**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI**

# EFFECT OF PRESERVATIVES ON THE MICROBIAL KEEPING QUALITY OF THE BURKINA DRINK



**BY**

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## DECLARATION

I hereby declare that this submission is my own research work and that, it contains no materials previously published by another person or material which has been accepted or concurrently

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**DEDICATION**

This work is dedicated to my parents.

## ABSTRACT

Food or the food industry has been a major driving force behind the growth of most economies and the "Burkina" drink which is gradually becoming a popular drink in major cities of this country. This study was carried out to evaluate the effect of sodium benzoate and potassium sorbate on the microbial keeping quality of the Burkina drink as well as to identify pathogens present in the drink during its storage period. The sample was obtained from local vendors within the Ayigya community and treated with varying concentrations of preservatives: sodium benzoate (0.05, 0.1 %) and potassium sorbate (0.1, 0.2 %) with the samples with no added preservatives serving as controls. Samples were stored at room temperature and refrigeration temperature for 15 days and assayed at three days intervals. Total aerobic count, total coliform count and yeast count were determined as stipulated by (ISO 4833-1:2003). The pH of the samples was recorded over the study period with the microbial quality test featuring the aforementioned with the inclusion of *Escherichia coli* and *Staphylococcus aureus*. The initial outcome indicated the product to be of low microbial quality with the total aerobic count, total Coliform count and yeast counts of $1.24\times10^{6}\pm5.66$ cfu/ml, $1.64\times10^{4}\pm4.95$cfu/ml and $1.23\times10^{6}\pm7.07$cfu/ml respectively exceeding the safe limits. The pathogenic profile indicated the presence of *Escherichia coli* ($9.4\times10^{4}\pm7.07$cfu/ml), *Staphylococcus aureus* (0 cfu/ml) and *Enterococci* ($6.9\times10^{4}\pm3.54$ cfu/ml). Probing into the identity of the isolated microorganisms revealed a total of five species of which four could be identified fully namely *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus* and *Alcaligenes faecalis* all of which are food pathogens associated with food spoilage. At the end of the study, there was no significant difference ($p > 0.05$) in the pH of the samples with regards to the treatments and the storage conditions. There was a significant difference ($p < 0.05$) in the total aerobic count of the samples with regards to the effect of the preservatives though there was no difference ($p > 0.05$) with the different temperatures. The yeast count in the samples were significantly different ($p < 0.05$) with regards to both the treatment and storage condition. At the end of the study, 0.2 % of potassium sorbate proved to be more effective against microbial growth compared with the other preservatives and concentrations used.


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# CHAPTER ONE

## 1.0 Introduction

### 1.1 Background

The “Burkina” drink is a popular drink usually sold on the streets of some of the major cities in the Ghana and has become a growing business which has come to add to other locally produced beverages. The “Burkina” drink originated from Burkina Faso hence its name but its original name is “deger”. “Burkina” as it is commonly referred to in Ghana is made from millet grain and its sweet flavour from fresh or powdered milk, sugar and salt. In most African countries, millet or millet made foods are key components of local diets (Obilana and Manyasa, 2002; Amadou et al., 2011). Milk or milk products provide significant amounts of protein and other relatively minor nutrients such as magnesium, calcium, vitamin A, phosphorus, iodine, zinc and potassium (Anita, 2001). Milk and milk products are excellent source of high-quality proteins and carbohydrates. The complex biochemical composition of milk makes it an excellent medium for microbes (Okonkwo, 2011) hence its low keeping quality especially at room temperature. According to local producers or manufacturers, freshly produced “Burkina” drink has a very short lifespan of only 24 hours under normal room temperature and at most 4-5 days under refrigeration conditions especially for the ones made with fresh cow´s milk without the addition of any preservative. Spontaneous microbial spoilage occurs in raw milk at room temperature within a few days thereby turning the product sour (Wouters et al., 2002). The number of microbes especially lactic acid bacteria present in the milk post-pasteurization has an effect on the rate of spoilage.

Tawiah, (2015) studied the microbiological and proximate composition of the “Burkina” drink in some parts of Accra and observed high levels of *Staphylococcus aureus*, yeasts, molds, and *E. coli* compared with the limits set by the Ghana Standards Authority. Even though fermented

milk products keep better than raw milk, they are still predisposed or susceptible to deterioration by spoilage organisms, mainly, yeasts and molds after a longer period of time.

Preservatives are defined as a collection of chemical compounds intentionally and carefully added to food or that seem to arise in a food as a result of pre-processing treatment, processing or storage (Prescott et al, 2002). Potassium sorbate and sodium benzoate are some of the popular chemical additives used in the food industry on a variety of food materials. They basically inhibit the growth and reproduction of microorganisms including bacteria, yeasts and molds. Chemical preservatives are added to food and pharmaceutical preparations not only to stop microbial spoilage of the products but also to significantly reduce the likelihood of the consumer getting an infection when the preparations are consumed (Sean and Eileen, 2004). Food quality is improved, food waste is minimized and generally consumer acceptability for food products is enhanced when chemical preservatives are applied to foods (Freizer, 1989).

### 1.2 Problem Statement

The rich nutrient content of the milk component of the "Burkina" drink makes it an excellent medium for microbes thereby making it difficult for local producers and vendors to store or preserve. Activities by microorganisms such as *Staphylococcus aureus, Escherichia coli* and other yeast cells present in the drink usually render it unfit for consumption thereby reducing its shelf life. The drink begins to deteriorate rapidly 24 hours after production if not refrigerated. In spite of its growing popularity, the fast deterioration rate can affect large scale production of the Burkina drink. Therefore, the goal of this study is to determine the effect of the addition of preservatives on the microbial keeping quality of the "Burkina" drink at different temperatures.

### 1.3 Justification

Microbial activity is a major contributory factor with regards to food spoilage. Inhibition or reduction of microbial growth or activity in the "Burkina" drink is therefore a major factor to

consider with regards to shelf life extension of the drink. The successful use of preservatives and refrigeration temperature to reduce microbial activity in the drink will provide a solution to the relatively short shelf life of the product thereby increasing or enlarging market reach and finally enhancing the economic benefits of the drink.



### 1.4 Objectives

The objective of the study is to assess the effect of preservatives on the microbial keeping quality of the "Burkina" drink at different temperatures.

#### 1.4.1 Specific Objectives

- To determine the levels of microbial population (bacteria and yeasts) of the drink with or without preservatives (sodium benzoate and potassium sorbate) at different concentrations during storage at room and refrigeration temperatures.
- To identify microorganisms present in the Burkina drink that influence the rate of deterioration of the drink by conducting a number of biochemical tests.

## CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Origin of Burkina drink

The Burkina drink, made from mainly milk (raw or powdered) and millet, originated from the neighboring West African country Burkina Faso, where it is called "deger" but in Ghana it is usually referred to as the Burkina drink because of where the drink originated from.

In Ghana it is usually produced in the Muslim communities or inner cities popularly referred to as Zongos where the drink is produced under unhygienic conditions. The production and sale of the Burkina drink is a fast growing venture in Ghana especially in big cities.

## 2.2 Microbial Quality and Spoilage of Fermented Milk Products

The Codex Alimentarius standard for fermented milk products allows for a sum total of microorganisms in the final product to the level of $10^7$ cfu/g. This only applies to products that are not heat treated after fermentation (Codex Standard for Fermented Milks, 2003). In Ghana, the standard for milk and milk products recommend that no cells of coliforms, *E. coli* or *Salmonella* be detected in the final milk product (Ghana Standard 337-2003). The initial microbial load of milk, post pasteurization treatment contamination and the processing conditions control or affect the microbiological quality of milk and milk products (Richter et al., 1992). In the dairy industry, microorganisms like coliforms, gram-negative psychrotrophs, lactic acid bacteria, yeasts and molds play a key role in the deterioration and damage of dairy products. Undesirable and pathogenic microorganisms like *Listeria monocytogenes, Yersinia enterocolitica, Salmonella spp., Campylobacter jejuni*, pathogenic strains of *Escherichia coli* and *Staphylococcus aureus* which can be found or isolated in many dairy products are of a huge health concern to consumers and the general public (Tatini and Kauppi, 2003).

The growth of microbes in foods is influenced by innate factors such as nutrient availability, redox potential, pH, water activity (aw) and antimicrobial constituents, and external factors including temperatures under which foods are stored and the availability of oxygen (Mossell and Ingram, 1955). These factors combine to give a synergistic effect in influencing the proliferation of the microbes in foods. The reduction in the use of preservatives and the processing of foods allow for growth of microorganisms, particularly if the food is stored improperly (Waites and Arouthnott, 1990). Molds and yeasts have been implicated as the main

microorganisms involved in the spoilage of bakery products and to some extent bacteria (Earle and Putt, 1984). Due to the high acid contents of acid fruit products, microorganisms like yeasts which are extremely tolerant to acidic environment control the spoilage of such products. For fruit juices, microbial spoilage is influenced by yeasts, molds, and lactic acid bacteria (Deak and Beuchat, 1993). Development of off-flavors and unfavourable smell, increase in the turbidity of the solution and the production of gas is associated with the spoilage of juice (Jay and Anderson, 2001).

The shelf life of the Burkina drink at room temperature is between 24 – 48 hours and between 10 and 12 days under refrigeration conditions. For fermented milk products like yoghurts, when they exceed their shelf-life periods, yeasts and molds are the microbes mainly involved in the spoilage or deterioration of the product (Lacroix and Lachance, 1990). The addition of other ingredients like sugar to the Burkina drink increases the richness of the drink environment thereby increasing its support for a vast variety of microbes especially yeast species. Also, milk products stored over a lengthy period of time under refrigeration conditions are damaged by psychrotrophic bacteria and their enzymes which causes deterioration of the products resulting in the production of off-flavours and change of colour (Cousin, 1982). For fermented dairy products when the yeast cells reach a level between $10^6 10^7$ cfu/g, the product begin to develop undesirable yeasty off-flavours and gassy appearance.

A substantial number of these yeasts cells produce or generate alcohol and $CO_2$, resulting in fermented dairy products like cheese developing a yeasty taste (Horwood et al., 1987).

Another way by which dairy products deteriorate is as a result of the degradation of vital component of the product through the action of microbial enzymes such as proteases, phospholipases, and lipases. A large number these extracellular enzymes are produced by psychrotrophic bacteria which ranges from $10^6$ to $10^7$ cfu/ml and these extracellular enzymes

affect the quality of the milk and this is detected through sensory tests (Fairbairn and Law, 1987). Microbial enzymes like proteases affect the quality of milk products by primarily producing bitter or undesirable peptides whereas lipases have been associated with the development of rancid flavors in ultra-high temperature milk (Adams and Brawley, 1981). When milk and milk products go bad or spoil, they begin to develop undesirable organoleptic properties such as bitterness caused as a result of proteolysis and lipolysis, sourness caused by *Lactococcus lactis*, development of burn or caramel flavour as a result of the action of *Lactococcus lactis*, production of volatile fatty acids by the action of coliforms and *Clostridium*, as well as undesirable physical changes like the change in colour or appearance of the product.

**2.3 Millet**

Millet (Peninsetum americanum) refers to a wide range of small-seeded grains which are part of the family Poaceae (true grass) and are staple foods for humans (Zhu, 2014). According to ICRISAT/FAO (1996), the total world production of millet is 28.38 million tonnes from nine main species out of which Africa produces 11.36 million tonnes representing 40% of the total world production number. Millet is regarded as a vital source of nutrients and an absolutely necessary food for a number of people in developing countries in Africa and Asia (Amadou et al., 2013; Mridula and Sharma, 2015). The most cultivated species of millet is the pearl (Pennisetum glaucum), mostly grown in West Africa and in some parts of Asia. Other species include finger millet, mostly grown in Eastern Africa and India, proso millet, widely cultivated Russia, Ukraine and Australia and the foxtail millet which is mostly cultivated in some parts of Asia and Southern Europe (Baltensperger and Cai., 2004).

Millet has good productivity when grown or cultivated on marginal lands, brief cultivation cycle or season and the ability to modify or adapt to a wide range of climate conditions such as high temperature and dry conditions, making millet a very suitable food crop for food security

in some parts of Asia and Africa (Taylor, 2004). This makes the production of millet a very excellent staple food for people in poor or developing countries or generally people with lower socioeconomic status. The different farming regions include mainly the semi-arid tropics (SAT) and sub-tropics of Africa, and also covers other areas that are susceptible to drought with other climatic conditions like relatively high humidity and medium-high altitude (ICRISAT/FAO., 1996). According to (FAO 2014), India is the top producer of millet followed by Nigeria and Niger. In Africa, millet production is particularly high in the West African sub-region with Nigeria, Niger, Burkina Faso and Mali being the top four producers across the continent.

Millet is rich in vitamins B, lipids, dietary fibre, polyphenols and minerals (calcium, iron, phosphorus) (Saleh et al., 2013: Shahidi and Chandrasekara., 2013). They also contain comparatively high proportions of protein (up to 9.5 g/100g for fonio and teff), potassium, ash and zinc (Obilana and Manyasa., 2002). Millet also comprises of 28-32% glutelin and glutelin-like proteins, 22-28% albumins and globulins, while its prolamin content ranges from 22-35% (Leder., 2004). Millet also provides an abundant source of phytochemicals, soluble and insoluble antioxidants (Taylor and Duodu, 2015; Muthamilarasan et al., 2016).

The fatty acids contained in millet are highly polyunsaturated and contain high amounts of non-starchy polysaccharides (Hegde and Chandra 2005; Muthamilarasan et al., 2016). They are also known to gradually free sugars that results in it having a reduced glycemic index (Bala-Ravi, 2004). It is also gluten free, which indicates that it will be an excellent option for persons or invalids suffering from celiac diseases caused due to gluten intolerance or extreme sensitivity to gluten (Taylor et al., 2006). When compared with other cereals like maize, millet possesses much more superior nutritional qualities. Millet possesses vital amino acids, quality micro and macro nutrients and higher protein (Shobana and Malleshi, 2007; Saleh et al., 2013). Due to their anti-inflammatory, antioxidant, antimicrobial, antiviral, anticancer and antiplatelet

aggregation properties, millet has become a rich source of nutraceutical and functional food ingredients when consumed and generally helps to improve the health of consumers (Chandrasekara and Shahidi, 2011; Taylor and Duodu, 2015; Muthamilarasan et al., 2016)

Traditionally, millets are used to produce a wide variety of foods and beverage products such as beer, non-alcoholic drinks, flour, porridge, fermented and non-fermented flatbreads (Baltensperger and Cai, 2004). In Africa, many native or traditional foods and drinks are made from flour and malt of the millets.

### 2.3.1 Food made from millet in Africa

In Burkina Faso, ben-saalga which is a thin and fine porridge is produced by cooking the fermented sediment of pearl millet in water (Nout, 2009). Ogi which is an indigenous porridge-like food is cooked with fermented millet or sorghum/maize (Blandino et al., 2003). It is mostly consumed by inhabitants of the West African sub-region.

Koko which is a very common breakfast meal or snack in-between meal is a porridge made from millet (Lei and Jakobsen, 2004). This is prepared by fermenting the slurry and subsequently boiling the top water and addition of sedimented lower part to get the desired constituency and uniformity. Another popular staple food in some West African states like Ghana, Nigeria and Burkina Faso is fura which is a semi-solid ball of dough.

*Kaffir* or *bantu* is an alcoholic beverage made from fermented millet grains and are mostly consumed by inhabitants of some West, Central and East African nations. In Ghana and Nigeria, pito which is an alcoholic beverage with a light brown colour is produced from fermented millet or sorghum (Ajiboye et al., 2014). “Couscous” which is a steam cooked food product made from millet is common in some of the French speaking countries in West Africa (Obilana, 2014). People from the northern part of Ghana are those that consume food made from millet.

Food like koko, tuo (stiff porridge) and fura are popular millet made food products in the northern part of Ghana.

**2.4 Milk**

Milk from cows is the most popular, commercially used and consumed milk type in the world and has been associated with good health when eaten. Other sources of milk for commercial purposes include milk from goats, sheep, donkeys, horses and buffalo. Milk is produced by mammals including humans and is used to feed their offspring. Milk is normally whitish in colour, liquid in form and produced by the mammary glands of mammals. Cow milk which accounts for close to 80% of the total milk produced in the world refers to a lacteal secretion, which is colostrum free and its acquired through the milking of cows in good physical condition. Nowadays, beverages made from milk are pasteurized and should comprise of milk fat of more than 3.25% and milk solid of more than 8.25%.

The production of milk is an important source of cash flow for households in developing countries with close to 150 million households around the world involved in this venture. As at 2012, the total world milk production stood at 769 million tonnes with countries like India, United States of America, China, Pakistan and Brazil completing the top five producers respectively. The European continent put together produced the highest amount of milk with regards to unions or regions as at 2013. United States of America is the highest producer of cow´s milk which is the most commercially used milk type. Table 1 below shows the top ten producers of cow´s milk in the world and their corresponding production numbers in tonnes. Also, as at 2011, Ireland had the highest per capita consumption of raw milk with 135.6 litres followed by Finland, United Kingdom, Australia and Sweden to complete the top five (Goff, 2014).

**Table 1: Top ten producers of cow milk worldwide**

| COUNTRY | PRODUCTION QUANTITY (MT) |
|---|---|
| United States of America | 91,271,058 |
| India | 60,600,000 |
| China | 60,600,000 |
| Brazil | 34,255,236 |
| Germany | 31,122,000 |
| Russia | 30,285,969 |
| France | 23,714,357 |
| New Zealand | 18,883,000 |
| Turkey | 16,655,009 |
| United Kingdom | 13,941,000 |

Source: FAO (2013)

Milk which is a very nutritious food product deteriorates or is damaged within a short period of time due to microbial activity especially if left untreated thus processing milk enhances its storage and creates different products (Nebedum and Obiakor., 2007). Due to its short shelf life, some of the preservation methods adopted or used to improve its shelf life include refrigeration, freezing, addition of preservatives, dehydration, pasteurization, heat sterilization and fermentation. It also serves as a suitable growth medium or environment for a wide range of microbes (Rajagopal et al., 2005). Microorganisms like *Staphylococcus aureus* find the milk environment to be excellent for their growth.

