina, & Molina-Maldonado, 2012). Lemon grass is considered as a natural antimicrobial and was added purposely because of the antimicrobial properties found in the grass. Lemon grass was considered due to readily ability and easy cultivation of the plant. Vendors can cultivate at their backyard. A new processing flow diagram was developed which discourages the soaking of the leaves without boiling and proposes the use of lemon grass which serves as a good antibacterial. In order to test the efficacy of lemon grass, one vendor was selected and a quantity of lemon grass was given to her for the preparation, all other conditions remained unchanged. Samples were taken during early afternoon for microbial analysis and no viable count was recorded for total plate count, enterobacteraceae and yeast. This is comparable to other studies done on lemon grass oil and its antimicrobial effect in foods, lemon grass and thermal treatment were used to reduce the spoilage of yeast specifically S. *cerevisiae*. There was complete inhibition of S. *cerevisiae* on sampling after two days and there was no growth even after seven days, and the combination of lemon grass oil and thermal treatment provided better juice preservative with minimal impact on the organoleptic properties of juices (Tyagi *et al*., 2014). Another work was done on antifungal activity of lemon grass essential oil against key postharvest pathogens by (Tzortzakis & Economakis, 2007).


Hibiscus calyces
Sort
Rinse in clean water
Lemon grass (10 grams in 100 grams)
Soaking for 8 hours

