KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

Microbiological and Proximate Composition of "Burkina" Drink

A Case Study in Accra

By

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DECLARATION

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

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ABSTRACT

―Burkina‖, a drink originating from Burkina Faso, is currently in high competition with beverages such as ice ―Kenkey‖, ―Fula‖, ―Nunu‖, and other cereal based beverages sold on the streets of Ghana. It is considered as a complete food because it is made up of major ingredients such as millet which is one of the world‘s oldest cereal, and cow‘s milk. The present study was undertaken to microbiologically characterize pathogenic microbes associated with ―Burkina‖ drink, and also to determine pH and proximate composition. Thirty six samples were aseptically purchased from Nima, Maamobi, and 37 Military Hospital and Accra Mall areas within Accra Metropolitan Assembly and analyzed based on standard laboratory methods. Results from the study revealed high microbial loads ranging from 6.86 ± 0.13 to 7.94 ± 0.21 log cfu/ml for the Total Viable bacteria, 3.78 ± 0.84 to 4.68± 0.08 log cfu/ml for *Staphylococcus aureus*, 2.65 ± 0.12 to 4.31 ± 0.57 log cfu/ml for the yeasts and moulds and 12 to >110MPN/ml for the *Escherichia coli*. The pHs of the ―Burkina‖ drink were all within the acidic range, of 3.79± 0.01 to 4.82± 0.02. The percentage mean proximate composition was 81.67 ± 0.77% for the moisture, 11.55 ± 1.24% for carbohydrates, 3.79 ± 0.71% for proteins, 2.46 ± 0.82% for total fats, and 0.43 ± 0.03% for total ash contents. The study observed high nutritional composition for the ―Burkina‖ drink; however, the microbial counts from most of the sources studied exceeded the maximum acceptable limits making the samples microbiologically unsafe for human consumption.

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LIST OF ABBREVIATIONS AND ACRONYMS

AMA	Accra Metropolitan Assembly
ANOVA	Analysis of variance
AOAC	Association of Analytical Communities
A_w	Water activity
CFR	Code of Federal Regulation
CFU	Colony Forming Units
DASA	Dairy Association of South Australia
DUNK	Developing unity, through nurturing knowledge
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FDA	Food and Drugs Administration
FSA	Food Science Australia
GRAS	Generally Recognized as Safe
GSA	Ghana Standard Authority
HCL	Hydrochloric acid
ISO	International Organization for Standardization
KBS	Kenya Bureau of standard
LI	Legislative Instrument
MMDA	Metropolitan Municipal and District Assembly
NAOH	Sodium Hydroxide
NRSC	National Road and Safety Commission
OECD	Organization for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
RTE	Ready to Eat

TFTC	Too Few to Count
TNTC	Too numerous to count
TSI	Triple Sugar Iron
TVC	Total Viable Count
USDA	United States Department of Agriculture
VOCTA	Voluntary Consumers Training and Awareness Society
WFLO	World Food Logistics Organization
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholate

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CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND INFORMATION

Street vended foods contribute significantly to food security and nutrition and are physically and economically accessible to most people. ―Burkina‖ is a street vended drink that has its root from Burkina Faso. It is made from millet, fermented fresh cow‘s milk or powdered milk, water and sugar with salt added to taste. Optional ingredients used include; spices, flavour, margarine, ―Fan‖ ice cream and roasted groundnuts. In Burkina Faso, its original name is ―Dèguè‖. In Ghana, it is produced mostly in the Muslim communities in small quantities; however, some few private individuals produce it in commercial quantities. In Accra, the Capital City of Ghana, production of ―Burkina‖ drink has become a fast growing business competing strongly with other locally prepared beverages such as ―local malt‖, ―ice kenkey, ―fula‖, ―nunu‖, ―atadwe milk‖ among many other snacks sold on the streets of Accra. The use of street vended food has currently become an integral part of convenient food preparation patterns all over the world including Ghana. The growing urbanization of developing countries‘ populations emphasizes the need for safe, convenient, nutritious foods, for use by persons of all ages, in a society in which women as well as men frequently work outside the home and have little opportunity for traditional food preparation. The consumption of these ready-to-eat foods has, however, been reported to be associated with serious health problems including infections with *Listeriosis monocytogenes* (Adams and Moss, 2000; FDA, 2001). Though street food vendors are considered useful segment of the economy, consumers are not well educated about the safety of their products. The complex biochemical composition of milk makes it an excellent medium for both pathogenic and spoilage microorganisms (Okonkwo, 2011) hence, the use of fresh cow‘s milk as part of the ingredients in the preparation of the product makes it very critical for attention to be paid into its production. Wouters *et al.,* (2002) stated that raw

milk has low keeping quality and at room temperature, spontaneous microbial spoilage occurs turning the product sour for some few days through the activities of lactic acid bacteria. The growth of these microorganisms in the food could make the food unsafe and harmful to consumers. Outbreaks of milk-borne diseases have occurred despite pasteurization, as a result of either improper pasteurization or product recontamination much more in their fresh state (Nebedum *et al.*, 2007). ―Burkina‖ drink has come to add to other street vended foods in the country and provide a source of affordable nutrients to the majority of consumers especially the low-income group in most developing countries (Muzaffar *et al.*, 2009). It is one of the commonly patronized street vended foods in Accra; however, its production is prone to microbial growth, probably, because of its nutrient-rich raw materials. This study, thus, focuses on the pH, proximate composition and the microbiological quality of ―Burkina‖ drink sold in the Accra Metropolis.

1.2. PROBLEM STATEMENT

According to Shekar (2010) street vended foods contribute significantly by providing employment for many, while providing nutritious, inexpensive ready-to-eat food to millions of workers. Unfortunately, FAO (2013) has stated that, some serious concerns exist about the safety of street vended foods. According to Rane (2011) poor knowledge and improper food handling of street vendors in basic food safety measures and poor knowledge and awareness among consumers with certain foods could explain the health and safety issues that street vended foods pose.

Most of the manufacturers of these products do not conform to Good Manufacturing Practices (GMPs) nor are well informed about the safety hazards associated with the product. Additionally, most of the products are sold on the streets without labelling and even when labelled, do not meet the labelling requirements in Ghana (GSA, 1992). This may be coupled with inappropriate storage conditions especially for diary based product. Consequently, the

absence of a label on the product does not provide consumers with full information to aid them to make informed choices when purchasing nor provide them with full information about the composition and nutritional value nor meet local or international standard. Hence, the need to investigate the pH, proximate composition and microbial quality of ―Burkina‖ drink, a street vended drink.

1.3. JUSTIFICATION

The documentation of the nutritional value of the product will enhance its popularity as well as providing information to consumers to make informed choices based on the quality of the product. The database from this result will encourage regulatory bodies to come out with a standard for the product which could be used by manufacturers in the country since there is currently no standard for the product. Results from the study will indicate whether the product also meet appropriate labelling requirement.

1.4. AIM

To assess the pH, proximate composition and the microbial quality of street vended ―Burkina‖ drink sold in four areas in the Accra Metropolis.

1.5. SPECIFIC OBJECTIVES

1. To determine the pH of each sample.
2. To determine the proximate composition of the street vended ―Burkina‖ drink.
3. To determine the microbial load and types of microbes associated with street vended ―Burkina‖ drink.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. STREET VENDED FOODS IN GHANA

Street vended foods are foods and beverages prepared and/or sold by vendors on the street and other public places for immediate consumption or consumption at a later time without further preparation or processing (FAO/WHO, 2006). There is a perpetual advancement in urbanization in many developing countries, and governments‘ face a major threat in ensuring that city dwellers are able to have enough food. The selling of snacks and whole meals on the streets of Ghana has become an important way to obtain income for both the young and old. The street food stratum is an undeniable aspect associated to urban growth, which is making cooking at home challenging due to certain constraints such as; time factor, vehicular traffic, longer travelling distance between residences and working places. Hence, urbanization and its related societal and physical changes have caused the need for street vended foods to increase. This situation has resulted in street vended foods accounting for a key part of our day-to-day diet and so adds towards meeting nutritional needs, despite its variations. FAO (2007) has stated that, an approximated 2.5 billion of the world populace eat street vended foods daily. Urbanization and relocation of people have drastically altered the way of life and eating habits of people. The accessibility of all kinds of foods in the streets of cities has tremendously relieved workers, faced with long periods of absence from home. According to FAO/WHO (2012), street vended foods are quite economical as compared to restaurant meals and offer wide variety, which can be an alternative to home cooked food.

In Accra, Ghana, street vended foods were estimated to have a turnover of $100 million and to employ more than 60,000 people (NRI, 2003). In Ghana, as in most of Africa, nearly everyone form their foods nearly to some extent on large number of starchy or carbohydraterich foods.