Milk is a rich source of energy and protein, providing 34 to 61 kcal (depending on fat content) and approximately 3.2 g of protein per 100 g. Cow´s milk contains about 4.5 g lactose per 100 g of milk with lactose being the basic carbohydrate component of milk. Raw milk also has a

pH of about 6.4. After pasteurization, raw milk can be kept under refrigeration temperatures for about 14-20 days even though raw milk is acted on by psychrotrophic bacteria. Cow´s milk contains a lot of important nutrients which makes it a complete meal when consumed. These vital nutrients include vitamins B, calcium, B vitamins, casein and minerals like iodine, potassium and phosphorus. Milk fat which is a major component of milk consists of triglycerides (98%), diacetyl glycerol, phospholipids, and fatty acids.

Milk also contain lipids which provide the human body a lot of energy and are significant or relevant contributors to both suitable and objectionable flavours in milk and dairy products. Milk is a rich source of vitamins especially vitamins A, D, E and K. For adults, minors and infants, vitamins from milk plays a very important role in their growth. Vitamins A and E, thiamin, riboflavin, folic acid and vitamin B12 can also be found in milk. However, human milk is richer in vitamins E and some vital acids compared to cow milk. Milk is also rich in calcium and phosphorus which are essential for tooth and bone formation in minors and infants and also help prevent osteoporosis in old people. Milk supplies the human body with high levels of magnesium, zinc and iodine when ingested or consumed.

The major component of dairy products is milk. Some popular dairy products include cheese (soft, ripened, cottage and fresh), butter, yoghurt, dried or powdered milk and concentrated milk. The stability of these products on the shelf is influenced by intrinsic factors like pH of the product or medium, moisture content and external factors like storage temperature and processing parameters. Due to the rich nutrient content of milk, it serves as an excellent medium for the growth and proliferation of a wide range of microorganisms including *Staphylococcus aureus*.

## 2.5 Fermentation

Fermentation is one of the most used ancient technique of producing and extending the shelflife of beverages and foods (Bilings, 1998). It is simply an activity performed to alter or convert substrates into fresh products as a result of the action of microbes. It also helps to destroy objectionable and undesirable components and microorganisms, enhances the nutritive quality and appearance of the food and also improves safety of the food (Simango, 1997). Food fermentation results in the activation of enzymes, modification of pH and an improvement in the action of some enzymes such as amylases, proteases and hemicellulases. Fermented food products are mostly derived from milk, meat, cereals and grains, vegetables and fruits (Hirahara, 1998).

The fermentation process improves the shelf-life of foods and beverages (Onweluzo and Nwabugwu, 2009), enhances protein content and digestibility, improves nutritive quality, releases minerals, functional properties and reduces the concentration of phytic acid while improving sensory properties or attributes and imparting antimicrobial qualities on the food product (Jay et al., 2005; Inyang and Zakari, 2008). It also maximizes or improves the levels of some of the vital nutrients and further reduces the dietary bulk in cereals (Akinhanmi et al., 2008). Cooking time has been reported to reduce when dealing with fermented products (Jay et al., 2005; Sasikumar, 2014). Also, fermentation has been identified to enrich the sensorial qualities of food products as a result of the production of various or diverse flavors, aromas and textures (Jay et al., 2005; Kohajdova and Karovicova, 2007).

In Africa, a wide variety of foods undergo fermentation to produce new products and they include dairy products (cheese, yoghurt), cereals (rice, sorghum and maize), legumes (soybean), roots and tubers (cassava), meat, fruits and vegetables (grapes, pickles) and others which include coffee, tea, cocoa etc. With most fermented foods in Africa, the dominant

microorganisms involved in the fermentation process are lactic acid bacteria and yeasts (Halm et al 1993; Amoa - Awua 1996). Lactic acid bacteria do not only help to preserve food products by reducing its pH, removing the microbe nutrient source and producing antimicrobials but in recent times they are also used to create food varieties by altering food flavor, texture, aroma and the general look of the food product. The fermentation process in recent times is used to create desirable food products from raw commodities (Daeschel 1989).

### 2.5.1 Milk fermentation

Milk is an extremely perishable product and thus the principal or prime intent of milk fermentation is to lengthen or extend its shelf-life and in addition preserve the nutritious constituents of the milk. Fermentation has been used to make good or quality products with generally accepted organoleptic properties or attributes.

Milk fermentation can either be self-generated or inoculated with starter cultures with lactic acid bacteria being the most widely used and studied microorganism with regards to milk fermentation (Olson, 1990; Urbach, 1995). The microorganisms reduce the pH of the medium by their actions which assists to reduce the proliferation of other harmful pathogens (Schnurer and Magnussen, 2005). Some also act as probiotics.

Four main genera of microorganisms belonging to the lactic acid bacteria group namely *Pediococcus, Lactococcus, Lactobacillus* and *Leuconostoc* are mostly involved in lactic acid fermentation of foods like milk especially in Africa. The microorganisms can be added to the milk medium to begin the fermentation process or may already exist in the medium. Large scale production of fermented milk products is usually carried out under controlled fermentation conditions.

### 2.5.2 Fermented milk products in Africa

Some fermented milk based products commonly found in Africa include *ayib* (Ethiopia), *leben* (Morocco), *zabadi* (North Africa) and *nono* (West Africa). *Kishk* which is a fermented milk product mixed with wheat is popular in some parts of Africa Oyewole (1997) and the Middle East.



### 2.5.3 Nutritional Quality of Fermented Milk Products

For fermented milk products, the composition of the raw milk used influences the nutritional values of the fermented milk product even though the ingredients used, fermentation and manufacturing processes also affects the constituents and concentration of nutrients.

The Codex Alimentarius standards recommend that fermented milk product must be composed of milk protein with a minimum percentage of 2.7 % (% m/m) and a maximum of 10% (m/m) for milk fat.

## 2.6 Burkina Drink Production

Burkina drink is originally produced from raw cow milk but due to recent complaint by consumers with regards to the unhygienic manner in which raw milk is handled, most producers currently use powdered milk for the preparation of the drink. Also, another factor such as the inexpensive nature of the powdered milk compared to the raw milk makes it an excellent choice for producers. Some producers however add small quantities of the raw milk to the powdered fermented milk in order to still give it that distinct flavour.

### 2.6.1 Millet Balls Preparation

Initially, the millet is sorted out to take away any additional or undesirable material that might result in the contamination of the millet. The millet is then washed with water to remove all forms of dirt and later soaked in water overnight. Water is drained out of it and the millet sent

to the mill to grind into a rough or course texture the next day after soaking. A cooking pot containing water is placed on fire to boil. The millet is then placed on a mesh and rolled vigorously by hand in order to form tiny balls. When the water heats up, the tiny balls of millet are placed in a colander and placed as a lid on the cooking pot. A thick paste made up of the powdered millet and water is used to plaster the pot to ensure that vapour is retained in the pot. The colander is then covered and the steam from the boiling water allowed to engulf the tiny millet balls to soften it. After the softening of the tiny millet balls, they are then poured into a basin. Subsequently, the tiny millet balls in the basin are kept at room temperature until they are needed.

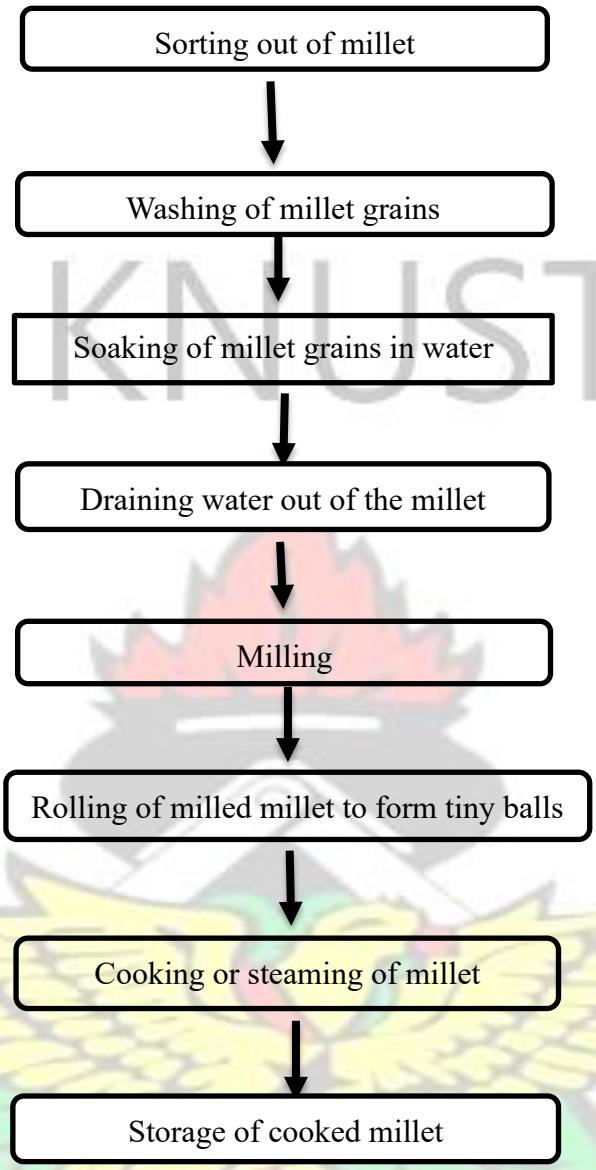


**Figure 1: A flowchart summarizing the preparation of millet**

### 2.6.2 Preparation of Raw Milk for the Processing of "Burkina" Drink

Even though most of the producers of the drink currently do not use raw milk for the production of the drink for commercial purposes, some households especially in the Zongo areas still use it for the production of the drink for domestic use. With the raw milk, the milk is poured into a container and heated for some time in order to reduce the microbial load even though it is not performed at a specific temperature or a given time duration. After heating the raw milk, it is then transferred into another container and allowed to cool. Previously fermented milk product

is then added to the raw milk to serve as starter culture to ferment the raw milk. It is then stored at room temperature for close to 24 hours to allow for fermentation to take place. With regards to the fermentation of the raw milk for the production of the Burkina drink, there is no specific module or procedure for the fermentation process. The indigenous or innate way of fermenting dairy products is the procedure that has been adopted for the fermentation of the raw milk.

### 2.6.3 Preparation of Powdered Milk for the Processing of "Burkina" Drink

With the use of powdered or dehydrated milk, hot water is added to the powdered milk and stirred in order for it to completely dissolve and form a homogenous mixture. The mixture of water and powdered milk is then allowed to cool for some time. A previously fermented milk product or an already processed Burkina drink is added to mixture in order to begin the fermentation process. The mixture is then kept at room temperature for about 24 hours to enable the fermentation process to take place.

## 2.7 Effect of Storage Temperature

The temperature at which milk products are stored affects the shelf-life of the product. When milk products are stored at refrigeration temperatures 4 °C, it is expected that its microbial quality is preserved over a much longer length of time compared with products stored at room temperature 25 °C. Generally, foods kept at room temperature over a relatively long length of time tend to support microbial growth faster leading to the occurrence of food poisoning outbreaks (Adesiyun and Balbirsingh, 1996). According to (Mortazavian et al., 2007), the viability of *Lactobacillus acidophilus* in a yoghurt sample stored at 2 °C for 5 days was much lower compared with the samples stored under relatively higher temperatures that is 5 °C and 8 °C. Again, when fermented milk products like yoghurt are stored under relatively low temperatures like 5 °C or lower, it is anticipated that the product maintains its desirable microbiological, physiochemical and organoleptic or sensory qualities for close to a month

whilst withstanding deterioration by yeast (Davis, 1975). However, when dairy products are stored under very low temperatures, it can result in the creation of a disorder in the milk fat globule membrane exposing it and making makes it extremely likely to be affected by lipolysis. Psychrotrophic bacteria act on milk and milk products under relatively low temperatures and the effect on these products can be reduced by limiting the contamination levels. Generally, the keeping quality of dairy products is enhanced or maintained by reducing the microbial load present in the medium. Normally, Pseudomonas species account for a majority of psychrotrophic bacteria found or isolated in raw milk owing to their ability to grow and reproduce at reduced temperatures between (3–7◦C) and also their ability to make use or process relatively large molecules of proteins and lipids for growth.

**2.8 Use of Chemical Preservatives**

A chemical preservative is defined as any chemical substance that when incorporated into food or any preparation, reduces or stops microbial spoilage of the food or a specific product such as a pharmaceutical product that helps to reduce the possibility of a consumer getting an infection when the product is ingested (Sean and Eilean, 2004). Chemical preservatives ordinarily do not consist of sugars, common salts, vinegars, spices or oils extracted from spices. Chemical preservatives can also be anti-oxidizing agents, anti-stalling compounds, anti-browning compounds or chelating agents. Chemical food preservatives include benzoates, nitrites, sulphites and sorbates. However, in recent years the use of chemical compounds in food products has become a cause of concern to consumers and the general public (Sofos and Busta, 1981).

The chemical agents affect or inhibit growth of microorganisms by interacting and disrupting cell factors like damaging the cell membrane, denaturing important cell proteins or affecting protein synthesis and also interfering and affecting the genetic material or nucleic acids thus

affecting cell reproduction and growth (Lansing et al., 2002). Some chemical preservatives affect the cell wall of microorganisms and also affect metabolic enzymes. The inhibitory action of most chemical preservatives depends on the pH of the medium, concentration of preservative and initial load or species of microorganism present. Lowering the pH or increasing the acidity of the medium with increase in the concentration of the preservative increases the inhibition ability of the preservative (Restaino *et al*., 1981).

Due to the low keeping quality of milk and milk products, the use of preservatives has also been adopted in the dairy industry. Even though fermented dairy products are generally more stable in terms of their microbial keeping quality as a result of the action of microbes leading to the reduction of pH and production of bacteriocins, preservatives are sometimes added to fermented dairy products in order to further extend its shelf life. Chemical preservatives like sorbic and propionic acid have been applied on dairy products like cheese and yoghurt to prevent the growth of molds on the surface of the product.

### 2.8.1 The Use of Potassium Sorbate as a Food Preservative

Potassium sorbate, other sorbic acids and its salts are collectively called sorbates. The reason for the widespread use of sorbates as a preservative is founded on their capability to hinder, restrain or delay the growth of large numbers of microorganisms including yeast, molds and bacteria. They act on microorganisms by inhibiting cell metabolism. They inhibit specific enzymes, inhibit nutrient uptake and affect various biological processes like the transport system of the microorganism, cell metabolism, growth and replications of the microorganism. Potassium sorbate produces sorbic acid when dissolved in water. Sorbic acid has a molecular mass of 112.13 g and the molecular formula $C_6H_8O_2$.

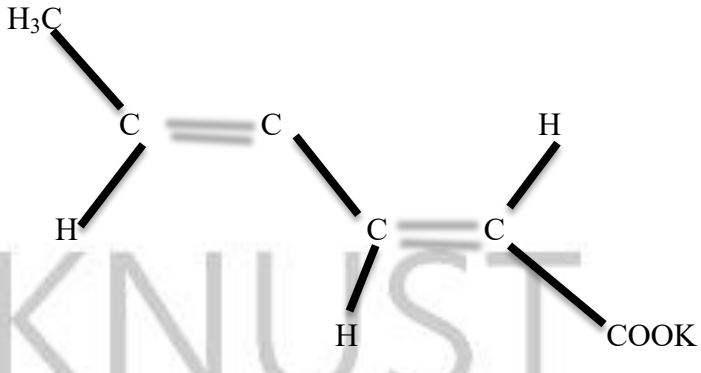


**Figure 2: A chemical structure of Potassium sorbate**

Sorbates are effective up to a pH of 6.5 even though they are more effective at relatively lower pH level. The effectiveness of the microbial inhibition action of sorbates also depend on species or strains of microorganisms, additives, temperature of storage and concentration of sorbate. It has been reported that potassium sorbate has a bacteriostatic effect when its concentration is below 0.3% and also has a bactericidal effect if its concentration reaches or is above 0.3% (Sofos and Busta 1993). Potassium sorbate is also used to preserve or keep food items like cakes, cheeses and syrup unchanged with its effective concentration being 0.2% (Sofos and Busta., 1981).

"Kerosene" which is an undesirable off-flavour or odour can arise in fermented dairy products like cheese that contain sorbate or have been stored with sorbate. Molds which are associated with spoilage of fermented dairy products act on the sorbate compounds and degrades it further into trans 1,3-pentadiene, which results in the production of the undesirable flavour and odour.

### 2.8.2 The Use of Sodium Benzoate as a Food Preservative

Sodium benzoate is the most frequently or widely used chemical preservative in the food and beverage sector. Sodium benzoate exists either as a white substance with a grainy or coarse structure or a white substance in a crystalline powder form with a molecular weight of

144.12. Sodium benzoate acts as a very potent antimicrobial performer by getting involved and affecting the permeability of vital ions, metabolites and nutrients through plasma membranes, leading to cell death due to the lack of nutrient (Freese et al., 1973). They destroy the proton motive force of the microorganism. The inner part of the microbe cell is acidified as a result of the diffusion of benzoic acids that have not been dissociated. They move into the cell of the microbe without interference or restriction and then produce protons that ionize the inner or enclosed part of the cell. When used in foods, they may sometimes impart into the food or beverage undesirable flavours (Chipley, 1983).

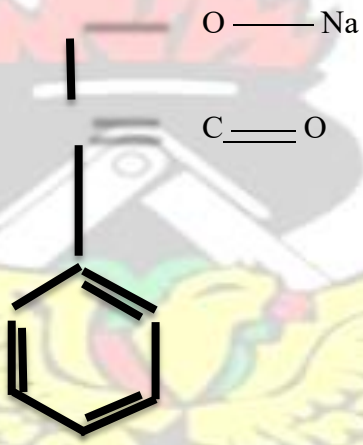


**Figure 3: A chemical structure of Sodium benzoate**

Benzoic acid exists naturally in foods like fruits but sodium benzoate is much preferred for use as an additive since it is much more soluble in water. In use, sodium benzoate is converted to benzoic acid, the compound with antimicrobial properties or active compound (Furia., 1972). The benzoic acids are antifungal agents that is they are effective against yeast and molds and their sodium salt is more dissolvable than other forms with its range not exceeding 0.1% for countries like the United States of America (James et al., 2000). As at

1995, the limit for the use of sodium benzoate in the *European* market ranges from 0.015% to 0.5% (EC, 1995). Sodium benzoate like most preservatives depend on pH for its effectiveness.

