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SOURCES OF MICROBIAL CONTAMINATION DURING *SOBOLO* DRINK PRODUCTION

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DECLARATION

I declare that I have undertaken the study reported herein under the supervision of Dr. Francis Alemawor and that except portions where references have been duly cited, this dissertation is the outcome of my own research.

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Date **Signature**

Certified by Head of Department:

……. **Mr. John Barimah** **……..…….**

Date **Signature**

DEDICATION

I dedicate this work to God Almighty for His great mercy and provisions for my life. Without Him it is impossible. I also dedicate the success of this work to my lovely and supportive husband, Mr. Wumbilla Salifu; I thank God for your life.

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ABSTRACT

Beverages are liquid preparations specifically made for human consumption. Studies have shown that the consumption of beverages made from plants is increasing. *Sobolo*, one of these drinks which is also referred to as the Hibiscus tea, is prepared from the magenta leaves of *Hibiscus sabdariffa*. Nonetheless, regulatory institutions hardly check the quality of the drinks to ensure that it meets the Ghana Standards in terms of nutrition and microbial load and quality. Previous studies have focused on the microbial contamination of the hawked *Sobolo* drink. The study was therefore conducted to determine the microbial quality of the *Sobolo* drink and establish which stage(s) of the various unit operations involved in its preparation is/are prone to contamination. The snowball sampling method was employed in this study to locate the *Sobolo* producers. In all, twelve (12) producers were contacted for this survey. And fifty-two (52) samples were picked in triplicates across the different stages of the drink preparation processes: steeping stage, boiling stage, seizing and dilution stage and formulation stage. Overall, this study showed that the products that underwent the steeping stage was associated with a high mean microbial count (15cfu/100ml) as compared to those that did not undergo the steeping stage (9cfu/100ml) which was statistically significant (p-value = 0.021). Also, the study revealed that high microbial counts in raw water (18cfu/100ml) reduced significantly (p-value = 0.001) after boiling (6cfu/100ml), then increased slightly after sieving and dilution (7cfu/100ml), and also increased steeply after the final formulation stage (15cfu/100ml). This shows that the activities in the intermediate stages after boiling to the formulation stage increases the level of microbial contamination. It is therefore recommended that only boiled water is used to dilute the mixture if required.

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ABBREVIATIONS

ACC……Aerobic Colony Count

ADI……Adequate Daily Intake

cfu……..Colony-forming unit *E.*

coli…….*Escherichia coli*

FDA……Food and Drugs Authority

GWCL……..Ghana Water Company Limited

GWSC……Ghana Water and Sewerage Corporation

HPA……Health Protection Agency

HPC……Heterotrophic Plate Count

LDL……Low Density Lipoprotein

MPN……...Most Portable Number

RWD……..Rural Water Department

SD……..Standard Deviation

UNICEF…….United Nations International Children Fund

WHO…….World Health Organization

WSD……..Water Supply Division

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Beverages are liquids normally made for human consumption. These beverages are generally alcoholic and non-alcoholic drinks and they include juice, soft drinks, carbonated drinks, and alcoholic drinks. All the beverages have some form of water in them. Beverages can also be termed as liquid refreshment. Beverages are mostly made from fruits, malts and barley, corn, millet, sorghum and plants parts (Bedford, 2002).. According to Locke (2014), making drinks from plants and trees is the new emerging beverage trend. Examples include the coconut water, birch water, maple water and cactus water.

In Africa, many local drinks are prepared by the indigenous people for refreshment and other reasons. Some of these include the "amasi", "umqombothi" and "rooibos" by South Africa; "kunu", "zobo", "burukutu" and "adoyo" by Nigeria; and palm wine, "pito", "zomkuoom" and "asaana" in Ghana. Some local beverages are common to some countries and these include the "sorrel" drink by Guinea also known as bissap in Cote d'Ivoire, *Sobolo* in Ghana and "zobo" in Nigeria. Other common beverages include pito, "burukutu", palm wine and coconut water.

Sobolo, as it is commonly known in Ghana, is a tisane refreshing non-alcoholic drink made from the magenta coloured *calyces* of *Hibiscus sabdariffa*. *Hibiscus sabdariffa* is a herbaceous plant from the family of Malvaceae that is mainly cultivated in tropical and

subtropical areas. This local drink is known in several countries by different names; these include “Guinea sorrel” or “bissap” by the people of Senegal, “roselle” or “sorrel” by the people of Asia, “karkadé” by the people of North Africa and “flora” by the people of Jamaica in Central America (Cisse, 2010). The calyces are also known as ‘bito’ or ‘veo’ in Upper East Region of Ghana (Atoagye, 2012). The Roselle calyces are edible and have been used in the preparation of tea and fermented drinks in several countries and communities where it is found.

The *Sobolo* drink, taken either hot or cold, is widely known for its nutritional and therapeutic benefits (Amusa *et al*., 2005). The human body is made of about 70% of water and therefore requires regular intake of water to prevent dehydration. Soft drinks and other beverages are a common replacement of water when one is thirsty. The sales and rate of consumption of this locally made beverage is increasing since the drink is gaining high popularity and acceptance (Amusa *et al*., 2005; Owureku-Asare *et al*., 2014). *Sobolo* drinks, like many others locally prepared drinks, are extensively consumed by persons of all ages and religion in Ghana.

Generally, the *Sobolo* drink is prepared by boiling the calyces in water with spices to form syrup. The calyces are then removed by sieving through a colander. The syrup is then diluted with water. Ginger, sugar and fruit (pineapple is commonly used) are then added to formulate the drink to taste. The drink is then packaged in transparent polythene bags or plastic bottles. The packaged drinks are then refrigerated and sold.

Most homes in Ghana rely on pipe borne water for the day-to-day activities. However, a study has shown evidence of microbes in tap water which is contrary to the perception that

pipe borne water system is devoid of microbes (Cobbina *et al*., 2009). Since water is a major component in the preparation of the *Sobolo* drink, it may be contaminated with microbes if it is not prepared under hygienic conditions.

1.2 JUSTIFICATION OF THE STUDY

Although enough research has not been done on *Sobolo*, Owureku-Asare *et al*., (2014) observed that many people are into *Sobolo* business in Sunyani and Ghana as a whole leading to the drink having a high number of consumers. Due to the non-alcoholic nature of *Sobolo*, it has a number of consumers ranging from children to adults. Individuals involved in this business are not monitored as well as the drinks being produced.

However, a study has shown that hawked *Sobolo* drink has numerous microbes associated with it (Musah *et al*., 2014). The preparation process could account for the microbial contamination. If the drink does not meet the required standard in terms of microbial quality a lot of consumers will be affected. This could lead to an increase in sickness and mortality rate, therefore, the need to conduct this research in order to determine the microbial quality of the drink as well as the sources of the microbial contamination. This will help to take the necessary precautions in those stages during the production of the drink. Additionally, this will help reduce the microbial contamination of the final product, reduce sickness and mortality rate.

1.3 PROBLEM STATEMENT

The *Sobolo* drink is a locally manufactured drink in Ghana from *Hibiscus sabdariffa* calyces, sugar, ginger and a fruit flavouring of choice with water being the major component. Many people are into this business in the Sunyani Municipality and Ghana as a whole since the drink has a number of consumers. Additionally, Owureku-Asare *et al*., (2014) observed an increasing consumer base for the *Sobolo* drink in Ghana.

Nonetheless, regulatory institutions hardly check the quality of the drinks to ensure that it meets the Ghana Standards in terms of nutrition and microbial load and quality. Also many individuals engaged in the production of the drink do not have the requisite permission note or certificate from the FDA. The use of non-boiled water in the preparation of the *Sobolo* drink poses a potential health problem to the high number of consumers of the *Sobolo* drink within the Sunyani Municipality.

Previous studies have focused on the microbial contamination of the hawked *Sobolo* drink. The present study sought to evaluate the microbial quality of the *Sobolo* drink (in the Sunyani Municipality) and determine which stage(s) of the various unit operations involved in its preparation is/are prone to contamination.

1.4 HYPOTHESES OF THE STUDY

All the stages involved in the preparation of *Sobolo* drink contributes to its contamination.

1.5 OBJECTIVES OF THE STUDY

The main objective of this study was to determine the *Sobolo* drink preparation stages prone to microbial contamination and the consumptions characteristics of the *Sobolo* drink in the Sunyani Municipality of Ghana.

The specific objectives of the study were:

- To determine the levels of bacterial coliforms and *Escherichia coli* at each preparation stage of *Sobolo* drink produced in the Sunyani Municipality
- To assess consumption characteristics of the *Sobolo* drink among consumers in the Sunyani Municipality

CHAPTER TWO

LITERATURE REVIEW

2.1 AN OVERVIEW OF *HIBISCUS SABDARIFFA*

Three hundred varieties of the hibiscus are spread in tropical and subtropical zones across the world. *Hibiscus sabdariffa* (Roselle) is part of the family Malvacea which often survives in relatively poor soils (Cisse, 2010).

Hibiscus sabdariffa is a wood-based sub shrub, it can grow from two (2) to two and half (2.5) meters tall. The leaves are deeply three to five lobes, 8-15 cm long, arranged alternately in the stems. The flowers are 8-10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a thick fleshy calyx at the base, 1-2 cm wide, expansion of 3-3.5 cm, fleshy and bright red as a ripe fruit (Mohammed *et al*., 2011). The calyces are gathered for sale either fresh or dried.

2.2 MEDICINAL BENEFITS OF *HIBISCUS SABDARIFFA*

The human body has natural enzyme complex systems and non-enzymatic antioxidant defences which counter the damaging effects of free radicals and other oxidants. The human body's defences against free radicals can be improved through the consumption of high dietary antioxidants (Vertuani *et al*., 2004).

*Hibiscus sabdariffa (*Roselle) is reported to be used for the management and treatment of several diseases such as hypertension and urinary tract infections. It is also associated with traditional/ local medicine (Odigie *et al*., 2003).

Early works has shown that *Hibiscus sabdariffa* (Roselle) is able to relax the uterus; control indigestion (control of diarrhoea) and manage loss of appetite, colds, respiratory problems and circulation disorders. Roselle has antibacterial and anti-oxidant properties which reduces the blood pressure (hypotensive effect) and also possess antispasmodic effect. Roselle significantly reduces serum cholesterol and preventing LDL oxidation.

Roselle light is used as a laxative and digestive remedy and abscesses (Ali *et al*., 1991). Roselle is also use to prevent atherosclerosis in humans, because of its effect on antiultra-lipid and anti-oxidation of LDL. Thus, it may be in the prevention of a number of cardiovascular diseases, which plays an important role in the useful cholesterol (Lee *et al*., 2002).

2.3 NUTRITIONAL COMPOSITION OF *HIBISCUS SABDARIFFA* CALYX

Minerals, vitamins and bioactive compounds, such as polyphenols, organic acids, and phytosterols can be sourced from the Roselle plant. Some of them have antioxidant properties (Mgaya, 2014). The composition of *H. sabdariffa* calyx is highly variable with high, low and average values of the different properties of *Hibiscus sabdariffa* calyx (Cisse, 2010). The nutritional composition is shown in **Table 2.1**.

Table 2.1: Nutritional Composition of Hibiscus sabdariffa calyx

	Units	Minimum	Average	Maximum
Moisture	g.100g-1	84.5	86.3 (8)	89.5
Protein	g.100g-1	0.9	6.6 (8)	17.9
Lipid	g.100g-1	0.1	2.3 (7)	3.9

Fibres	g.100g-1	2.5	8.8 (6)	12.0
Ash	g.100g-1	4.5	5.6 (5)	6.8
Carbohydrates	g.100g-1	3.3	8.1 (4)	12.3
Malic acid	g.100g-1	0.12	1.36 (3)	2.70
Calcium	mg.100g-1	1.3	94.0 (9)	213.0
Iron	mg.100g-1	2.9	17.2 (9)	37.8
Phosphorus	mg.100g-1	40.0	191.1 (6)	312.6
Ascorbic acid	mg.100g-1	6.7	72.0 (6)	141.1
Anthocyanin	mg.100g-1	150	350 (5)	1500

( ) refers to number of values taken into account in calculating the average
(Source: Cisse, 2010)

2.4 UTILIZATION OF *HIBISCUS SABDARIFFA* IN FOOD PRODUCTION

Roselle calyces in tropical Africa and neighbouring countries are used for many purposes. Many parts of the Roselle plants, including seeds, leaves, fruits and roots are used in various food products. The fleshy red calyces are the most popular among them. The fresh calyces are used for making fruit juices, syrups, new wines, puddings, jams, ice cream, jellies, gelatines, cakes and flavours whereas the dried calyces are used to make spices, tea, sauces, butter, pies and other desserts. Roselle tender stems/stalks and leaves are either consumed raw in salads or cooked alone as vegetable soups/ stews or in combination with meat and other vegetables (Qi *et al*., 2005; Atoagye, 2012).

The calyces are also gathered for sale, either fresh or dried. Dried vials are used in Europe to flavour extracts for liqueurs. A drink is also produced from calyces' infusion called

"Zobo" in Nigeria, "Bissap" or *Sobolo* in Ghana, which is used for refreshments and entertainment at home and public meetings.

2.5 ACCESS TO PORTABLE DRINKING WATER

Conferring to the World Health Organization (WHO) in 2015, access to improved drinking water sources was associated with 91 percent of the world population whereas
76 percent in 1990 had access to improved drinking water sources (Gorchev and Ozolins, 2011a). Globally, a minimum of 180 billion people are accessing drinking water from a contaminated faecal source (Gorchev & Ozolins, 2011a). Diseases such as typhoid, diarrhoea, polio, cholera and dysentery can be transmitted through contaminated water. The ingestion of contaminated drinking water has been predicted to be the cause of about 502,000 diarrheal deaths annually. Ghana Health Service 2009 annual report, cases of diarrhoea in hospitalized children under 5 years, pneumonia the third cause of most medical centres, is the fourth cause of death of under five years children. WHO further estimates that by 2025, about half (50%) the world's population will have limited access to portable water and will be in water-stressed conditions.

Initiation of development of the public supply of water in Ghana was done in Cape Coast, 1928. From 1928 to 1965, the Department of Public Works of the water distribution was in charge of public water supply. Supply of public water was initiated by the Hydraulics

Department of the Department of Public Works (PWD). They were responsible for water supply in urban and rural areas.

Rural Water Department (RWD) was established in 1948, to take charge for rural water supply. After independence in 1957, Water Supply Division (WSD) was established under the ministry of Works and Housing to be responsible for the supply of water to both urban and rural areas. Works and Housing Ministry is responsible for procurement responsibilities in urban and rural water (GWLC, 2013).

Severe water shortages in 1959 prompted the Ghana Government to call the World Health Organization (WHO) to assess the situation of water supply in Ghana. The results steered the creation of the Ghana Water and Sewerage Corporation (GWSC), a subset of the Water Supply Division (WSD). Ghana Water and Sewerage Corporation (GWSC) therefore was founded in 1965 as enshrined in Act 310, to provide, distribute and conserve both urban and rural supply of water in Ghana for optimal use by the public, domestic and industrial institutions. The GWSC was also mandated to establish, operate and control sewerage systems. Ghana Water and Sewerage Company in accordance with statutory law, 1993 (Act 461) amendments are converted into a limited liability company and now referred to as the Ghana Water Company Limited (GWCL). For the purpose of this study, water sourced from GWCL is referred to as municipal water and tap water interchangeably (GWLC, 2013).