Grains such as millet, sorghum, rice, and maize (corn) and tubers such as yams and cassava (manioc), for example, form the main component of majority of known meals in Africa and make available the major part of the regular caloric intake. These popular starches are customarily eaten with protein-rich legumes and by smaller quantities of foods that add both flavour and nutrition, such as vegetables, oils, spices, and meat or fish. The existence of street food vendors is widely spread, functioning from all vantage or any available site at all times of day and night, serving customers with spicy foods, attractive beverages, and snacks that are time convenient and affordable. Most of these street vended foods provide occupation to many, while providing wholesome, economical and ready-to-eat food to millions of workers (Shekar, 2010). In addition, some local Ghanaian dishes require long method of cooking, so when the cost of the ingredients and the cooking fuel is factored into the time spent shopping and cooking, it is often economical to buy these foods from street vendors, who prepare them in large quantities. The eating habits of most Ghanaian are drastically changing along with the way and demands of our day-to-day life, particularly in the capitals. Additionally, most of the populace, especially career women, who cook at home in our part of the world commute long distances away from their homes to work or to attend school. Ghanaian women play a key part in the economy, managing a large share of the market enterprise and product trading (Johnson and Yawson, 2000). Hence, they and their households rely on street vended foods for virtually their daily meals throughout the country. Observation made on some of the street vended foods offered for sale range from the traditional dishes such as ―fufu‖, ―banku‖, ―ampesi‖, among others to continental dishes such as ―shawarma‖, pizza, fried rice (―check check‖), among many others. Different varieties of street vended foods exist greatly between countries, cultures and vendors and it is observed that in most countries, the types of foods purveyed are not documented, yet they are often unique and are an important source of nutrients for the population (Muzaffar *et al*., 2009; Tambekar *et al*., 2011). In addition, report by FAO (2001) on street vended foods, noted that street vended foods are different from fast foods because

street vendors specialize in fewer foods and use frying as a main food preparation method and that the diversity of preparation methods used impacts differently on the nutritional value of the food and has increased its acceptability among consumers.

Sadly, the occurrence of these unofficial food ventures can cause health problems if foods are not prepared and handled properly. Although, the potential benefits of street vended foods cannot be overlooked, street vended foods are prepared mostly by those with minimal enlightenment of food safety practices and in surroundings that can mar the hygienic preparation, storage and sale of the food. Street food vendors are mostly unauthorized and unskilled in food hygiene or sanitation and work under very primitive and unhealthy conditions (Barro *et al.*, 2002; Muinde and Kuria, 2005). According to Codex Alimentarius Commission (2014), street vended foods are perceived to be a major public health risk due to lack of fundamental infrastructure and services, difficulty in regulating the massive numbers of street food vending operations because of their variety, movability and provisional nature. The emphasis of the need for safer street food has received media attention in recent years, as a result of many diarrhoeal diseases such as cholera, enterobacteriosis and enterovirosis (Estrada-Garcia *et al.*, 2004; Cardinale *et al.*, 2005). Foodborne bacterial microorganisms mostly found in street vended foods are *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens* and *Salmonella* spp, typically among them is the *Salmonella typhi*, a major problem associated with public health hazards (Mosupye and von-Holye, 2000). Sadly enough, although people know that food borne diseases could be acquired through the eating of street vended foods, greater part disregard these health hazards.

In Ghana, national and municipal administrators such as the Food and Drugs Authority (FDA), Ghana Standard Authority (GSA) and Metropolitan Municipal and District Assemblies (MMDAs) control the street food sector in the cities and towns (Johnson and

Yawson, 2000). These controls take various forms due to their varying mandates. Organization for Economic Co-operation and Development (OECD, 2000) has, however, revealed that these regulatory authorities are unsuccessful to carry out their duties efficiently, mainly due to failures of administrative capacity, the limits of authority of regulatory agencies, among others. Urbanization and population growth, especially in developing countries, are expected to continue into the next century and street-vended foods, which are largely but not exclusively an urban phenomenon will consequently expand (Garode and Waghode, 2012). WHO (2006), stated that street vendors provide affordable meals for thousands of Accra citizen but concerns over foodborne disease have led to efforts by Ghanaian authorities to enhance food safety and urge vendors to adopt more hygienic practices. According to FAO/WHO, (2012), street food vendors, as a class, usually come from poor background, with very little knowledge about food safety and hygiene practices. Moreover, their literacy level being low is an additional handicap. Thus, though they serve a large section of the society, their food is not always conforming to the norms and procedures of food safety and hygienic conditions for human consumption. For instance, people who buy street vended foods, have been reported to be affected by food borne diseases such as diarrhoea, cholera, typhoid fever and food poisoning (FAO/WHO, 2012). Foodborne illnesses associated with microbes are a major worldwide health predicament to food safety and are the result of death in developing countries (WHO, 2002). The consumers who turn to such foods are more interested in its accessibility than the question of its safeness, quality and hygiene (Mensah *et al.*, 2002). Hence, the need to microbiologically characterize pathogenic microbes associated with ―Burkina‖ drink sold on the streets of the Accra Metropolis.

2.2. SOURCES OF CONTAMINATION OF STREET VENDED FOODS

Potential source of contamination might start right from the "farm to fork" along the supply chain, that is, the first contamination of raw foods with disease causing bacteria to successive

contamination by vendors during preparation and to the final consumer. Some sources of contamination of street vended foods are discussed below.

2.2.1. Vending Location and Food Handling

Barro *et al.*, (2006), states that the circumstances under which some street vendors cook are revealed to be inappropriate for the preparation and selling of foods. Most of these locations/sites for food preparation are on the street side without any form of enclosure, hence exposing the food to all forms of contamination. Even when it is enclosed, it is not made up of the appropriate fencing or building materials but rather materials such as used wood, polythene bags, and used roofing sheets among many others. Due to the ready-to-eat nature of these street vended foods coupled with the hastiness on the side of consumers, vendors emphasize more on providing quick service to consumers; however, food sold by street vendors could cause serious health problems due to the lack of essential food safety and hygienic practices. Main sources attributing to microbial contamination are the place of establishment, utensils for cooking and serving, raw materials, temperature and time used for preparing and the personal hygiene of vendors (FAO/WHO, 2012). Furthermore, because preparation sites are not properly enclosed, it exposes food to houseflies and dirt, which may harbour foodborne microbes. The improper location or site may lack good sanitary facilities and coupled with the unsanitary handling of food by these vendors can result in them being carriers of disease causing organisms such as *Staphylococcus aureus, Salmonella, Campylobacter, Shigella* and *Escherichia coli*.

2.2.2. Waste Disposal

Wastes generated from these sites are kept within the same premises in inappropriate waste disposal bins rather than to have them covered, thus, exposing the food to all source of contamination. In addition, because of the unavailability of amenities for sewerage and garbage disposal, wastes are discharged into nearby streets and gutters generating aerosol

contamination. Such environments act as dwellings for rodents, breeding points for flies and media for growth of microorganisms. A study by Muinde and Kuria, (2005), on the hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya, in Africa showed that 85% of the population of these vendors prepared foods such as fish, fruit salads, roasted maize and chips in insanitary surroundings. According to Barro *et al.*, (2002), on his report on studies carried out on street vended foods in Africa, vendors of food have tremendous unlimited and unregulated growth and as such have placed a severe strain on city resources, such as water, sewage system and interferences with the city plan through overcrowding and untidiness which is seriously affecting daily life.

2.2.3. Water

According to (FAO/WHO, 2012), the quality of water that is used in the preparation of food by street vendors is also of questionable nature and that studies have shown that unhygienic water consumption has been the main cause for epidemics for the public at large. Water is a critical raw material and is used in many street-vended operations as drinking water, for washing of foods, incorporated in the food as an ingredient or may be used for washing equipment, utensils and hands. Commonly, these places are not properly sited hence there is absence of potable water for various undertakings at the vending site which pose a major worry. Thus, many vendors re-use the same water over and over again, notably for washing utensils and used dishes.

Water, like food, is a medium for the spread of many causes of disease and continues to cause major outbreaks of diseases in developed and developing countries internationally. It was revealed as the source of, among others, the vilest outbreak of *Escherichia coli* 0157:H7 in Canada (Kondro, 2000). According to WHO (2013), diarrhoeal diseases is the third main cause of death in low income countries accounting for 1.8 million deaths world-wide in the year 2005 alone. Generally, many of the cases result from the use of contaminated water and food. Climat

(2013), stated that one of the biggest problems which play a role in the contamination of street vended foods is the water supply and that the local water supply may not have an acceptable quality, may be in insufficient amounts for drinking, washing, cleaning and for other uses. ―Burkina‖ drink production and processing require sufficient amounts of water as it is used in washing the millet and preparing the fermented milk, among others, however, most locations where these ―Burkina‖ drinks are prepared do not have constant supply of water (Personal observation), and thus the question of the quality of water used in producing the ―Burkina‖ drink in these areas.

2.2.4. Personal Hygiene of the Vendors/Food Handlers

According to Zain *et al.,* (2002), food handlers play key function in guaranteeing food safety all through the chain of production, processing, storage and preparation. Although it is stated by Medeiros *et al.,* (2001), that an unknown percentage of foodborne disease could be prevented by actions taken by the consumers themselves food handlers are sources of food borne illnesses and the cause of food borne illness outbreaks (Guzewich and Ross, 1999). This is because, according to Barro *et al.,* (2002) and Mensah *et al*., (2002), considerable difference exists when it comes to problems of food safety in the developed countries and those of developing countries. In developing countries, poor personal hygiene of food handlers, local methods of processing and packaging, inappropriate holding temperature are still used during food marketing and technology in contrast to what happens in developed

countries.

2.2.5. Storage

Adesiyun and Balbirsingh, (1996), reported that keeping foods at room temperatures for long durations have been reported to be a major factor to the occurrence of food poisoning outbreaks. Foods are often observed being held for several hours after cooking and those prepared

overnight for the following day also held at ambient temperatures until sold. In addition, some of the foods are held in the cooking pot in which they are cooked, until sold or reheated, all contributing to longer holding time, making it a conducive for the growth of foodborne pathogens.