It is most effective in foods and beverages with relatively low pH values between 4-4.5 (El-Gazzar and Marth, 1987).

This preservative causes no deleterious effects in humans when consumed in small amounts (Chichester and Tanner, 1972). This involves detoxifying mechanisms where benzoate is assimilated or sucked up from the intestine and put into action or actuated by linkage with coenzyme A (CoA) to produce benzoyl coenzyme A. The benzoyl coenzyme A then reacts with glycine to form a water soluble compound called Hippuric acid which is readily excreted by the kidney (Ihekoronye and Ngoddy, 1995). Sodium benzoate has activity against yeast, mold and bacteria. Due to their action on yeast cells, they cannot be used in foods that employs the use or action of yeast cells for processing or fermenting that particular food product (Friedman & Greenwald, 1994).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODOLOGY

### 3.1 Source of Burkina drink

Freshly prepared „Burkina" drink was obtained from a vendor at Ayigya, a suburb in Kumasi (near Kwame Nkrumah University of Science and Technology, KNUST) and transported on ice immediately to the Microbiology laboratory of the Department of Biochemistry and Biotechnology.

### 3.2 Chemical Reagents and Media preparation

The reagents used in the study were hydrogen peroxide from Care$^{+}$ LOT SY68 and agars for the culture and isolation of microbes as well as biochemical assay. The agars used were products of OXOID Laboratories, Basingstoke Hampshire, England. They included Plate Count Agar used for the isolation of total viable count; Brilliant *E.coli* agar for *Escherichia coli*; Violet Red Bile Lactose agar for the isolation of Coliforms; Mannitol Salt Agar for isolation of *Staphylococcus* and Malt Extract agar for yeast and molds.

#### 3.2.1 Preparation of Plate Count Agar

Plate Count Agar (Nutrient agar) was prepared by suspending 23.5 grams in 1000 ml (1 liter) distilled water and heated to boil to dissolve completely. It was sterilized at 121 °C for 15 minutes in a sealed bottle. The sterilized agar was left to cool at 50 °C then poured into sterile Petri plates. The prepared plates were allowed to set and incubated for 24 hours at 37 $^{0}$C prior to use for sterility validation (ISO 4833-1, 2003).

#### 3.2.2 Preparation of *Escherichia coli* media

The presence of *E.coli* in the samples was confirmed and enumerated on the Brilliant E. coli agar which is a coliform selective medium. The agar was prepared according to the directive

of the manufacturer (28.1 g in a litre of distilled water and bring to boil no further sterilization is needed). The agar was cooled to 50ºC and poured into sterile agar plates after which sterility check was conducted after 24 hours. (ISO 7251, 2006 [E])

### 3.2.3 Preparation of Mannitol Salt Agar

Agar powder of Mannitol salt (111 g) was suspended in 1 liter of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121 °C for 15 minutes. The prepared plates were validated after 24 hours of preparation for sterility/ prior to use. (ISO 6888-1, 1999[E]).

### 3.2.4 Preparation of Violet Red Bile Lactose agar (VRBLA)

The agar was prepared by dissolving 38.5 g of agar powder in a liter of distilled water and heated in a water bath at 100 $^0$C for 20 minutes to melt. The agar was allowed to cool and poured into sterile Petri dishes to set after which sterility checks were conducted after a 24 hour incubation period at 37 $^0$C. (ISO 7251, 2006 [E])

### 3.2.5 Preparation of Simmons Citrate agar

The agar tubes were prepared by dissolving 23.3 g of agar powder in a liter of distilled water and melting in water bath at 100 $^0$C to completely dissolve after which the agar was distributed into test tubes with each tube containing a final volume of 6 ml of agar. The tubes were autoclaved at 121 $^0$C for 15 minutes and brought to cool by slanting to obtain agar slants. The slants were kept incubated at 37 $^0$C for 24 hours to assess the sterility.

### 3.2.6 Preparation of Triple Sugar Iron agar

A weight of 64.6 g of the agar powder was dissolved in distilled water and boiled in a water bath at 100 $^0$C to completely dissolve after which the agar was distributed into test tubes with each tube containing a final volume of 6 ml of agar. The tubes were autoclaved at 121 $^0$C for

15 minutes and brought to cool by slanting to obtain agar slants. The slants were kept incubated at 37 $^{0}$C for 24 hours to assess the sterility.

### 3.3 Sample preparation

The Burkina drink sample was divided into two sets (refrigerated and non-refrigerated) and each set comprised ten (10) different containers with 100 ml of sample in each container. For each treatment and storage condition, the samples (100 ml) were placed in two different containers in order to reduce the level of contamination. One pair of sample was left untreated without any preservative whereas the other 4 pairs were treated. The samples were treated as follows:

For the control sample, four containers were prepared and 100 ml of the sample were placed in each container. Four containers were treated with sodium benzoate at a concentration level of 0.05%; for the 100 ml sample in the container, 0.05 g of sodium benzoate was added to it. The next set of containers (4) were treated with sodium benzoate at a 0.1% concentration level; for the 100 ml sample in the container, 0.1 g of sodium benzoate was added to the sample. Another set of containers (4) were treated with potassium sorbate at a 0.1% concentration level; 0.1 g of the preservative was added to 100 ml of the sample in the container. The last set of containers (4) were also treated with potassium sorbate at a 0.2% concentration level; for every 100 ml sample in each container, 0.2 g of the preservative was added or treated with it.

**Table 2 Summary of preservative concentrations used in treatment of samples.**

| Treatment | Concentration (%) |
|---|---|
| Sodium benzoate | 0.05 , 0.1 |
| Potassium sorbate | 0.1 , 0.2 |
| No preservative | 0 |

The different treatments and storage conditions gave a sample size of ten (10) per each section for analysis resulting in a total of 50 samples across the period (5×2×5 factorial). The study period of 18 days was spread into five (5) sample sections thus a 3-day sample interval with each sample section featuring microbiological (aerobic count, yeast count, mold count and coliform count) and pH analysis. One set of the containers which is 5 containers with each containing different treatments (no preservative added, 0.05% and 0.1% sodium benzoate, 0.1% and 0.2% potassium sorbate) were stored at room temperature whiles the replicated set of containers were also stored in the refrigerator (4ºC).

**Table 3 Sample labelling**

| **Sample** | **Label** |
|---|---|
| Control (Non-refrigerated) | A |
| Control (Refrigerated) | B |
| 0.1% Potassium Sorbate (Non-refrigerated) | C |
| 0.2% Potassium Sorbate (Non-refrigerated) | D |
| 0.1% Potassium Sorbate (Refrigerated) | E |
| 0.2% Potassium Sorbate (Refrigerated) | F |
| 0.05% Sodium Benzoate (Non-refrigerated) | G |
| 0.1% Sodium Benzoate (Non-refrigerated) | H |
| 0.05% Sodium Benzoate (Refrigerated) | I |
| 0.1% Sodium Benzoate (Refrigerated) | J |

### 3.4 Assessment of the microbial quality and safety of product

The microbiological analyses were done for two objectives: the first was the assessment of the quality and safety of the product and the other being the estimation of the shelf life of the product under varying treatments.

The other part of the analysis featured pH determination across the study period. The pH was taken using a pH meter at a temperature of 28 $^0$C. The reading was done in triplicate.

The procedures described below were used to test for presence (qualitative) and enumerate (quantitative) the microorganisms in the samples. Colonies on selected plates were counted using a colony counter. The morphological characteristics of colonies such as colour, shape, margin, elevation and optical characteristics were examined to facilitate grouping and identification.

### 3.4.1 Diluent preparation and serial dilution

The diluent used in this study was buffered peptone water from Biolab which was prepared according to the manufacturer‟s instruction (Dissolve 10g of agar in a liter of distilled water and stir to dissolve completely and dispense into appropriate containers. Sterilize at 121$^0$C for 15 minutes and allow to cool).

The stock dilution was prepared by dissolving 10 ml of sample in 90ml of sterile diluent and shaking for 30 seconds. The subsequent dilutions were prepared by adding 1ml aliquot of the stock solution to 9ml of sterile diluent in succession to obtain a six-fold serial dilution. The dilutions were then inoculated unto the respective media.

### 3.4.2 Total Viable Count (TVC)

Total Viable Counts were isolated and enumerated by spread plate method and grown on Plate Count Agar (PCA). A volume of 100 µL of the dilutions were inoculated unto Petri dishes of plate count agar and spread evenly in triplicate. The inoculated plates were incubated at 37 $^0$C for 24 hours and 48 hours after which all white spot or spread were counted and recorded as total viable count using the colony counter (ISO 4833-1, 2003).

### 3.4.3 Enumeration of *Staphylococcus species*

*Staphylococcus species* were isolated and enumerated by spread plate method and grown on Mannitol Salt Agar (MSA). An inoculum volume of 100 µL was inoculated unto the agar plate and incubated at 35 ºC for 24 hours. After incubation yellow colonies were counted and recorded as *Staphylococcus* counts using the colony counter (ISO 6888-1, 1999[E]).

### 3.4.4 Enumeration of *Escherichia coli* and Coliform

To ascertain the safety and quality of the product, the presence of *E.coli* and Coliforms was determined on Brilliant *E. coli*$^{TM}$ and VRBLA respectively. The same inoculum volume used in earlier tests (100µL) was inoculated unto the respective agars and incubated at 37 $^{0}$C for 24 hours. Purple colonies were recorded and counted on the E.coli medium for positive test whereas red/pink colonies were recorded and counted for positive Coliform test (ISO 7251:2006(E)).

### 3.4.5 Enumeration of Yeast and Molds

An inoculum volume of 1 ml was inoculated unto prepared plates of malt extract agar and incubated at 25 $^{0}$C for 72 hours for yeast and 120 hours for molds. The presence of white bacterial-like colonies were recorded after 72 hours for yeast and the presence of relatively bigger colonies with mycelia and spores were recorded and enumerated for molds. (ISO 21527-1, 2008)

### 3.5 Isolation and characterization of pure isolates

The detected colonies on the plate count agar were isolated and characterized to determine the identities of the organisms. This was done by a series of tests after obtaining the pure colonies from sub-culturing. The tests include Gram staining, catalase, citrate and triple sugar iron tests which were conducted on the pure colonies.

### 3.5.1 Isolation and sub-culturing of pure colonies

The PCA plates were observed for the presence of growth after the incubation period not only for quantitative measures but qualitative as well. The observed colonies were differentiated on the merit of their morphological properties which featured colour, shape/form, elevation, margin and optical characteristics. The colonies that were judged different were labeled and sub-cultured in to pure colonies.

### 3.5.2 Sub-culturing of isolated colonies

The isolated colonies were sub-cultured unto fresh nutrient agar plates by streak plating. The individual colonies were picked by the aid of a sterile inoculating loop and streaked unto the sterile agar plates. The inoculated plates were incubated for 24 hours at $37^0$C and pure colonies were observed along the lines of streak. The streaked plates were then refrigerated awaiting further biochemical tests.

### 3.5.3 Identification of Pure colonies

The pure colonies were subjected to Gram staining, catalase, citrate and triple sugar iron tests to identify the class of organism isolated.

### 3.5.4 Gram staining of isolated colonies

The colonies were fixed in physiological saline drops unto the slide by flaming and cooling. The fixed slides were then stained with the Grams crystal violet solution for 2 minutes after which the stain was washed off with distilled water. Grams iodine solution was then added unto the slide for another minute and washed off with ethanol. The slide was finally stained with safranin for 2 minutes and washed off with distilled water after which the slides were allowed to air dry and observed under the microscope for the Gram reaction, shape and arrangement of the cells.

### 3.5.5 Catalase test

The catalase test was carried out by adding the cells to drops of hydrogen peroxide on a slide. The formation of bubbles upon the addition of the cells was recorded as positive and the absence of bubbles as negative (ISO 6888-1, 1999[E]).

### 3.5.6 Citrate and Triple sugar iron test

The pure colonies were streaked unto the citrate agar at the slant and stabbed at the base in the agar tubes. The tubes were incubated at 37 $^{0}$C for 24 hours and the results were recorded for color changes in both the slant and butt/base. A color change from green to blue was recorded as positive whereas a green color after the incubation period was recorded for a negative test (ISO 6579, 2002 [E]).

### 3.6 Microbial and pH study

The microbial analyses for this study included the total aerobic count, yeast count, mold count and total Coliform count. The tests were conducted as described above in section 3.3 under their various headings and specifications with no modifications.

### 3.7 Statistical Analysis

The results were analyzed using Statsgraphics centurion software edition IV and the data was subjected to a two-way analysis of variance (ANOVA) to ascertain the effect of both the storage temperature and the usage of the preservatives on total aerobic count, yeast count and pH. All graphs were drawn using Microsoft Excel 2016.

## CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Microbial Quality of „Burkina drink"

The safety and quality of the milk drink was evaluated on the merit of the microbial population both qualitatively and quantitatively. The results showed that the product was unsafe for direct consumption without further processing based on the initial microbial load. Total aerobic count of the stock product was recorded as $1.24\times10^6 \pm 5.66$ cfu/ml which exceeded the acceptable limit of $1.0\times10^4$ cfu/ml. The fungi assay showed no mold presence, however a significant amount of yeast was observed in the sample with the yeast count reaching as high as $1.23\times10^6 \pm 7.07$cfu/ml whereas the acceptable and safe limit is pitched at $1.0\times10^2$ cfu/ml.

The pathogenic profile featured *Escherichia coli, Staphylococcus aureus, Enterococci* and total Coliforms. The results indicated that *Escherichia coli* and *Enterococc*i spp were present in the sample with counts exceeding the acceptable limit of 0cfu/ml. The *E.coli* count recorded was $9.4\times10^4 \pm 7.07$cfu/ml while the *Enterococci* spp. count was averagely $6.9\times10^4 \pm 3.54$ cfu/ml. The total coliform was determined to be $1.64\times10^4 \pm 4.95$cfu/ml. All the microbial counts detected were beyond the acceptable limit as per these known food pathogens, the expected tolerable limit is nil (0), signifying total absence.

**Table 4: Microbial quality of freshly prepared Burkina drink prior to treatment for study**

| Test | Results (cfu/ml) | Specification (cfu/ml) ((GS337: 2003). | Inference |
|---|---|---|---|
| Total aerobic count | $1.24\times10^6 \pm 5.66$ | $1.0\times10^4$ | Not safe |
| Yeast count | $1.23\times10^6 \pm 7.07$ | $1.0\times10^2$ | Not safe |
| Mould count | None detected | $1.0\times10^1$ | Safe |
| Total coliform count | $1.64\times10^4 \pm 4.95$ | 0 | Not safe |
| *Escherichia coli* count | $9.4\times10^4 \pm 7.07$ | 0 | Not safe |

| | | | |
|---|---|---|---|
| *Enterococci* spp. count | $6.9\times10^4\pm3.54$ | 0 | Not safe |
| *Staphylococcus aureus* | None detected | 0 | Safe |

cfu - Colony Forming Unit

### 4.2 Characterization of isolated colonies

The identities of the organisms isolated from the PCA plates were determined after obtaining pure colonies from sub-culturing. A total of 7 colonies were isolated and purified. These colonies differed in some morphological characteristics as observed under white light on the agar plates with the unaided eye. The details of their morphological profile are provided in Table 4.

#### 4.2.1 Morphological characterization of isolated colonies

All the colonies were similar in color (cream) and elevation (flat) but differed in form, margin and optical characteristics. The forms fluctuated between circular and irregular with most being circular with entire margins whereas the irregular colonies had undulate margins. Most of the isolated colonies (86%) were opaque in appearance.

**Table 5 Morphological characterization of isolated colonies from freshly prepared Burkina drink**

| Code | Color | Form | Elevation | Margin | *Opt. char. |
|---|---|---|---|---|---|
| **1** | Cream | Circular | Flat | Entire | Opaque |
| **2** | Cream | Irregular | Flat | Undulate | Opaque |
| **3** | Cream | Circular | Flat | Entire | Opaque |
| **4** | Cream | Circular | Flat | Entire | Opaque |
| **5** | Cream | Circular | Flat | Entire | Opaque |
| **6** | Cream | Irregular | Flat | Undulate | Translucent |

| | | | | | |
|---|---|---|---|---|---|
| **7** | Cream | Circular | Flat | Entire | Opaque |

*Opt. char – Optical Characteristics

### 4.2.2 Gram staining and microscopy of isolated colonies

The seven isolates were exposed to Gram stain to determine their Gram reaction, shapes and cellular arrangements and the outcome indicated four (4) different classes of microorganisms on the merit of the above mentioned Gram parameters. Isolates 1, 4 and 5 were observed to be similar in their Gram properties being Gram positive short rods with chain cellular arrangements. Isolate 2 was the only microorganism with a different cell shape being circular and thus coccus specifically though Gram positive by reaction with singular arrangements. Isolates 3, 6 and 7 were entirely different by reaction being Gram negative rods with singular arrangement.

**Table 6 Gram staining and microscopy of isolated colonies from fresh milk drink**

| Sample ID | Gram Reaction | Shape | Arrangement | Inference |
|---|---|---|---|---|
| **1** | Positive | Rods | Chains | Bacillus |
| **2** | Positive | Coccus | Single | Coccus |
| **3** | Negative | Short rods | Single | Coccus-bacillus |
| **4** | Positive | Rods | Chains | Bacillus |
| **5** | Positive | Short rods | Chains | Bacillus |
| **6** | Negative | Rods | Single | Bacillus |
| **7** | Negative | Rods | Single | Bacillus |

ID – Identification

### 4.2.3 Biochemical profile of isolated colonies

The biochemical profile of the isolated colonies featured the catalase, citrate and fermentation of three sugars; glucose, sucrose and lactose. The results indicated all the isolates to be catalase positive but differing in their response to citrate and sugar fermentation. The citrate test showed 43% of the isolates to be nonresponsive to citrate thus citrate negative and the other 57% being citrate positive.