According to a survey conducted by Mohammed (2012) on the Water quality deterioration in piped water and its effect on usage and customers perception, it showed that about 4 out of 10 Ghanaian households only within the piped areas of the urban sectors are GWCL customers (Mohammed, 2012).

2.6 TAP WATER

Tap water also known as running water, city water or municipal water is water supplied through a valve or faucet (Wikipedia, 2015). Its uses are numerous and include washing, drinking, cooking, and the flushing of toilets. Tap water is required to be of high quality, non-contaminated and safe. Tap water is known specifically in our nation as pipe borne water from Ghana Water Company Limited (GWCL). This is the commonest source of portable water in Ghana.

Ofosu *et al*., (2014) indicates that the urban population in Ghana having access to improved water supply reduced from 86% in 1990 slightly to 79% in 2006 and further down to 59% in 2009. About 60% of households in the Sunyani Municipality have access to treated piped water from the Ghana Water Company Limited. Out of this, 48% have a pipe connection to their households whereas the remaining 52% obtained water from either public or private commercial standpipes. However, less than 30% of people with a pipe connection in their homes have water supply every day.

2.7 PIPE SYSTEM AND ITS DEFICIENCIES

Water supply systems generally include source water, transfer or conveyance of drinking water to the community and this is referred to as drinking water distribution system in the pipeline transportation with accessories such as (valves, hydrants, meters, tanks). The network is expected to transport water at a satisfactory pressure in the desired quantity to the consumers in the utility service area.

According to Mohammed (2012), the water is sourced from either a well or surface water by relevant conventional treatment methods. He further describes water distribution system as a system comprised of large diameter water transmission pipelines.

Mohammed (2012) further explains that the distribution network comprises of small to medium sized pipes that are normally located alongside the streets so residents can easily tap the service line. The engineering features (hydraulic and engineering design) of water supply distribution system targets the following:

- Production of water in acceptable quantities and quality to meet the needs of the population.
- Adequate pressures and velocity within the network.
- The appropriate engineering design that would guarantee the access to potable water and efficiency in transporting water to the community.
- Storage facilities are designed and located in a manner that would ensure access to water even in times of emergencies

However, Cobbina *et al*., (2009) assessed the quality of municipal water supplied to the western part of Accra by the Weija head works. Out of 135 samples analysed total coliform bacteria was detected in three-quarters of the samples and faecal coliforms were detected in one quarter of the samples.

2.8 WATER INTAKE

Water is the largest component of the human body and is crucial for cellular homeostasis and good health. Water intake includes potable water, beverage and water forming part of

the food. An adequate daily intake (ADI) for the total water intake is set to stop damaging effects, such as acute dehydration, metabolic and functional abnormalities (Gorchev & Ozolins, 2011; Popkin & Rosenberg, 2011).

Plasma osmolality is the primary measure of hydration status. Because hydration is kept within the normal inlets, (water, drinks and food as a combination) of the total water consumption data for the ADI, is largely defined by the American research on median total water intake. ADI for total water intake for young men aged between nineteen (19) years to thirty (30) years is 3.7 L and that for young women aged between nineteen (19) years to thirty (30) years is 2.7 L per day (Gorchev *et al*., 2011).

According to the U.S. survey (USDA, 2015), fluids (drinking water and beverages) consumed delivered 3.0 L (101 fluid oz.; 13 cups) and 2.2 L (74 fluid oz.; ≈ 9 cups) per day for nineteen (19) years to thirty (30) year-old men and women, respectively. This is about 81 per cent of total water intake in the U.S. survey. Food consumed contained nearly nineteen (19) per cent of total water intake. [Conversion factors: 1 L = 33.8 fluid oz.; 1 L = 1.06 qtr. 1 cup = 8 fluid oz.].

2.9 DRINKING WATER QUALITY AT SOURCE AND AT POINT-OF-USE

The safety of drinking water at source and point-of-use is essential in communities worldwide where drinking water is sourced from communal supplies. The microbial quality of drinking water at source could be high but the modes of transportation and distribution of water could promote development of biofilms and pose contamination risks at the point-of-use. At the point-of-use, one may store drinking water temporary to use; this storage

practices coupled with poor sanitation practices may introduce and promote habitation of microbes (UNICEF, 2015).

2.10 MICROBIAL CONTAMINATION

Coliforms biofilm can grow or regenerate in the distribution system. Age, low residual disinfectant, warm climate, relatively high levels of total organic carbon, the old iron pipe, and inadequate washing of the dead ends contribute to the growth of the biofilm, sometimes bacteria, including *E. coli*, which it is released in the water. Biofilm may support the regrowth of virulent bacteria, occurring in a factory treatment failure

(Payment, 1999).

Typically, the microorganisms' grow on the water contact surface as biofilms and water. Growth following treatment of drinking water is often called "regeneration" or "regrowth". The growth reflects generally higher water samples measuring different bacteria plate count (HPC) values. High levels of HPC systems occur mostly in the distribution pipeline, the pipeline in the country, in the portion in water bottle and stagnant connection pipes in the apparatus, such as softeners, carbon filters (Bartram *et al*., 2004). Contamination by microorganisms may also happen through wrongly installed and/ or through concealed leaks in the piped water system.

In a research conducted by Amusa *et al*., (2005), the calyces of *H. sabdariffa* used were found to harbour *Aspergillums Niger, A. flatus, A. tamari, Penicillium oxalicum, Fusarium oxysporum* and *Rhizopus* spp. whereas the hawked *Sobolo* drinks harbored *Bacillus cereus, B. subtilis, Proteus* spp., *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli;* and the freshly processed *Sobolo* drinks harbored no bacteria.

In a related research Musah *et al*., (2014), found seven different isolates in hawked *Sobolo* drinks, namely *Aspergillums sp., Bacillus sp., Klebsiella sp., Pseudomonas sp., Streptococcus sp., Staphylococcus aureus and Escherichia coli.*

2.11 MICROBIOLOGICAL INDICATORS IN DRINKING WATER CONTROL

Enteric microorganisms exist in different types numbering hundreds and these are known to infect microbes in the human gut. Enteric organisms excreted in the infected human's or animal's faeces, can directly or indirectly contaminate water for human consumption (Edberg *et al*., 2000). Due to drinking water disinfection practices, the incidence of waterborne diseases has been greatly reduced. Indicator organisms are used for various purposes and these include as an indicator for:

- faecal contamination in surveillance monitoring and verification;
- the validation efficiency of filtration or disinfection;
- operational monitoring of integrity and cleanliness of distribution systems (Ashbolt *et al*., 2001) .

The notion of using organisms such as *E. coli* as indicators of faecal pollution is a wellestablished practice in the assessment of drinking-water quality. The faecal indicators that are used should not be pathogens themselves and they should:

- be present universally in faeces of humans and animals in huge numbers;
- not increase in natural waters;
- persist in water in a similar manner to faecal pathogens;
- be present in higher numbers than faecal pathogens;

- respond to treatment processes in a similar fashion to faecal pathogens; be readily detected by simple, inexpensive culture methods.

The above stated criteria reflect an assumption that one particular (same) organism may well be used as an indicator of both treatment/process efficacy and faecal pollution. However, the table below shows that one indicator cannot appropriately fulfil these two roles and that a variety of organisms ought to be considered for different purposes (**Table 2.2**).

Heterotrophic bacteria may be used as indicators of cleaning disinfection, operating efficiency and distribution system; efficacy of treatment can be done by use of *Clostridium perfringens* and coliphage (Odonkor & Ampofo, 2013). Traditionally, *Escherichia coli* have always been used to monitor the quality of drinking water; this part remains an essential monitoring activity conducted as part of the monitoring or verification parameter. Thermo tolerant coliforms can be used as an alternative to testing E. *coli* in the circumstances. Water meant for human consumption must be free from faecal indicator organisms. However, a high degree of safety is anticipated because monitoring of *E. coli* or thermo tolerant coliform bacteria provides a because of its large number in contaminated waters (Odonkor & Ampofo, 2013).

Table 2 2. 2 Use of indicator organisms in monitoring

Microorganism(s)	Type of monitoring	Validation of process Operational	Verification and surveillance
E. coli (or thermotolerant coliforms)	Not applicable	Not applicable	Faecal indicator
Total coliforms	Not applicable	Indicator for cleanliness and integrity of distribution systems	Not applicable

Heterotrophic plate counts	Indicator for effectiveness of disinfection of bacteria	Indicator for effectiveness of disinfection processes and cleanliness and integrity of distribution systems	Not applicable
Clostridium perfringens	Indicator for effectiveness of disinfection and physical removal processes for viruses and protozoa	Not applicable	Not applicable
Coliphages *Bacteroides fragilis* phages Enteric viruses	Indicator for effectiveness of disinfection and physical removal processes for viruses	Not applicable	Not applicable

(Source: WHO, 2011)

2.11.1 Total Coliform Bacteria

General Description

Total coliform bacteria includes a wide variety of Gram-negative, aerobic and facultative anaerobic, non-spore forming bacilli capable of growing in the presence offers relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 hours at 35–37 °C. The group of coliform bacteria is more heterogeneous and includes a wider variety of genera, such as *Hafnia* and *Serratia*. This is contrary to the traditional belief that coliform bacteria belonged to the genera *Enterobacter, Escherichia, Klebsiella* and *Citrobacter*. *Escherichia coli* are thermo tolerant coliforms, which can ferment lactose at higher temperatures, are a subset of the total coliform group. Enzyme β-galactosidase is produced by total coliforms as part of lactose fermentation process. The total coliform group therefore includes both environmental and faecal species (WHO, 2011).

Value of Indicator

Total coliforms include the survival and growth of aquatic organisms. Therefore, they are not used as indicators of faecal pathogens, but they can be used to assess the possible cleanliness and integrity of distribution systems and biofilm. But, there exist an advanced indicator for these purposes. Total coliform bacteria have been recommended as a sterilization indicator. This notwithstanding, the total coliform test is much slower and undependable direct measurement of residual disinfectant. Additionally, the ratio of total coliforms is extremely sensitive to sterilization as compared to enteric viruses and sensitive protozoa. HPC readings identify a bigger span of microorganisms; it is routinely viewed as a better pointer of the quality and cleanliness (WHO, 2011).

Source and Occurrence

With the exception of *E. coli*, in both sewage and natural waters, total coliform bacteria can be found in them. Most of the coliforms are heterotrophic and increase steadily in water and soil environments. A number of these bacteria can be traced to human and animal excreta. The availability of biofilms is conducive environment for the survival, growth and distribution of total coliforms (WHO, 2011).

Application in Practice

A 100 ml sample of water is typically used to measure the total coliforms. A couple of comparatively not difficult ways are in existence on how to produce on acid from lactose or to produce β-galactosidase from the enzyme. The methods involve membrane filtration subsequently by the incubation of the membranes on selective media at 35–37 °C and counting of colonies after 24 hours. The most probable number procedures using tubes or

microtitre plates and presence/absence test is another method to carry out this test (WHO, 2011).

Significance in Drinking-Water

It is expected that once disinfection is carried out, total coliforms should not be detected; however the presence of total coliform is a pointer that the treatment was insufficient. The expose of regrowth and possibly biofilm formation or contamination through ingress of foreign material, including soil or plan is a manifestation of the existence of total coliforms in distribution systems and stored water supplies (WHO, 2011).

2.11.2 Escherichia Coli and Thermo Tolerant Coliform Bacteria

General Description

The total coliforms that have the capacity to cause the fermentation of lactose at 44–45 °C are referred to as thermo tolerant coliforms. The abundant genus in most water is *Escherichia*, however, the *Citrobacter*, *Klebsiella* and *Enterobacter* are some kinds that are also referred to as thermo tolerant (Feng *et al*., 2002). The differentiating feature of *Escherichia coli* from other thermo tolerant coliforms is its capacity to produce indole from tryptophan or by the production of the enzyme β-glucuronidase. *Escherichia coli* is found in extremely high quantities in human and animal faces and is seldomly found in the absence of faecal pollution, although there is some proof of its growth in tropical soils. Besides *E. coli* other thermo tolerant coliform species can include environmental organisms (WHO, 2011).

Value of Indicator

The most appropriate pointer of faecal contamination is the presence of *Escherichia coli*. Invariably, in majority of situations, within the populations of thermo tolerant coliforms, the most common is the *E. coli*; therefore, this group is viewed as not been dependable but acceptable indicator of faecal pollution. For thermo tolerant coliforms, the *Escherichia col*i is the first organism of choice in monitoring programmers for verification, including surveillance of drinking-water quality. In addition, these organisms serve as disinfection indicators, but testing is very slow and less dependable than direct measurement of disinfectant residual. Furthermore, *E. coli* is considered comparatively very sensitive to disinfection than are enteric viruses and protozoa (WHO, 2011).

Source and Occurrence

Escherichia coli is prevalent in high quantities in human and animal faces, sewage and water polluted with faecal material. An environment which is likely not to promote the growth of these organisms is water temperatures and nutrient condition existent in drinking water and water distribution system (WHO, 2011).

Application in Practice

Escherichia coli are generally measured in 100 ml samples of water. Simple procedures exist to show the presence of *E. coli* based on the production of the enzyme βglucuronidase or the production of acid and gas from lactose. These procedures include membrane filtration and the most probable method. The membrane filtration when done is followed by incubation of the membranes on selective media at 44–45 °C and counting of colonies

after 24 hours whereas most probable number procedures is conducted using tubes or microtitre plates for presence/absence tests (WHO, 2011).

Significance in Drinking-Water

Faecal contamination is proof of the existence of *E. coli* (or, alternatively, thermo tolerant coliforms), and its subsequent detection should consequently serve as a basis for the consideration of further action, which could include further sampling (WHO, 2011).

2.11.3 Heterotrophic plate counts (HPC)

General Description

HPC measurement notices a broad range of heterotrophic microorganisms, including bacteria and fungi. This is made easy based on the capacity of the microorganisms to grow on growth media that is enriched, without selective or inhibitory agents, over a specified incubation period and at a defined temperature. The range of organisms identified by HPC testing includes organisms that are resistant to disinfection, such as the spore formers; microorganisms that are sensitive to disinfection processes, including coliform bacteria; and microorganisms that quickly multiply in treated water which lack residual disinfectants (Stephen *et al*., 2004; WHO, 2002).

Depending on the method and conditions applied, the tests will identify/ notice only a little portion of the microorganisms present in the water. The population recovered will differ

based on the test method and conditions applied. There is no single universal HPC measurement even though standard methods have been developed. A broad spectrum of media is accessible; incubation temperatures used per the test method may vary from 20 °C to 37 °C and the incubation times varies from a few hours to 7 days or more. The actual microorganisms the HPC testing recovers can also vary widely between seasons, between locations and between consecutive samples at a single location (Bartram *et al*., 2004).