2.3. "BURKINA" DRINK AS STREET VENDED FOOD

―Burkina‖ drinks are sold as ready to eat foods, mostly packaged in transparent plastic bottles of different shapes and sizes while others are sold by mixing the ingredients based on the amount requested by the customer in transparent polythene bags as ―take away‖ or in plastic bowls and drank on site. The prices of ―Burkina‖ drink sold on the streets range between GH¢1.00 and GH¢2.00 depending on the ingredients, quantity and producer. The bottled drinks are kept in transparent plastic containers with ice blocks to keep it at a low temperature and it is best served when chilled. Consumption of ―Burkina‖ drink is virtually multiethnic, as it is now sold and eaten by migrants and travelers, and has become familiar far beyond the regions where they were mainly produced. Hence, the sale of ―Burkina‖ drinks cuts across the length and breathe of Ghana from the Greater Accra region through to Eastern region (Koforidua), Ashanti region (Kumasi), and to the Central region (Swedru).

2.3.1. Processing of "Burkina" drink

―Burkina‖ drink is a complete meal, rich in protein and very good for all groups, but could also cause serious food poisoning when prepared and packaged under unhygienic conditions. Nutrients from plant origin are mostly used in the food industry and cereal as a plant constitute a major source of dietary nutrients world-wide (Amadou *et al.*, 2011; Izadi *et al*., 2012). Foods and beverages obtained from millet are known internationally and are still part of key diets in most African countries (Obilana and Manyasa, 2002; Amadou *et al.*, 2011). Millets are often milled into flour, rolled into large balls, parboiled and then eaten as porridge with milk, however, sometimes millets are used for beverages (Issoufou *et al.*, 2013) such as

—Burkina‖. Fermented milk products such as yoghurt have been eaten for more than thousands of years. Fermented milk products, like the milk from which they are made, are rich in protein, vitamins and minerals, however, in addition to the purely nutritional properties, there is increasing awareness for a number of other health benefits (Buttriss, 2007). The use of fresh milk as part of its ingredients in the preparation of the product makes it very critical for attention to be paid into its production. Wouters *et al.,* (2002) states that, raw milk has low keeping quality and at room temperature spontaneous microbial spoilage occurs turning the product sour some few days later. Depending on its mode of production, preservation, packaging and distribution along the supply chain, microorganisms such as lactic acid bacteria and others could be found in it, to cause contamination (Wouters *et al.*, 2002). Different microorganisms including fungi, bacteria, rickettsia and viruses could also be found in milk since the udder of the animal could harbour organisms while others come as contaminants due to poor handling. Thus, pathogenic organisms that might have gained access into the product have enough time to multiply and/or produce harmful metabolites in the rich environment. Most of the food-borne illnesses associated with milk consumption are linked to post-pasteurization contamination (Olsen *et al.*, 2004). Post-pasteurization contamination of milk is mostly by contaminated hands of dairy workers, unsanitary utensils and polluted water supply (Pantoja *et al.*, 2009). Although fermented milk helps in its organoleptic properties, the fermentation process results in a drop in pH which may not be able to inhibit the growth of the acidophilic microbes and thus carried to the consumer.

2.4. MILLET

The most widely available grain, and the grain most frequently purchased when farmers‘ own production is exhausted, is millet (Brown, 2009). There are many different types of millets. The four main types include; pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), proso or white millet (*Panicum miliaceum*), and foxtail millet (*Setaria italica*) (Yang *et al.*,

2012). According to FAO (2009), global production of millet reached about 32 million tonnes with the top producing countries being India (10,610,000), Nigeria (7,700,000), Niger (2,781,928), China (2,101,000), Burkina Faso (1,104,010), Mali (1,074,440), Sudan (792,000), Uganda (732,000), Chad (550,000) and Ethiopia (500,000).

2.4.1. Millet Food Products in Africa

Across West Africa, the major food dishes obtained from millet vary. The stiff or thick porridges (—Tuwo‖ or —Tộ‖) are well known and mostly eaten in all the Sahelian countries across the region (Obilana, 2014). The steam-cooked product _Couscous‘ and thin porridge —bouillie‖ are popularly consumed in the French speaking countries such as Senegal, Mali, Guinea, Burkina Faso, Niger and Chad (Obilana, 2014). In Nigeria and Niger the thin porridge —Fourra‖ is very common —Soungouf,‖ —Sankhal‖ and —Araw‖ are well noted in Senegal (Obilana, 2014).

In Ghana, millet is one of the traditional cereal staple of people living in the three Northern regions. Due to urban migration this staple cereal has found its way to other regions within the country especially within the Greater Accra region in the south. It is used in preparing foods such as —Tuo‖ (a stiff porridge), —Hausa Koko‖ (a thin porridge), —Masa‖ (a fried cake) and —Fura‖ (a beverage) predominately by the Muslim community within the country. Virtually, these millet products are sold in all streets in the country because in recent times, the trend has changed as its usage has become multiethnic, however, because millet is expensive, most people have substituted millet with maize for the preparation of foods such as —Hausa Koko‖ and —Tuo‖.

2.4.2. Nutritional Value of Millet

The presence of all the essential nutrients in millets makes them suitable for the manufacturing of food products such as baby foods, snack foods and dietary supplements and more millet

products have become part of the daily diet (Subramanian and Viswanathan, 2007; Liu *et al.,* 2012). According to Truswell (2002), millets has been noted as a functional food and nutraceuticals due to its dietary fibers, proteins, energy, minerals, vitamins and antioxidants needed for human health. Millets are also rich in phytochemicals and micronutrients (Mal *et al*., 2010; Singh *et al*., 2012).

For instance, pearl millet was found to be rich in resistant starch, soluble and insoluble dietary fibers, minerals, and antioxidants (Ragaee *et al.,* 2006). Approximately, it is made up of 92.5% dry matter, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali *et al.,* 2003). Again, foxtail millet protein characterization showed that its protein concentrate is a potential functional food ingredient and the essential amino acid pattern suggests possible use as a supplementary protein source to most cereals because it is rich in lysine (Munyeme *et al.,* 2010).

Finger millet is also known to have health benefits due to its polyphenol contents (Chethan and Malleshi, 2007). Approximately, it has carbohydrate content of 65-76%, protein content of 5-8%, dietary fiber content of 15-20%, and mineral content of 2.5-3.5% similar to other cereals. Its crude fiber and mineral contents are significantly higher than those of wheat (1.2% fiber content, 1.5% mineral content) and rice (0.2% fiber content, 0.6% mineral content). In addition, black finger millet contains 8.71 mg/g dry weight fatty acid and 8.47 g/g dry weight protein (Glew *et al.,* 2008).

2.4.3. Health Benefits of Millets

In India and some other countries, sprouted (malted) grains are commonly used as weaning foods for infants and as easily-digested foods for the elderly and infirm (Platel *et al.,* 2010). A study at the Central Food Technological Research Institute in Mysore, India, measured the changes caused by malting finger millet, wheat and barley (Platel *et al*., 2010). They found that,

malting millet increased the bioaccessibility of iron (>300%) manganese (17%), and calcium (―marginally‖), while reducing bioaccessibility of zinc and making no difference in copper.

A study carried out in Memorial University of Newfoundland in Canada, on the antioxidant activity and phenolic content of several varieties of millet, reported that, kodo millet has the highest phenolic content with proso millet showing the least. All varieties showed high antioxidant activity, in both soluble and bound fractions (Chandrasekara and Shahidi, 2010).

A study in Seoul, South Korea, on rats fed a high-fat diet for 8 weeks to induce hyperlipidemia, and then randomly divided the rats into four diet groups: white rice, sorghum, foxtail millet and proso millet for four weeks. The study revealed that, triglycerides were remarkably lower in the two groups consuming foxtail or proso millet, and levels of Creactive protein were lowest in the foxtail millet group. The researchers concluded that millet has the potential to prevent cardiovascular diseases (Lee *et al*., 2010).

A study in Sri Devaraj Urs Medical College in Tamaka, Kolar, India on the prevalence and awareness of diabetes in rural areas, in order to inform health policy revealed that, while there was widespread lack of awareness of the long term effects of diabetes and diabetic care, common perception favoured consumption of ragi, millet and whole wheat chapattis instead of rice, sweets and fruit (Muninarayana *et al.*, 2010).

The National Institute of Nutrition in Hyderabad, India, also carried out a study on the total phenolic content and antioxidant activity of various pulses, legumes and cereals, including millets. Finger millet and ―Rajmah‖ (a type of bean) were highest in antioxidant activity, while finger millet and black gram dhal (a type of lentil) had the highest total phenolic content (Sreeramulu *et al*., 2009) . Thus, millet has got a lot of health benefits that must be tapped into.

2.5. MILK

Milk is naturally, rich nutrient and economical product, in addition to its high nutrient to energy ratio. Milk and dairy products are key food groups in many national dietary guidelines and may play a part in dietary quality (Miller *et al.,* 2007). Milk fats add distinct characteristics to the appearance, texture, flavour and satiability of dairy foods. In addition, it‘s a rich source of energy, essential fatty acids, fat-soluble vitamins, and several other components, such as conjugated linoleic acid and sphingolipids. Milk is also rich in high quality protein. Bánóczy *et al.,* (2009), added that the main carbohydrate found in milk is lactose and that Cow‘s milk contains about 4.5g of lactose per 100g milk and lactose is the least carcinogenic of the known dietary sugars. In addition, various other components of milk have the potential to prevent dental caries. Cow‘s milk provides a lots of vital nutrients to the diet and whilst milk as a good source of calcium, B vitamins, B2 (riboflavin) and B12, and the minerals iodine, potassium and phosphorus (Food Standards Agency, 2002). The consumption of milk has been termed a marker for a complete healthy diet because it boost nutrient intake (Marshall *et al.*, 2005).