The fermentative profile showed rather varying responses with some fermenting only glucose (43%), other fermenting all sugars (28%) and the remainder not fermenting any of the test sugars (28%). Only isolate 7 showed the production of gas with none of the isolates producing $H_2S$.

The reconciliation of the biochemical profiling with the Gram and morphology shows typically five different colonies to have been isolated from the milk drink. Of the isolated 7 colonies, six could be identified fully with the exception of 1. The identities of the isolates colonies are thus featured in Table 8 below with a summary of the biochemical profile obtained.

**Table 7 Biochemical profile of isolated colonies from fresh milk drink prior to treatment**

| Colony ID | Catalase | Citrate | | Triple Sugar | | Gas | $H_2S$ |
|---|---|---|---|---|---|---|---|
| | | Slant | Butt | Butt | Slant | | |
| **1** | Positive | B | G | R | Y | No gas | Negative |
| **2** | Positive | G | G | Y | Y | No gas | Negative |
| **3** | Positive | B | G | R | R | No gas | Negative |
| **4** | Positive | B | B | R | Y | No gas | Negative |
| **5** | Positive | G | G | R | Y | No gas | Negative |
| **6** | Positive | B | G | R | R | No gas | Negative |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **7** | positive | G | G | Y | Y | Gas | Negative |

KEY: B – Blue Y – Yellow G
– Green R – Red

**Table 8 Morphological, staining and metabolic characteristics of isolated colonies**

| Organisms | Gram stain, shape & arrangements | Culture characteristics on agar plate | Glucose fermentation | Sucrose fermentation | Lactose fermentation | Citrate | Catalase | Gas | $H_2S$ |
|---|---|---|---|---|---|---|---|---|---|
| ***E.coli*** | - rods Single | White opaque and moist growth | + | + | + | - | + | + | - |
| ***B.cereus*** | + rods | White, opaque and irregular growth | + | + | - | + | + | - | - |
| ***E.faecalis*** | + rods Chains | White opaque and moist growth | + | + | + | - | + | - | - |
| ***A.faecalis*** | - rods Single | White opaque and moist growth | _ | - | - | + | + | - | - |
| **Unknown** | + rods Chains | Cream opaque and moist growth | + | - | - | - | + | - | - |

+ Positive - Negative

**Table 9 Identification of isolated colonies from Burkina drink**

| Colony ID | Name |
|---|---|
| ¼ | *Bacillus cereus* |
| 2 | *Enterococcus faecalis* |
| 3/6 | *Alcaligenes faecalis* |
| 7 | *Escherichia coli* |



### 4.3 Microbial keeping quality determination of the Burkina drink

The microbial growth study was done for a period of 15 days comprising 6 sample reading days while conducting total aerobic count, yeast count, mold count, total coliform count and pH on each reading day. The tests were mainly microbiological and (pH).

#### 4.3.1 pH determination

The pH showed a downward progression over the study period with a slight reduction in each sample section towards a more acidic pH profile. This phenomenon was observed across all samples analyzed though the reduction was more profound in some as opposed to others. The overall pH ranged in the regions of 4-3 pH units thus putting the samples in the acidic region.

**Table 10 pH of Burkina drink over storage period under varying conditions**

| Sample | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 |
|---|---|---|---|---|---|
| **A** | 4.26±0.01 | 3.96±0.00 | 3.70±0.00 | 3.71±0.00 | 3.57±0.06 |
| **B** | 4.85±0.01 | 4.31±0.00 | 3.93±0.00 | 3.98±0.01 | 3.89±0.02 |
| **C** | 4.39±0.01 | 4.04±0.00 | 3.76±0.00 | 3.78±0.01 | 3.72±0.01 |
| **D** | 4.46±0.01 | 4.10±0.00 | 3.81±0.00 | 3.81±0.01 | 3.75±0.01 |
| **E** | 4.91±0.01 | 4.39±0.00 | 4.09±0.01 | 4.10±0.00 | 3.95±0.01 |
| **F** | 5.04±0.01 | 4.46±0.01 | 4.20±0.04 | 4.21±0.01 | 4.11±0.01 |

| | | | | | |
|---|---|---|---|---|---|
| **G** | 4.32±0.00 | 3.97±0.00 | 3.69±0.01 | 3.75±0.01 | 3.70±0.04 |
| **H** | 4.44±0.00 | 4.11±0.04 | 3.82±0.01 | 4.15±0.00 | 4.05±0.01 |
| **I** | 4.89±0.00 | 4.42±0.01 | 4.07±0.00 | 4.10±0.00 | 3.98±0.01 |
| **J** | 4.92±0.01 | 4.44±0.01 | 4.16±0.01 | 3.81±0.01 | 3.72±0.01 |

Comparing the refrigerated with non-refrigerated samples, the trend was a slightly lower pH (acidic) in the non-refrigerated sample as opposed to the refrigerated ones though the difference is not statistically significant at $p > 0.05$ using 95% CI as can be observed in Figure 4 to 6.

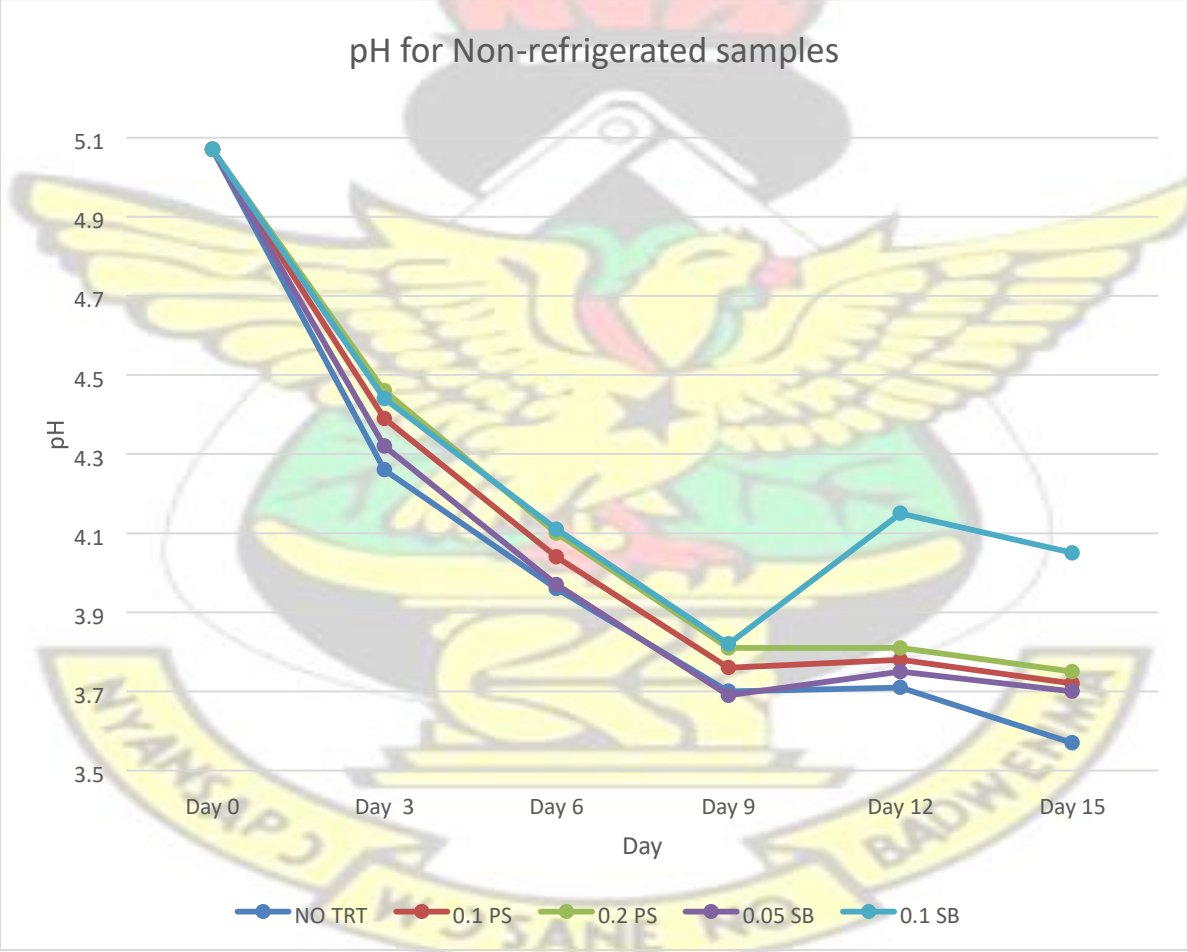


**Figure 4 pH trend over the study period for non-refrigerated samples**

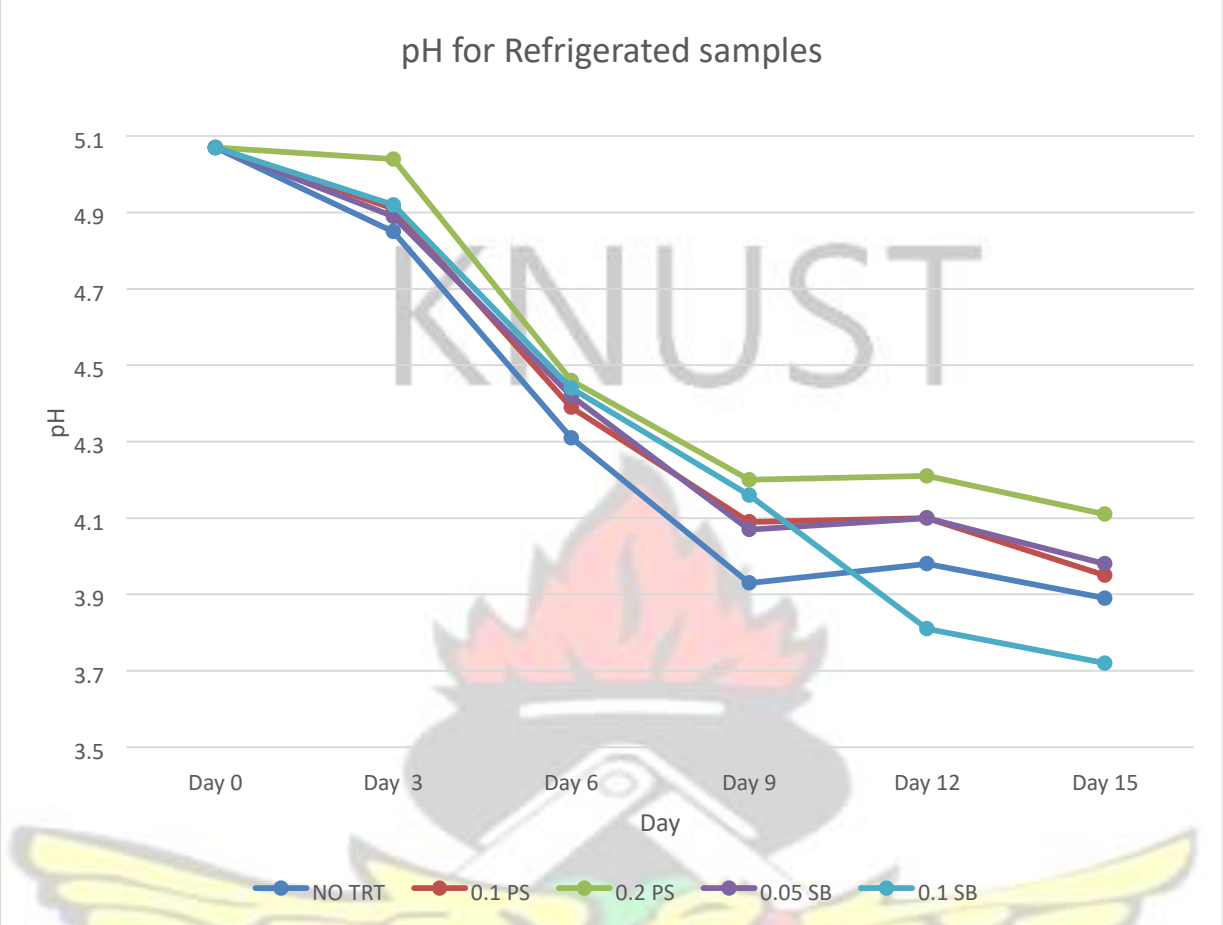


**Figure 5 pH trend over the study period for refrigerated samples**

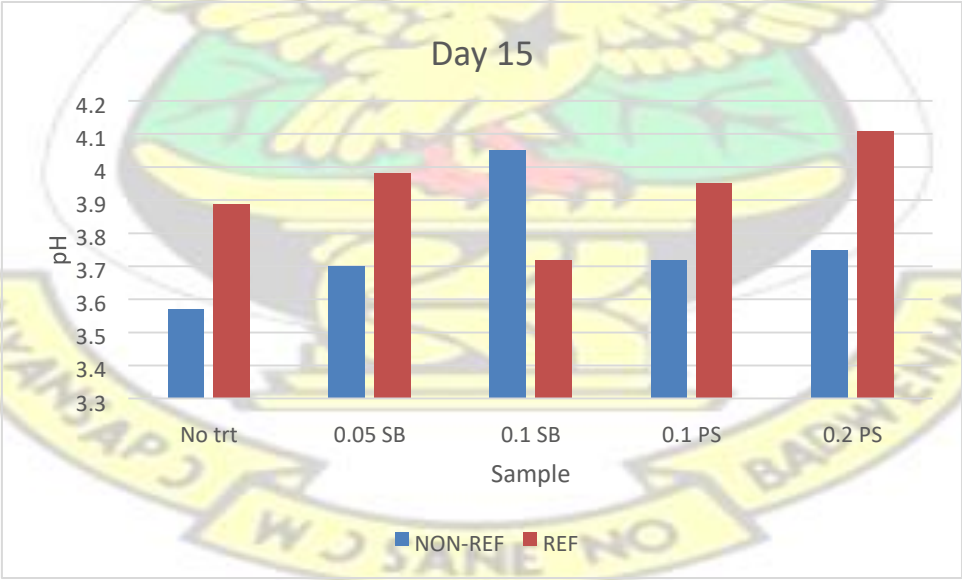


**Figure 6: pH of refrigerated and non-refrigerated samples after study period**

The analysis of the trend in pH across the samples over the study period showed a constant decline in pH with each sample session for both refrigerated and non-refrigerated samples. This produced a graph where the plots decline towards a more acidic region as the storage period prolong though it is more profound in the non-refrigerated samples.

The statistical analysis of the trend over time indicated no significant difference in the pH of the different concentrations of preservatives as well as the different storage conditions (refrigerated and non-refrigerated) at $p > 0.05$ using 95% CI.

### 4.3.2 Total aerobic count

The general feature of the aerobic count was a downward progression as the days lengthen resulting in a gradual reduction in the population of aerobic microorganisms colonizing the product though there were some few outliers. At the end of the study period, sample A, B and E recorded counts that exceeded the safe or acceptable limit of $1.0\times10^4$ cfu/ml rendering it unsafe for consumption. The other samples, C, F, G, H, I and J were in regions close to the safe limit though slightly exceeding the threshold. Sample D at the close of the study period was within the safe limit and thus was considered safe for consumption. The samples with preservatives added generally recorded lesser counts over the study period as opposed to the untreated samples as observed in Figure 7 to 9.

**Table 10 Total aerobic count of refrigerated and non-refrigerated samples of milk drink.**

| Sample | Day 3(cfu/ml) | Day 6 (cfu/ml) | Day 9 (cfu/ml) | Day 12(cfu/ml) | Day 15(cfu/ml) |
|---|---|---|---|---|---|
| **A** | $1.2\times10^7\pm7.55$ | $6.7\times10^6\pm5.51$ | $1.21\times10^6\pm3.51$ | $6.4\times10^6\pm4.36$ | $8.3\times10^6\pm5.68$ |

| | | | | | |
|---|---|---|---|---|---|
| **B** | $9.9\times10^{6}\pm10.39$ | $6.4\times10^{6}\pm17.00$ | $7.5\times10^{6}\pm5.51$ | $8.6\times10^{6}\pm4.04$ | $1.1\times10^{7}\pm6.51$ |
| **C** | $9.7\times10^{4}\pm6.51$ | $1.39\times10^{4}\pm2.52$ | $3.2\times10^{4}\pm3.06$ | $1.16\times10^{4}\pm8.71$ | $1.3\times10^{4}\pm4.16$ |
| **D** | $4.9\times10^{4}\pm2.08$ | $4.6\times10^{3}\pm6.11$ | $3.2\times10^{3}\pm3.51$ | $3.6\times10^{3}\pm3.51$ | $2.8\times10^{3}\pm5.86$ |
| **E** | $1.2\times10^{6}\pm7.51$ | $6.7\times10^{5}\pm6.51$ | $5.2\times10^{5}\pm3.06$ | $1.4\times10^{5}\pm3.00$ | $1.0\times10^{5}\pm6.51$ |
| **F** | $8.7\times10^{5}\pm4.00$ | $3.5\times10^{5}\pm4.04$ | $1.2\times10^{5}\pm6.11$ | $1.2\times10^{4}\pm6.03$ | $1.1\times10^{4}\pm9.29$ |
| **G** | $1.6\times10^{6}\pm5.68$ | $6.7\times10^{5}\pm4.16$ | $5.9\times10^{4}\pm4.51$ | $1.3\times10^{4}\pm5.57$ | $1.1\times10^{4}\pm7.54$ |
| **H** | $1.1\times10^{6}\pm6.03$ | $3.8\times10^{5}\pm6.51$ | $7.8\times10^{4}\pm4.04$ | $1.5\times10^{4}\pm9.29$ | $1.7\times10^{4}\pm6.00$ |
| **I** | $1.1\times10^{5}\pm5.51$ | $7.2\times10^{5}\pm5.51$ | $2.9\times10^{5}\pm5.86$ | $1.1\times10^{5}\pm4.58$ | $1.35\times10^{4}\pm5.03$ |
| **J** | $1.5\times10^{6}\pm5.51$ | $8.7\times10^{5}\pm4.51$ | $7.9\times10^{5}\pm3.00$ | $1.32\times10^{5}\pm2.51$ | $1.52\times10^{4}\pm6.03$ |