Value of Indicator

As an indicator of the presence of pathogen, the test has minimal value but can be convenient in operational monitoring. Where the purpose is to keep numbers as low as possible, the HPC test can be used as a treatment and disinfectant indicator. In addition, HPC measurement can be useful in evaluating the integrity and cleanliness of distribution systems as well as the presence of biofilms (WHO, 2011).

Source and Occurrence

Heterotrophic microorganisms include (generally harmless) natural micro-organisms present in the water between the biological environment and biological flora members of locations within HPC test detection range by the large difference between successive samples. Some treatment methods for drinking water such as coagulation and sedimentation reduce the number of HPC aquatic organisms. However, other methods of treatment support biological proliferation, such as activated carbon and a biological sand filtration. There is significant reduction of organisms by chlorination, ozone and ultraviolet radiation (Pepper *et al*., 2004).

However, without the water sterilization process, under appropriate conditions no residual, disinfectant HPC organisms can grow quickly. HPC organisms in water, with water as a contact surface can increase and develop biofilms. Growth or "regeneration" of the main determining factor is the availability of temperature, nutrients, including assimilable organic carbon, lack of disinfectant and residual stagnation (WHO, 2011).

Application in Practice

Based on the procedure used, results on simple aerobically incubated agar plates are obtainable within hours to days of conducting the test.

Significance in Drinking-Water

After disinfection, for most users of HPC test results, quantities of microorganisms are likely to be low. However, actual results are of less value compared to changes in numbers at particular locations. In distribution systems, increasing numbers can point to deterioration in cleanliness, possible stagnation and the potential development of biofilms (WHO, 2011). Microorganisms that remain after HPC tests usually are those that are of natural (typically non-hazardous) microbiota of water (Bartram, 2013).

2.11.4 pH

The pH of the water is an indicator of acidic or basic conditions. pH ratio of the value in the range of 0-14; 7 shows a neutral point. The normal range is 6.5 to 8.5 pH of drinking water. pH is largely the result type in the field of local natural mineral and geological conditions found in the rock. This can also affect the pH of acid rain. The pH value of water

of less than 7 is acidic and tends to corrosion. Acidified water (low pH) from the pipe system may lead to leaking of the metal. Leach (lead pipe brass copper or lead) can also cause health problems. pH water of greater than 7 indicates alkalinity, and tends to affect the taste of water (Mccaffrey, 1997).

The taste of alkaline drinking water may be similar to "soda". The result of natural geological conditions at the site and the type of mineral found in the local rock. The pH also can be affected by acid rain. Water with a pH value below 7 is acidic and tends to be corrosive. The acidic water (low pH) can leach metals from piping systems, which can cause leaking pipes. Leach metals pipes (lead from lead pipes or copper pipes copper) can also cause health problems. Water with a pH value greater than 7 indicates alkalinity and tend to affect the taste of water. Alkaline drinking water may taste like "soda" (Hoehl *et al*., 2010; Koseki *et al*., 2007).

2.12 MICROBIOLOGICAL GUIDELINES FOR READY-TO-EAT FOODS

Ready-to-eat foods are foods that have been partly or completely processed that upon purchase no further processing is done to it before consumption (Health Protection Agency, 2009).

The Centre for Food Safety, Food and Environmental Hygiene Department, Hong Kong has amended the 2002 Food Standards Code by the Australian New Zealand. This amendment was done by a multidisciplinary expert committee. The standards were set on Microbiological Criteria for Ready-to-eat Food in General, Aerobic Colony Count (ACC) and Hygiene Indicator Organisms for ready-to-eat foods, Specific Foodborne Pathogens and Microbiological Criteria for Specific Food Items (Gruber *et al*, 2003).

Table 2.3: Guidance on the interpretation of results for Aerobic Colony Count levels [30°C/48 hours] in various ready-to-eat foods (Results are in cfu/ml)

Food Category	Examples	Satisfactory	Borderline	Unsatisfactory
Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham requiring refrigeration, pasteurised foods including fruit juice and soups; desserts	$<10^4$	$-<10^7$	$\geq 10^7$

(Source: Centre for Food Safety, 2014)

According to the classification, *Sobolo* drink can be categorized under section 3 which has a satisfactory Aerobic Colony Count (ACC) limit of $<10^4$ cfu/ml.

Additionally, the Ghana Standards Authority has developed standards for Microbiological analysis of foods-sampling and microbiological criteria, GS 955:2013 2nd Edition under which *Sobolo* drink is categorized. The details are as follows:

Parameter	**Limit**
Aerobic Plate Count (APC)	$<1.0 \times 10^3$
Coliforms	$<1.0 \times 10^2$
Yeast/ Moulds	$<15 \times 10^1$

(GSA, 2013)

2.13 SOFT DRINKS INTAKE

Beverages, especially the non-alcoholic beverages, are the best replacement for water when one is thirsty. Some people even consume soft drinks not only because of thirst, but also when one is hungry and as an after meal dessert. Soft drinks are also served at all types of functions and events such as social gatherings, amongst others; at market places, school premises and lorry stations (Amusa *et al*., 2005).

A survey by the US Department of Agriculture in 2004 showed that soft drink consumption is increasing. It noted that consumption between 1947 and 2001, per capita soft drink more than tripled while consumption of milk beverages decreased by almost half. In 1947, Americans consumed nearly 11 gallons of soft drinks milk drinks 40 gallons. In 2001, per capita consumption of milk has fallen to 22 gallons, and consumption of soft drinks has increased to 49 gallons (Harnack *et al*., 1999; Vartanian *et al*., 2007).

Soft drinks are in various kinds and these include those of foreign and local origin. Soft drinks of foreign origin include Cola cola, Pepsi, Don Simon fruit juice, etc. Local beverages consumed in Ghana include asana or maize beer, *Sobolo* drink, *akpeteshie*, coconut juice, palm wine, *zomkoum* or toasted millet flour in water, pito - a locally brewed beer made from millet and fula mashed in water, milk, ginger and sugar (Ghanaian Food, 2015).

The local drinks identified in Ghana are most predominantly consumed in specific communities such as *zomkoum* in the northern sector and coconut juice in the western region. However, *Sobolo* drink is a universal drink and is consumed within almost every community.

2.14 *SOBOLO* DRINK

Sobolo drink, also referred to as Hibiscus tea is a tisane refreshing drink from magenta coloured leaves of Roselle, scientifically known as *Hibiscus sabdariffa* from the family Malvaceae. *Hibiscus sabdariffa* L. is an herbaceous plant, grown commonly in tropical and subtropical areas. Depending on the area it is found, it has different names. For instance, it

is called "*sorrel*" in Guinea or "*bissap*" in Senegal, "*roselle*" or "*sorrel*" in Asia, "*karkadé*" in North Africa, and "*flora*" of Jamaica in Central America, "*zobo*" and "*soborodo*" in Nigeria and *Sobolo* in Ghana (Amusa *et al*., 2005; Cisse, 2010). In many cultures, the Hibiscus tea due to its nutritional and therapeutic properties is viewed as a health drink (Amusa *et al*., 2005; Da-Costa-Rocha *et al*., 2014).

Following a survey carried out by Owureku-Asare *et al*., (2014) on consumer perceptions, knowledge and consumption patterns. It was disclosed that 81% of respondents purchase the *Sobolo* drink out of which 50.9% purchase from street vendors and hawkers.

Plate 2.1: Fresh calyces
(Source: Inside Journeys, 2013)

Plate 2.2: *Sobolo* drink
(Source: Suntemple food, 2014)

2.15 PREPARATION OF *SOBOLO* DRINK

Hibiscus sabdariffa is grown predominantly for its calyx even though the fresh stalks/ stems and leaves can also be used in the preparation of sauces and soups. The traditional processing activities of the calyces constitute mainly for the production of jam, concentrates and mainly of drinks or beverages (Cisse *et al*., 2009).

The drink is generally prepared by aqueous extraction of phytochemicals, colour and aroma from a solid-to-solvent ratio. The extraction operation is carried out at the temperatures of

25–100 °C. After extracting the aqueous solution, filtration is carried out, sugar and other additives, artificial flavourings and fruit juices or fruit pieces (pineapple, strawberry and ginger) may be added (Cisse, 2010).

2.15.1 Extraction

Extraction is an approach to separate a preferred element from an object when it is mixed with other substances. A suitable solvent is brought into contact with the mixture. The solvent should be one that the element of interest is soluble in and insoluble to the other substances present within. Extraction includes Liquid-liquid phase *extraction*, and Solidliquid phase *extraction* (Stafilov *et al*, 2006; Wells, 2003).

During preparation some producers use cold water for extraction whereas others extract in hot water during boiling. A study on optimization of hot water extraction and sweetness level in beverage production by Bolade *et al*., (2009) revealed that extraction period of 30 minutes at a constant temperature of 100±2 °C is the best stage of extraction. Phytochemicals that are extracted include flavonoids, alkaloids, anthocyanins, steroids, saponins, sterols and tannins are present in petals of the *H. sabdariff*a (Dai & Mumper, 2010; Obouayeba *et al*., 2014).

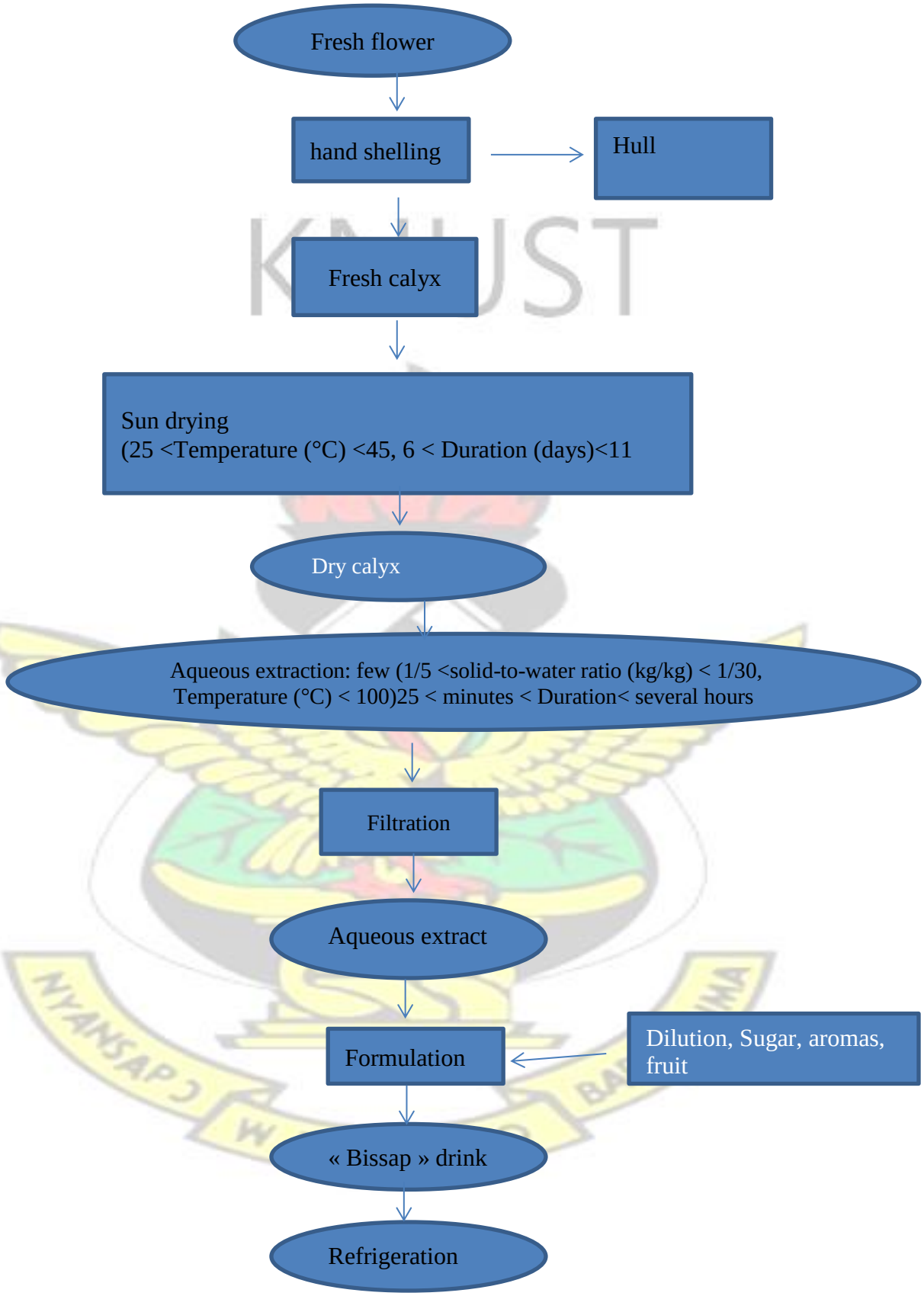
Fresh flower
hand shelling
Hull
Fresh calyx
Sun drying
(25 <Temperature (°C) <45, 6 < Duration (days)<11
Dry calyx
Aqueous extraction: few (1/5 <solid-to-water ratio (kg/kg) < 1/30,
Temperature (°C) < 100)25 < minutes < Duration< several hours
Filtration
Aqueous extract
Formulation
Dilution, Sugar, aromas, fruit
« Bissap » drink
Refrigeration

Figure 2.1: Flow diagram for the traditional processing of *Hibiscus sabdariffa* drink in Senegal (Source: Cisse, 2010)

CHAPTER THREE

METHODOLOGY

3.1 STUDY DESIGN

The cross-sectional study design was employed in this study. Sampling units were the producer's raw water (i.e. tap water, mechanized borehole water or hand dug well), steeped calyces in water, freshly boiled *Sobolo* syrup before dilution, freshly diluted *Sobolo* drink and *Sobolo* drink that has been formulated with flavourings (i.e. pineapple, ginger, sugar). The samples were collected in triplicates and transported on ice to the laboratory for analyses within three hours of collection.

3.2 STUDY SITE

The study was carried out in the Sunyani Municipality of the Brong Ahafo Region of Ghana. The Sunyani Municipal Assembly covers a total land area of 506.7 km^2. It is located at the heart of Brong Ahafo region lying between latitudes 7^0 20' N and 7^0 05'N and longitudes 2^0 30'W and 2^0 10'W. It is bordered on the north by Sunyani West District; west by Dormaa East District; Asutifi District to the South and Tano North District to the east. The Municipality falls within the wet Semi-Equatorial climatic zone of Ghana. According to the 2010 Housing and Population Census, the population of the Sunyani Municipality stands at 123,224 (Ghana Statistical Service, 2014).

3.3 STUDY POPULATION AND SAMPLE SIZE

All producers of the *Sobolo* drink with the Sunyani Municipality, between the ages of 18 and 60 years, were considered and included in the study. A total number of Twelve (12) participants were recruited for this study from the study population. In all, fifty-two (52) samples were picked in triplicates across the different stages of the drink preparation processes: steeping stage, boiling stage, sieving and dilution stage and formulation stage, for laboratory analysis. Also, three hundred and eight three (383) *Sobolo* consumers were recruited for a consumer survey.