Raw untreated milk is still used by large number of farm families and workers and by a growing segment of the general population who believe that even though raw untreated milk is not safe, the fresh milk imparts beneficial health effects that are destroyed by pasteurization. A zoonotic bacterial agent present in raw milk is of great public health and economic significance. As well as causing serious economic problems concerning the dairy industry, they constitute a major impediment to the trade of animals and animal products, and this can lead to obstruction of social and economic progress, especially in developing countries in Africa (Munyeme *et al.,* 2010). Moreover, the level of cultural awareness among farmers about the importance of economic and public health from zoonotic diseases in most of these countries is low and this increases the effort required to control these diseases Munyeme *et al.,* (2010). One product that is commonly distributed in raw form is milk and is usually colonized by a variety of many

zoonotic pathogens such as *Campylobacter jejuni, Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica* (Marco and Wells-Bennik, 2008). These pathogens in milk have been linked to the environment in the farm, mixing clean milk with mastitis milk and from livestock (Marco and Wells-Bennik, 2008). The natural raw milk obtained from the mammary gland of healthy animal is usually low in microbes and the application of hygienic measures during milking prevents milk from contaminating as well. A microbe can get access to the milk through colonization of the teat canal or an infected udder (clinical and subclinical mastitis) or from milk utensils or water supply used (Hayes *et al.,* 2001). Milk contaminated by high levels of bacteria usually becomes unsuitable for further processing (Nanu *et al.,* 2007) since the presence of these bacteria may affect the quality and safety of milk and its products (Szteyn *et al.,* 2005).

E. coli is a normal inhabitant of the intestines of animals and humans but its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to severe gastrointestinal disturbance. Other toxigenic strains such as *E. coli* O157:H7 may cause life threatening syndromes (Soomro *et al.,* 2002; Kawano *et al.,* 2008).

Milk is a suitable media for the growth and proliferation of *Staphylococcus aureus*. The organism is accounts for approximately 30% to 40% of all mastitis cases (Asperger *et al.,* 2003). Insanitary measures, contaminated equipment, mammary gland infected with *S. aureus* and hands of milkers during handling and processing of raw milk are said to be the main cause of milk contamination with *S. aureus* (Scherrer *et al.,* 2004). *S. aureus* is noted as the most important cause of food borne illnesses world-wide (Gwida and El-Gohary, 2013).

2.6. FERMENTATION

Fermentation is among the ancient biotechnologies used in food production. Fermentation results in desirable properties such as extended shelf-life and good organoleptic properties in foods (Smid and Hugenholtz, 2010). In the fermentation of carbohydrates, it is oxidized to give a wide range of products which are mostly organic acids, alcohol and carbon dioxide (Ray and Panda, 2007). According to Gaggia *et al.,* (2011), fermentation helps in the preservation of food by forming inhibitory metabolites such as organic acid, lacticacetyl, reutrin, and bacteriocins, often with decreased water activity by drying or by the use of salt (Gaggia *et al.,* 2011). Fermentation improves food safety by inhibiting pathogens (Adams and Nicolaides, 2008), and also improves the nutritional value (Poutanen *et al.,* 2009, Van *et al.,* 2010) as well as the organoleptic quality of food (Lacroix *et al.,* 2010; Sicard and Legras, 2011). Most of these fermentation processes involve lactic acid bacteria, yeasts or mixture of these as the functional microorganisms. Cereal-based fermented products such ―Kenkey‖ in Ghana, ―Poto-poto‖ in Congo (Blandino *et al*., 2003), ―Ogi‖ in Nigeria (Osungbaro, 2009)

―Injera‖ in Ethiopia (Abiyu *et al.,* 2013), ―Kisra‖ in Sudan (Magdi *et al.,* 2010) and ―Togwa‖ (Mugula *et al.,* 2001) in Tanzania are found in Africa.

2.6.1. Fermentation of Milk

Fermented foods are vital to human beings world-wide with about 20-40% of food obtained from fermented foods (Amadou *et al.,* 2011). Fermented milk and yoghurt have nutritional advantages in comparison to raw milk. For example, yoghurt as a fermented milk product has higher levels of calcium and potassium (199/255mg/100g) than raw milk (123/166mg/100g) and that fermentation plays important role in the nutrition of infants and young children as it is used for the preparation of complementary foods in many African countries (Yasmine, 2000). Fermented foods look not only tasty and healthy, but has special characteristic such as

enjoyable flavour, aromas, textures, and improved cooking and processing properties (Holzapfel, 2002). According to FAO/WHO (2011), in fermenting milks, specific combinations of microorganisms, which are defined according to national or international rules or industrial specifications, are used. These microorganisms possess the status of been _Generally Recognized as Safe‘ (GRAS) in United State of America and _Qualified Presumption of Safety‘ (QPS) in the European Union, due to its lasting history of been safe for use in food production and to an absence of pathogenicity and potentially harmful metabolites (Bernardeau et al., 2008; Delorme, 2008). In the case of ―Burkina‖ drink, however, it is done traditionally without any specification.

2.7. PRODUCTION OF "BURKINA" DRINK

Originally, ―Burkina‖ is prepared using raw cow‘s milk, however, due to the expensive nature of fresh cow‘s milk, vendors reduce cost by using powdered milk for the milk fermentation but add small amount of fresh cow‘s milk as starter culture to give it the characteristic taste when fresh cow milk is used.

2.7.1. Millet Preparation

―Burkina‖ is prepared in small quantities according to traditional procedures. A brief procedure is described below;

The millet is initially sorted out to remove any foreign matter that might cause any contamination to the initial raw material. It is then washed three times to remove all forms of dirt after which it is air-dried and pounded in a mortar to remove the first coat (epiderm) before grinding into a powdery form. Salt is dissolved in water, sprinkled on the dough and uniformly mixed. Using steam as a method of cooking the milled millet is steamed to cook until compact dough is formed. The compact dough is allowed to cool after which it is mashed manually to break the compacted dough into smaller granular coarse gravels using a masher. Margarine is

rubbed in the granular coarse gravels for it to form uniformly and to add some taste to the granular millet. It is then kept in a container, covered and stored at room temperature until needed.

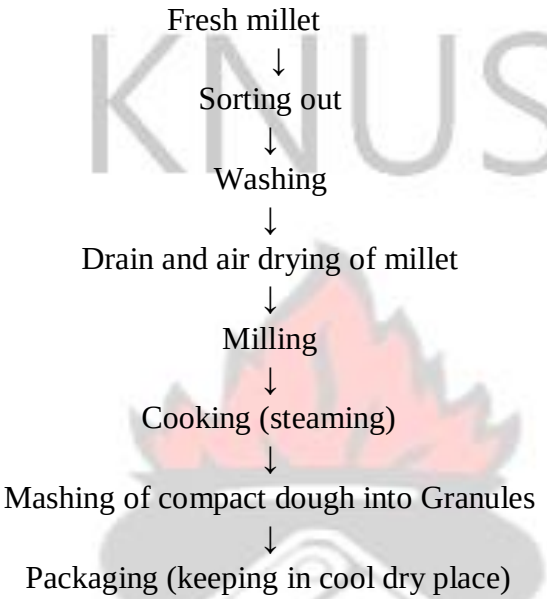
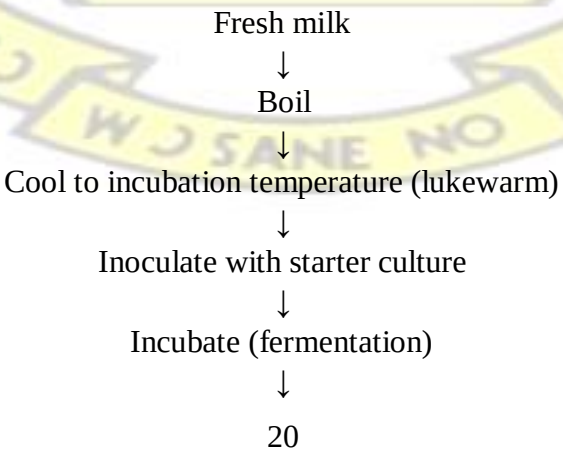


Figure 1: Summary of Millet Preparation

2.7.2. Fermentation of Raw (fresh) Cow's Milk in the Preparation of "Burkina" Drink

In the fermentation of fresh milk for ―Burkinal drink, no particular standard is used but rather it is done traditionally based on indigenous knowledge. Raw milk is brought to a boil and transferred into a plastic basin. The boiled milk is allowed to attain a lukewarm temperature. The lukewarmed milk is inoculated with starter cultures from previously fermented milk and covered overnight to ferment and then stored.



Package and store

Figure 2: Summary of the Fermentation of Fresh Cow's Milk into "Burkina" Drink

2.7.3. Fermentation of Powdered Milk in the Preparation of "Burkina" Drink

The powdered milk is uniformly dissolved in a reasonable amount of water in a plastic bucket or bowl. Water is boiled and added to the dissolved powdered milk. The resulting mixture is allowed to cool to ambient temperature. Inoculation is done using a starter culture of old cow's milk and uniformly stirred to obtain the desired taste of a cow's milk. It is covered and allowed to undergo fermentation overnight.

2.8. pH AS AN INTRINSIC FACTOR FOR MICROBIAL GROWTH

The pH measurement is one of the key parameters in the quality control of foodstuffs and it gives knowledge about the safety of natural products such as citrus fruits, juices or dairy product (Nor, 2010). Additionally, pH, temperature, pressure and reactant concentration also play important role on getting a desired product. McGlynn, (2011) stated that microorganisms such as moulds, yeasts and bacteria are sensitive to the pH of foods. Although the optimal pH value for most microorganisms is around 7, nearly all foods are acidic in nature, having pH value less than 7.0. pH value of a specific food may have a significant effect on its method of processing in order to preserve it, for example, *S. aureus* grows at a pH of 4.5 to 9.3 (EFSA, 2011).