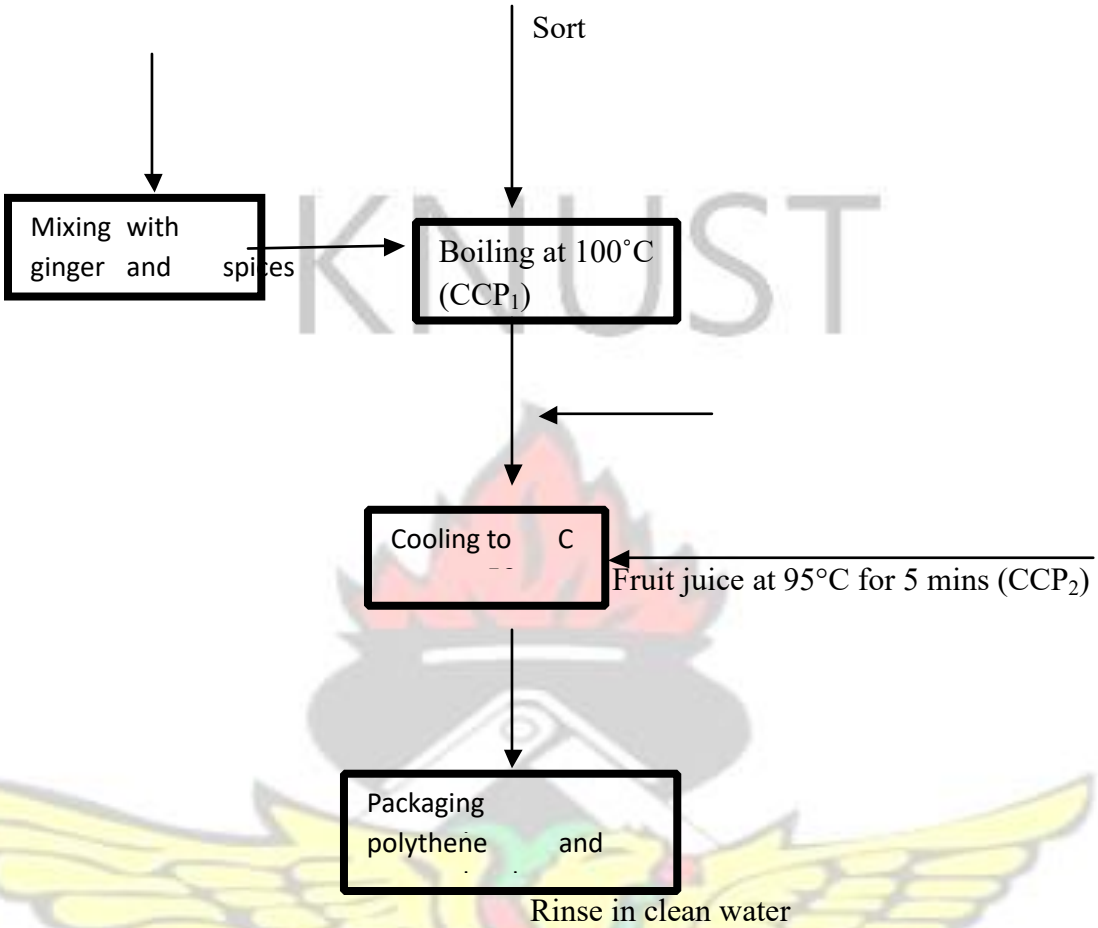


Figure 4.1: Proposed Flow Diagram for *sobolo* Drink

4.7.1 Good Manufacturing Practices to be observed by *sobolo* producers and vendors

- The raw materials, calyces of hibiscus, spices, sugars and flavors used in the preparation should be kept in a clean, cool dry place.
- Separate utensils and equipment should be used for the production of the drink and not for household chores and they should be thoroughly washed before the start of the production.
- Personal hygiene such as washing of hands before, during and after production, use of clean and designated clothes for the production of the drink.
- Preparation of the drink should be done in an enclosed environment.
- Preferably all water used during production should be pre boiled.

4. 7.2 Critical Control Points (CCP) for the proposed *sobolo* production

The critical control points of the proposed *sobolo* production is seen at the boiling of the calyces and the pasteurization of the fruit extract.

4.7.3 Control measure for the CCP's for the proposed *sobolo* production

During the production of the drink a possible microbial contamination could arise from the calyces used and to curtail this hazard sorting and rinsing of the calyces are to be done before they are

soaked in clean water. Contamination of drink from water used in cooking could also present microbial hazard. The water used in the production of the at any stage of the process should be boiled to 100 °C. At the boiling stage of the drink the boiling should be done for the required minutes to eliminate all pathogenic organisms to the barest minimum.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECCOMMENDATIONS

6.1 Summary

For total plate count the mean viable counts showed no significant differences among three forms of packaging materials irrespective of the time of sampling. Similar results were seen also for enterobacteraceae counts and yeast counts. There were significant differences with respect to time of sampling for these samples; MSP and LSP for polythene baggies, ASB, ASSB and MSB for used bottles, RSC, ISC and QSC for cups for total plate count analysis. For enterobacteraceae analysis, samples LSP and ASP also showed significant differences for the different times of sampling as in total plate count. Similar results were seen for used bottles, whereby samples ASB, ASSB and MSB showed significant differences with respect to time of sampling. All samples from cups showed significant differences with respect to time.

Samples MSP and LSP showed significant difference with time for yeast determination. For samples collected from used bottles ASSB and MSB are the ones that showed significant differences with respect to time of sampling. Lastly all samples collected from cups showed significant differences with respect to time of sampling.

The pH of the samples collected was within the range of 2- 4, which was seen stated as pH for *sobolo* drink in literature. Samples MSP, HSP, ASSB and SSC showed significant differences among the time of sampling. All other samples showed no significant differences with time.

Generally, there were decrease in pH with respect to time but this was not seen for samples HSP,

ASSB, ISC and QSC. The total soluble solids recorded different values for all samples, the least value recorded as 6.40 and highest was 12.90. There were significant differences with respect to time of sampling for all samples collected.

6.2 CONCLUSION

The preparation and sales of *sobolo* drink observed in Kumasi Metropolis were generally not prepared and sold under hygienic conditions. Pathogenic microbes were not analysed from the samples collected but high counts were seen in total plate count, enterobacteraceae and yeast. From the observational studies, producers and vendors showed poor working conditions in terms of cleanliness and hygiene. Food hygiene practices as well as personal hygiene practices are to be taught and ensured by institutions like Food and Drugs Authority. There were no significant differences among the three forms of packaging materials used under this study. From the study *sobolo* is considered to be very acidic with a sour taste requiring sugars and fruit juices for sweetening. The addition of sugars was seen to be based on preference of the producers. A proposed diagram developed under this study discourages soaking of the calyces without boiling. Also lemon grass was added during the boiling stage which showed significant difference in the reduction of microbial counts.

6.3 RECCOMMENDATIONS

- Samples to be analysed with respect to packaging material should be collected from the same vendors.
- Specific pathogenic microorganism should be analysed.

REFERENCES

Abou-arab, A. A., Abu-salem, F. M., & Abou-arab, E. A. (2011). Physico- chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (Hibiscus subdariffa), *7*(7), 445–456.