3.4 SELECTION OF PARTICIPANTS

The snowball sampling method was used in the recruitment of the participants for this study. This technique was employed because it is useful for this study design. The producers were contacted and those who were prepared to participate in this study were recruited.

3.5 DATA COLLECTION

Questionnaires were used in the collection of data from the consumers of the *Sobolo* drink (Appendix 1). The selection of consumers was done by the purposive sampling technique. Microbial data on presence of coliforms were obtained through laboratory analysis of samples picked along different stages of the *Sobolo* drink preparation process.

3.6 MICROBIOLOGICAL TESTS

3.6.1 Total and Faecal coliform

The Most Probable Number (MPN) method was used to determine total and faecal coliforms in the samples. MacConkey Broth and Brilliant Green Bile 2% broth media were used for total coliforms and faecal coliforms respectively. Standard laboratory procedures were employed.

Prior to sterilization, glassware to be used in the analysis were washed thoroughly with deionized water and allowed to dry. This was then sterilized in a hot oven at 160°C for at least 3 hours (Harrigan & McCance, 1976). Instruments such as loops, forceps and spoons were sterilized by flaming directly after dipping in spirit. These media and chemicals were used to detect and enumerate different types of microorganisms according to Harrigan (1998). The total viable count of bacteria was carried out by using the pour plate count method.

Procedure for Total Coliform Bacteria

MacConkey broth (35g) was suspended in 1L of distilled water and heated until completely dissolved. 10mls of the media was dispensed into MacCantey bottles with inverted duraham tubes and was then autoclaved at 121^{o}C for 15 minutes with Automatic autoclave LS-2D. Then 10mls of the samples picked from survey sampling units were added to the media (in 5 replicates) and subsequently incubated at 37^{o}C for 24 hours with Gallenkamp Economy Incubator with fan, size 2. Eventually the presence of gas and colour change close to yellow was used as indicator for the presence of Total Coliform Bacteria. Counts per 100 milliliters were calculated from the Most Probable Number tables (HACH, 2015).

Procedure for Faecal Coliform

An amount of 40 g of Brilliant Green Bile 2% broth was suspended in 1L of distilled water and allowed to soak for 10 minutes and then swirled to mix. 10mls of the media was dispensed into MacCantey bottles with inverted duraham tubes and was then autoclaved at 115 ^{o}C for 15 minutes with Automatic autoclave LS-2D. Then 10mls of the samples picked from survey sampling units were added to the media (in 5 replicates) and subsequently incubated at 44 ^{o}C for 48 hours with Gallenkamp Economy Incubator with fan, size 2. Following this, the presence of gas was used as indicator to show presence of Faecal coliform. Counts per 100 millilitres were then calculated from the Most Probable Number tables (HACH, 2015).

3.6.2 *Escherichia coli* (Thermo tolerant Coliforms)

Media used was Peptone water. Instruments used included autoclave and incubator.

Peptone water (15 g) was dispersed in 1L of distilled water and allowed to soak for 10minutes and then swirled to mix. 10mls of the media was dispensed into MacCantey bottles and was then autoclaved at 121^{o}C for 15 minutes with Automatic autoclave LS2D. Then 10mls of the samples picked from survey sampling units were added to the media (in 5 replicates) and subsequently incubated at 44^{o}C for 48 hours with Gallenkamp Economy Incubator with fan, size 2. Later a drop of Kovacs reagent was added to the sample. The development of brown ring colour was used as indicator to denote the presence of Thermo tolerant coliform (*Escherichia coli*) (HACH, 2015).

3.7 DATA ANALYSIS

The data was entered on Microsoft Excel and further analysed using the SPSS (Statistical Package for Social Scientist) Statistical Software (v.19.0 of SPSS Inc., Chicago, IL).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 EVIDENCE OF COLIFORM CONTAMINATION FROM THE INDIVIDUAL *SOBOLO* PRODUCERS

The purpose of this study was to determine the stages of microbial contamination in the production process of the *Sobolo* drink, and also to assess the consumption characteristics

of consumers in the Sunyani Municipality. Twelve (12) *Sobolo* producers were selected based on the snowball sampling methodology for the study, and liquid samples were collected in triplicates across the different stages of the drink preparation. Also, the raw water used in the preparation was sampled. Therefore, a total of one hundred and fifty six (156) samples were collected from the producers for analysis.

In summary, the *Sobolo* drink is mainly prepared by first steeping the calyces, followed by boiling the steeped calyces with additives (spices), sieving the boiled mixture and diluting with water to obtain an extract, and formulating the extract with sugar and flavours. But, the initial steeping stage can be skipped to obtain the same end product. A study has shown that the boiling of the calyces at a temperature of 100 °C could also serve the same purpose of the steeping stage and with low microbial count (Bolade *et al*., 2009). Hence, most of the producers skipped the steeping stage, with the exception of producers 3, 4, 5 and 7 (**Table 4.1**).

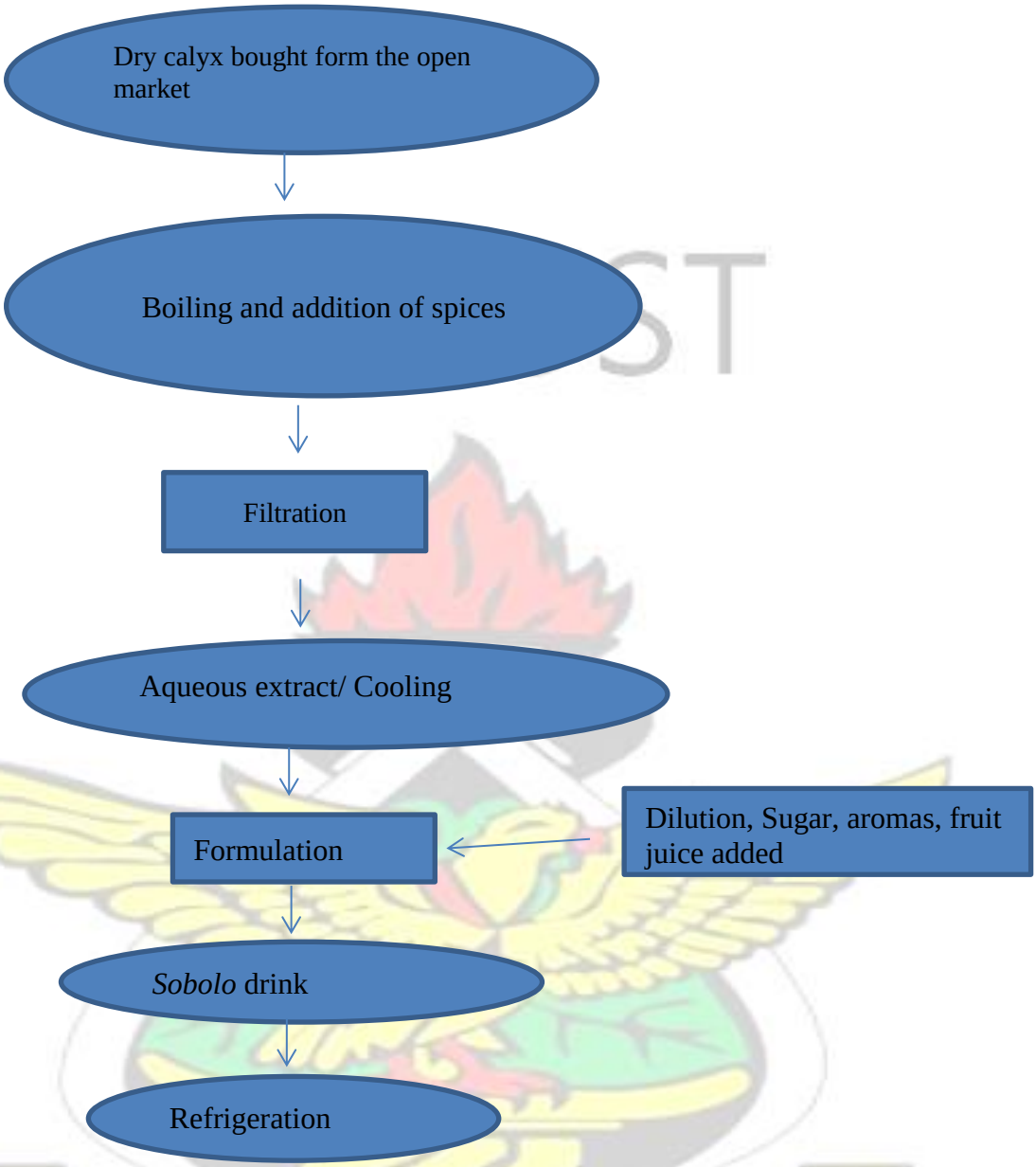

Figure 4.1: General preparation process of *Sobolo* drink practiced in Sunyani Municipality (Source: Survey)

There was evidence of coliform contamination in most of the samples collected from the producers as shown in **Table 4.1**. The samples from producer 4 recorded the highest presence of coliform contamination across all the preparation stages of the *Sobolo* drink and also from the water used in the preparation whereas producer 12 recorded the least

presence of coliform contamination (**Table 4.1**). These microbes are suspected to have been present in the environment in which the drink was prepared, on the materials and tools used in the preparation of the drink and possibly from the individual producers since no defined measures were taken to prevent contaminating the drink by the producers.

Producer 2 recorded the highest mean coliform bacteria count (17±5.23cfu/100ml) with producers 7 and 12 recording no coliform bacteria count (**Table 4.1**) from the water used in the preparation of the *Sobolo* drink. Likewise, producer 3 recorded the highest mean faecal coliform count (5±0.87cfu/100ml) whereas producers 7 and 12 recorded no faecal coliform count as shown in **Table 4.2**. The difference in the microbial load of the water from the individual producers could be due to the difference in their water sources as well as how the water is being stored. The contamination could also come from the tools and equipment used at this stage.

With regards to the producers who used the steeping stage in their preparation, producer 4 recorded the highest mean coliform bacteria count (17±8.24cfu/100ml) while producer 3 recorded no coliform bacteria count (**Table 4.1**). Similarly, producer 4 recorded the highest mean faecal coliform count (5±0.87cfu/100ml) whereas producers 3 and 5 recorded no faecal coliform count (**Table 4.2**). Producer 3 recorded the highest mean coliform bacteria count (9±4.04cfu/100ml) in the liquid samples collected form the boiled calyces and additives whereas producers 2, 9 and 11 recorded no total coliform bacteria count (**Table 4.1**). Also, producer 4 recorded the highest mean faecal coliform count (2±1.85cfu/100ml) with producers 2, 9, 11 and 12 recording no faecal coliform count as shown in **Table 4.2**. The contamination could be due to the substrates and tools and materials used. Even though

producer 7 recorded no coliform bacteria count and faecal coliform count in the raw water used, coliform bacteria and faecal coliform were recorded during the steeping/ soaking stage and all the subsequent stages. This may be attributed to the calyces purchased from the open market as revealed by Atoagye in 2012 that dry Roselle leaves sold on the market are high in total coliform (Atoagye, 2012).

Table 4.1: Mean coliform bacteria count (cfu/100ml) from the individual *Sobolo* producers

Producer	Water	Steeped calyces	Boiled calyces and additives	Sieved and diluted mixture	Formulated drink
1	15±6.18	*	9±1.16	13±3.46	15±1.73
2	17±5.23	*	0.00	0.00	4±5.77
3	16±4.39	0.00	9±4.04	10±1.16	11±2.52
4	9±3.12	17±8.24	8±8.00	8±1.73	5±4.04
5	8±8.09	3±4.62	6±4.62	13±1.73	14±3.12
6	7±2.89	*	4±5.77	12±4.16	9±6.23
7	0.00	7±6.56	7±5.29	7±5.29	13±4.04
8	14±3.46	*	8±1.73	8±1.73	8±6.81
9	17±4.23	*	0.00	0.00	14±4.62
10	3±4.62	*	3±4.62	0.00	3±4.62
11	11±9.24	*	0.00	0.00	0.00
12	0.00	*	3±4.62	3±4.62	2±2.89
Mean	7±4.82	5±2.58	10±4.53	6±3.84	8±4.89

The data are expressed as mean ± standard deviation, * **:** producer did not include the stage unit: cfu/100ml

Table 4.2: Mean faecal coliform count (cfu/100ml) from the individual *Sobolo* producers

Producer	Water	Steeped calyces	Boiled calyces and additives	Sieved and diluted mixture	Formulated drink
1	5±3.58	*	1±0.64	1±1.27	3±2.08
2	2±1.27	*	0.00	0.00	2±2.08
3	5±0.87	0.00	1±1.27	2±0.64	2±1.85
4	2±2.94	5±0.87	2±1.85	1±0.64	1±0.64
5	2±2.08	0.00	1±0.64	3±1.21	2.30±1.25
6	1±0.64	*	1±0.64	2±1.85	2±1.10
7	0.00	2±1.03	1±0.64	1±0.64	3±2.07
8	3±0.81	*	1±0.64	1±0.64	2±1.10
9	4±0.81	*	0.00	0.00	2±1.10

10	1±0.64	*	1±0.64	0.00	1±0.64
11	3±2.62	*	0.00	0.00	0.00
12	0.00	*	0.00	0.00	0.00
Mean	1.84±1.28	2±0.72	1±0.31	1±0.46	2±1.08

The data are expressed as mean ± standard deviation, * **:** producer did not include the stage, unit: cfu/100ml

After sieving the boiled mixture and diluting with water to obtain the extract, producer 5 recorded the highest mean coliform bacteria count (13±1.73cfu/100ml) in the liquid samples collected, though producers 2, 9, 10 and 11 recorded no total coliform bacteria count (**Table 4.1**). Similarly, producer 5 recorded the highest mean faecal coliform count (3±1.21cfu/100ml) with producers 2, 9, 10, 11 and 12 recording no faecal coliform count as shown in **Table 4.2**.

Producers 1, 3, 4, 5, 6, 7 and 8 diluted their boiled mixture with just non-heat treated water which is in contrast to producers 2, 9, 10, 11 and 12 who boiled their calyces after sieving in another water to obtain a different extract which was used in the dilution stage. Hence with the exception of producer 12, the other producers who used the boiled extract as a diluent recorded no coliform bacteria count as shown in **Tables 4.1**.

Also, after the final formulation to obtain the *Sobolo* drink, producer 1 recorded the highest mean coliform bacteria count (15±1.73cfu/100ml) while producer 11 recorded no coliform bacteria count (**Table 4.1**). Also, producer 7 recorded the highest mean faecal coliform count (3±2.07cfu/100ml) whereas producers 11 and 12 recorded no faecal coliform count as shown in **Table 4.2**.

4.2 TOTAL MICROBIAL COUNTS ACROSS THE STAGES OF PREPARATION

The mean microbial count across all the stages of the preparation of the *Sobolo* drink for all the producers was 26±44.91 cfu/100ml with the coliform bacteria and faecal coliform accounting for 7±6.16cfu/100ml and 1±1.43cfu/100ml respectively as shown in **Table 4.3**. This shows that there was at least the presence of microbial contamination across some of the stages in the preparation of the drink from the producers. The high occurrence of microbial contamination encountered in this study may be mostly due to the unsanitary, and largely the unhygienic nature of the drink preparation areas. Microbial contamination of food and drinks are good indicator of the state of environment in which they are prepared or served (Omemu *et al*., 2006).