cfu - Colony Forming Units

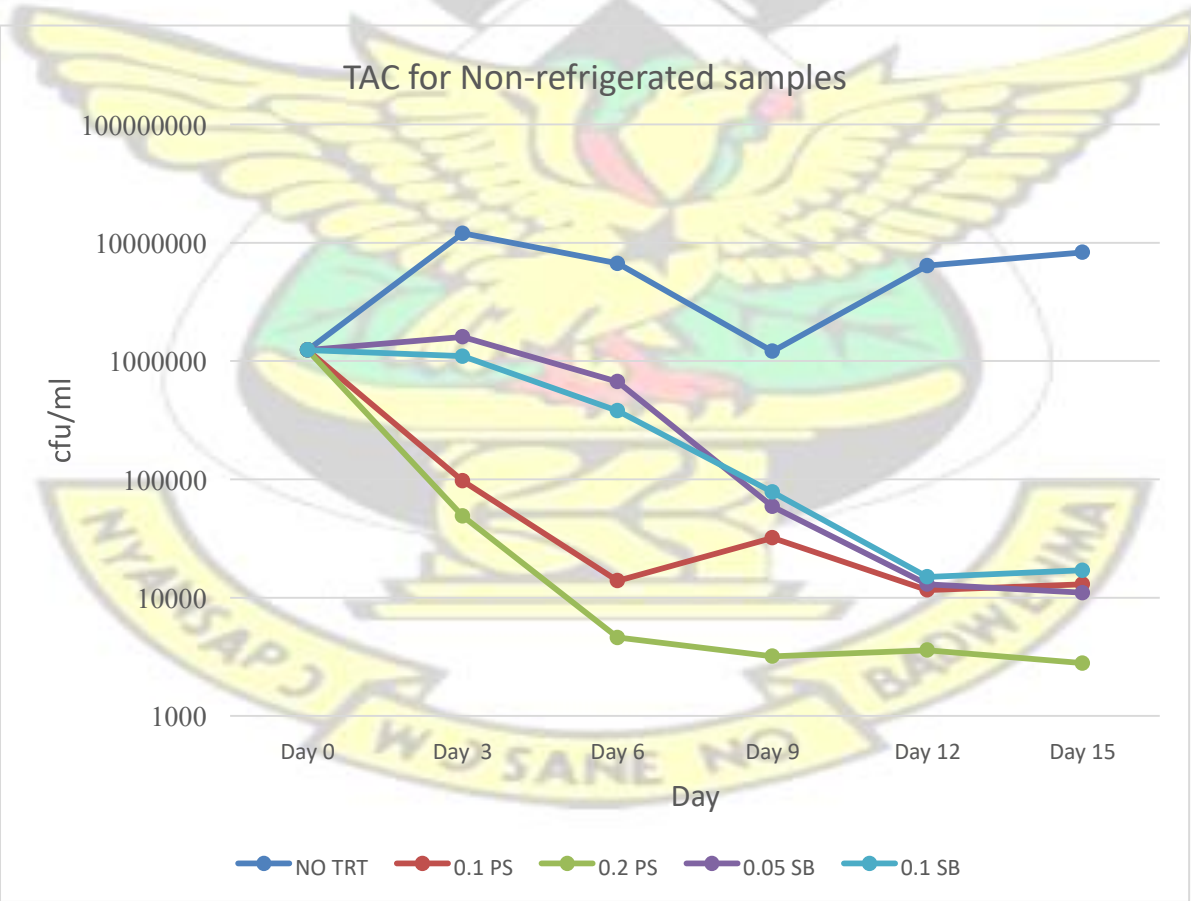


**Figure 7: Total aerobic count trend of non-refrigerated samples over study period**

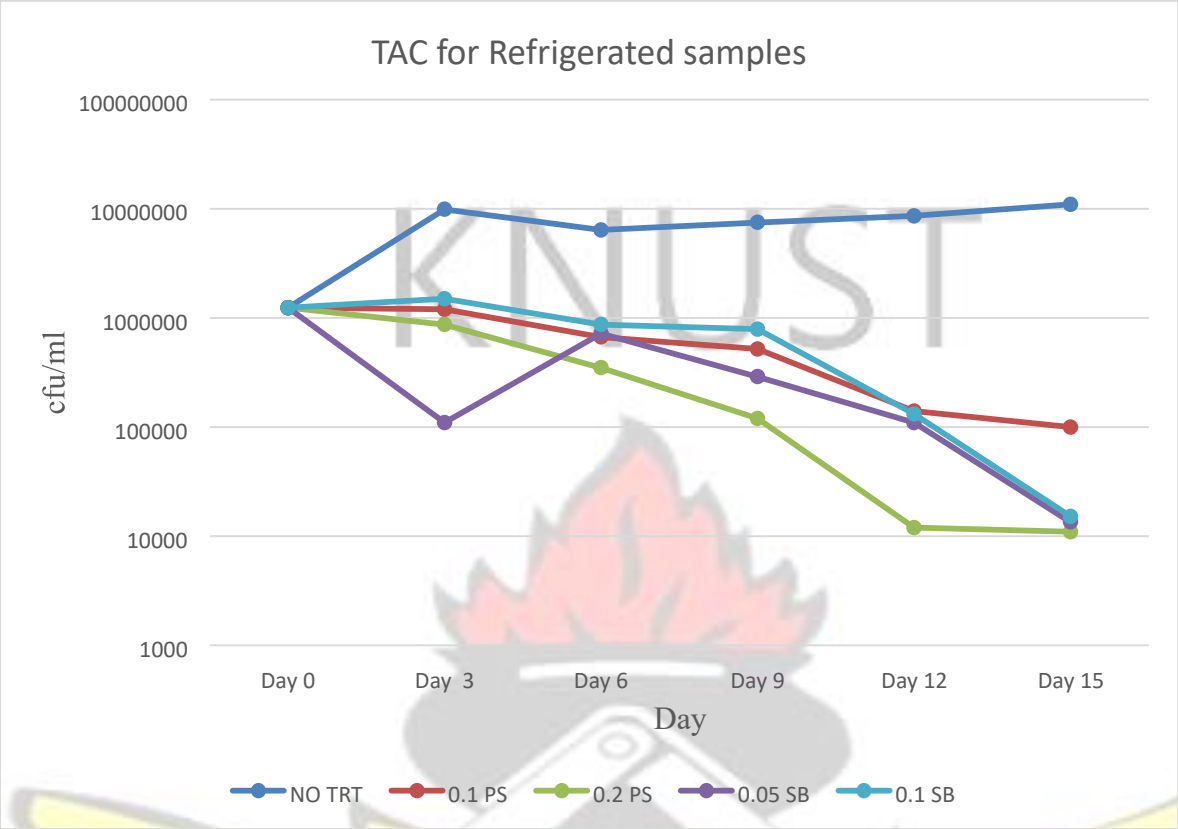


**Figure 8: Total aerobic count trend of refrigerated samples over study period**

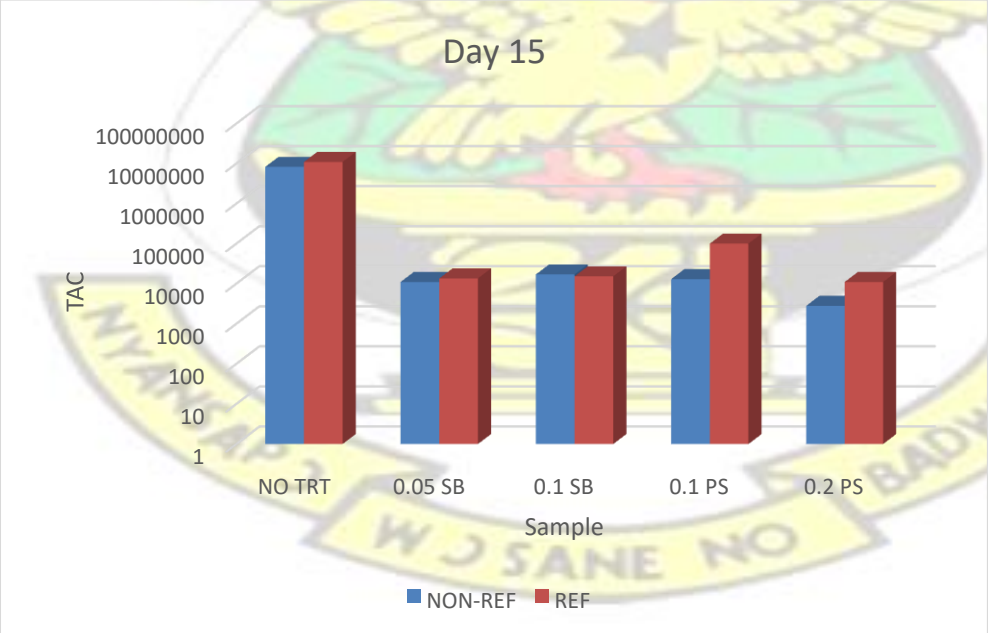


**Figure 9: Total aerobic count of refrigerated and non-refrigerated Burkina drink after study period.**

### 4.3.3 Total Coliform count

The total coliform count rather reflected an interesting phenomenon where the numbers reduced significantly by the second sample section and totally died out by the third day as observed in Figure 10 and 11. This produces a rather sharp and steep slope as opposed to the aerobic count which gave a shallow slope. The phenomenon occurred across all samples, both treated and untreated as such.

**Table 11: Total Coliform count of milk drink stored under varying conditions over time**

| Sample | Day 3 (cfu/ml) | Day 6(cfu/ml) | Day 9(cfu/ml) | Day 12 (cfu/ml) | Day 15 (cfu/ml) |
|---|---|---|---|---|---|
| **A** | $1.2\times10^{4}\pm5.51$ | $5.7\times10^{3}\pm4.51$ | $7.7\times10^{1}\pm5.13$ | - | - |
| **B** | $9.9\times10^{4}\pm1.53$ | $8.6\times10^{3}\pm7.02$ | $7.6\times10^{1}\pm6.24$ | - | - |
| **C** | $8.7\times10^{2}\pm3.51$ | - | - | - | - |
| **D** | $7.0\times10^{2}\pm2.08$ | - | - | - | - |
| **E** | $8.1\times10^{2}\pm3.06$ | - | - | - | - |
| **F** | $7.1\times10^{2}\pm2.08$ | - | - | - | - |
| **G** | $6.7\times10^{2}\pm6.03$ | - | - | - | - |
| **H** | $5.7\times10^{2}\pm4.16$ | - | - | - | - |
| **I** | $6.5\times10^{2}\pm3.51$ | - | - | - | - |
| **J** | $6.0\times10^{2}\pm3.51$ | - | - | - | - |

cfu - Colony Forming Units - = TFTC

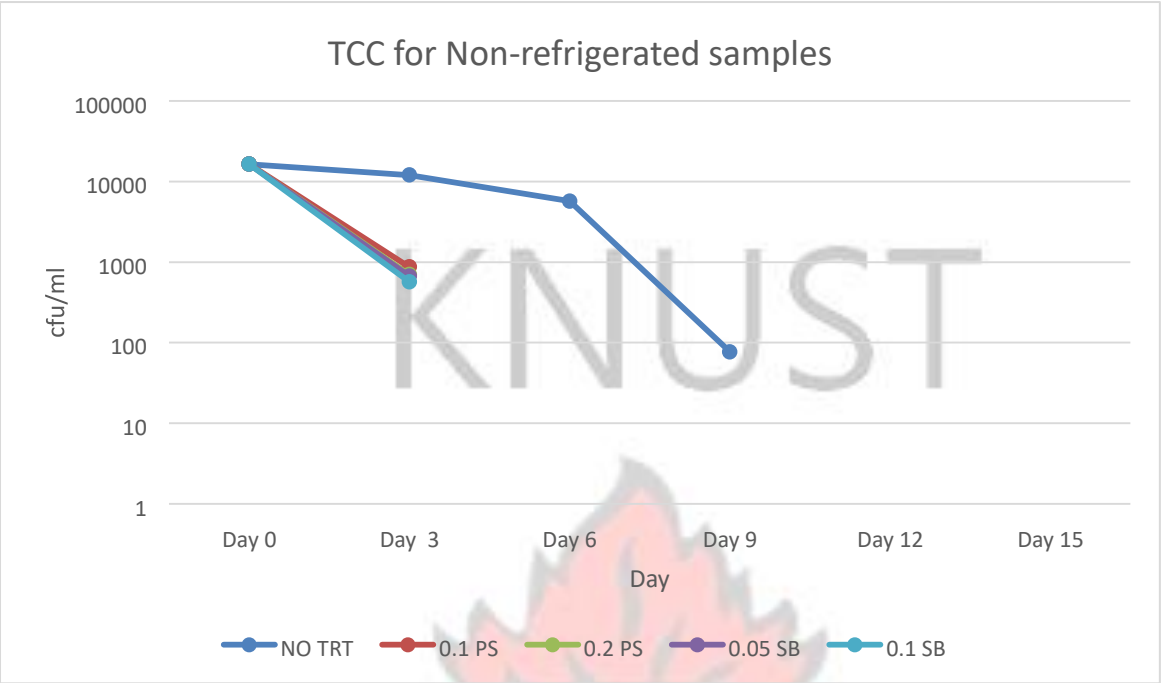


**Figure 10: Total Coliform count trend of non-refrigerated samples over study period**

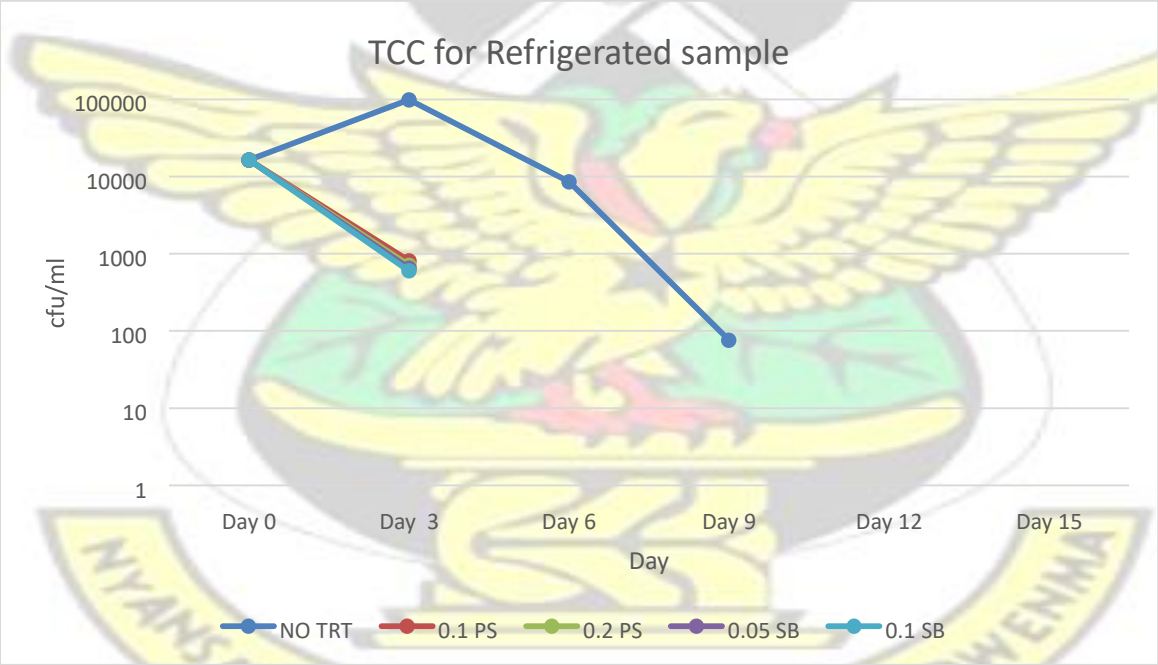


**Figure 11: Total Coliform count trend of non-refrigerated samples over study period**

### 4.3.4 Yeast and mold count

The assay into the yeast and mold profile of the stored drinks showed no different pattern from the total aerobic count and total Coliform count with a decline in yeast population over the study period as no molds were isolated and detected in any of the samples. The highest counts

were detected in samples A and B which are the untreated samples. The samples with preservatives appeared to show a significant decrease in yeast population over the time period as observed in Figure 12 to 14. The details are provided below in Table 13. The recorded counts all exceeded the acceptable and safe limit of $1.0\times10^2$ cfu/g with the least being $1.3\times10^2\pm1.53$ cfu/g at the end of the study period. The statistics shows significant difference with regards to the various storage conditions (refrigerated and non-refrigerated) as well as the different treatments at $p < 0.05$ using 95%.