Adak, G. K., Meakins, S. M., Yip, H., Lopman, B. A., & O'Brien, S. J. (2005). Disease risks from foods, England and Wales, 1996-2000. *Emerging Infectious Diseases*, *11*(3), 365–72. http://doi.org/10.3201/eid1103.040191

Adanlawo, I. G., & Ajibade, V. A. (2006). Nutritive Value of the Two Varieties of Roselle (Hibiscus sabdariffa) Calyces Soaked with Wood Ash, *5*(6), 555–557.

Adebayo-Tayo, B., & Samuel, U. (2009). Microbial quality and proximate composition of dried Hibiscus sabdariffa calyxes in Uyo, Eastern Nigeria. *Malaysian Journal of Microbiology*. Retrieved from http://mjm.usm.my/uploads/issues/162/research3.pdf

Adegunloye, B. J., Omoniyi, J. O., Owolabi, O. A., Ajagbonna, O. P., Sofola, O. A., & Coker, H. A. (1996). Mechanisms of the blood pressure lowering effect of the calyx extract of Hibiscus sabdariffa in rats. *African Journal of Medicine and Medical Sciences*, *25*(3), 235–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10457797

Ademiluyi, A. O., & Oboh, G. (2013). Aqueous extracts of Roselle (Hibiscus sabdariffa Linn.) varieties inhibit α-amylase and α-glucosidase activities in vitro. *Journal of Medicinal Food*, *16*(1), 88–93. http://doi.org/10.1089/jmf.2012.0004

Afele, M. (2006). Street food boom in Ghana spurs calls for better hygiene. *Bulletin of the World Health Organization*, *84*(10), 772–773. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=2009340849&lang=es&site=ehost-live

Akwa, U., & State, I. (2009). Microbial quality and proximate composition of dried Hibiscus sabdariffa calyxes in Uyo , Eastern Nigeria, *5*(1), 13–18.

Allison L. Hopkins, Marnie G. Lamm, J. F. and C. R. (2014). Hibiscus sabdariffa L. in the treatment of hypertension and hyperlipidemia: a comprehensive review of animal and human studies. *Fitoterapia*, *85:*, 84–94. http://doi.org/10.1016/j.fitote.2013.01.003.Hibiscus

Amusa, N. A., Ashaye, O. A., Aiyegbayo, A. A., Oladapo, M. O., Oni, M. O., & Afolabi, O. O. (2005). Microbiological and nutritional quality of hawked sorrel drinks (soborodo) (the Nigerian locally brewed soft drinks) widely consumed and notable drinks in Nigeria, *3*(October), 47–50.

Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. (2014). Microbes Associated with Freshly Prepared Juices of Citrus and Carrots. http://doi.org/10.1155/2014/408085

Angulo, F. J., Tippen, S., Sharp, D. J., Payne, B., Collier, C., Hill, J. E., Swerdlow, D. L. (1997). A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. *American Journal of Public Health*. http://doi.org/10.2105/AJPH.87.4.580

Asiedu-Addo, S. (2014, September 21). Cholera kills 12 in Cape Coast. *General News*. Cape Coast. Retrieved from http://www.graphic.com.gh/news/general-news/31018-cholera-kills12-in-cape-coast.html

Azeredo, D. R. P., Alvarenga, V., Sant'Ana, A. S., & Sabaa Srur, A. U. O. (2016). An overview of microorganisms and factors contributing for the microbial stability of carbonated soft drinks. *Food Research International*. http://doi.org/10.1016/j.foodres.2016.01.024

Babalola, S., Babalola, A., & Aworh, O. (2001). Compositional attributes of the calyces of roselle (Hibiscus sabdariffa L.). *The Journal of Food Technology in Africa*. http://doi.org/10.4314/jfta.v6i4.19306

Baird-Parker, A., & Kooiman, W. (1980). Soft drinks, fruit juices, concentrates and fruit preserves. *Microbial Ecology of Foods*. Retrieved from https://scholar.google.com/scholar_lookup?title=Soft+drinks%2C+fruit+juices%2C+concentrates+and+food+preserves&author=ICMSF&publication_year=2005#0

Barro, N., Bello, A., Savadogo, A., Ouattara, C., Iiboudo, A., & Traoré, A. (2006). Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology*. Academic Journals (Kenya). Retrieved from http://www.ajol.info/index.php/ajb/article/view/42982