The presence of faecal coliform in the samples indicates faecal contamination. The isolation of the coliform bacteria in the finally formulated *Sobolo* drink samples makes these drinks unsafe for human consumption. The isolation of faecal coliform in the *Sobolo* drinks indicates the presence of faecal or sewage contaminants introduced into the food through the use of contaminated water or contamination from the unsanitary environment and equipment (Pelczar *et al*, 2005).

Table 4.3: Two-sample T-test performed on overall coliform count in the preparation of the *Sobolo* drink

Indicator of microbial contamination	Mean ± SD	95% confidence interval for mean		Difference between bacterial and faecal coliform (p-value)
		Lower	Upper	
Coliform Bacteria	7±6.16	5.44	7.38	0.067
Faecal Coliform	1±1.43	0.74	1.19	
Total Colony Count	26±44.91	18.78	32.99	

SD: Standard deviation; difference in the parameters statistically significant ($p<0.05$) unit: cfu/100ml

From **Table 4.3,** p-value of 0.067, the difference between bacterial and faecal coliforms is greater than 0.05. In this case the null hypothesis is accepted which means that all stages associated with the production of *Sobolo* contribute to the microbial contamination. Although, the raw water sourced by all twelve (12) producers was said to be from GWCL (home and stand pipes), the study revealed that the raw water used by the producers in the preparation of the *Sobolo* drink had the highest level of mean total coliform count (coliform bacteria and faecal coliform) of 18±41.08cfu/100ml. This supports report by Cobbina *et al*., (2009) that pipe borne water is not without microbes. However, the levels of the mean total coliform count significantly deceased ($p = 0.001$) to a mean count of 6±16.03cfu/100ml after boiling the calyces and additives as shown in **Figure 4.1** and **Table 4.4**.

The mean total coliform count increased slightly after sieving and diluting the extract from 6±16.03cfu/100ml to 7±11.93cfu/100ml although the difference in the mean counts is not statistically important (1cfu/100ml, $p = 0.822$) (**Figure 4.1** and **Table 4.4**). The increase in the mean total coliform count after the boiling stage is due to the fact that some of the producers diluted their boiled mixture with non-heat treated raw water, which has high levels of microbial contamination (18±41.08cfu/100ml).

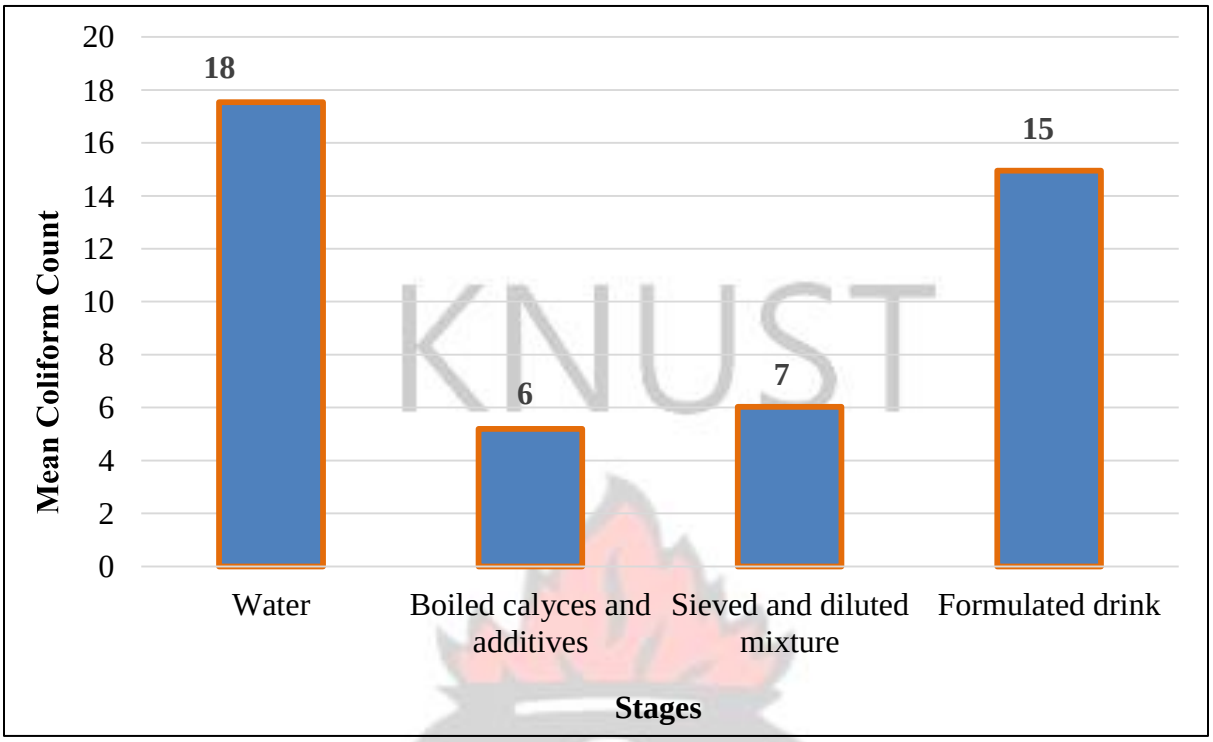

Figure 4.2: Average total coliform bacteria counts (cfu/100ml) across the stages in the preparation of the *Sobolo* drink

Also, after the final formulation stage, the levels of the mean total coliform count increased sharply from 7±11.93cfu/100ml to 15±30.62cfu/100ml with the difference in the mean counts statistically significant (9cfu/100ml, p = 0.018) as shown in **Figure 4.1** and **Table 4.4**). It can therefore be hypothesised that, there was high introduction of the microbes in the average formulation stages. This was because during the formulation stage, there were introduction of various utensils for stirring the extract during the addition of sugar and flavours. The additives could also account for the high microbial counts from the final stage.

Table 4.4: Multiple comparisons of the average total coliform bacteria counts from the stages in the preparation of the of *Sobolo* drink

	G	H

Sampling Stage	Mean ± SD	Formulated drink 15±30.62	Sieved and diluted mixture 7±11.93	Boiled calyces and additives 6±16.03
Water	18±41.08	3ɤ (0.492)	11† ɤ (0.002)	12† ɤ (0.001)
Boiling of calyces and additives	6±16.03	-9† ɤ (0.009)	-1 ɤ (0.822)	
Sieving and dilution of mixture	7±11.93	-8† ɤ (0.018)		

SD: Standard deviation, the data are expressed as the difference in means with the pvalues in the brackets; **†:** mean difference in the parameters are statistically significant ($p<0.05$); ɤ refers to mean difference expressed as G-H unit: cfu/100ml

Overall, the result shows that, the highest microbial contamination occurred from the introduction of raw water. However, the number of microbes were greatly minimised due to the boiling step but the other intermediate stages increased the level of microbial contamination again accounting for the high mean total coliform count from the final formulated *Sobolo* drink.

Contamination of the *Sobolo* drink can occur during cooling of the hot extract, addition of flavours and sweetener, or dispensing of the extract into polythene sheets and bottles. The utensils and water used during the post heating stages can also serve as source of contamination. A study has shown that, the major source of contamination of locally made drinks is the water used in the processing (Okeke *et al*., 2000).

4.3 TOTAL MICROBIAL COUNTS IN THE PRODUCTS WITH AND WITHOUT THE STEEPING STAGE

There was a considerable variation ($p = 0.021$) between the mean total coliform count from the products with the steeping stage (15±29.86cfu/100ml) as compared to the products without the steeping stage (9±26.96cfu/100ml) as shown in **Table 5**. The SD values are far

varied from the mean values which show that the individual values of the mean total coliform count are far apart. The difference between steeping (soaking) and no steeping is $p<0.05$ (p-value = 0.021) therefore the introduction or absence of the steeping stage does not significantly affect the microbial load of the final product.

Table 4.5: Two-sample T-test performed on total coliform count in the samples with and without the steeping stage

Stage	Mean ± SD	95% confidence interval for mean		Difference between steeping and no steeping (p-value)
		Lower	Upper	
Steeping (n = 60)	15±29.86	10.51	19.29	
No steeping (n = 120)	9±26.96	5.58	11.83	0.021†
Total	12±28.24	8.52	13.65	

SD: Standard deviation, **†:** difference in the parameters statistically significant ($p<0.05$)
unit: cfu/100ml

Also, there was a consistent higher level of the mean total coliform count across all the stages after the steeping stage in the preparation as compared to the preparation process without the steeping stage as shown in **Figure 4.2**. The consistently higher levels of the microbial count in the preparation process with steeping stage might be due to the fact that, the steeping of the calyces may create an enabling environment or medium for bacteria survival, hence, the increase in the microbial load during steeping. Boiling as a method of water purification reduces the microbial load to minimum level but does not eliminate the microbial content completely due to; the boiling time, temperature stability, types of microbes and the reproductive element present such as spores and cyst

(Cheesbrough, 2005).

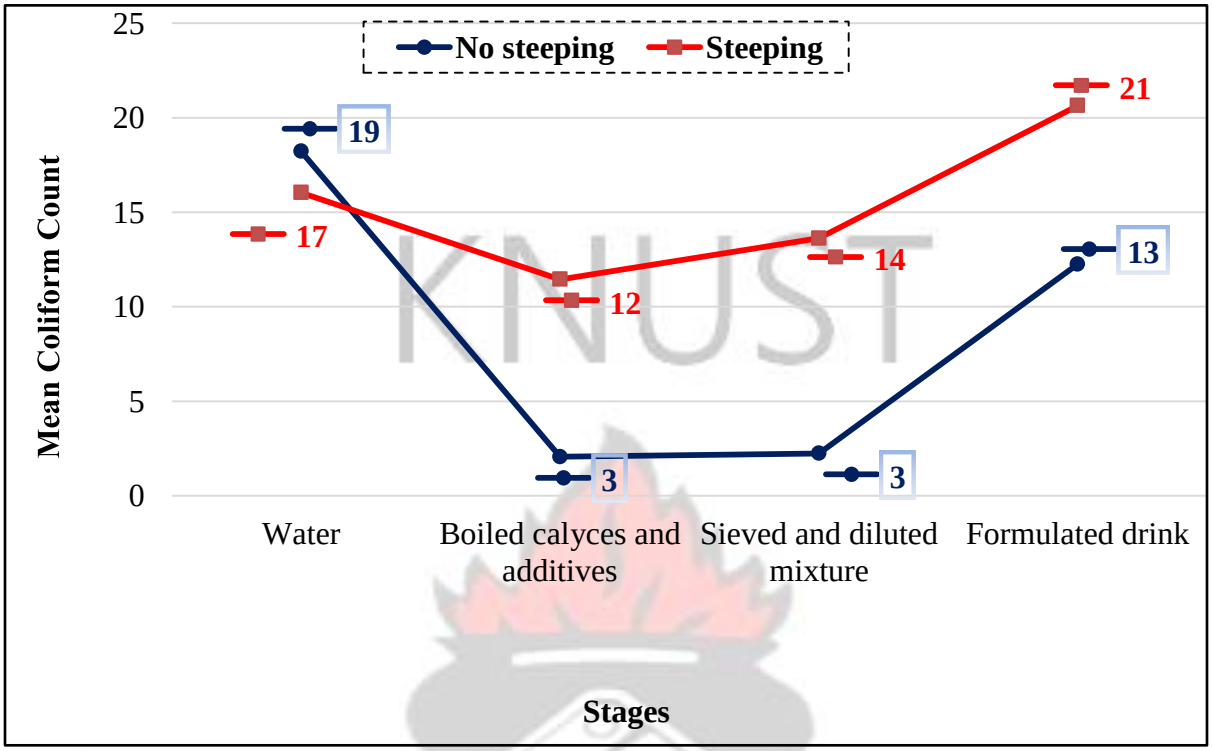

Figure 4.3: Mean total coliform counts (coliform bacteria and faecal coliform) in the other preparation stages with and without the steeping stage

4.4 CONSUMPTION OF *SOBOLO* DRINK IN THE SUNYANI MUNICIPALITY

Assessment of the consumption characteristics of the *Sobolo* drink in the Sunyani Municipality was done by administering questionnaires to a total of three hundred and eight three (383) *Sobolo* consumers. With regards to the quantity of the *Sobolo* consumers drink, 82.3% of the consumers indicated they consume at least 330ml or more of the drink per serving whereas only 2.1% indicated they consume other unspecified quantities of the *Sobolo* drink as shown in **Table 4.6**. Almost half (44.9%) of the consumers indicated that they consume the drink thrice per week. However, 9.4% of the consumers indicated that they consume drink only once per week (**Table 4.6**).

Table 4.6: Consumption characteristics of the *Sobolo* drink by consumers

Variable		Frequency	Percentage (%)
Quantity of *Sobolo* drink consumers purchase	Up to 250ml	60	15.7
	330ml	158	41.3
	More done 330ml	157	41.0
	Others	8	2.1
Number of times consumers drink *Sobolo* per week	Once	36	9.4
	Twice	109	28.5
	Thrice	172	44.9
	More than thrice	66	17.2
Number of years consumers have being drinking *Sobolo*	1 – 3	246	64.2
	4 – 6	102	26.6
	7 – 9	12	3.1
	More than 9	23	6.0
Reasons for the consumption of *Sobolo*	Health benefits	184	48.0
	Taste, colour and aroma	57	14.9
	Quenching of thirst	107	27.9
	Full meal/snack	35	9.1

Also, with respect to the number of years of consumption, more than half (64.2%) of the consumers indicated that they have being consuming the drink for 1 to 3 years whereas 9.1% of the consumers indicated that they have being consuming drink for 7 years and more (**Table 4.6**). Almost half (48.8%) of the consumers indicated that they consume the *Sobolo* drink for its health benefits. There are reports (Ali *et al*., 1991; Lee *et al*., 2002; Owureku-Asare *et al*., 2014) confirming that most people consume the *Sobolo* drink for health related issues. Again, 14.9% of the consumers indicated that they consume the *Sobolo* drink for its taste, colour and aroma, 27.9% for quenching of thirst, and 9.1% as a full meal/snack as shown in **Table 4.6**.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS 5.1 CONCLUSION

Sobolo is a local drink that is prepared from *Hibiscus sabdariffa.* This drink has gained a lot of market in Sunyani and Ghana as a whole. Many researches have shown microbial contamination of the hawked product, however the source of contamination was not known. This research revealed that, there was microbial contamination at each step during the preparation process. The boiling stage recorded the least contamination with the formulation stage (ie. the sieving, dilution and addition of sugar) recording the greatest contamination. However, the microbial load of the drink in terms of coliform bacteria and faecal coliforms did not reveal an alarming food safety concern. There was no E. coli in

any of the samples; however coliform bacteria and faecal coliforms were recorded in all the stages. Since all the stages contributed to the microbial contamination of the drink, there is the need to take the necessary precautions during the preparation of the drink in order to reduce the microbial load of the final product.