CHAPTER THREE

3.0. MATERIALS AND METHODS

This chapter discusses the outline of Laboratory Analysis, Methods and Techniques and basic equipment, materials as well as chemicals (media, reagents, among others) that were used in this research.

3.1. MATERIALS

3.1.1 Study area

This study was carried out within the Accra Metropolis from August 2014 to October 2014.

The Accra Metropolitan Assembly (AMA) is among the ten (10) District Assemblies in Greater Accra Region and also among the One Hundred and seventy (170) Districts within the Country. Accra occupies a special position in Ghana because it is the Metropolitan, Regional and National Capital. Geographically, the Accra Metropolis covers an area of 173 sq. km. It is bounded to the South by the Gulf of Guinea stretching from Gbegbeyese to La.

To the East it is bounded by Ledzokuku-Krowor Assembly. To the North it is bounded by Ga East and Ga West and to the West by Ga South Districts (AMA, 2012).

The Accra Metropolitan Assembly (AMA) is the center of Ghana. The Assembly has full economic opportunities and potentials that must be exploited by its populace. The Metropolis has great capability for the development of eco-tourism, hospitality and commerce. However, low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes are among the major challenges faced by AMA due to the high density of the population in the city and has even resulted in demolition exercise in some areas such as Sodom and Gomorrah in recent times.

3.1.2. Sample and Sample Collection Technique

Commercially processed ―Burkina‖ drinks packaged in plastic containers were bought from street vendors in the Accra Metropolis (Nima, Maamobi, and 37 Military Hospital and Accra Mall areas) and its environs and aseptically carried on ice in a sterile ice chest and sent to the laboratory for pH, proximate composition and microbiological analysis immediately on arrival. Five producers and twelve vendors were interviewed. Triplicate samples were purchased from each of these vendors interviewed for the analysis.

3.1.3. Chemicals, Media and Reagents

Alcohol, Boric acid, Concentrated Sulphuric acid, Bromocresol green- methyl Red Indicator solution, Sodium Hydroxide solution, Standard Sulphuric acid solution and hydrogen peroxide were all from British Drug House VWR International Limited, England. Baird Parker Agar, Bismith Sulphate Agar, Brilliant Green Bile Broth, Dichloran Rose-Bengal Chloramphenicol Agar, *Escherichia coliform* Broth, Kovac's reagent, Lauryl Tryptone Broth, Maximum Recovery Diluent, Peptone water Plate Count Agar, Rappaport Vassiliadi Soya Broth, Rabbit Plasma Fabrinogen, Tetrathionate broth, Triple sugar Iron, Tryptone water, Urea Agar Xylose Lysine Desoxycholate were all from Oxoid limited, Basingstroke, Hampsire, England.

3.2. METHODS

3.2.1. Proximate analysis

3.2.1.1. Determination of Moisture Content

In determining the percentage Moisture content of the samples, the method by the Association of Analytical Communities (AOAC, 2005) was used. A metal dish was conditioned at 105 C for 30 minutes in ^{0} an oven and then cooled in a desiccator to room temperature. The weight of metal dish was taken to the nearest 0.01g. About 5g of the test sample was measured into the

metal dish and the total weight of dish and content taken. In the oven, the metal dish with its content was heated at 105 ^{0}C for about 5 hours until no further reduction in weight was observed. The metal dish with its content were covered and placed in a desiccator to cool for about 30-45 minutes after which it was removed and the final weight taken.

The Moisture content was expressed as a percentage by mass of the product received;

$$\frac{\text{Loss of weight} \times 100}{\text{Mass in grams of test sample taken}}$$

This implies that $\frac{M_2 - M_3 \times 100}{M_2 - M_1}$

Where;

M_1 = Initial weight of empty glass crucible.
M_2 = weight of empty glass crucible + wet sample. M_3 = weight of empty glass crucible + dry sample.

3.2.1.2. Determination of Total Ash Content of Whole Grains

In determining the percentage Total Ash content, AOAC 945.46 (2005) method was used. A crucible was conditioned in a furnace at 550 ^{0}C for 30 minutes and then cooled in a desiccator to room temperature and the weight of the crucible was taken to the nearest 0.01g. About three grams (3g) of the test sample was weighed into the crucible and heated for about 20 minutes over boiling water bath till they were visibly dry. The total weight of crucible with its content was taken in grams. In a furnace, the crucible was heated at 660 C for about two ^{0} hours and together with its content placed in a desiccator to cool for about 30-45 minutes. It was then removed and the final weight taken.

The Total Ash was expressed as a percentage (%) by mass of the product received;

$$\frac{\text{Weight of Ash} \times 100}{\text{Mass in grams of test sample taken;}}$$

This implies $\frac{M_3 - M_1 \times 100}{M_2 - M_1}$

Where;

M_1 = Initial weight of empty glass crucible.

M_2 = weight of empty glass crucible + wet sample.
M_3 = weight of empty glass crucible + ash

3.2.1.3. Determination of Crude Protein Content

In determining the percentage of protein content, AOAC 991.20 (2005) method was used. About 0.25g of the samples was placed in a Kjeldahl digestion flask also containing a Selenium based catalyst and 25ml of concentrated sulphuric acid (H_2SO_4) added in a fume chamber. The flask was swirled gently to effect proper mixing and heated in a digestion chamber until digestion was completed after 5 hours. The digest was allowed to cool and transferred into a 100ml volumetric flask and topped up to the mark using distilled water. About 10ml of the diluted digest was put in the steam distillation unit, which was previously flushed with distilled water. About 18ml of 40% Sodium Hydroxide (NaOH) was then added to the solution in the steam distiller after which about 25ml of 2% boric acid was pipetted into a conical flask and two drops of bromocresol green-methyl red mixed indicator added. This mixture was placed under the condenser outlet of the distillation system, with the tip of the condenser completely immersed in it. The distillation was carried out until the boric acid solution turned from pink to yellow-green. The solution in the conical flask was titrated against 0.1 Hydrochloric acid (HCL) solutions and the end point recorded. The distillation and titration processes were done with triplicate samples of the diluted digest. A blank was taken through the same procedure using distilled water in place of the sample. The crude protein content was then calculated using a factor of 6.25.

$$\text{Nitrogen \%} = \frac{(V_S - V_b)\ 1.407 \times 100 \times N_A \times 100}{M_s \times 10}$$

Where;

Protein = % Total Nitrogen × 6.25
V_s= Titre value of acid titration against digested sample solution
V_b = Titre value of acid titration against digested blank
N_A= Normality of acid (0.1N HCL)
M_s = Initial mass of sample= Density of sample × volume

6.25= General Protein conversion factor

3.2.1.4. Determination of Total Fat Content

In determining the Total Fat content, AOAC 989.05, (2005) method was used. About 100g of the sample was poured into a previously weighed glass Petri dish and dried over a water bath till most of the water had evaporated. The sample was then transferred to an oven and further dried at 105 ^{0}C till a constant weight is obtained. The weights of water lost and dried solids obtained were determined by subtracting and later used to calculate the total amount of fat on the wet weight basis.

Five grams (5g) of the dried sample was weighed into each two paper thimbles. The thimbles were sealed and placed in soxhlet extractors. About 150 ml of petroleum ether was poured into each of the two previously dried and weighed round bottom flask attached to the extractor. Extraction was carried out for 16 hours. After this period, the petroleum ether was recovered and placed in an oven (with the door partially closed) for the ether to completely evaporate. The flasks were cooled in a dessicator, weighed and the fat content calculated on the weight basis using the water content after drying the wet sample.

$$\text{Fat} = \frac{M_F - W_D}{M_S - W_T} \times 100$$

Where;

M_F = mass of the fat extracted
W_D = mass of total dried sample
M_S = mass of the dried sample taken for extraction
W_T = mass of wet sample originally taken and weighed

3.2.1.5. Carbohydrate Determination by Difference Method

The percentage of carbohydrate was determined using Harold *et al.,* (1981). Carbohydrate percentage was expressed in percentage (%) by mass of the product, as the amount obtained

after subtracting the moisture content, total ash, protein content and fat content of the test sample from 100.

That is, %Carbohydrate = 100-(Moisture content + Total Ash + Protein content + Fat content)

3.2.1.6. Determination of pH

The pH meter was calibrated with standard buffer solutions of 4, 7 and 10. About 30ml of the drink was aseptically poured into three different 50ml beakers and the pH determined using a digital pH meter (Mettler Toledo Seven Compact). The pH meter was dipped into the sample and recording taken after about four minutes of stability. The pH meter was recalibrated after readings were taken.

3.2.2. MICROBIOLOGICAL ANALYSIS

3.2.2.1. Enumeration of Yeasts and Moulds

About ten milliliters (10 ml) each of test sample was aseptically added to 90ml sterile Maximum Recovery Diluent in sterile stomacher bag (10^{-1} dilution). A 1ml inoculum from the 10^{-1} dilution was aseptically pipetted into 9ml Maximum Recovery Diluent in sterile McCartney bottle (10^{-2} dilution). This procedure was repeated until a dilution of 10^{6} was obtained. About 0.1 ml each of the dilutions was aseptically pipetted unto sterile Dichloran Rose-Bengal Choramphenical Agar plates and spread with a glass spreader. The Petri dishes were incubated at 25° C ±1 ^{0}C for 3-5 days.