**Table 12: Yeast and mold count of milk drink stored under varying conditions and treatments**

| Sample | Day 3 (cfu/ml) | Day 6 (cfu/ml) | Day 9 (cfu/ml) | Day 12 (cfu/ml) | Day 15 (cfu/ml) |
|---|---|---|---|---|---|
| **A** | $1.2\times10^{6}\pm2.08$ | $1.5\times10^{6}\pm7.55$ | $1.1\times10^{6}\pm4.72$ | $9.7\times10^{5}\pm5.13$ | $7.5\times10^{5}\pm5.13$ |
| **B** | $9.4\times10^{5}\pm5.03$ | $1.1\times10^{6}\pm7.21$ | $9.1\times10^{5}\pm5.51$ | $6.9\times10^{5}\pm5.29$ | $4.5\times10^{5}\pm2.29$ |
| **C** | $7.7\times10^{4}\pm5.51$ | $7.3\times10^{4}\pm4.51$ | $3.4\times10^{3}\pm2.51$ | $1.3\times10^{2}\pm3.21$ | $1.4\times10^{2}\pm2.65$ |
| **D** | $4.8\times10^{4}\pm1.53$ | $5.1\times10^{4}\pm6.00$ | $2.7\times10^{3}\pm2.08$ | $1.5\times10^{2}\pm2.00$ | $1.3\times10^{2}\pm1.53$ |
| **E** | $3.8\times10^{4}\pm4.00$ | $9.9\times10^{3}\pm2.31$ | $9.0\times10^{3}\pm2.65$ | $6.5\times10^{3}\pm5.13$ | $4.8\times10^{3}\pm4.16$ |
| **F** | $5.7\times10^{4}\pm3.51$ | $5.9\times10^{4}\pm4.04$ | $3.9\times10^{3}\pm5.57$ | $1.5\times10^{3}\pm2.52$ | $1.3\times10^{3}\pm2.08$ |
| **G** | $1.2\times10^{5}\pm3.06$ | $7.7\times10^{4}\pm5.57$ | $7.5\times10^{4}\pm7.21$ | $6.8\times10^{4}\pm5.57$ | $3.8\times10^{4}\pm5.13$ |
| **H** | $1.7\times10^{5}\pm5.51$ | $6.6\times10^{4}\pm6.24$ | $5.1\times10^{4}\pm5.51$ | $3.7\times10^{4}\pm4.04$ | $2.4\times10^{4}\pm4.16$ |
| **I** | $7.9\times10^{5}\pm5.69$ | $5.0\times10^{4}\pm6.11$ | $2.9\times10^{4}\pm2.65$ | $1.4\times10^{4}\pm2.52$ | $1.3\times10^{4}\pm2.00$ |
| **J** | $4.8\times10^{5}\pm8.02$ | $6.2\times10^{4}\pm5.13$ | $4.5\times10^{3}\pm2.52$ | $3.1\times10^{3}\pm3.06$ | $2.5\times10^{3}\pm4.04$ |

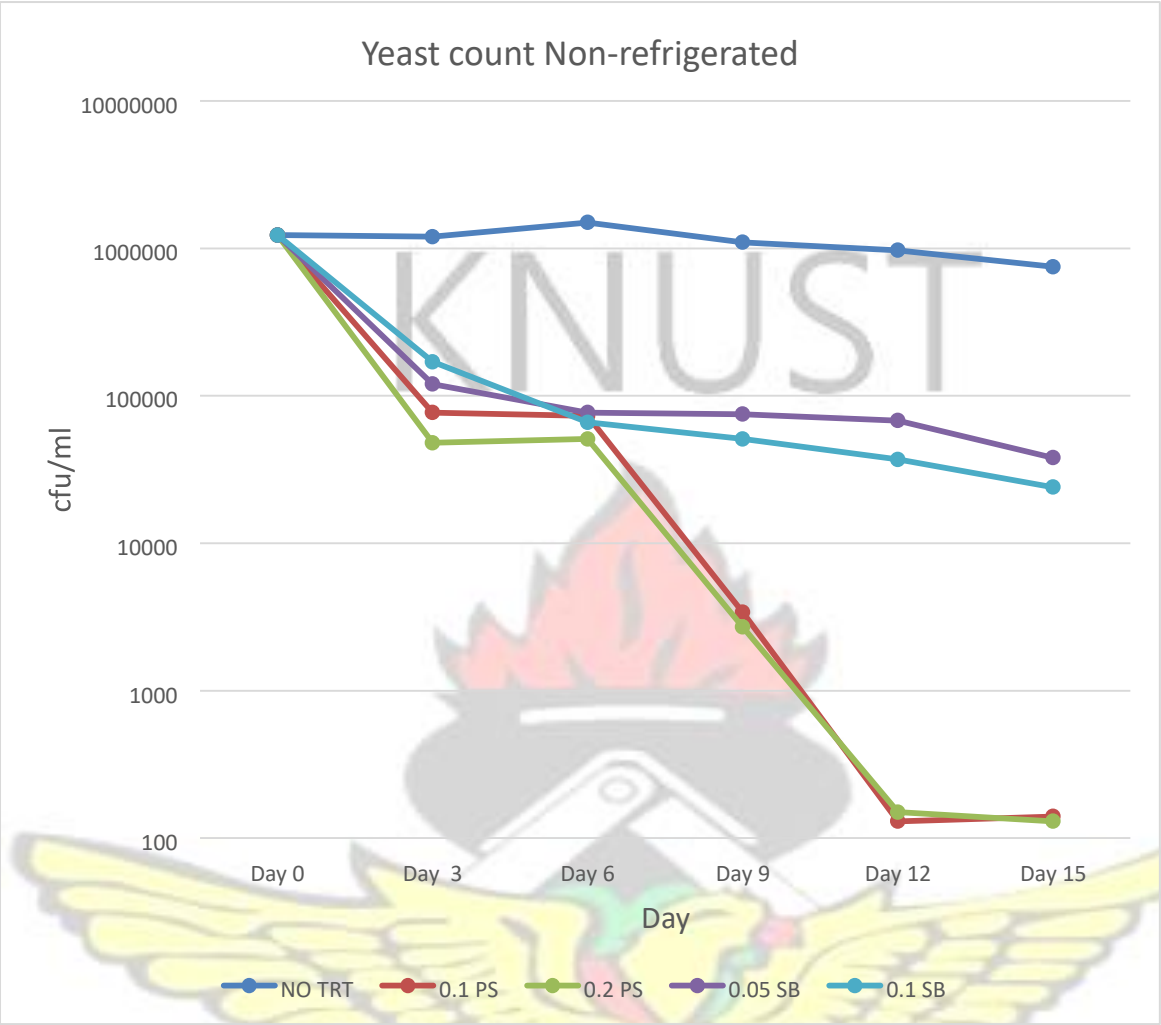


**Figure 12: Yeast count of non-refrigerated samples over study period**

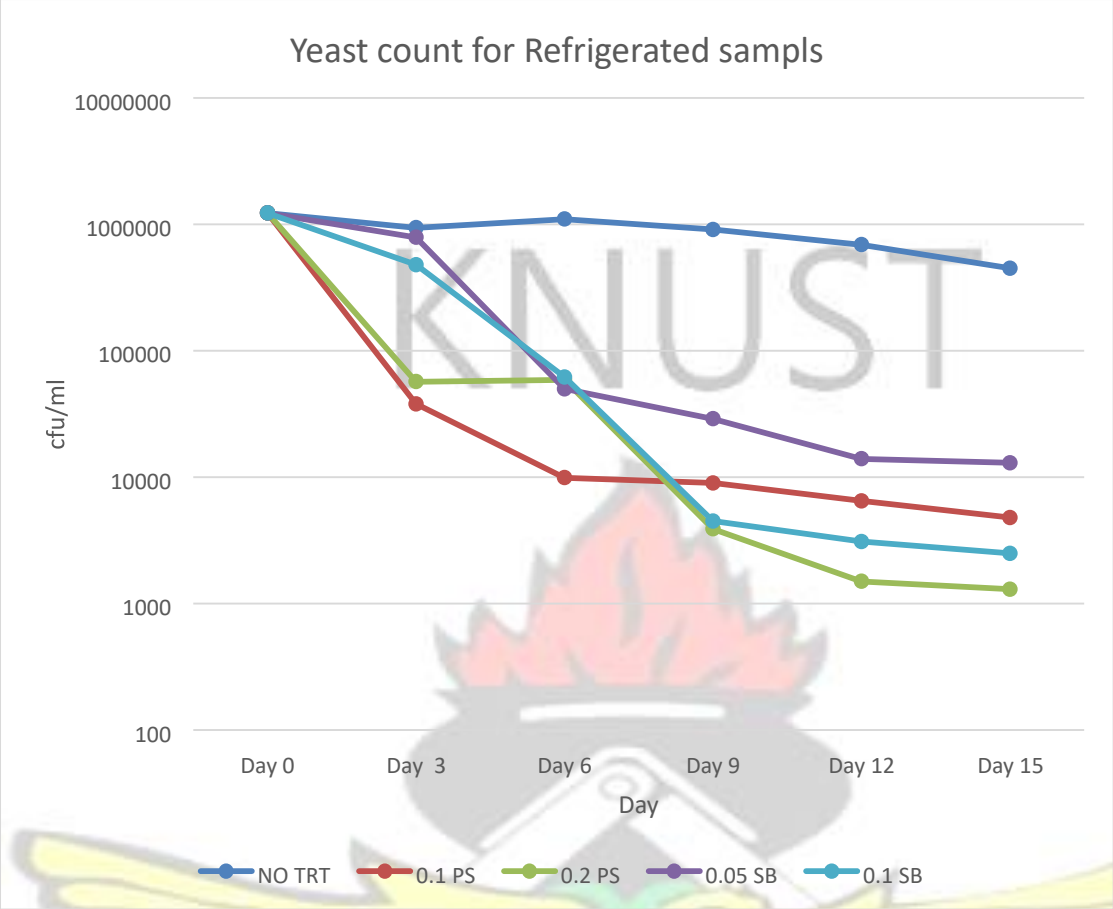


**Figure 13: Yeast count of non-refrigerated samples over study period**

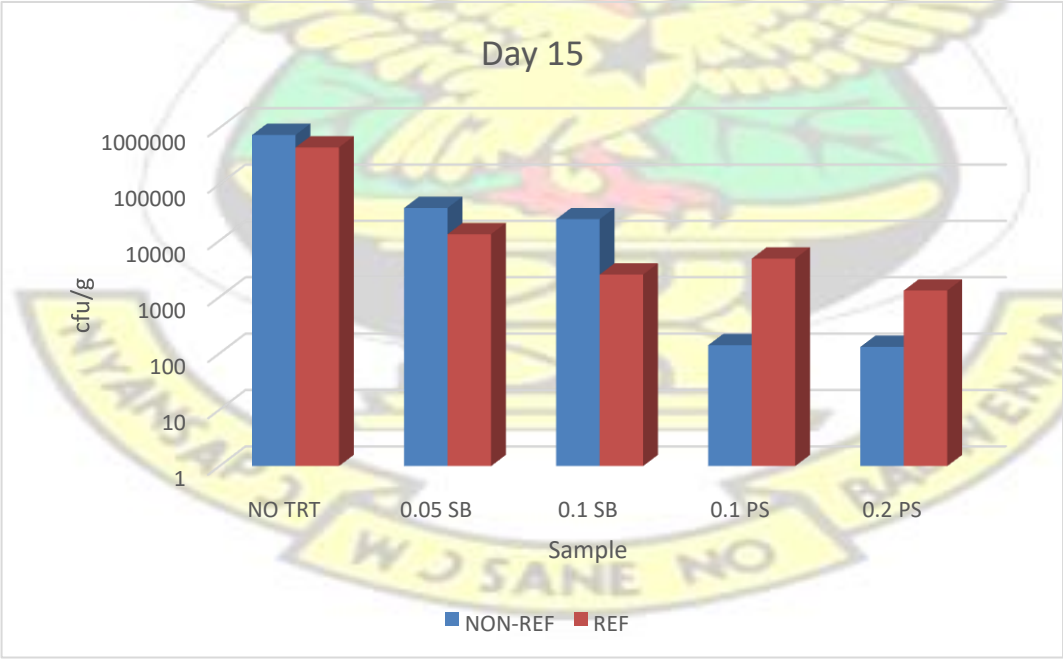


**Figure 14: Yeast count of non-refrigerated (A) and refrigerated (B) Burkina drink after study period.**

**CHAPTER FIVE**

## 5.0 DISCUSSION

Milk and milk based foods, despite their nutritional prowess, serve as rich substrates for the proliferation of microbes both pathogenic and non-pathogenic. This has been the basis for the development of food products like Burkina drinks that undergo fermentation just like popular dairy products such as yoghurt.

The results obtained upon assaying for the microbial quality of the „Burkina" drink indicated a fairly poor and unsafe product for public consumption due to the detection of pathogenic organisms such as *Escherichia coli* and *Enterococci* which were by numbers ($9.4\times10^4 \pm 7.07$ cfu/ml and $6.9\times10^4 \pm 3.54$ cfu/m for *E.coli* and *Enterococc*i respectively) way beyond the acceptable limit of 0 cfu/ml (GS337: 2003). The observed aerobic count of $1.24\times10^6 \pm 5.66$ cfu/ml exceeded the acceptable limit of $1.0\times10^4$ cfu/ml with the yeast assay also indicating the presence of significant amount of yeast reaching as high as $1.23\times10^6\pm7.07$cfu/ml ((GS734:2003, GS337:2003). The initial pH level was slightly acidic 5.07. The total Coliform count was determined to be $1.64\times10^4\pm4.95$ cfu/ml which also exceeded the safe limit of 0 cfu/ml.

This highly contaminated nature of the product could be attributed to a couple of factors ranging from the raw materials through processing to packaging. According to Abdalla *et al*, (2009), foods are contaminated by pathogens that emerge from three main sources namely the personnel who come into contact with the food, working area and finally raw materials. At these local markets, food and food products are exposed to the immediate environment and atmosphere harboring all manner of insects and rodents which are agents of contamination. The handling and storage of these food products is highly unhygienic as the vendors unprotected hand is used in handling without proper sanitary measures such as good hand washing practice

and the use of gloves and sanitizers. This exposes the food items to direct contamination from precontaminated hands.

Generally, the pH of all the samples decreased as the storage period lengthens though the difference is not statistically significant at $p > 0.05$ among the samples with regards to both the effect of the formulation or treatment applied and the storage temperature. The initial pH level for the sample was appropriate for the action by the two different preservatives since they work well in a slightly acidic medium (El-Gazzar and Marth., 1987). The reduction in the pH of all the samples as the storage period lengthens can be associated with the action of microorganisms that exist in the sample or drink leading to fermentation and subsequently the production of certain acids. Reduction in the pH for the non-refrigerated samples was faster and lower as compared to the refrigerated samples. According to Mortazavian *et al,* (2007), the viability of *Lactobacillus acidophilus* in a yoghurt sample stored at 2°C for 5 days was much lower as compared with samples stored under relatively higher temperatures that is 5°C and 8°C. The reduction in the viability of microorganisms especially since most of them are mesophilic in nature under relatively low temperatures like refrigeration temperatures (4ºC) contributed to the lower rate of proliferation of the microorganisms in the refrigerated samples as compared to that of the non-refrigerated samples for each treatment. This phenomenon inherently slowed the rate at which the pH of the refrigerated samples is reduced. The reduction in pH for the samples not treated with any preservative was much faster as compared to the other samples treated with preservative. This clearly indicates the effect or action of the preservatives on the samples. According to Ukwo *et al* (2010), antimicrobials have been used to effectively improve the shelf life of fruit products. Growth activities of the microorganisms result in the production of secondary metabolites which affect the immediate environment. One of such typical phenomena is the reduction in pH with the increase in microbial population. This phenomenon

is needful in fermentative processes where an acidic medium is desired to influence taste as in yoghurt production. This is due to the production of acids, particularly lactic acid in milk base foods such as Burkina drink by the organisms as they go by the fermentative pathway of metabolism. Despite the desirable aspects of pH reduction on taste, this phenomenon can be disadvantageous as the pH may drop to levels beyond the tolerable limits of the organisms particularly in a mixed culture thus inhibiting and killing the organisms by a phenomenon known as negative feedback or inhibition.

Statistical analysis indicated that the effect of the formulations or treatments applied to the samples contributed to a significant difference ($p < 0.05$) in the total aerobic count of the samples whereas there was no significant difference ($P>0.05$) with that of the storage condition or temperature. The total aerobic count of the samples reduced as the days of storage prolonged with a few exceptions especially when it got to the latter days. In some samples the total aerobic count reduced faster in the refrigerated samples as compared to the non-refrigerated samples for that particular treatment. This corroborates an earlier work by Mortazavian et al, (2007) that *Lactobacillus acidophilus* grew better in a relatively higher temperature as compared to a lower temperature. Most microorganisms tend to grow well under conditions or temperatures similar to room temperature especially in the tropical areas however some are psychrotrophic in nature which means they can survive low temperatures. The samples that were not treated with any preservative showed a much lower rate of reduction in numbers of the total aerobic count as compared with the samples that were treated with preservatives. This finding therefore gives an indication that the addition of preservative to the Burkina drink might help enhance the shelf life of the product. Combination of antimicrobials like benzoates with low temperatures have been used to improve the shelf-life of freshly prepared juices (Uma et al., 2011). These antimicrobials alter or attack microorganisms by interrupting important cell factors by causing

cell membrane damage, preventing cell replication and altering cell proteins (Lansing et al., 2002).

With regards to the total coliform count (TCC), the drop in numbers of coliforms was particularly sharp especially after the initial two reading days. In most cases after the first 3 days, the total coliform count reduced drastically or they completely died out. The reduction in the pH of the samples was as a result of the development of certain acids like lactic acids which creates a very uncomfortable condition for the growth of these coliforms. According to Ray and Bhunia, (2013), the proliferation, growth or survival of coliforms at low pH values is very minimal. This accounts for the sudden drop in coliform count. The creation of the unfavourable condition in the sample can also be attributed to the reduction in the temperature for the refrigerated samples. Howell et al, (1996) reported that faecal coliform increased in growth in warmer conditions. According to FDA (2012), the lowest temperature for the proliferation of coliforms like *E. coli* is 7 °C. Coliforms are a class of organisms which are of prime importance in the area of food safety as they have been implicated in a couple of food poisoning and contamination cases recorded across the globe. This makes coliforms pathogenic and of concern to the health and safety of consumers of meat. The family coliforms consist of microorganisms such as *Escherichia coli* which is also responsible for diarrhea experienced from food contamination. Other Coliforms are *Klebsiella pneumoniae* which causes pneumonia, *Enterococci* spp., *Hafnia* and *Citrobacter*. This has caused most countries to pay more attention to having Coliforms in foods with the standards ranging from 0 to 10 cfu/g as the tolerable limits but in Ghana the Coliform must be zero (GS337:2003). The results obtained upon the assessment of the Burkina drink showed high Coliform counts (Table 4), which is clearly an indication of the poor hygiene and sanitary conditions of the immediate environment of production.