5.2 RECOMMENDATIONS

1. A well-defined and protected area should be provided for the preparation of the *Sobolo* drink. *Sobolo* drink is food product that is consumed by persons of all ages; the preparation area should not be associated with any potential food safety hazard.
2. It is also recommended that the Food and Drugs Authority will educate all producers and traders of *Sobolo* drink as well as ready-to-eat food products.
3. Only boiled water should be used to dilute the mixture if required; however the required volume of drink needed can also be boiled during the preparation stage.

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APPENDICES

Appendix 1

QUESTIONNAIRE FOR CONSUMER SURVEY OF *SOBOLO* DRINK CONSUMPTION IN SUNYANI AND TECHIMAN MUNICIPALILITIES

SECTION A: Socio-Economic Characteristics

1.0 PERSONAL INFORMATION

1.1 Sex of respondent (Gender) □Male □Female

1.2 Age of respondent (specify in years).....years (Age)

1.3 Location……

1.4 Body weight (kg)…….

1.5 Ethnicity

……

1.6 Educational level of respondent

☐ None

□Primary education

□Junior high school/middle education
□Senior high education

□Vocational/Technical education

□Tertiary

□Others (specify).....

1.7Marital status

□Married

□Single

□Divorced/ Separated

□Other (specify).....

SECTION B. General Questions About *Sobolo* Drink Consumption

1.8 Where do you buy your *Sobolo* Drink? Street hawkers ☐ Shop ☐

1.9 What quantity of *Sobolo* drink do you consume daily?

☐ ≤250ml ☐ 330ml ☐ >330ml ☐ Other…….

2.0 How often do you consume the *Sobolo* drink in a week?

☐ Once ☐ Twice ☐ Thrice ☐ Other

2.1 For how many years now have you been consuming *Sobolo* drink?

(specify)…….....

2.2 What quality attribute do you check before buying the *Sobolo* Drink?

☐ Taste ☐ Colour ☐ Expiry date Packaging ☐ All of above

☐ Other (specify).....

☐ None,why?.....……

…….

2.3 Why do you drink *Sobolo* Drink?……..

……

2.4 Do you have any concerns towards *Sobolo* drink? State.

……

……

2.5 Can you suggest the way forward…….....

……

……

Appendix 2

Table 101a: Microbial indicators presence/ absence and counts across the stages of *Sobolo* preparation

	Calyces in Water			Boiled Leaves			Tap water			Sieved & Diluted Mixture			Formulated Drink		
	TC	FC	TPC	TC	FC	TPC	TC	FC	TPC	TC	FC	TPC	TC	FC	TPC
Producer 1				10	1.1	18	16	6.9	138	16	2.2	4	13	3.6	58
				8	0	18	16	0	130	10	0	5	13	3.6	35
				8	0	16	16	5.1	149	10	0	10	16	0	60
Producer 2				0	0	0	16	2.2	28	0	0	0	0	0	3
				0	0	0	16	0	35	0	0	0	10	3.6	3
				0	0	0	16	0	40	0	0	2	0	0	7
Producer 3	0	0	0	5	0	2	16	5.1	147	8	1.1	42	8	0	30
	0	0	1	8	0	2	16	5.1	152	10	2.2	52	10	1.1	38
	0	0	2	13	2.2	5	16	3.6	140	10	1.1	46	13	3.6	35

Producer 4	16	5.1	120	16	3.6	102	8	0	3	8	1.1	26	5	0	2
	16	3.6	110	0	0	90	0	0	1	8	1.1	30	8	1.1	2
	16	3.6	120	8	1.1	96	16	5.1	14	5	0	20	0	0	2
Producer 5	0	0	0	8	0	6	0	0	0	10	2.2	40	16	3.6	74

	0	0	0	0	0	0	5.9	0	2	13	2.2	40	10	2.2	63
	8	0	10	8	1.1	9	16	3.6	2	13	2.2	70	13	1.1	70
Producer 6				0	0	0	5	1.1	3	10	1.1	6	16	2.2	20
				10	1.1	0	10	1.1	4	16	3.6	7	0	0	20
				0	0	0	5	0	2	8	0	3	8	1.1	22
Producer 7	13	2.2	7	10	1.1	3	0	0	0	10	1.1	3	16	5.1	96
	8	1.1	6	0	0	0	0	0	0	0	0	0	13	2.2	90
	0	0	0	8	1.1	3	0	0	0	8	1.1	3	8	1.1	90
Producer 8				8	0	4	16	2.2	20	8	0	4	0	0	0
				5	0	4	16	2.2	30	5	0	4	13	2.2	15
				8	1.1	5	10	3.6	20	8	1.1	5	10	1.1	10

Producer 9				0	0	0	16	3.6	180	0	0	0	16	2.2	160
				0	0	0	16	2.2	170	0	0	0	16	1.1	150
				0	0	0	16	3.6	180	0	0	0	8	0	140
Producer10				0	0	0	8	1.1	4	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	8	1.1	3
				8	1.1	6	0	0	0	0	0	0	0	0	0
Producer11				0	0	0	16	3.6	120	0	0	0	0	0	0
				0	0	0	16	5.1	130	0	0	0	0	0	0
				0	0	0	0	0	120	0	0	0	0	0	0
Producer12				0	0	0	0	0	0	0	0	0	0	0	0
				8	0	0	0	0	0	8	0	4	0	0	0
				0	0	0	0	0	0	0	0	0	5	0	2
Mean	6.417	1.33	31.33	4.36	0.41	10.81	9.44	1.84	54.56	5.61	0.65	11.8	7.556	1.192	36.11
SD	7.204	1.851	51.62	4.76	0.78	26.56	7.19	2.12	67.67	5.29	0.95	18.5	6.162	1.45	45.97

Table 101b: Microbial indicators presence/ absence and counts across the stages of *Sobolo* preparation

	Calyces in Water			Boiled Leaves			Tap water			Sieved & Diluted Mixture			Formulated Drink		
	TC	FC	TPC	TC	FC	TPC	TC	FC	TPC	TC	FC	TPC	TC	FC	TPC
Producer 1				10	1.1	18	16	6.9	138	16	2.2	4	13	3.6	58
				8	0	18	16	0	130	10	0	5	13	3.6	35
				8	0	16	16	5.1	149	10	0	10	16	0	60
Mean				8.667	0.367	17.33	16	4	139	12	0.733	6.3333	14	2.4	51
SD				1.155	0.635	1.155	0	3.579	9.539	3.464	1.27	3.2146	1.732	2.078	13.892
Producer 2				0	0	0	16	2.2	28	0	0	0	0	0	3
				0	0	0	16	0	35	0	0	0	10	3.6	3

				0	0	0	16	0	40	0	0	2	0	0	7
Mean				0	0	0	16	0.733	34.33	0	0	0.6667	3.333	1.2	4.3333
SD				0	0	0	0	1.27	6.028	0	0	1.1547	5.774	2.078	2.3094
Producer 3	0	0	0	5	0	2	16	5.1	147	8	1.1	42	8	0	30
	0	0	1	8	0	2	16	5.1	152	10	2.2	52	10	1.1	38
	0	0	2	13	2.2	5	16	3.6	140	10	1.1	46	13	3.6	35
Mean	0	0	1	8.667	0.733	3	16	4.6	146.3	9.333	1.467	46.667	10.33	1.567	34.333
SD	0	0	1	4.041	1.27	1.732	0	0.866	6.028	1.155	0.635	5.0332	2.517	1.845	4.0415

Producer 4	16	5.1	120	16	3.6	102	8	0	3	8	1.1	26	5	0	2
	16	3.6	110	0	0	90	0	0	1	8	1.1	30	8	1.1	2
	16	3.6	120	8	1.1	96	16	5.1	14	5	0	20	0	0	2
Mean	16	4.1	116.7	8	1.567	96	8	1.7	6	7	0.733	25.333	4.333	0.367	2

SD	0	0.866	5.774	8	1.845	6	8	2.944	7	1.732	0.635	5.0332	4.041	0.635	0
Producer 5	0	0	0	8	0	6	0	0	0	10	2.2	40	16	3.6	74
	0	0	0	0	0	0	5.9	0	2	13	2.2	40	10	2.2	63
	8	0	10	8	1.1	9	16	3.6	2	13	2.2	50	13	1.1	70
Mean	2.667	0	3.333	5.333	0.367	5	7.3	1.2	1.333	12	2.2	43.333	13	2.3	69
SD	4.619	0	5.774	4.619	0.635	4.583	8.091	2.078	1.155	1.732	0	5.7735	3	1.253	5.5678
Producer 6				0	0	0	5	1.1	3	10	1.1	6	16	2.2	20
				10	1.1	0	10	1.1	4	16	3.6	7	0	0	20
				0	0	0	5	0	2	8	0	3	8	1.1	22
Mean				3.333	0.367	0	6.667	0.733	3	11.33	1.567	5.3333	8	1.1	20.667
SD				5.774	0.635	0	2.887	0.635	1	4.163	1.845	2.0817	8	1.1	1.1547
Producer 7	13	2.2	7	10	1.1	3	0	0	0	10	1.1	3	16	5.1	96

	8	1.1	6	0	0	0	0	0	0	0	0	0	13	2.2	90

	0	0	0	8	1.1	3	0	0	0	8	1.1	3	8	1.1	90
Mean	7	1.1	4.333	6	0.733	2	0	0	0	6	0.733	2	12.33	2.8	92
SD	6.557	1.1	3.786	5.292	0.635	1.732	0	0	0	5.292	0.635	1.7321	4.041	2.066	3.4641
Producer 8				8	0	4	16	2.2	20	8	0	4	0	0	0
				5	0	4	16	2.2	30	5	0	4	13	2.2	15
				8	1.1	5	10	3.6	20	8	1.1	5	10	1.1	10
Mean				7	0.367	4.333	14	2.667	23.33	7	0.367	4.3333	7.667	1.1	8.3333
SD				1.732	0.635	0.577	3.464	0.808	5.774	1.732	0.635	0.5774	6.807	1.1	7.6376
Producer 9				0	0	0	16	3.6	180	0	0	0	16	2.2	160
				0	0	0	16	2.2	170	0	0	0	16	1.1	150
				0	0	0	16	3.6	180	0	0	0	8	0	140

Mean				0	0	0	16	3.133	176.7	0	0	0	13.33	1.1	150
SD				0	0	0	0	0.808	5.774	0	0	0	4.619	1.1	10
Producer10				0	0	0	8	1.1	4	0	0	0	0	0	0
				0	0	0	0	0	0	0	0	0	8	1.1	3
				8	1.1	6	0	0	0	0	0	0	0	0	0
Mean				2.667	0.367	2	2.667	0.367	1.333	0	0	0	2.667	0.367	1
SD				4.619	0.635	3.464	4.619	0.635	2.309	0	0	0	4.619	0.635	1.7321
Producer11				0	0	0	16	3.6	120	0	0	0	0	0	0
				0	0	0	16	5.1	130	0	0	0	0	0	0
				0	0	0	0	0	120	0	0	0	0	0	0
Mean				0	0	0	10.67	2.9	123.3	0	0	0	0	0	0
SD				0	0	0	9.238	2.621	5.774	0	0	0	0	0	0

Producer12				0	0	0	0	0	0	0	0	0	0	0	0
				8	0	0	0	0	0	8	0	4	0	0	0
				0	0	0	0	0	0	0	0	0	5	0	2
Mean				2.667	0	0	0	0	0	2.667	0	1.3333	1.667	0	0.6667
SD				4.619	0	0	0	0	0	4.619	0	2.3094	2.887	0	1.1547

Table 102: Evidence of coliform from the individual *Sobolo* producers

Producer	Water		Steeped calyces		Boiled calyces and additives		Sieved and diluted mixture		Formulated drink	
	CB	FC	CB	FC	CB	FC	CB	FC	CB	FC
1	+	+	*	*	+	+	+	+	+	+
2	+	+	*	*	-	-	-	-	+	+
3	+	+	-	-	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	-	+	+	+	+	+	+
6	+	+	*	*	+	+	+	+	+	+
7	-	-	+	+	+	+	+	+	+	+

8	+	+	*	*	+	+	+	+	+	+
9	+	+	*	*	-	-	-	-	+	+
10	+	+	*	*	+	+	-	-	+	+
11	+	+	*	*	-	-	-	-	-	-
12	-	-	*	*	+	-	+	-	+	-

CB : Coliform bacteria, **FC :** faecal coliform, **+ :** Presence of coliform, **- :** absence of coliform, *** :** Producer did not include the stage

Table103: Mean Microbial Counts per Producer

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Producer1	36	9.4028	14.50234	2.41706	4.4959	14.3097	.00	60.00
Producer2	36	5.0500	10.20883	1.70147	1.5958	8.5042	.00	40.00
Producer3	36	23.5861	40.06647	6.67775	10.0296	37.1427	.00	152.00
Producer4	36	13.4194	26.38012	4.39669	4.4937	22.3452	.00	102.00
Producer5	36	14.0861	21.94568	3.65761	6.6608	21.5115	.00	74.00
Producer6	36	5.1750	6.45146	1.07524	2.9921	7.3579	.00	22.00
		10.3833				18.9477		

Producer7	36	6.7083	25.31211	4.21869	1.8189	9.0920	.00	96.00
Producer8	36	30.0194	7.04485	1.17414	4.3247	50.6671	.00	30.00
Producer9	36	1.1194	61.02430	10.17072	9.3718	1.9512	.00	180.00
Producer10	36	11.4083		.40972	.2877	23.0684		
Producer11	36		2.45832	5.74357	-.2517		.00	8.00
			34.46139				.00	130.00
Producer12	36	.7500	2.08909	.34818	.0432	1.4568	.00	8.00
Total	432	10.9257	27.92679	1.34363	8.2848	13.5666	.00	180.00

Table 104: Multiple Comparisons of mean microbial counts in all sampled producers

(I) Sampled Producers	*Sobolo* (J) Sampled Producers	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Producer1	Producer2	4.35278	6.36516	.494	-8.1588	16.8643
	Producer3	-14.18333*	6.36516	.026	-26.6949	-1.6718
	Producer4	-4.01667	6.36516	.528	-16.5282	8.4949
	Producer5	-4.68333	6.36516	.462	-17.1949	7.8282
	Producer6					