Colonies on media were counted using the colony counter and recorded. Where there was no or scanty growth it was recorded as too few to count (TFTC) and where too numerous, it was recorded as too numerous to count (TNTC). For TNTC results, test samples were repeated using higher dilutions. The unit for the count was recorded as CFU/ml (colony forming units per milliliter). (ISO 21527-1, 2008)

3.2.2.2. Total Viable Counts

About ten milliliters (10ml) of test sample was aseptically added to ninety milliliters (90ml) of sterile Maximum Recovery Diluent in stomacher bag (10^{-1} dilution). One milliliter (1ml) of the inoculum from the first dilution was aseptically pipetted into 90ml Maximum Recovery Diluent in sterile McCartney bottle (10^{-2} dilution). About 1ml each of the dilutions was pipetted into sterile Petri dishes and molten plate count agar at 45 C poured into each Petri dish and then carefully swirled to mix inoculum and media before being allowed to set. Plates were then inverted and incubated 30 C for 72 ± 3 hours.

Colonies on media were counted using the colony counter and recorded after the incubation period. When there was no growth, it was recorded as too few to count (TFTC) and when too numerous it was recorded as too numerous to count (TNTC). For TNTC results, test samples were repeated using higher dilutions. The unit for the count was CFU/ml (colony forming units per milliliter) (ISO 4833-1, 2003).

3.2.2.3. Identification of Staphylococcus aureus

About ten milliliters (10ml) of sterilized distilled water was aseptically pipetted into one vial of rabbit plasma fibrinogen and shaken to dissolve until a clear solution was obtained. This solution was then poured aseptically into 90ml of sterilized Baird Parker Agar at 45°C±2°C. About ten milliliters (10ml) of test sample was aseptically added into ninety milliliters (90 ml) of sterile Maximum Recovery Diluent in a stomacher bag (10^{-1} dilution). One milliliter (1 ml) of inoculum from the first dilution was aseptically pipetted into a sterile Petri dish and molten sterilized Baird Parker Agar poured on it. Inoculum was carefully mixed with the media and allowed to solidify. Petri dish was inverted and incubated at 37°C for 48 hours. Black shinning and convex colonies with a clear surrounding or ring formed around it on the media indicated the presence of *Staphylococcus aureus.* Where there was no growth or few growth, it was

recorded as too few to count (TFTC) (< 10) and when too numerous it was recorded as too numerous to count (TNTC). The unit for the count was CFU/ml (colony forming units per milliliter). The presence of the rabbit plasma fibrinogen supplement added to the Baird parker agar caused coagulation when *S. aureus* was present (test). To confirm whether it was *S. aureus*, few drops of hydrogen peroxide was added to the organisms (growth) on the Baird parker agar. Formation of bubbles determined the presence of *S. aureus* (Catalase test) (ISO 6888-1, 1999[E]).

3.2.2.4. Identification of Salmonella typhi

About twenty five milliliters (25 ml) of test sample was aseptically pipetted into stomacher bags. About 225ml of already prepared sterilized buffered peptone water was aseptically added and incubated at 37°C for 24 hours after which selective enrichment followed. In the selective enrichment, 1ml of the inoculum was transferred into 10ml freshly prepared tetrathionate broth in McCartney bottle and 0.1ml transferred to 10ml Rappaport Vassiliadi Soya broth in McCartney bottle. The tetrathionate broth was incubated at 37 C for 24 hours and the Rappaport Vassiliadi soya was incubated at 41.5 oC in a water bath for 24hours. Samples from both tetrathionate and Rappaport Vassiliadi soya broth were streaked on bismith sulphate agar and xylose lysine desoxycholate plates and incubated at 37C for 24 hours. Controls were run alongside the test samples.

Comparison of streaked test sample was done with that of the streaked control organisms (stock cultures). For a positive result, grey colonies with metallic sheen are formed for typical *Salmonella typhi* on bismith sulphate agar. For a negative result, no growth is observed on the bismith sulphate agar. For a positive result on xylose lysine desoxycholate (XLD), pink colonies with black centres formed were regarded as positive for *Salmonella typhi*.

Positive colonies were picked into nutrient agar and incubated for 37 C for 24 hours.

Colonies formed were streaked on the surface of slant and also stabbed on triple sugar iron (TSI) agar in sterile Marcarthy bottles and incubated at 37 $_{o}$C for 18 to 24 hours. To confirm the presence of *Salmonella typhi*, colonies formed on the nutrient agar were streaked on urea agar in sterile Marcarthy bottle and incubated at 37C ^{0} for 18 to 24 hours. A yellow colourization or unchanged colourization of the medium confirmed the presence of *Salmonella typhi* (ISO 6579, 2002 [E]).

3.2.2.5. Test for Escherichia coli

About 10ml of test sample was aseptically weighed into 90ml sterile Maximum Recovery Diluent in stomacher bag (10^{-1} dilution). About 1ml of inoculum from the 10^{-1} dilution was aseptically pipetted into 9ml Maximum Recovery Diluent in sterile McCartney bottle (second dilution). A 1ml volume of the second dilution was pipetted into another 9ml maximum recovery diluent in sterile McCartney bottle (third dilution). About 1ml each of the three dilutions that is 10^{-1}, 10^{-2} and 10^{-3} were aseptically pipetted into three sets of labelled test tubes with inverted Durham tubes containing 10ml each of Lauryl tryptone broth for single strength tubes. For double strength tubes, 10ml of the first dilution was pipetted into three sets of double strength tubes. Test tubes were then incubated at 37°C for 48 hours. The presence of gas trapped in the Durham tubes and turbidity indicated positive tubes. These positive tubes were transferred aseptically into *E. coli* broth and incubated for 48 hours in a water bath. Positive tubes from the *E. coli* broth were transferred using an inoculating loop into tryptone water and incubated at 44C for 48 ^{o} hours in a water bath. Kovacs reagent was added to the tryptone after incubation. A red-ring colouration at the top was a positive indication of the presence for *E. coli* (ISO 7251, 2006 [E]).

3.3. STATISTCAL ANALYSIS

All analysis was done in triplicates so as to reduce the margin of error as much as possible. The results were analyzed using Microsoft Excel 2007 and the data was subjected to one way

analysis of variance (ANOVA) and the significance difference between the means of the four sampling areas for the parameter for the proximate composition, pH and temperature. Results were tabulated for easy interpretation.

CHAPTER FOUR

4.0. RESULTS

4.1. pHs OF THE SAMPLES ANALYZED

The pH of the ―Burkina‖ drink ranged from 3.80 ± 0.05 to 4.82 ± 0.02. In decreasing order of magnitude, the highest pH value was obtained in samples from the vending areas of the 37 Military hospital (4.82 ± 0.02), Nima (3.84 ± 0.05), Maamobi (3.83 ± 0.05) and the Accra Mall (3.79 ± 0.01) vending areas (Table 1). From table 1, it appears differences exist between the pH values of the ―Burkina‖ drink among the four locations.

Table 1: pHs of "Burkina" Drink from the Four Sampling Areas

VENDING AREA	A	B	C	Avg ± SD
Nima	3.90±0.06	3.83±0.06	3.80±0.01	3.84±0.05
Maamobi	3.89±0.00	3.80±0.01	3.80±0.00	3.83±0.05
37 Military Hospital	4.83±0.03	4.82±0.01	4.80±0.01	4.82±0.02
Accra Mall	3.80±0.12	3.78±0.01	3.80±0.02	3.79±0.01