Molds were not detected in any of the samples however yeast were detected in the samples. The detection of yeast in the samples means all the samples did not meet the required standard according to the Codex standard (2003). Earlier work by Tawiah, (2015) on the microbial composition of the Burkina drink also reported relatively high counts of yeasts. Statistically, there were significant differences ($p < 0.05$) in the different storage conditions and the treatment or formulations applied to the samples with regards to yeast counts. In most of the samples yeasts count for the samples not treated preservatives were much higher as compared to the samples treated with preservatives at the end of the study. Since the antimicrobial preservatives used also has antifungal properties (James, 2000), the relatively fast reduction in the yeasts count of the samples treated with preservatives might be due to the action of these preservatives. These antimicrobials mostly affect yeast cells by inhibiting cell transport. An earlier work by Warth, (1988) concluded that benzoic acids affect the growth rate of yeast cells in the presence of energy source by prompting the yeast cells to use the energy available for the reduction in cytoplasmic benzoic concentration and also to maintain pH. The unavailability of an energy source reduces the growth of the yeast cells. In most of the samples, the drop in yeast count was much greater in the refrigerated samples as compared to their corresponding non-refrigerated samples. Fungal species like other microorganisms are affected by extrinsic factors like temperature. According to Viljoen et al, (2003), the number of yeast isolated and enumerated when a yoghurt sample was stored for 30 days at 25ºC was highest reaching up to $10^5$ and $10^6$ as compared to the same sample stored under refrigeration temperature (5 ºC) for the same number of days with yeast count reaching $10^2$ and $10^3$. According to Heard and Fleet, (1988), temperature affects the growth of yeast cells during fermentation process in a fruit juice. Therefore, the low temperature might have been the reason why the reduction in the yeast count was faster in the refrigerated samples as compared to the non-refrigerated samples for most treatments. For fermented milk products, yeast has been reported to be the major spoilage agent

since it can survive and grow in an acidic medium with pH between 4 and 5 (Vetier *et al*., 2003). Some of the main reasons for the high presence of yeast in the sample might be due to the raw materials used or inability of producers to adhere to good manufacturing practices.

The identification of isolated colonies concluded on *Escherichia coli, Enterococcus faecalis, Bacillus cereus* and *Alcaligenes faecalis*. These organisms are food pathogens whose presence are mostly deemed unacceptable and render the food unwholesome.

One typical Coliform that is of significance in food safety is *Escherichia coli* and is mostly used as the indicator for faecal contamination and human induced contamination. The detection of *E.coli* strains particularly the pathogenic strains in food is deemed a high alert factor thus the acceptable or tolerable limit of *E.coli* being set at 0 cfu/g by the (ISO 7251:2006(E)). They mostly reside in the intestines of animals. The factors resulting in *E.coli* contamination could be attributed to the human interface and factors such as poor sanitary practices of improper hand washing which could transmit these organisms from the contaminated human to the food material. When ingested by humans, they mostly cause diarrhoea whilst other strains cause anemia or some kidney diseases.

*Bacillus cereus* which was identified after the biochemical tests is a pathogenic microorganism that affects food products and its processing in the food industry. It is a type of bacteria that causes food poisoning as a result of producing enterotoxins that contaminate the food material. Even though most strains of *Bacillus cereus* are harmful, a few other strains are beneficial to animals and humans. They affect food positively by contributing to the probiotic nature of some foods for animals and humans. Possible causes of *bacillus cereus* poisoning include improperly prepared food or the improper handling of foods.

Some strains of *Enterococcus faecalis* are beneficial to humans and animals since they compete with other pathogenic bacteria in the body and prevents the colonization of the body of the host by these pathogenic bacteria hence they are mostly placed under probiotic organisms. Also, the production of bacteriocins by *Enterococcus faecalis* further enhances its probiotic properties which has led to it being adopted in the dairy industry for the production of a number of dairy products including cheeses and natural milk.

A careful look at the profile of the aerobic count, yeast and Coliform count shows a general decline in population with increasing period of storage for the treated samples whilst the control either maintained its microbial population or reduced marginally. This clearly highlights the effect of the preservatives at regulating or slowing down the rate of microbial proliferation even to the point of killing them. The addition of preservatives especially comes in handy to the local producers who might not have access to refrigeration facilities and thus had to run at losses when the product is not sold immediately after production. It even becomes better with refrigeration facilities and this will be to the benefit of the producer in terms of income generation and reducing losses. This assertion is supported by the statistical output indicating a significant ($p < 0.05$) contribution of the preservatives to the microbial load of the products. Interestingly the mode of storage contributed insignificantly ($p > 0.05$) to the microbial population especially that of the total aerobic count but that cannot be the same with regards to yeast count since the contribution of the storage condition is statistically significant ($p < 0.05$).

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATION

## 6.1 Conclusion

The study indicates the unwholesome nature or state of the Burkina drink sold by the particular vendor or producer where the sample was collected from since it failed to meet microbiological standards for consumable foods of such nature either internationally or locally (Ghana).

The pathogenic profile featured *Escherichia coli, Staphylococcus aureus, Enterococci* and total Coliforms and the results indicated some relatively high levels of *Escherichia coli* and *Enterococci* spp. and coliforms to be present in the sample. Probing into the identity of the isolated microorganisms revealed a total of seven species of which four could be identified fully namely *Escherichia coli, Enterococcus faecalis, Bacillus cereus* and *Alcaligenes faecalis* all of which are food pathogens associated with food spoilage.

From the results, 0.2 % of potassium sorbate proved to be more effective against microbial growth as compared to the other preservatives whether the samples were stored at room or refrigeration temperatures. All the samples treated with preservatives except the refrigerated 0.1 potassium sorbate sample showed an aerobic count that was within acceptable limit after day 15. The addition of potassium sorbate (0.1 and 0.2%) to the non-refrigerated reduced the yeast count to a level below the acceptable limits of $1.0\times10^2$ cfu/ml.

## 6.2 Recommendations

The findings on the microbial quality of the Burkina drink analyzed indicates the risks the consumer is exposed to upon consuming this product. In view of the direction of this study thus requiring the use of a smaller sample size, it is recommended that further research be carried out on a larger sample size to establish the quality of the Burkina drinks available on the market both microbiologically and nutritionally.

The market for the Burkina drink is ever increasing thus requiring the optimization of the traditional mode of preparation to high scale commercial production with the quality guaranteed. This calls for optimization studies into the subject matter.

Due to a lack of resources, the characterization and isolation of contaminating microorganisms was short circuited requiring further genomic and typical biochemical characterization to the specie and strain level to further establish the safety of consuming the product.

## REFERENCES

**Abdalla** M. A., Suliman S. E. and Bakhiet A. O., (2009). Food safety knowledge and practices of street food vendors in Atbara City (Naher Elneel State Sudan). *African Journal of Biotechnology*, 8:24.

**Adams,** D. M., Brawley, T. G. (1981) Factors Influencing the Activity of a Heat_ Resistant Lipase of *Pseudomonas. Journal of Food Science*. Vol 46. 677-680

**Adesiyun**, A. A., Balbirsingh, V. (1996). Microbiological analysis of „black pudding", a Trinidadian delicacy and health risk to consumers. *International Journal of Food Microbiology*. 31: 283-299

**Ajiboye**, T. O., Iliasu, G. A., Adeleye, A. O., Abdussalam, F. A., Akinpelu, S. A., Ogunbode, S. M., Jimoh, S. O., and Oloyede, O. B. (2014). Nutritional and antioxidant dispositions of sorghum/millet beverages indigenous to Nigeria. *Food Science and Nutrition*. 2(5): 597-604.

**Akinhanmi**, F. T., Arogundade, A. L., Tiamiyi, O. M., and Oloruntoba, E. (2008). Protein fractions of legumes and cereals consumed in Nigeria. *International Journal for Agricultural Science Environmental Technology*. 7(1): 54-62.

**Amadou** I., Gbadamosi O. S. Guo-Wei L., (2011). Millet-based traditional processed foods and beverages- A review. *Cereal Food World,* 56(3):115-121

**Amadou**, I., Mahamadou, E. G., and Guo-Wei, L. (2013). Millets: nutritional composition, some health benefits and processing – a review. *Emirates Journal of Food Agriculture* 25(7): 501-508

**Amoa-Awua**, W. K. A., Appoh, F. E., Jakobsen, M. (1996). Lactic acid fermentation of cassava dough into agbelima. *International Journal of Food Microbiology.* 8: 87-98

**Anita**, S.W. (2001). The role of milk in the British diet. I*nternational Journal of Dairy Technology*. 54:130

**Bala-Ravi**, S. (2004). Neglected millets that save the poor from starvation. Low external input sustain. *Indian Journal of Agricultural Sciences* 6(1): 34-36.

**Baltensperger**, D., and Cai. Y. Z (2004). Millet/minor. In C. W. Wrigley, H. Corke, & C. E Walter (Eds.), *Encyclopedia of grain science* (pp.261-268). Oxford: Elsevier

**Blandino**, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero D., and Webb, C. (2003). Cerealbased fermented foods and beverages. *Food Research International*. 36(6): 527-543.

**Billings**. T. (1998). On fermented foods. Available: http://www. living-foods.com. (2002)

**Chipley**, J. R (1983). Sodium benzoate and benzoic acid. In: Branen AL, Davidson PM, eds. Antimicrobials in foods. New York, NY, M. Decker, pp. 11–35.

**Chandrasekara,** A., and Shahidi, F. (2011). Bioactivities and antiradical properties of millet grains and hulls. *Journal of Agriculture and Food Chemistry.* 59: 9563–9571.

**Chichester**, D. F., and R. W. Tanner. 1972. Antimicrobial food additives. pp. 115-184. In T. E. Furia (ed.), Handbook of food additives. CRC Press, Cleveland, Ohio.

**Codex standard for fermented milk**, (2003). Codex Standard 243 http:// www.codexalimentarius.org/input/download/standards/.../CXS_243e.pdf. (Assessed 22nd July, 2015)

**Cousin**, M. A. (1982). Presence and activity of psychrotrophic microorganisms in milk and dairy products. *Journal of Food Protection*. Vol 45. Issue 2.

**Daeschel**, M. A. (1989). Antimicrobial substances from lactic acid bacteria for use food preservatives. *Food Technology*. 43: 164-167

**Davis**, J. G. (1975). The microbiology of yoghurt. In J. G. Carr, C. V. Cutting, & G. C. Whitting (Eds.), Lactic acid bacteria in beverages and food (pp. 245–266). London: Academic Press.

**Deak,** T., and Beuchat L.R. (1993). Yeast Associated with Fruit Juices Concentrates. *Journal for Food Protection.* 56: 777-782.

**Earle**, M. D., and Putt, G. J. (1984). Microbial spoilage and use of sorbate in bakery products. *Food Technology in New Zealand.*

**EC** (1995) European Union Directive 95/2/CE from 20.02.1995 on food additives, colourants and sweeteners. European Commission.

**El-Gazzar,** F. E., and E. H. Marth. 1987. Sodium benzoate in the control of growth and aflatoxin production by Aspergillus parasiticus. *Journal for Food Protection*. 50:503-509.

**FAO** (Food and Agricultural Organization of the United Nations) (2014). The statistical division. (Assessed in June, 2014).

**Fairbairn**, D. J., Law, B. A. (1987). The effect of nitrogen and carbon sources on proteinase production by *Pseudomonas fluorescens. Journal of Applied Microbiology*. Vol 62. Issue 2. 105-113.

**Food and Agriculture Organization** *UN. (2013).* Milk, whole fresh cow producers Retrieved September 21, 2017.

**Food and Drugs Administration** (2012). Foodborne pathogenic microorganisms and natural toxins handbook, 2nd edition, US Food and Drug Administration, Silver Spring, Pp. 87-92.

**Friezer** W. C. (1989). Contamination, Spoilage and Preservation of Yoghurt, AVI Publishers, U.S.A., p. 13-29.

**Friedman**, L. J, Greenwald C. G (1994) Food additives. In: Kroschwitz J, Howe-Grant M, eds. Kirk-Othmer: *Encyclopedia of chemical technology*. 11: 805–833.

**Freese**, E.C., Sheu, C.W., and Galliers, E. 1973. Function of lipophilic acids as antimicrobial food additives. *Nature* 241: 321-325.

**Furia**, T. E. (ed.). 1972. Handbook of food additives, 2nd ed. CRC Press, Cleveland, Ohio.

**Ghana Standard**; GS337: (2003). Milk and milk product specification for yoghurt and sweetened yoghurt. Ghana Standard Authority Library, Accra. P. 1-3

**Ghana Standard**; GS734: (2003). Cereals for whole or decorticated millet grains. Ghana Standard Authority Library, Accra. Pp. 1-4.

**Goff**, D. Introduction to Dairy Science and Technology: Milk History, Consumption, Production, and Composition: World-wide Milk Consumption and Production". *Dairy Science and Technology. University of Guelph*.

**Halm,** M., Lillie, A., Sorensen, A. K., and Jakobsen, M. (1993). Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. *International Journal of Food Microbiology*, 19: 135-143

**Heard**, G. M. and Fleet, G. H. (1988). The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. *Journal of Applied Bacteriology* 65:23-28.

**Hegde**, P. S., and Chandra, T. S. (2005). ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (Paspalum Scrobiculatum) compared to other millets. *Food Chemistry*. 92: 177–82

**Hirahara**. T. (1998). Functional food science in Japan. In T. Matilda-Sandholm and K. Kaupilla. (Eds). Functional food research in Europe Finland: Julkaisija-Utgivare. (pp. 19-20)

**Horwood**, J. F., Stark, W., and Hull, R. R. (1987). A fermented, yeasty flavour defect in cheddar cheese. *Australian Journal of Dairy Technology* 42, 25-26.

**Howell**, J. M., Coyne, M. S., and Cornelius, P. L. (1996). Effect of Sediment Particle Size and Temperature on Fecal Bacteria Mortality Rates and the Fecal Coliform/Fecal Streptococci Ratio. *Journal of Environmental Quality*. 25:1216-1220

**ICRISAT/FAO**. (1996). The world sorghum and millet economies: facts, trends and outlook. ICRISAT, Patancheru, India and FAO, Rome. 68 pp.

**Ihekoronye**, A.I. and Ngoddy, P.O. (1995). Integrated Food Science and technology for the Tropics. Macmillan Educational LTD. pp. 121-147.

**Inyang,** C. U., and Zakari, U. M. (2008). Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant "fura"- a Nigerian cereal food. *Pakistan Journal of Nutrition.* 7: 9-12.

**ISO** 21527-1:2008. Microbiology of food and animal feeding stuff -Horizontal method for the enumeration of yeast and moulds. Colony count technique in products with water activity greater than 0.95. Pp. 1-8.

**ISO** 4833-1:2003. Microbiology examination of food and animal feeding stuff - Horizontal method for the enumeration micro-organisms colony count at 30°C. Pp.1-9.

**ISO** 6579:2002(E). Microbiology of food and animal feeding stuffs -Horizontal method for the detection of Salmonella spp. Pp. 1-27.

**ISO** 6888-1:1999[E]). Microbiology of food and animal feeding stuff -Horizontal method for the enumeration of coagulase positive Staphylococci (Staphylococcus aureus and other species) - part 1: Technique using Baird-Parker agar medium. Pp. 1-6.

**ISO** 7251:2006(E). Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of presumptive Escherichia coli (Most probable number). Pp. 1-27.

**James**, M.J., 2000. Modern Food Microbiology. 6th Edn., Aspen Pub. Frederick, Md.

**Jay**, J. M., Loessner, M. J., and Golden, D. A. (2005). Modern Food Microbiology, 7th ed. Springer, India.

**Jay**, S., & Anderson, J. (2001). Fruit juice and related products. Spoilage of processed foods: causes and diagnosis (pp. 189). Waterloo NSW: AIFST Inc. (NSW Branch) Food Microbiology Group

**Jay**, J. M., Loessner, M. J., and Golden, D. A. (2005). Modern Food Microbiology, 7th ed. Springer, India.

**Kohajdova**, Z., and Karovicova, J. (2007). Fermentation of cereals for specific purpose. *Journal of Food Nutrition Research.* 46(2): 51-57.

**Lacroix**, C and Lachance O. (1990). Effect of Various Humectants and Aw on Proteolysis,

Yeast and Mold Growth and Shelf-Life During Cold Storage of Yogurt. *Canadian Institute of Food Science and Technology Journal*, 23: 101-108

**Lansing**, M.P., P.H. John and A.K. David, 2002. Microbiology. 5th Edn., McGraw-Hill, Comp.

**Leder**, I. (2004). Sorghum and millets. In: Cultivated Plants, Primary as Food Sources Encyclopedia of Life Support Systems, Fuleky, G, Ed., EOLSS Publishers, Oxford, UK.

**Lei**, V., and Jakobsen, M. (2004). Microbiological characterization and probiotic potential of koko and koko sour water, African spontaneously fermented millet porridge and drink. *Journal of Applied Microbiology*. 96(2): 384-397.

**Mortazavian.** A. M, Ehsani. M. R, Mousavi. S. M, Rezaei. K, Sohrabvandi. S, Reinheimer. J. A. (2007). Effect of refrigerated storage temperature on the viability of probiotic microorganisms in yogurt. *International Journal of Dairy Technology*. 60: 123-127

**Mossell.** D. A. A. and Ingram. M. (1955). The physiology of microbial spoilage. *Journal of Applied Bacleriology.* 18: 232-268

**Mridula,** D., and Sharma, M. (2015). Development of non-dairy probiotic drink utilizing sprouted cereals, legume and soymilk. LWT - *Food Science and Technology*. 62: 482-487.

**Muthamilarasan**, M., Dhaka, A., Yadav, R., and Prasad, M. (2016). Exploration of millet models for developing nutrient rich graminaceous crops. *Plant Science*. 242: 89-97.

**Nebedum**, J.O and Obiakor, T. (2007). The effects of different preservation methods on the quality of nunu, a locally fermented Nigerian dairy product. *African Journal of Biotechnology.* 6(4): 454-458,

**Nout,** M. J. R., (2009). Rich nutrition from the poorest- cereal fermentations in Africa and Asia. *Food Microbiology*. 26: 685-692.

**Obilana** A. B., (2014). Overview: Importance of millets in Africa. http:// www.afripro.org.uk/papers/paper02obilana.pdf. (Assessed 21st July, 2015).