Bevilacqua, A., & Corbo, M. (2011). Shelf life prolongation of fruit juices through essential oils and homogenization: a review. ... *Current Research and …*. Retrieved from http://www.formatex.info/microbiology3/book/1157-1166.pdf

Biesalski, H. K., Dragsted, L. O., Elmadfa, I., Grossklaus, R., M??ller, M., Schrenk, D., … Weber, P. (2009). Bioactive compounds: Definition and assessment of activity. *Nutrition*. http://doi.org/10.1016/j.nut.2009.04.023

Campbell, P. T. (2011). Assessing the knowledge, attitudes and practices of street food vendors in the City of Johannesburg regarding food hygiene and safety. University of the Western Cape. Retrieved from http://etd.uwc.ac.za/xmlui/handle/11394/1827

Cardinale, E., Perrier Gros-Claude, J. D., Tall, F., Guèye, E. F., & Salvat, G. (2005). Risk factors for contamination of ready-to-eat street-vended poultry dishes in Dakar, Senegal. *International Journal of Food Microbiology*, *103*(2), 157–165. http://doi.org/10.1016/j.ijfoodmicro.2004.12.023

Clarence, S. Y., Nwinyi, O., & Chinedu, S. N. (2009, June 1). Assessment of bacteriological quality of ready to eatfood (Meat pie) in Benin City metropolis, Nigeria. *African Journal of Microbiology Research*. Academic Journals. Retrieved from http://eprints.covenantuniversity.edu.ng/53/1/5.pdf

Clydesdale, F. M., Main, J. H., & Francis, F. J. (1979). Roselle (Hibiscus sabdariffa L.) Anthocyanins as Colorants for Beverages and Gelatin Desserts. *Journal of Food Protection*, *3*, 196–276. Retrieved from

http://www.ingentaconnect.com/content/iafp/jfp/1979/00000042/00000003/art00003

Darimani, A., Akabzaa, T. M., & Attuquayefio, D. K. (2013). Effective environmental governance and outcomes for gold mining in Obuasi and Birim North Districts of Ghana. *Mineral Economics*, *26*(1), 47–60. http://doi.org/10.1007/s13563-013-0036-2

Davidson, P. M. (2006). FOOD ANTIMICROBIALS: BACK TO NATURE. *Acta Horticulturae*, (709), 29–34. http://doi.org/10.17660/ActaHortic.2006.709.3

Donkor, E. S., Kayang, B. B., Quaye, J., & Akyeh, M. L. (2009). Application of the WHO keys of safer food to improve food handling practices of food vendors in a poor resource community in Ghana. *International Journal of Environmental Research and Public Health*, *6*(11), 2833–2842. http://doi.org/10.3390/ijerph6112833

Doyle, M. P., Busta, F. F., Donnelly, C. W., Hocking, A. D., Montville, T. J., & Tompkin, R. B. (2009). Food Microbiology and Food Safety Series Editor FOOD MICROBIOLOGY AND FOOD SAFETY SERIES. *Ynes R. Ortega*. Retrieved from http://www.springer.com/series/7131

Duhain, G. L. M. C. (2011). Occurrence of Cryptosporidium spp. in South African irrigation waters and survival of Cryptosporidium parvum during vegetable processing.

Duke, J. A. (2001). *Handbook of medicinal herbs*. *Herbal reference library*.

Duke, Y. A. (1985). *Handbook of medicinal herbs. 13th Ed.* (L. G. Ltd, Ed.) (13th Ed.). Edinburgh. Retrieved from https://www.crcpress.com/Handbook-of-Medicinal-HerbsSecond-Edition/Duke/9780849312847

Egbuta, M. A., Mwanza, M., Njobeh, P. B., Phoku, J. Z., Chilaka, C. A., & Dutton, M. F. (2014). Isolation of Filamentous Fungi Species Contaminating Some Nigerian Food Commodities. *Journal of Food Research*, *4*(1), 38–50. http://doi.org/10.5539/jfr.v4n1p38 Elia,

H. (2016). AOAC 1990.

Eslaminejad, T., & Zakaria, M. (2011). Morphological characteristics and pathogenicity of fungi associated with Roselle (Hibiscus Sabdariffa) diseases in Penang, Malaysia. *Microbial Pathogenesis*, *51*(5), 325–37. http://doi.org/10.1016/j.micpath.2011.07.007