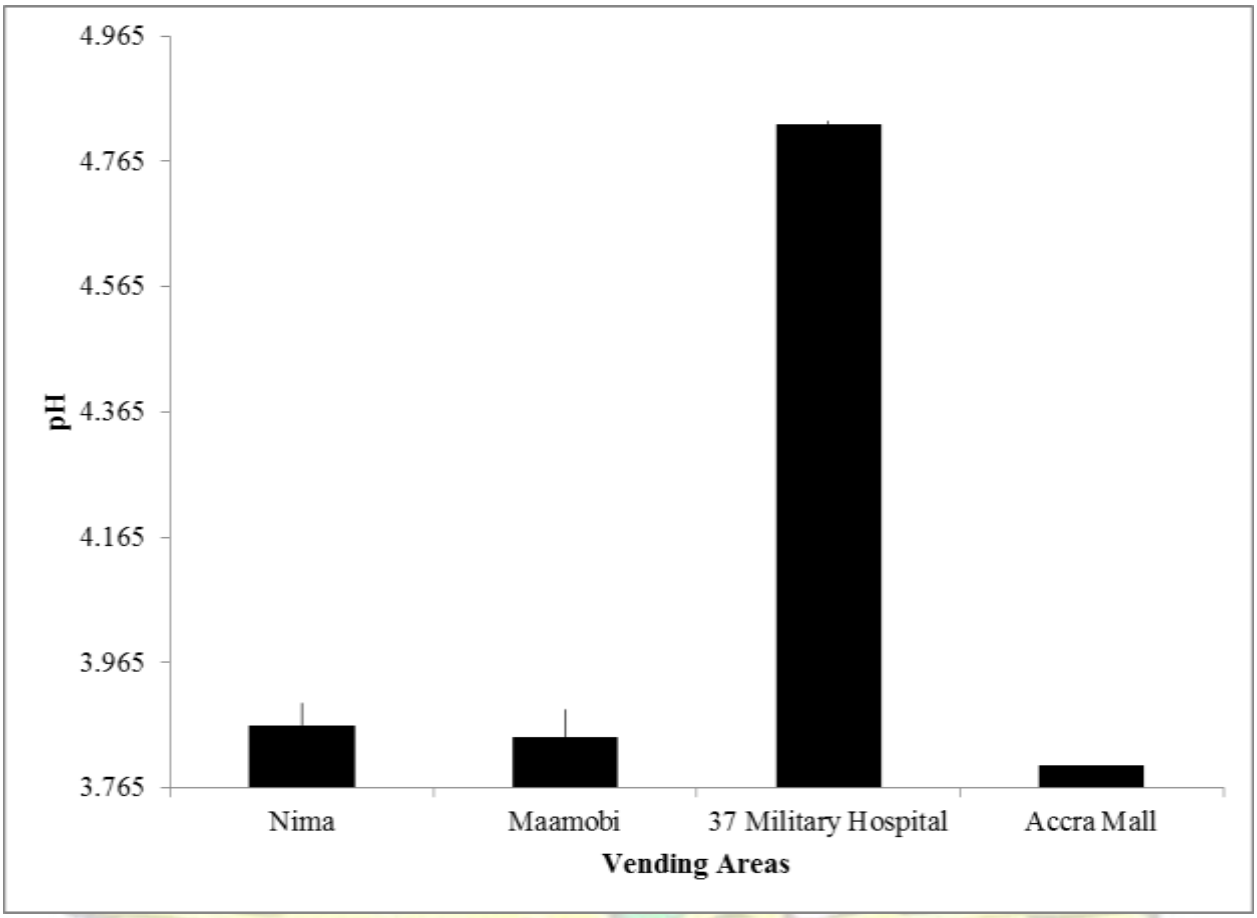


Figure 3: pHs of "Burkina" drink in the four sampling areas

A One Way Analysis of Variance table (ANOVA) test was conducted to compare the variance of the four locations to determine whether we can infer that the pH values of the ―Burkina‖ drinks differ and that at least two of the pH means differ. A multiple comparison test was conducted to identify the locations with differences in pH for ―Burkina‖ drink.

The null hypothesis H_o: pH means of the ―Burkina‖ drink is the same across the four locations

Alternative hypothesis H_1: At least two of the pH means of the ―Burkina‖ drink differ across the four locations.

From the table ANOVA table (8), the test statistic F = 521.425 and the critical value (F crit) =

4.066, which is less than the test statistic. It therefore appears that the data might be calling for the rejection of the null hypothesis. The p-value is 0.00, which falls into the rejection region provides overwhelming evidence calling for the rejection of the null hypothesis. At 95% confidence level, it can be said that the pH value of the ―Burkina‖ drink differs among all four areas where the survey was conducted. Having concluded that at least two of the pH means differ for the ―Burkina‖ drink, we need to know which of vending locations are responsible for these differences. A simple way of determining whether differences exist between each pair of the pH means is to compare the absolute value of the difference between their two sample means and the Post Hoc Tests, that is, (LSD, HSD and Scheffe). If the mean difference is greater than (LSD, HSD or Scheffe), the difference is considered significant. The differences that are significant are shown in red as seen in the post Hoc (Table 9).

From the post Hoc (Table 9), the difference between Nima, Maamobi and 37 Military Hospital were greater than Sheefe‘s 0.088 hence the difference is significant. Hence at 95% confidence interval that the pH in the ―Burkina‖ drink among vendors at 37 Military Hospital is significantly higher than vendors in Nima and Maamobi.

4.2. PROXIMATE CONTENT OF THE SAMPLES

Moisture content ranged from 80.76±0.51% to 82.40±0.84%. In an increasing order of magnitude; moisture content in samples from the Maamobi vending area was 80.76 ± 0.51%, 37 Military Hospital vending area was 81.31 ± 1.20%, Accra Mall vending area was 82.19 ± 0.76% with Nima vending area having 82.40 ±0.84% (Table 2). It therefore appears from the table that a difference does not exist in moisture content of the ―Burkina‖ drink among the various vending locations. From the ANOVA table (10), since the test statistic F = 2.790 is less than the critical value (F crit) of 4.066, this suggests that the null hypothesis (The mean of the moisture content of the ―Burkina‖ drink is the same across the four vending areas) is true and so needs not to be rejected.

The p-value 0.109 lends credence to the earlier assertion. Hence there is not enough evidence to infer that differences exist in the moisture content of the ―Burkina‖ drink among vending locations.

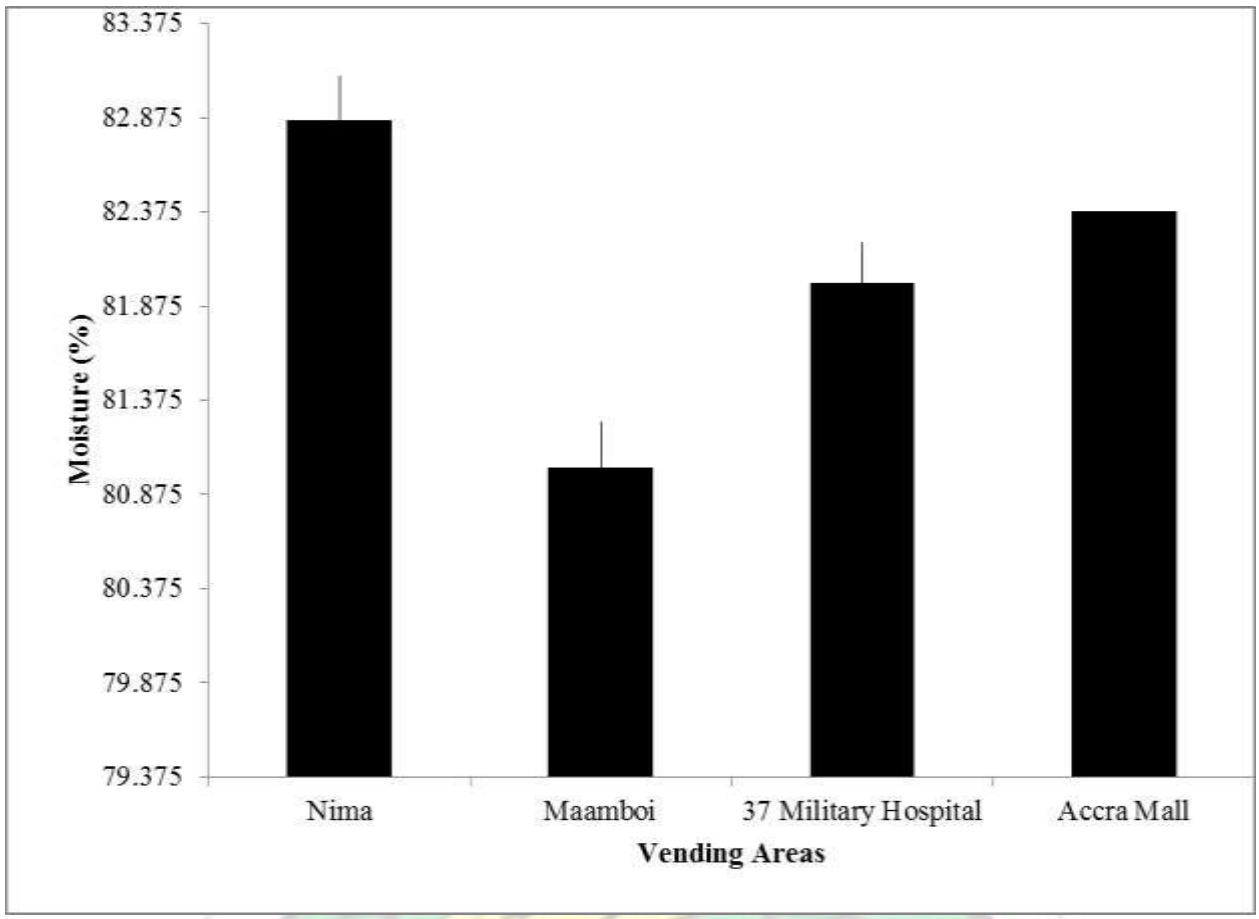


Figure 4: Moisture content of "Burkina" drink in the four sampling areas

In the case of the carbohydrate content (in a decreasing order of magnitude), samples from the Accra Mall vending area, had the highest carbohydrate content of 12.73 ± 0.20%, followed by samples from the Maamobi vending area with 12.45 ± 1.27%, Nima vending area had 10.80 ± 1.22%, whilst samples from the 37 Military Hospital vending area had the lowest value of 10.20±1.21% (Table 2). To determine whether significant difference exist in the carbohydrate content of ―Burkina‖ drink, a One Way Analysis of Variance (ANOVA) was used with the following hypothesis;

The null hypothesis H_0: The mean of carbohydrate content in the ―Burkina‖ drink is the same across the four vending areas

Alternative hypothesis H_1: At least two of the carbohydrate means differ. The test statistic is F = 4.304 and the critical value (F crit) = 4.066 which is less than the test statistic (Table 11). The data might be calling for the rejection of the null hypothesis. The p-value is 0.04, which slightly falls in the rejection region and so the test is quite significant and supports the rejection of the null hypothesis. At 95% confidence level, it can be said that the carbohydrate content of the ―Burkina‖ drink differ at least at two of the vending areas. In order to determine the vending areas where the carbohydrate content in the ―Burkina‖ drink differ, the Post Hoc Test above was conducted. If the mean difference is greater than (LSD or Scheffe's), the difference is considered significant. The colored cells seen in (Table 12) are significant since their mean is greater than (LSD and Scheffe). In conclusion at 95% confidence interval that the carbohydrate content in the ―Burkina‖ drink among vendors at Accra Mall is significantly higher than those at 37 Military Hospital and Nima. There is also enough evidence to conclude that the carbohydrate content in the ―Burkina‖ drink sold at Maamobi is more than the ones sold at 37 Military Hospital.