	Producer7	4.22778	6.36516	.507	-8.2838	16.7393
	Producer8	-.98056	6.36516	.878	-13.4921	11.5310
	Producer9	2.69444	6.36516	.672	-9.8171	15.2060
	Producer10	-20.61667*	6.36516	.001	-33.1282	-8.1051
	Producer11	8.28333	6.36516	.194	-4.2282	20.7949
	Producer12	-2.00556	6.36516	.753	-14.5171	10.5060
		8.65278	6.36516	.175	-3.8588	21.1643
Producer2		-4.35278	6.36516	.494	-16.8643	8.1588
		-18.53611*	6.36516	.004	-31.0477	-6.0246
		-8.36944	6.36516	.189	-20.8810	4.1421
	Producer1	-9.03611	6.36516	.156	-21.5477	3.4754
	Producer3	-.12500	6.36516	.984	-12.6365	12.3865
	Producer4	-5.33333	6.36516	.403	-17.8449	7.1782
	Producer5	-1.65833	6.36516	.795	-14.1699	10.8532
	Producer6	-24.96944*	6.36516	.000	-37.4810	-12.4579
	Producer7	3.93056	6.36516	.537	-8.5810	16.4421
	Producer8	-6.35833	6.36516	.318	-18.8699	6.1532
	Producer9	4.30000	6.36516	.500	-8.2115	16.8115
	Producer10					
	Producer11					
	Producer12					
Producer3		14.18333*	6.36516	.026	1.6718	26.6949
	Producer1	18.53611*	6.36516	.004	6.0246	31.0477
	Producer2	10.16667	6.36516	.111	-2.3449	22.6782
	Producer4	9.50000	6.36516	.136	-3.0115	22.0115
	Producer5	18.41111*	6.36516	.004	5.8996	30.9227
	Producer6	13.20278*	6.36516	.039	.6912	25.7143
	Producer7	16.87778*	6.36516	.008	4.3662	29.3893
	Producer8	-6.43333	6.36516	.313	-18.9449	6.0782
	Producer9					
	Producer10					
	Producer11					

		22.46667*	6.36516	.000	9.9551	34.9782
		12.17778	6.36516	.056	-.3338	24.6893
	Producer12	22.83611*	6.36516	.000	10.3246	35.3477
Producer4		4.01667	6.36516	.528	-8.4949	16.5282
		8.36944	6.36516	.189	-4.1421	20.8810
		-10.16667	6.36516	.111	-22.6782	2.3449
	Producer1	-.66667	6.36516	.917	-13.1782	11.8449
	Producer2	8.24444	6.36516	.196	-4.2671	20.7560
	Producer3	3.03611	6.36516	.634	-9.4754	15.5477
	Producer5	6.71111	6.36516	.292	-5.8004	19.2227
	Producer6	-16.60000*	6.36516	.009	-29.1115	-4.0885
	Producer7	12.30000	6.36516	.054	-.2115	24.8115
	Producer8	2.01111	6.36516	.752	-10.5004	14.5227
	Producer9	12.66944*	6.36516	.047	.1579	25.1810
	Producer10					
	Producer11					
	Producer12					
Producer5		4.68333	6.36516	.462	-7.8282	17.1949
		9.03611	6.36516	.156	-3.4754	21.5477
		-9.50000	6.36516	.136	-22.0115	3.0115
	Producer1	.66667	6.36516	.917	-11.8449	13.1782
	Producer2	8.91111	6.36516	.162	-3.6004	21.4227
	Producer3	3.70278	6.36516	.561	-8.8088	16.2143
	Producer4	7.37778	6.36516	.247	-5.1338	19.8893
	Producer6	-15.93333*	6.36516	.013	-28.4449	-3.4218
	Producer7	12.96667*	6.36516	.042	.4551	25.4782
	Producer8	2.67778	6.36516	.674	-9.8338	15.1893
	Producer9	13.33611*	6.36516	.037	.8246	25.8477
	Producer10					
	Producer11					
	Producer12					
Producer6	Producer1	-4.22778	6.36516	.507	-16.7393	8.2838

	Producer2	.12500	6.36516	.984	-12.3865	12.6365
	Producer3	-18.41111*	6.36516	.004	-30.9227	-5.8996
	Producer4	-8.24444	6.36516	.196	-20.7560	4.2671
	Producer5	-8.91111	6.36516	.162	-21.4227	3.6004
	Producer7	-5.20833	6.36516	.414	-17.7199	7.3032
	Producer8	-1.53333	6.36516	.810	-14.0449	10.9782
	Producer9	-24.84444*	6.36516	.000	-37.3560	-12.3329
	Producer10	4.05556	6.36516	.524	-8.4560	16.5671
	Producer11	-6.23333	6.36516	.328	-18.7449	6.2782
	Producer12	4.42500	6.36516	.487	-8.0865	16.9365
Producer7		.98056	6.36516	.878	-11.5310	13.4921
	Producer1	5.33333	6.36516	.403	-7.1782	17.8449
	Producer2	-13.20278*	6.36516	.039	-25.7143	-.6912
	Producer3	-3.03611	6.36516	.634	-15.5477	9.4754
	Producer4	-3.70278	6.36516	.561	-16.2143	8.8088
	Producer5	5.20833	6.36516	.414	-7.3032	17.7199
	Producer6	3.67500	6.36516	.564	-8.8365	16.1865
	Producer8	-19.63611*	6.36516	.002	-32.1477	-7.1246
	Producer9	9.26389	6.36516	.146	-3.2477	21.7754
	Producer10	-1.02500	6.36516	.872	-13.5365	11.4865
	Producer11	9.63333	6.36516	.131	-2.8782	22.1449
	Producer12	-2.69444	6.36516	.672	-15.2060	9.8171
Producer8		1.65833	6.36516	.795	-10.8532	14.1699
	Producer1	-16.87778*	6.36516	.008	-29.3893	-4.3662
	Producer2	-6.71111	6.36516	.292	-19.2227	5.8004
	Producer3	-7.37778	6.36516	.247	-19.8893	5.1338
	Producer4					
	Producer5					
	Producer6					

	Producer7	1.53333	6.36516	.810	-10.9782	14.0449
	Producer9	-3.67500	6.36516	.564	-16.1865	8.8365
	Producer10	-23.31111*	6.36516	.000	-35.8227	-10.7996
	Producer11	5.58889	6.36516	.380	-6.9227	18.1004
	Producer12	-4.70000	6.36516	.461	-17.2115	7.8115
		5.95833	6.36516	.350	-6.5532	18.4699
Producer9		20.61667*	6.36516	.001	8.1051	33.1282
		24.96944*	6.36516	.000	12.4579	37.4810
		6.43333	6.36516	.313	-6.0782	18.9449
	Producer1	16.60000*	6.36516	.009	4.0885	29.1115
	Producer2	15.93333*	6.36516	.013	3.4218	28.4449
	Producer3	24.84444*	6.36516	.000	12.3329	37.3560
	Producer4	19.63611*	6.36516	.002	7.1246	32.1477
	Producer5	23.31111*	6.36516	.000	10.7996	35.8227
	Producer6	28.90000*	6.36516	.000	16.3885	41.4115
	Producer7	18.61111*	6.36516	.004	6.0996	31.1227
	Producer8	29.26944*	6.36516	.000	16.7579	41.7810
	Producer10	-8.28333	6.36516	.194	-20.7949	4.2282
	Producer11	-3.93056	6.36516	.537	-16.4421	8.5810
	Producer12	-22.46667*	6.36516	.000	-34.9782	-9.9551
Producer10		-12.30000	6.36516	.054	-24.8115	.2115
		-12.96667*	6.36516	.042	-25.4782	-.4551
	Producer1	-4.05556	6.36516	.524	-16.5671	8.4560
	Producer2	-9.26389	6.36516	.146	-21.7754	3.2477
	Producer3	-5.58889	6.36516	.380	-18.1004	6.9227
	Producer4	-28.90000*	6.36516	.000	-41.4115	-16.3885
	Producer5	-10.28889	6.36516	.107	-22.8004	2.2227

	Producer12	.36944	6.36516	.954	-12.1421	12.8810
Producer11		2.00556	6.36516	.753	-10.5060	14.5171
		6.35833	6.36516	.318	-6.1532	18.8699
		-12.17778	6.36516	.056	-24.6893	.3338
	Producer1	-2.01111	6.36516	.752	-14.5227	10.5004
	Producer2	-2.67778	6.36516	.674	-15.1893	9.8338
	Producer3	6.23333	6.36516	.328	-6.2782	18.7449
	Producer4	1.02500	6.36516	.872	-11.4865	13.5365
	Producer5	4.70000	6.36516	.461	-7.8115	17.2115
	Producer6	-18.61111*	6.36516	.004	-31.1227	-6.0996
	Producer7	10.28889	6.36516	.107	-2.2227	22.8004
	Producer8	10.65833	6.36516	.095	-1.8532	23.1699
	Producer9					
	Producer10					
	Producer12					
Producer12		-8.65278	6.36516	.175	-21.1643	3.8588
		-4.30000	6.36516	.500	-16.8115	8.2115
		-22.83611*	6.36516	.000	-35.3477	-10.3246
	Producer1	-12.66944*	6.36516	.047	-25.1810	-.1579
	Producer2	-13.33611*	6.36516	.037	-25.8477	-.8246
	Producer3	-4.42500	6.36516	.487	-16.9365	8.0865
	Producer4	-9.63333	6.36516	.131	-22.1449	2.8782
	Producer5	-5.95833	6.36516	.350	-18.4699	6.5532
	Producer6	-29.26944*	6.36516	.000	-41.7810	-16.7579
	Producer7	-.36944	6.36516	.954	-12.8810	12.1421
	Producer8	-10.65833	6.36516	.095	-23.1699	1.8532
	Producer9					
	Producer10					
	Producer11					

*. The mean difference is significant at the 0.05 level.

Table 105: ANOVA between the sampled Producers

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29843.947	11	2713.086	3.720	.000
Within Groups	306295.478	420	729.275		
Total	336139.425	431			

Means Plots

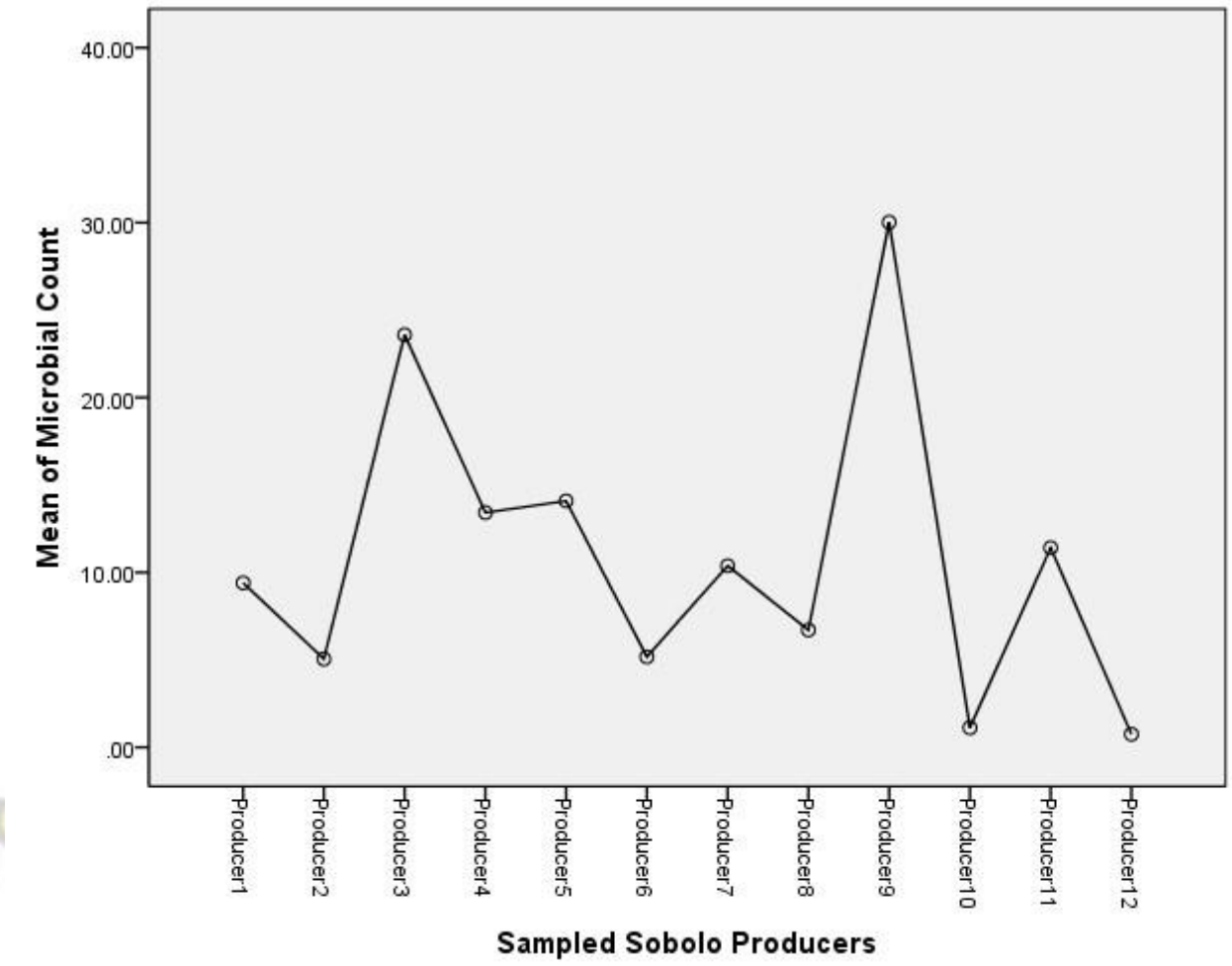

Figure 101: Multiple Comparisons of mean microbial counts in all sampled producers

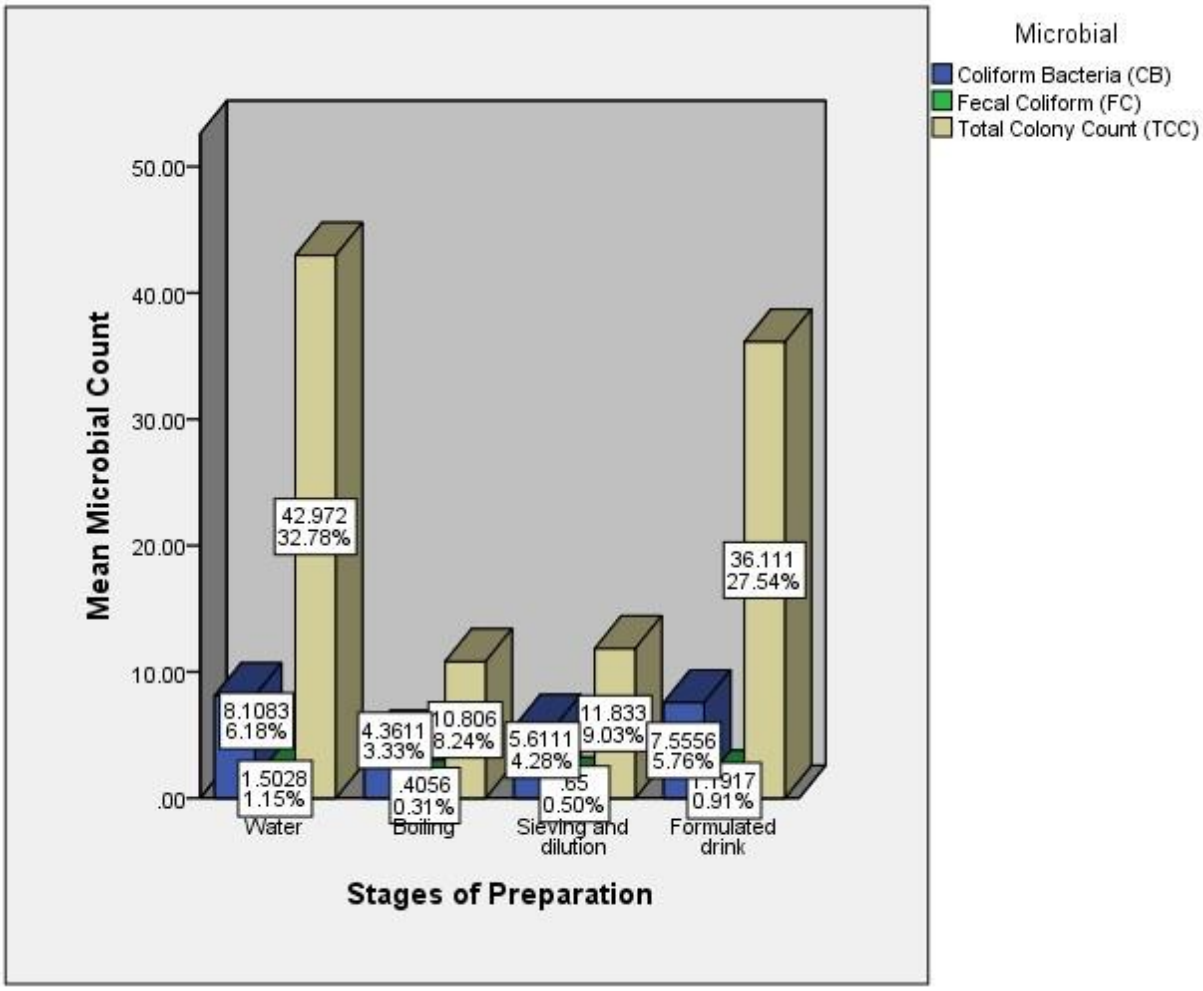

Figure 102: Mean microbial Counts of the Microbial Indicators

Table 106: Mean microbial Counts across all the stages of *Sobolo* Drink Preparation