Estrada-Garcia, T., Lopez-Saucedo, C., Zamarripa-Ayala, B., Thompson, M. R., GutierrezCogco, L., Mancera-Martinez, A., & Escobar-Gutierrez, A. (2004). Prevalence of Escherichia coli and Salmonella spp. in street-vended food of open markets (tianguis) and general hygienic and trading practices in Mexico City. *Epidemiology and Infection*, *132*(6), 1181–4. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2870212&tool=pmcentrez&rendertype=abstract

FAO. (2009). Good Hygienic Practices in the Preparation and Sale of Street Food in Africa. Retrieved January 27, 2016, from http://www.fao.org/docrep/012/a0740e/a0740e00.htm Fasoyiro, S. ., Ashaye, O. ., Adeola, A., & Samuel, F. . (2005). Chemical and storability of

fruitflavoured (Hibiscus sabdariffa) drinks. *World Journal of Agricultural Sciences*, *1*(2), 165–
168. Retrieved from http://www.idosi.org/wjas/wjas1(2)/11.pdf

Fasoyiro, S. B., Babalola, S. O., & Owosibo, T. (2005). Chemical Composition and Sensory Quality of Fruit-Flavoured Roselle (Hibiscus sabdariffa) Drinks. *World Journal of Agricultural Sciences*, *1*(2), 161–164.

Feglo, P., & Sakyi, K. (2012). Bacterial contamination of street vending food in Kumasi , Ghana. *Journal of Medical and Biomedical Sciences*, *1*(1), 1–8.

Frazier, W., & Westhoff, D. (1998). Food microbiology 4th ed. *International Edition, McGrawHill, Singapore*. Retrieved from https://scholar.google.com/scholar?q=+Frazier%2C+W.+C.+and+Westhoff%2C+D.+C.%2C+1998.+Food+Microbiology.+4th+ed.+International+edition.+Mc+GrawHill%2C+Singapore&btnG=&hl=en&as_sdt=0%2C19#0

Gaysinsky, S., & Weiss, J. (2007). Aromatic and spice plants: Uses in food safety. *Stewart Postharvest Review*, *3*(4). http://doi.org/10.2212/spr.2007.4.5

Ghana Statistical Service. (2012). 2010 Population and Housing Census. *Ghana Statistical Service*, 1–117. http://doi.org/10.1371/journal.pone.0104053

Gilbert, R., Louvois, J. De, & Donovan, T. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. *Public health/PHLS*. Retrieved from http://europepmc.org/abstract/med/11014026

Gracia-Valenzuela, M. H., Orozco-Medina, C., & Molina-Maldonado, C. (2012). Efecto antibacteriano del aceite esencial de orégano (Lippia berlandieri) en bacterias patógenas de camarón Litopenaeus vannamei. *Hidrobiologica*, *22*(3), 201–206. http://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Guardiola, S., & Mach, N. (2014). Therapeutic potential of Hibiscus sabdariffa: A review of the scientific evidence. *Endocrinología Y Nutrición (English Edition)*, *61*(5), 274–295. http://doi.org/10.1016/j.endoen.2014.04.003

Hanashiro, A., Morita, M., Matté, G. R., Matté, M. H., & Torres, E. A. F. S. (2005). Microbiological quality of selected street foods from a restricted area of São Paulo city, Brazil. *Food Control*, *16*(5), 439–444. http://doi.org/10.1016/j.foodcont.2004.05.004

Iloh, E. M. I. and A. C. (2007). Inhibition of Three Fungal Isolates from Sorrel Drink (Zobo) Using Hurdle Technique. *World Journal of Agricultural Sciences*, *3*(3), 339–343. Retrieved from http://www.idosi.org/wjas/wjas3(3)/12.pdf

Jung, E., Kim, Y., & Joo, N. (2013). Physicochemical properties and antimicrobial activity of Roselle (Hibiscus sabdariffa L.). *Journal of the Science of Food and Agriculture*. http://doi.org/10.1002/jsfa.6256

Käferstein, F., & Moy, G. (2003). food Safety- Its Role in Health and Development: The problems Related to Our Food Supply. *Journal of Medical Sciences*, *2*, 1–10.