**Obilana** A. B. and Manyasa E., (2002). Millet In: P.S Belton and J. R. N Taylor (Eds). Pseudo cereals and the common cereals: Grain properties and utilization potential Springer-Verlag: Newyork. Pp 177-217

**Okonkwo** O. I., (2011). Microbiological analysis and safety, evaluation of Nono: A fermented milk products consumed in most part of Northern Nigeria. *International Journal Diary*, 6:181-189

**Olson** N. F., (1990), "The Impact of Lactic Acid Bacteria on Cheese Flavor," FEMS Microbiology Reviews, 87: 131-148.

**Onweluzo**, J. C., and Nwabugwu, C. C. (2009). Fermentation of millet (Pennisetum americanum) and pigeon pea (Cajanus cajan) seeds for flour production: Effects on composition and selected functional properties. *Pakistan Journal of Nutrition*. 8: 737-744.

**Oyewole**, O.B. (1997). Lactic fermented foods in Africa and their benefits. *Food Control*. 8: 289-297,

**Prescott**, L.M, Harley, J.P, and Kleen, D.A. (2002). Microbiology, 5th Edition, McGraw Hill, New York. pp. 965-972.

**Rajagopal**, M., Werner, B. G., Hotchkiss, J, H. (2005). Low pressure $CO_2$ storage of raw milk: Microbiological Effects. *Journal of Dairy Science*. Vol 88: 3130-3138

**Ray**, B., and Bhunia, A. (2013). Fundamental food microbiology. Florida: CRC press.

**Restaino**, L. K., Komatsu K, and Syracuse M. J. (1981). Effects of acids on potassium sorbate inhibition on food–related microorganisms in culture media. *Journal of Food Science*, 47: 134-143.

**Richter**, R. L., Ledford R. A., Murphy, S. C. (1992). Milk and milk products. In; Vanderzant, C., Splittstoerr, D. F (Eds). Compendium of methods for the microbiological examination of foods. 3rd edition. American Public Health Association, Washington DC. Pp: 837-838

**Saleh**, A. S. M., Zhang, Q., Chen, J., Shen, Q. (2013). Millet grains: nutritional quality, processing and potential health benefits. Comprehensive *Reviews in Food Science and Food Safety*, 12: 281-295.

**Sasikumar**, R. (2014). Fermentation technologies in food production. In: Progress in Biotechnology for Food Applications. Lai, W. F., Ed., OMICS Group, California, USA.

**Schnurer**, J and Magnusson, J. (2005). Antifungal lactic acid bacteria as biopreservatives. *Trends Food Science Technology*. 16, 70–78

**Sean**, G. and S. Eileen, (2004). Chemical Disinfectants, Antiseptics and Preservatives In: Stepen, P.D., A.H Norman and P.G Sean (Eds.), *Pharmaceutical Microbiology* 7th Ed., Blackwell Science, Inc. Massachusetts, USA, pp: 285-305

**Shahidi,** F., and Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods*. 5: 570-581 **Shobana**, S., and Malleshi N. G. (2007). Preparation and functional properties of decorticated finger millet (Eleusinecoracana). *Journal of Food Engineering*. 79: 529-538.

**Simango,** C. (1997). Potential use of traditional fermented foods for weaning in Zimbabwe. *Journal of Social Science and Medicine*. 44: 1065-1068

**Sofos**, J. N. and Busta, F. F. (1981). Antimicrobial Activity of Sorbate. *Journal of Food Protection*. Vol. 44, No. 8, pp. 614-622

**Sofos,** J.N. and Busta, F.F. (1993). Sorbic acid and sorbates. Antimicrobials In Foods. (P.M. Davidson and L.A. Branen, eds.), Marcel Dekker, New York. pp. 49-94

**Tatini**, S. R., Kauppi, K. L. (2003). In: Encyclopedia of dairy sciences Roginski, H., Fuquay J.W., Fox, P.F. (Eds.), Vol. 1., Academic Press and Elsevier Science, Amsterdam, Pp. 74 79.

**Tawiah**, X. U. (2015). Microbiological composition of "Burkina" drink. A case study in Accra.

**Taylor**, J. R. N. (2004). Millet/pearl. In C. W. Wrigley, H. Corke, and C. E. Walker (Eds), Encyclopedia of grain science (pp. 253-261). Oxford: Elsevier

**Taylor**, J. R. N., and Duodu, K. G. (2015). Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. *Journal of Food Science and Agriculture*. 95: 225–237.

**Taylor**, J. R. N., Schober, T. J., and Bean, R. S. (2006). Novel food and non-food uses for sorghum and millets. *Journal of Cereal Science*. 44: 252-271.

**Ukwo** S. P, Ezeama C. F, Ndaeyo N. U. (2010). Growth of different yeast strain during fermentation of soursop (Annona muricata) juice as influenced by acetic acid bacteria. Natural Science. 8(10): 285-291

**Uma** T, Rama R. V., Khasim B. S. (2011). Preservation and shelf life extension of cashew apple juice. *Internet Journal of Food Safety* 13: 275-280

**Urbach,** G "Contribution of Lactic Acid Bacteria to Flavour Compound Formation in Dairy Products," *International Dairy Journal,* 5(8): 877-903.

**Vetier** N., Banon S., Chardot V. and Hardy J., (2003). Effect of Temperature and Aggregation Rate on the Fractal. *Journal of Dairy Science,* 78 (12): 2675 - 2682.

**Viljoen**, B. C., Lourens-Hattingh, A., Ikalafeng, B. and Peter, G. (2003). Temperature abuse initiating yeast growth in yoghurt. Food Research International. 36(2): 193-197

**Warth** A. D. (1988). Effect of benzoic acid on growth yield of yeasts differing in their resistance to preservatives. *Applied and Environmental Microbiology*. 2091-2095

**Waites**. W. M. and Arhuthno U, J. P. (1990) Foodborne illness, an overview. Lancet 336, 722-725.

**Wouters** J. T. M., Ayad E. H. E., Hugenholtz J. and Smit G., (2002). Microbes from raw milk for fermented dairy products. *International Dairy Journal*, 12(2002): 91-109.

**Zhu,** F. (2014). Structure, physicochemical properties, and uses of millet starch. *Food Research International*. 64: 200–211.

## APPENDIX

**Appendix 1: Total aerobic count on Burkina drink stored at room temperature over the study period from session 1(A) to session 5(E)**

**Appendix 1A**

| Sample | Rep | Dilutions | | | | | |
|---|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| | 1 | TNTC | TNTC | TNTC | 164 | 68 | 7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 2 | TNTC | TNTC | TNTC | 172 | 83 | 9 |
| | 3 | TNTC | TNTC | TNTC | 167 | 74 | 12 |
| B | 1 | TNTC | TNTC | TNTC | 146 | 53 | 5 |
| | 2 | TNTC | TNTC | TNTC | 137 | 48 | 7 |
| | 3 | TNTC | TNTC | TNTC | 141 | 42 | 9 |
| C | 1 | TNTC | 143 | 39 | 9 | - | - |
| | 2 | TNTC | 156 | 51 | 7 | 1 | - |
| | 3 | TNTC | 149 | 43 | 10 | - | - |
| D | 1 | 48 | 9 | 2 | - | - | - |
| | 2 | 51 | 11 | - | - | - | - |
| | 3 | 47 | 13 | - | - | - | - |
| E | 1 | TNTC | TNTC | 188 | 54 | 5 | - |
| | 2 | TNTC | TNTC | 195 | 39 | 7 | - |
| | 3 | TNTC | TNTC | 187 | 46 | 8 | - |
| F | 1 | TNTC | 321 | 83 | 12 | 1 | - |
| | 2 | TNTC | 297 | 91 | 10 | - | - |
| | 3 | TNTC | 317 | 87 | 14 | 2 | - |
| G | 1 | TNTC | TNTC | 240 | 74 | 21 | 1 |
| | 2 | TNTC | TNTC | 248 | 81 | 17 | 5 |
| | 3 | TNTC | TNTC | 237 | 72 | 19 | 3 |
| H | 1 | TNTC | TNTC | 156 | 51 | 13 | 1 |
| | 2 | TNTC | TNTC | 163 | 46 | 9 | 1 |
| | 3 | TNTC | TNTC | 168 | 53 | 10 | 2 |
| I | 1 | TNTC | 102 | 19 | 4 | - | - |
| | 2 | TNTC | 113 | 26 | - | - | - |
| | 3 | TNTC | 107 | 24 | 1 | - | - |
| J | 1 | TNTC | TNTC | 220 | 94 | 13 | 6 |
| | 2 | TNTC | TNTC | 212 | 83 | 17 | 2 |
| | 3 | TNTC | TNTC | 216 | 89 | 13 | - |

**Appendix 1B**

| Sample | Rep | Dilutions | | | | | |
|---|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| A | 1 | TNTC | TNTC | TNTC | 67 | 25 | - |
| | 2 | TNTC | TNTC | TNTC | 61 | 19 | 9 |
| | 3 | TNTC | TNTC | TNTC | 72 | 17 | 7 |
| B | 1 | TNTC | TNTC | TNTC | 51 | 16 | 5 |
| | 2 | TNTC | TNTC | TNTC | 83 | 14 | 3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | TNTC | TNTC | TNTC | 57 | 19 | 6 |
| C | 1 | 136 | 5 | - | - | - | - |
| | 2 | 141 | 7 | - | - | - | - |
| | 3 | 139 | 3 | - | - | - | - |
| D | 1 | 45 | 9 | - | - | - | - |
| | 2 | 53 | 10 | - | - | - | - |
| | 3 | 41 | 6 | - | - | - | - |
| E | 1 | TNTC | TNTC | 68 | 11 | - | - |
| | 2 | TNTC | TNTC | 61 | 9 | 1 | - |
| | 3 | TNTC | TNTC | 74 | 6 | - | - |
| F | 1 | TNTC | 201 | 36 | 5 | 1 | - |
| | 2 | TNTC | 197 | 39 | - | - | - |
| | 3 | TNTC | 206 | 31 | 1 | - | - |
| G | 1 | TNTC | 104 | 29 | 2 | - | - |
| | 2 | TNTC | 97 | 31 | 1 | - | - |
| | 3 | TNTC | 103 | 37 | 2 | - | - |
| H | 1 | TNTC | TNTC | 38 | 12 | 3 | - |
| | 2 | TNTC | TNTC | 45 | 8 | - | 1 |
| | 3 | TNTC | TNTC | 32 | 11 | - | - |
| I | 1 | TNTC | TNTC | 72 | 7 | - | - |
| | 2 | TNTC | TNTC | 67 | 12 | - | - |
| | 3 | TNTC | TNTC | 78 | 9 | - | - |
| J | 1 | TNTC | TNTC | 91 | 15 | - | - |
| | 2 | TNTC | TNTC | 82 | 7 | - | - |
| | 3 | TNTC | TNTC | 87 | 12 | - | - |

**Appendix 1C**

| Sample | Rep | Dilutions | | | | | |
|---|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| A | 1 | TNTC | TNTC | 121 | 17 | 2 | - |
| | 2 | TNTC | TNTC | 118 | 15 | - | 9 |
| | 3 | TNTC | TNTC | 125 | 12 | - | 7 |
| B | 1 | TNTC | TNTC | TNTC | 70 | 7 | - |
| | 2 | TNTC | TNTC | TNTC | 81 | 5 | - |
| | 3 | TNTC | TNTC | TNTC | 75 | 7 | - |
| C | 1 | TNTC | 35 | 9 | - | - | - |
| | 2 | TNTC | 29 | 5 | - | - | - |
| | 3 | TNTC | 33 | 7 | - | - | - |
| D | 1 | 35 | 7 | - | - | - | - |
| | 2 | 32 | 3 | - | - | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 28 | 6 | - | - | - | - |
| E | 1 | TNTC | TNTC | 53 | 11 | - | - |
| | 2 | TNTC | TNTC | 49 | 9 | 1 | - |
| | 3 | TNTC | TNTC | 55 | 13 | - | - |
| F | 1 | TNTC | 201 | 36 | 8 | 1 | - |
| | 2 | TNTC | 213 | 40 | 2 | - | - |
| | 3 | TNTC | 209 | 32 | 3 | - | - |
| G | 1 | TNTC | 54 | 9 | - | - | - |
| | 2 | TNTC | 59 | 3 | 1 | - | - |
| | 3 | TNTC | 63 | 7 | - | - | - |
| H | 1 | TNTC | 79 | 8 | 2 | - | - |
| | 2 | TNTC | 82 | 5 | - | - | 1 |
| | 3 | TNTC | 74 | 2 | - | - | - |
| I | 1 | TNTC | 298 | 23 | 7 | - | - |
| | 2 | TNTC | 281 | 27 | - | - | - |
| | 3 | TNTC | 287 | 29 | - | - | - |
| J | 1 | TNTC | TNTC | 76 | 5 | - | - |
| | 2 | TNTC | TNTC | 79 | - | - | - |
| | 3 | TNTC | TNTC | 82 | 2 | - | - |

**Appendix 1D**

| Sample | Rep | Dilutions | | | | | |
|---|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| A | 1 | TNTC | TNTC | TNTC | 66 | 5 | - |
| | 2 | TNTC | TNTC | TNTC | 59 | - | - |
| | 3 | TNTC | TNTC | TNTC | 67 | 47 | 7 |
| B | 1 | TNTC | TNTC | TNTC | 124 | 42 | 4 |
| | 2 | TNTC | TNTC | TNTC | 127 | 39 | 10 |
| | 3 | TNTC | TNTC | TNTC | 131 | 7 | - |
| C | 1 | 193 | 35 | 2 | - | - | - |
| | 2 | 187 | 49 | - | - | - | - |
| | 3 | 196 | 33 | 1 | - | - | - |
| D | 1 | 36 | 9 | - | - | - | - |
| | 2 | 32 | 3 | - | - | - | - |
| | 3 | 39 | 9 | - | - | - | - |
| E | 1 | TNTC | 135 | 23 | - | - | - |
| | 2 | TNTC | 141 | 29 | 5 | 1 | - |
| | 3 | TNTC | 138 | 25 | 3 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| F | 1 | 156 | 98 | 6 | 8 | 1 | - |
| | 2 | 149 | 87 | 4 | 2 | - | - |
| | 3 | 161 | 93 | 3 | 3 | - | - |
| G | 1 | 205 | 34 | 9 | - | - | - |
| | 2 | 214 | 49 | 3 | 1 | - | - |
| | 3 | 209 | 41 | 7 | - | - | - |
| H | 1 | 245 | 69 | 3 | 2 | - | - |
| | 2 | 253 | 52 | 1 | - | - | 1 |
| | 3 | 239 | 54 | 2 | - | - | - |
| I | 1 | TNTC | 108 | 3 | 7 | - | - |
| | 2 | TNTC | 111 | 7 | - | - | - |
| | 3 | TNTC | 117 | 9 | - | - | - |
| J | 1 | TNTC | 132 | 26 | 5 | - | - |
| | 2 | TNTC | 129 | 19 | - | - | - |
| | 3 | TNTC | 134 | 22 | 2 | - | - |

**Appendix 1E**

| Sample | Rep | Dilutions | | | | | |
|---|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| A | 1 | TNTC | TNTC | TNTC | 78 | 25 | - |
| | 2 | TNTC | TNTC | TNTC | 89 | 18 | - |
| | 3 | TNTC | TNTC | TNTC | 81 | 21 | 7 |
| B | 1 | TNTC | TNTC | TNTC | 164 | 52 | 4 |
| | 2 | TNTC | TNTC | TNTC | 157 | 59 | 10 |
| | 3 | TNTC | TNTC | TNTC | 151 | 57 | 8 |
| C | 1 | 121 | 25 | 2 | - | - | - |
| | 2 | 127 | 29 | - | - | - | - |
| | 3 | 129 | 23 | 1 | - | - | - |
| D | 1 | 21 | 4 | - | - | - | - |
| | 2 | 32 | 3 | - | - | - | - |
| | 3 | 30 | 1 | - | - | - | - |
| E | 1 | TNTC | 95 | 13 | - | - | - |
| | 2 | TNTC | 102 | 19 | - | 1 | - |
| | 3 | TNTC | 108 | 15 | 2 | - | - |
| F | 1 | 116 | 21 | - | - | 1 | - |
| | 2 | 114 | 27 | 4 | - | - | - |
| | 3 | 99 | 23 | 1 | - | - | - |
| G | 1 | 121 | 14 | - | - | - | - |
| | 2 | 97 | 19 | - | 1 | - | - |
| | 3 | 112 | 11 | - | - | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| H | 1 | 175 | 19 | 1 | - | - | - |
| | 2 | 163 | 22 | 1 | - | - | 1 |
| | 3 | 169 | 24 | 2 | - | - | - |
| I | 1 | 189 | 78 | 3 | 7 | - | - |
| | 2 | 191 | 81 | 7 | - | - | - |
| | 3 | 182 | 88 | 9 | - | - | - |
| J | 1 | 245 | 52 | 6 | - | - | - |
| | 2 | 251 | 59 | 7 | - | - | - |
| | 3 | 241 | 64 | 2 | - | - | - |

Statistical analysis of pH treated and untreated milk drink

| **Source of variation** | **% of total variation** | **P value** |
|---|---|---|
| Interaction | 6.67 | 0.562 |
| Column factor | 3.50 | 0.248 |
| Row factor | 89.26 | 0.882 |
| Residual | 4 | |

Column factor indicates the effect between the different modes of storage (refrigerated and non-refrigerated) whereas the row factor indicates the different formulations (varying concentrations of preservatives).

**Statistical analysis of total aerobic counts (refrigerated and non-refrigerated)**

| **Source of variation** | **% of total variation** | **P value** |
|---|---|---|
| Interaction | 6.67 | 0.562 |
| Column factor | 0.43 | 0.353 |
| Row factor | 98.03 | 0.001 |
| Residual | 16 | |

Column factor indicates the effect of the different modes of storage (refrigerated and nonrefrigerated) whereas the row factor indicates the effect of the different formulations (varying concentrations of preservatives).

Summary of statistical analysis of yeast count on milk drink

| **Source of variation** | **% of total variation** | **P value** |
|---|---|---|
| Interaction | 6.67 | 0.562 |
| Column factor | 25.62 | 0.001 |

| | | |
|---|---|---|
| Row factor | 64.49 | 0.001 |
| Residual | 16 | |

Column factor indicates the effect of the different modes of storage (refrigerated and nonrefrigerated) whereas the row factor indicates the effect of the different formulations (varying concentrations of preservatives).