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Water	108	17.5278	41.07874	3.95280	9.6918	25.3637	.00	180.00
Calyces in water	36	13.0167	32.12181	5.35363	2.1482	23.8851	.00	120.00
Boiling	108	5.1907	16.02613	1.54212	2.1337	8.2478	.00	102.00
Sieving and dilution	108	6.0315	11.92654	1.14763	3.7564	8.3065	.00	70.00
Formulated drink	108	14.9528	30.61388	2.94582	9.1130	20.7925	.00	160.00
Total	468	11.0865	28.23874	1.30534	8.5215	13.6516	.00	180.00

Table 107: ANOVA of Mean microbial Counts across all the stages of *Sobolo* Drink Preparation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12743.266	4	3185.816	4.101	.003
Within Groups	359654.820	463	776.792		
Total	372398.085	467			

Table 108; Multiple Comparisons of Mean microbial Counts across all the stages of *Sobolo* Drink Preparation

(I) Stages of Preparation	(J) Stages of Preparation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Water		4.51111	5.36378	.401	-6.0292	15.0515
	Calyces in water	12.33704*	3.79276	.001	4.8839	19.7902
	Boiling	11.49630*	3.79276	.003	4.0431	18.9495
	Sieving and dilution	2.57500	3.79276	.498	-4.8782	10.0282
	Formulated drink					
Calyces in water		-4.51111	5.36378	.401	-15.0515	6.0292
	Water	7.82593	5.36378	.145	-2.7144	18.3663
	Boiling	6.98519	5.36378	.193	-3.5552	17.5255
	Sieving and dilution	-1.93611	5.36378	.718	-12.4765	8.6042
	Formulated drink					
Boiling		-12.33704*	3.79276	.001	-19.7902	-4.8839
	Water	-7.82593	5.36378	.145	-18.3663	2.7144
	Calyces in water	-.84074	3.79276	.825	-8.2939	6.6124
	Sieving and dilution	-9.76204*	3.79276	.010	-17.2152	-2.3089
	Formulated drink					
Sieving and dilution		-11.49630*	3.79276	.003	-18.9495	-4.0431
	Water	-6.98519	5.36378	.193	-17.5255	3.5552
	Calyces in water	.84074	3.79276	.825	-6.6124	8.2939
	Boiling	-8.92130*	3.79276	.019	-16.3745	-1.4681
	Formulated drink					
Formulated drink		-2.57500	3.79276	.498	-10.0282	4.8782
	Water	1.93611	5.36378	.718	-8.6042	12.4765
	Calyces in water	9.76204*	3.79276	.010	2.3089	17.2152
	Boiling	8.92130*	3.79276	.019	1.4681	16.3745
	Sieving and dilution					

*. The mean difference is significant at the 0.05 level.

Means Plots

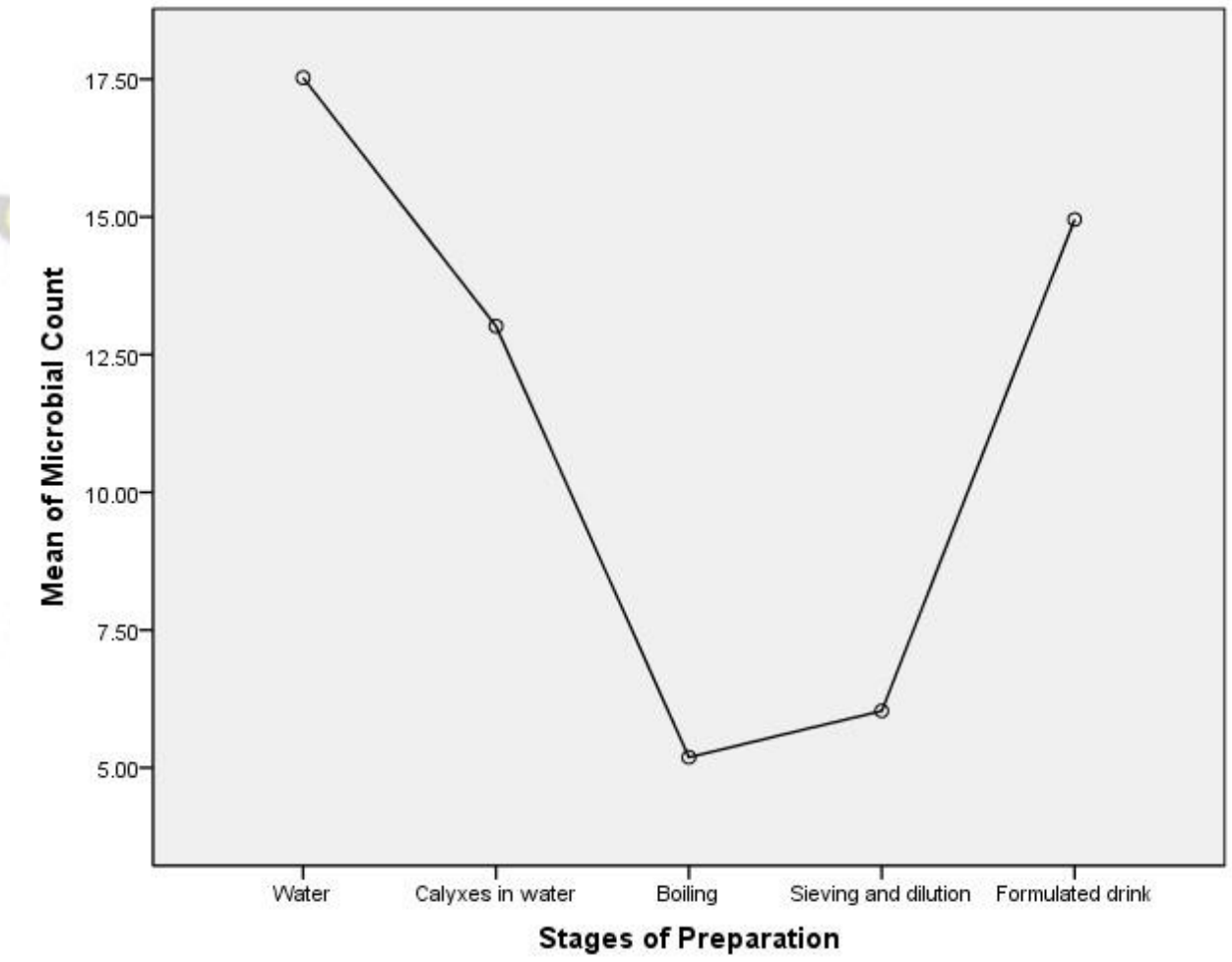

Figure 102: Mean microbial Counts across all the stages of *Sobolo* Drink Preparation

Table 109: Mean microbial Counts across all the stages of *Sobolo* Drink Preparation

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Water	108	17.5278	41.07874	3.95280	9.6918	25.3637	.00	180.00
Calyces in water	36	13.0167	32.12181	5.35363	2.1482	23.8851	.00	120.00
		5.1907	16.02613	1.54212		8.2478	.00	
Boiling	108	6.0315			2.1337	8.3065		102.00
Sieving and dilution	108	14.9528	11.92654	1.14763	3.7564	20.7925	.00	70.00
Formulated drink	108	11.0865	30.61388	2.94582	9.1130	13.6516	.00	160.00
Total	468		28.23874	1.30534	8.5215		.00	180.00

Table 110: ANOVA of mean microbial counts between the different stages of *Sobolo* preparation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12743.266	4	3185.816	4.101	.003
Within Groups	359654.820	463	776.792		
Total	372398.085	467			

(I) Stages of Preparation	(J) Stages of Preparation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Water	Calyces in water	4.51111	5.36378	.401	-6.0292	15.0515
	Boiling	12.33704*	3.79276	.001	4.8839	19.7902
	Sieving and dilution	11.49630*	3.79276	.003	4.0431	18.9495
	Formulated drink	2.57500	3.79276	.498	-4.8782	10.0282
Calyces in water	Water	-4.51111	5.36378	.401	-15.0515	6.0292
	Boiling	7.82593	5.36378	.145	-2.7144	18.3663
	Sieving and dilution	6.98519	5.36378	.193	-3.5552	17.5255
	Formulated drink	-1.93611	5.36378	.718	-12.4765	8.6042
Boiling	Water	-12.33704*	3.79276	.001	-19.7902	-4.8839
	Calyces in water	-7.82593	5.36378	.145	-18.3663	2.7144
	Sieving and dilution	-.84074	3.79276	.825	-8.2939	6.6124
	Formulated drink	-9.76204*	3.79276	.010	-17.2152	-2.3089
Sieving and dilution	Water	-11.49630*	3.79276	.003	-18.9495	-4.0431
	Calyces in water	-6.98519	5.36378	.193	-17.5255	3.5552
	Boiling	.84074	3.79276	.825	-6.6124	8.2939
	Formulated drink	-8.92130*	3.79276	.019	-16.3745	-1.4681
Formulated drink		-2.57500	3.79276	.498	-10.0282	10.0282

	Water	1.93611	5.36378	.718	-8.6042	4.8782
	Calyces in water	9.76204*	3.79276	.010	2.3089	12.4765
	Boiling	8.92130*	3.79276	.019	1.4681	17.2152
	Sieving and dilution					16.3745

Table 111: Multiple Comparisons of Mean microbial Counts across all the stages of *Sobolo* Drink Preparation

*. The mean difference is significant at the 0.05 level.

Means Plots

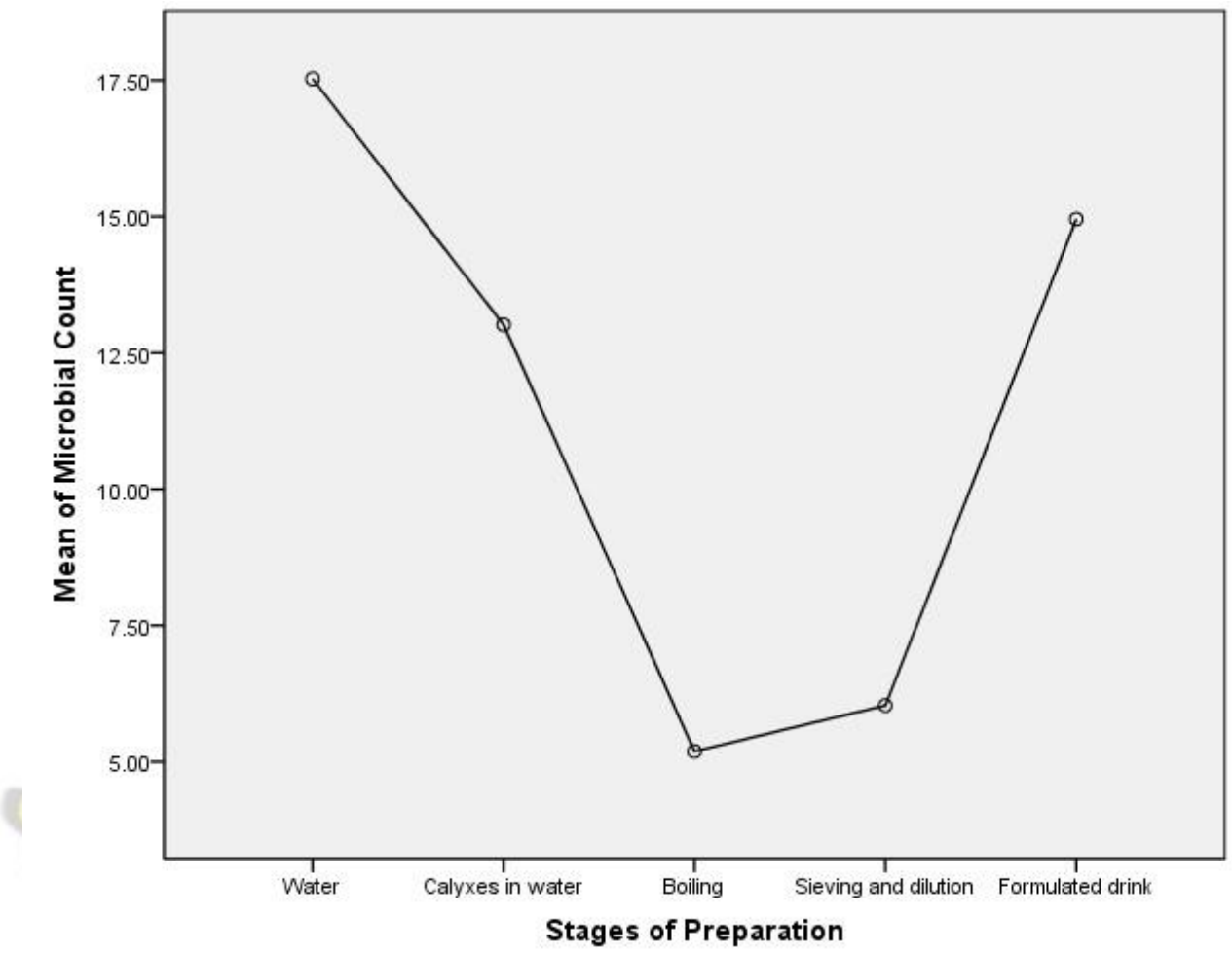

Figure 103: Mean microbial Counts against all the stages of *Sobolo* Drink Preparation