Kok, R., & Balkaran, R. (2014). Street Food Vending and Hygiene Practices and Implications for Consumers. *Journal of Economics and Behavioral Studies*, *6*(3), 188–193.

Lachance, M. A., Bowles, J. M., Chavarría Díaz, M., & Janzen, D. H. (2001). Candida cleridarum, Candida tilneyi and Candida powellii, three new yeast species isolated from insects associated with flowers. *International Journal of Systematic and Evolutionary Microbiology*. http://doi.org/10.1099/00207713-51-3-1201

Mbaeyi-Nwaoha, I., & Egbuche, N. (2012). Microbiological evaluation of sachet water and street-vended yoghurt and "Zobo" drinks sold in Nsukka metropolis. *International Journal of Biological and Chemical Sciences*, *6*(4), 1703–1717. http://doi.org/10.4314/ijbcs.v6i4.27

Mensah, P., Yeboah-Manu, D., Owusu-Darko, K., & Ablordey, A. (2002). Street foods in Accra, Ghana: how safe are they? *Bulletin of the World Health Organization*, *80*(7), 546–554. http://doi.org/10.1590/S0042-96862002000700006

Mohamed, R., Fernández, J., Pineda, M., & Aguilar, M. (2007). Roselle (Hibiscus sabdariffa) seed oil is a rich source of gamma-tocopherol. *Journal of Food Science*, *72*(3), S207–11. http://doi.org/10.1111/j.1750-3841.2007.00285.x

Morton, J. (1987). *Roselle*. Purdue University. Retrieved from https://www.hort.purdue.edu/newcrop/morton/roselle.html

Muinde, O., & Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods. *African Journal of Food Agriculture Nutrition and Development*, *5*(7), 1–15.

Musah, B. O., Nii-trebi, N. I., Nwabugo, M. A., & Asmah, R. H. (2014). Microbial Quality of Locally Prepared Hibiscus Tea in Accra. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *8*(11), 23–27. http://doi.org/10.9790/2402-081122327

New South Wales (NSW Food Authority). (2009). Microbiological quality guide for ready to-eat foods. A guide to interpreting microbiological results. *NSW Food Authority*, (July), 9.

Nivedita, U. A. and G. (2007). Anticancerous Effect of Hibiscus sabdariffa leaves on Hepatocellular Carcinoma Cell line Hep 3B. *Research Journal of Medicinal Plant*, *1*(3), 100–105.

Obire, O., Ramesa, R. Putheti., Dick, A. A and Okigbo, R. N. (2008). Biotechnology Influence for the Production Ofethyl. *E-Journal of Science and Technology*, *3*(3), 17–32.

Oboh, G., & Elusiyan, C. A. (2004). Nutrient Composition and Antimicrobial Activity of Sorrel Drinks (Soborodo). *Journal of Medicinal Food*, *7*(3), 340–342. http://doi.org/10.1089/jmf.2004.7.340

Odigie, I. P., Ettarh, R. R., & Adigun, S. A. (2003). Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1C

hypertensive rats. *Journal of Ethnopharmacology*, *86*(2-3), 181–185. http://doi.org/10.1016/S0378-8741(03)00078-3

Ogiehor, I. S., & Nwafor, O. E. (2008). Changes in the quality of zobo beverages produced from Hibiscus s abdarifa (Linn roscelle) and the effects of extract of ginger alone or in combination with refrigeration, *7*(8), 1176–1180.

Ohiokpehai, O. (2003). Nutritional Aspects of Street Foods in Botswana. *Pakistan Journal of Nutrition*, *2*(2), 76–81.

Oladipo, I. C., & Adejumobi, O. D. (2010). Incidence of antibiotic resistance in some bacterial pathogens from street vended food in Ogbomoso, Nigeria. *Pakistan Journal of Nutrition*. http://doi.org/10.3923/pjn.2010.1061.1068

Ologundudu, A., Ologundudu, A. O., Ololade, I. A., & Obi, F. O. (2009). Effect of Hibiscus sabdariffa anthocyanins on 2, 4- dinitrophenylhydrazine-induced hematotoxicity in rabbits. *African Journal of Biochemistry Research*, *3*(4), 140–144. Retrieved from http://www.academicjournals.org/AJBR

Omari, R., Quorantsen, E. K., Omari, P., Oppey, D., & Asigbee, M. (2014). Prevailing Food Safety Practices and Barriers to the Adoption of the WHO 5-Keys to Safer Food Messages in Rural Cocoa-Producing Communities in Ghana. *Internet Journal of Food Safety*, *16*, 36–44.

Omemu, a. M., & Aderoju, S. T. (2008). Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria. *Food Control*, *19*(4), 396–402. http://doi.org/10.1016/j.foodcont.2007.04.021

Omemu, A., Edema, M., Atayese, A., & Obadina, A. (2006). A survey of the microflora of Hibiscus sabdariffa (Roselle) and the resulting“ Zobo” juice. *African Journal of Biotechnology*, *5*(3), 254–259. http://doi.org/10.5897/AJB05.290

Onyenekwe, P. C., Ajani, E. O., Ameh, D. A., & Gamaniel, K. S. (1999). Antihypertensive effect of roselle (Hibiscus sabdariffa) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wistar rats. *Cell Biochemistry and Function*, *17*(3), 199–206. http://doi.org/10.1002/(SICI)1099-0844(199909)17:3<199::AIDCBF829>3.0.CO;2-2

Owulade, M.O.; Eghianruwa, K.I.; Daramola, F. O. (2004). Effects of aqueous extracts of Hibiscus sabdariffa calyces and Ocimum gratissimum leaves on intestinal transit in rats. *African Journal of Biomedical Research*, *7*, 31–33.

Rane, S. (2011a). Street Vended Food in Developing World: Hazard Analyses. *Indian Journal of Microbiology*. http://doi.org/10.1007/s12088-011-0154-x

Rane, S. (2011b). Street vended food in developing world: hazard analyses. *Indian Journal of Microbiology*, *51*(1), 100–6. http://doi.org/10.1007/s12088-011-0154-x

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., Soliva-Fortuny, R., & Martín-Belloso, O.

(2009). Control of Pathogenic and Spoilage Microorganisms in Fresh-cut Fruits and Fruit Juices by Traditional and Alternative Natural Antimicrobials. *Comprehensive Reviews in Food Science and Food Safety*, *8*(3), 157–180. http://doi.org/10.1111/j.1541-4337.2009.00076.x

Rheinländer, T., Olsen, M., Bakang, J. A., Takyi, H., Konradsen, F., & Samuelsen, H. (2008). Keeping Up Appearances: Perceptions of Street Food Safety in Urban Kumasi, Ghana. *Journal of Urban Health*, *85*(6), 952–964. http://doi.org/10.1007/s11524-008-9318-3

Safety, F., Authority, S., Health, M. O. F., Welfare, F., & Delhi, N. E. W. (2015). Manual of Methods of Analysis of Foods.

Salle, A. J. (1943). *Fundamental Principles Of Bacteriology*. *McGraw-Hill Book Company Inc*. Retrieved from https://archive.org/stream/fundamentalprinc029784mbp#page/n7/mode/2up

Sargeant, J. M., Torrence, M. E., Rajić, A., O'Connor, A. M., & Williams, J. (2006). Methodological quality assessment of review articles evaluating interventions to improve microbial food safety. *Foodborne Pathogens and Disease*, *3*(4), 447–56. http://doi.org/10.1089/fpd.2006.3.447

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M.-A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. http://doi.org/10.3201/eid1701.091101p1

Souza, E. L., Stamford, T. L. M., Lima, E. O., & Trajano, V. N. (2007). Effectiveness of Origanum vulgare L. essential oil to inhibit the growth of food spoiling yeasts. *Food Control*, *18*(5), 409–413. http://doi.org/10.1016/j.foodcont.2005.11.008

Sperber, W. H., & Doyle, M. P. (Eds.). (2009). *Compendium of the Microbiological Spoilage of Foods and Beverages*. New York, NY: Springer New York. http://doi.org/10.1007/978-14419-0826-1

Tambekar, D, Jaiswal, V, Dhanorkar, D, Gulhane, P, Dudhane, M. (2008). Madueke_Vol21.pdf. Retrieved January 28, 2016, from http://www.usa-journals.com/wpcontent/uploads/2014/01/Madueke_Vol21.pdf

Thilde Rheinländer, Mette Olsen, John Abubakar Bakang, Harriet Takyi, Flemming Konradsen, H. S. (2008). Keeping up Appearances: Perceptions of street Food .pdf. *Journal of Urban Health*, *85*(6), 952–964. Retrieved from http://download.springer.com/static/pdf/336/art%253A10.1007%252Fs11524-008-93183.pdf?originUrl=http%3A%2F%2Flink.springer.com%2Farticle%2F10.1007%2Fs11524008-9318-3&token2=exp=1445619018~acl=%2Fstatic%2Fpdf%2F336%2Fart%25253A10.1007%25252Fs11524-008-931

Tournas, V. H., Heeres, J., & Burgess, L. (2006). Moulds and yeasts in fruit salads and fruit juices. *Food Microbiology*, *23*(7), 684–8. http://doi.org/10.1016/j.fm.2006.01.003

Tseng, T.-H., Kao, T.-W., Chu, C.-Y., Chou, F.-P., Lin, W.-L., & Wang, C.-J. (2000). Induction of apoptosis by Hibiscus protocatechuic acid in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expression. *Biochemical Pharmacology*, *60*(3), 307–315. http://doi.org/10.1016/S0006-2952(00)00322-1

Tyagi, A. K., Gottardi, D., Malik, A., & Guerzoni, M. E. (2014). Chemical composition, invitro anti-yeast activity and fruit juice preservation potential of lemon grass oil. *LWT - Food Science and Technology*, *57*(2), 731–737. http://doi.org/10.1016/j.lwt.2014.02.004

Tzortzakis, N. G., & Economakis, C. D. (2007). Antifungal activity of lemongrass (Cympopogon citratus L.) essential oil against key postharvest pathogens. *Innovative Food Science and Emerging Technologies*, *8*(2), 253–258. http://doi.org/10.1016/j.ifset.2007.01.002

Umechuruba, C. I., & Biol, C. (1997). An annotated list of plant diseases in Nigeria. Retrieved from http://www.cabdirect.org/abstracts/20013037714.html

Umoh, V. J., & Odoba, M. B. (1999). Safety and quality evaluation of street foods sold in Zaria, Nigeria. *Food Control*, *10*(1), 9–14. http://doi.org/10.1016/S0956-7135(98)00149-2

Vertuani, S., Angusti, A., & Manfredini, S. (2004). The Antioxidants and Pro - Antioxidants Network : An Overview. *Current Pharmaceutical Design*, *10*, 1677–1694 The. http://doi.org/10.2174/1381612043384655

von Holy, A., & Makhoane, F. M. (2006). Improving street food vending in South Africa: achievements and lessons learned. *International Journal of Food Microbiology*, *111*(2), 89–92. http://doi.org/10.1016/j.ijfoodmicro.2006.06.012

Wong, P., Yusof, S., Ghazali, H. M., & Che Man, Y. B. (2002). Physico-chemical characteristics of roselle (Hibiscus sabdariffa L.). *Nutrition & Food Science*, *32*(2), 68–73. http://doi.org/10.1108/00346650210416994

World Health Organization. (1996). Essential safety requirements for street-vendors foods, 1–41.

Yapp, C., & Fairman, R. (2006). Factors affecting food safety compliance within small and medium-sized enterprises: implications for regulatory and enforcement strategies. *Food Control*, *17*(1), 42–51. http://doi.org/10.1016/j.foodcont.2004.08.007

APPENDIX I

Sobolo packaged in polythene bag

Sobolo packaged in used bottles

Sobolo packaged in cups

APPENDIX II

Total Plate count (Morning)

Tukey HSD

	N	Subset for alpha = 0.05
VAR00002		
		1
Cups	4	2227925.0000

Used bottles	4	16729157.5000
polythene	4	24913957.5000
Sig.		.601

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

TPC (Late afternoon)

Tukey HSD

VAR00002	N	Subset for alpha = 0.05
		1
Cups	4	31205.8325
Used bottles	4	208438.2500
polythene	4	1181600.0000
Sig.		.453

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Total Plate count (early Afternoon)

Tukey HSD

VAR00002	N	Subset for alpha = 0.05
		1
Cups	4	9573800.0000
Used bottles	4	13927575.0000
polythene	4	88914375.0000
Sig.		.548

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Enterobacteria (Morning)

Tukey HSD

VAR00002	N	Subset for alpha = 0.05
		1
Cups	4	159769.0000
Used bottles	4	204824.2500
polythene	4	6532425.0000
Sig.		.481

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.