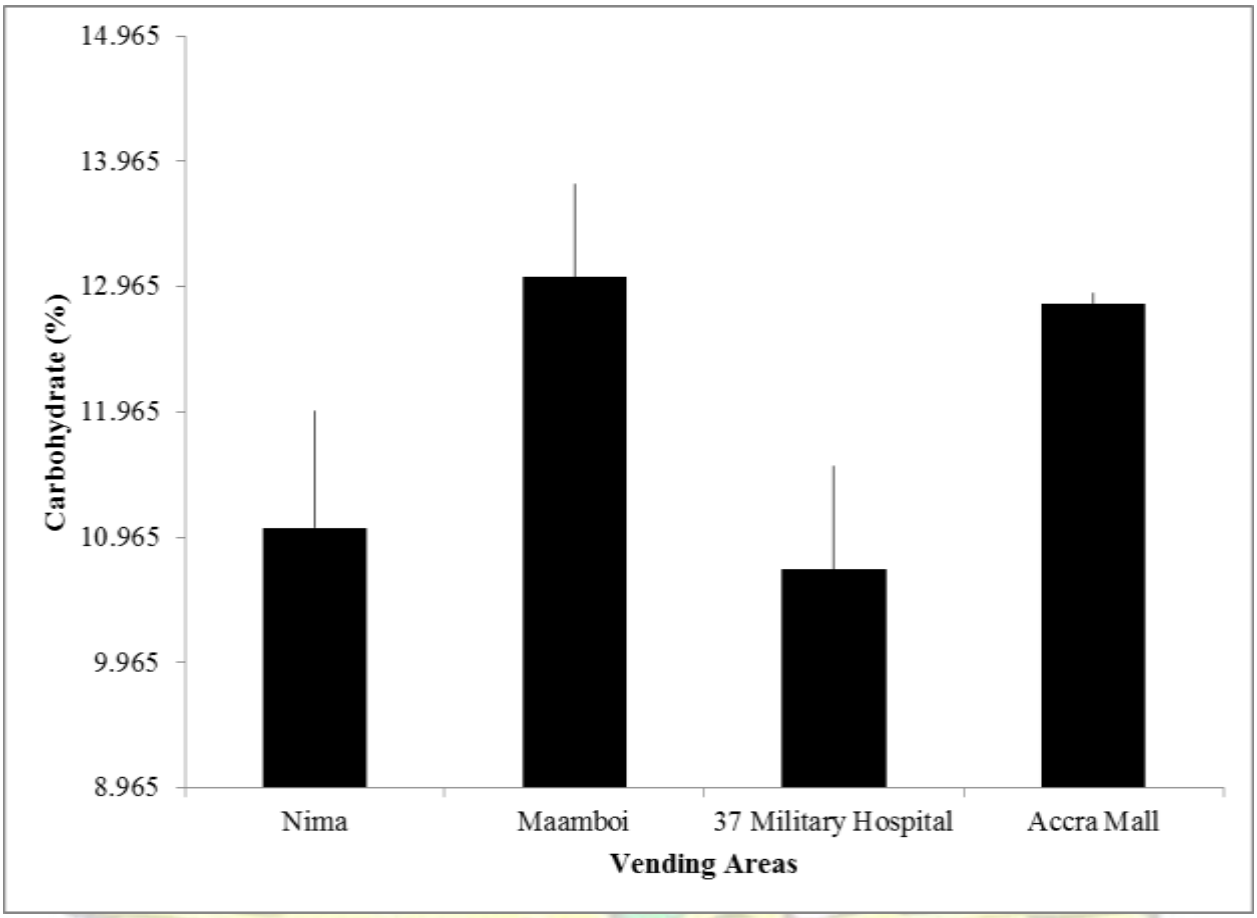


Figure 5: Carbohydrate content of "Burkina" drink in the four sampling areas.

Protein content of the drinks did differ significantly where the p- value was 0.02 (Table 13) from the various sampling areas, and ranged from 3.25±0.01% in samples from the Accra vending area to 4.83±0.53% from the 37 Military Hospital vending area. The highest value was obtained in samples from 37 Military Hospital vending area (4.83±0.53%), followed by samples from the Maamobi vending area (3.66±0.64%), 3.43±0.63% in samples from the Nima vending area, with the lowest obtained in samples from the Accra Mall vending area (3.25±0.01%) (Table 2). Using a One Way Analysis of Variance (ANOVA), the following hypothesis was used;

The null hypothesis H_0: The mean of protein content in the ―Burkina‖ drink is the same across the four vending areas

Alternative hypothesis H_1: At least two of the protein means differ.

From Table 13, the test statistic was F = 5.468 and the critical value was F critical = 4.066 which is less than the test statistic and so the test might be calling for the rejection of the hypothesis. The p-value of 0.024 indicates a strong evidence to infer that the alternative hypothesis is true. We, therefore, it can be concluded that differences exist in the protein content of ―Burkina‖ drink across the vending areas. From Table 14, it can be concluded at 95% confidence interval that the protein content of ―Burkina‖ drink sold at 37 Military Hospital is significantly more than the ones sold at Nima and Maamobi.

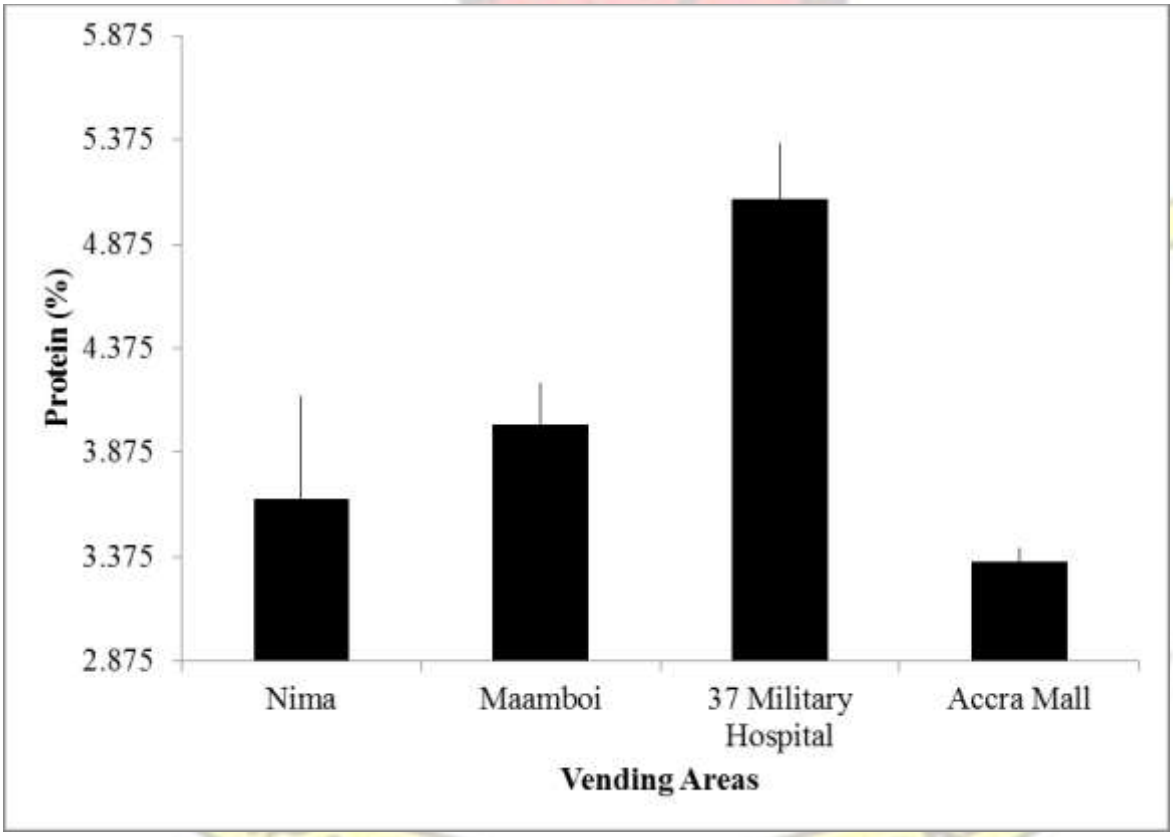


Figure 6: Protein content of "Burkina" drink in the four sampling areas

The total fat content of the ―Burkina‖ drink from the various vendors increased from 1.41±0.39% to 3.24±0.40% as shown in Table 2. The highest total fat content was obtained in samples from the 37 Military Hospital vending area (3.24±0.40%). This was followed by

2.95±0.26% in samples from the Nima vending area, (2.25±0.53%) in samples from Maamobi and 1.41±0.39% in samples from the Accra Mall vending area. To determine whether significant difference exist in the fat content of Burkina drink among vending areas, a One Way Analysis of Variance (ANOVA) is used with the following hypothesis;

The null hypothesis H_o: The mean of total fat content in the ―Burkina‖ drink is the same across the four vending areas

Alternative hypothesis H_1: At least two of the total fat means differ

From the (Table 15) above, the test statistic F = 12.988 and the critical value (F critical) = 4.066 which is less than the test statistic. This implies that the null hypothesis stands the chance of being rejected. The p-value is 0.002 also less than the significant value 0.05, this indicates that there is an overwhelming evidence to reject the null hypothesis and conclude that differences exist among the vending areas in terms of total fat content of ―Burkina‖ drink. From the (Table 16) above, we can conclude at 95% confidence interval that total fat content of ―Burkina‖ drink sold at 37 Military Hospital is significantly more than those sold at Accra Mall and Maamobi. There is also enough evidence to infer that the total fat content in the ―Burkina‖ drink sold at Nima is more than the ones sold at Accra Mall. Finally, the fat content in the ―Burkina‖ drink sold at Maamobi is more than that of Accra Mall.

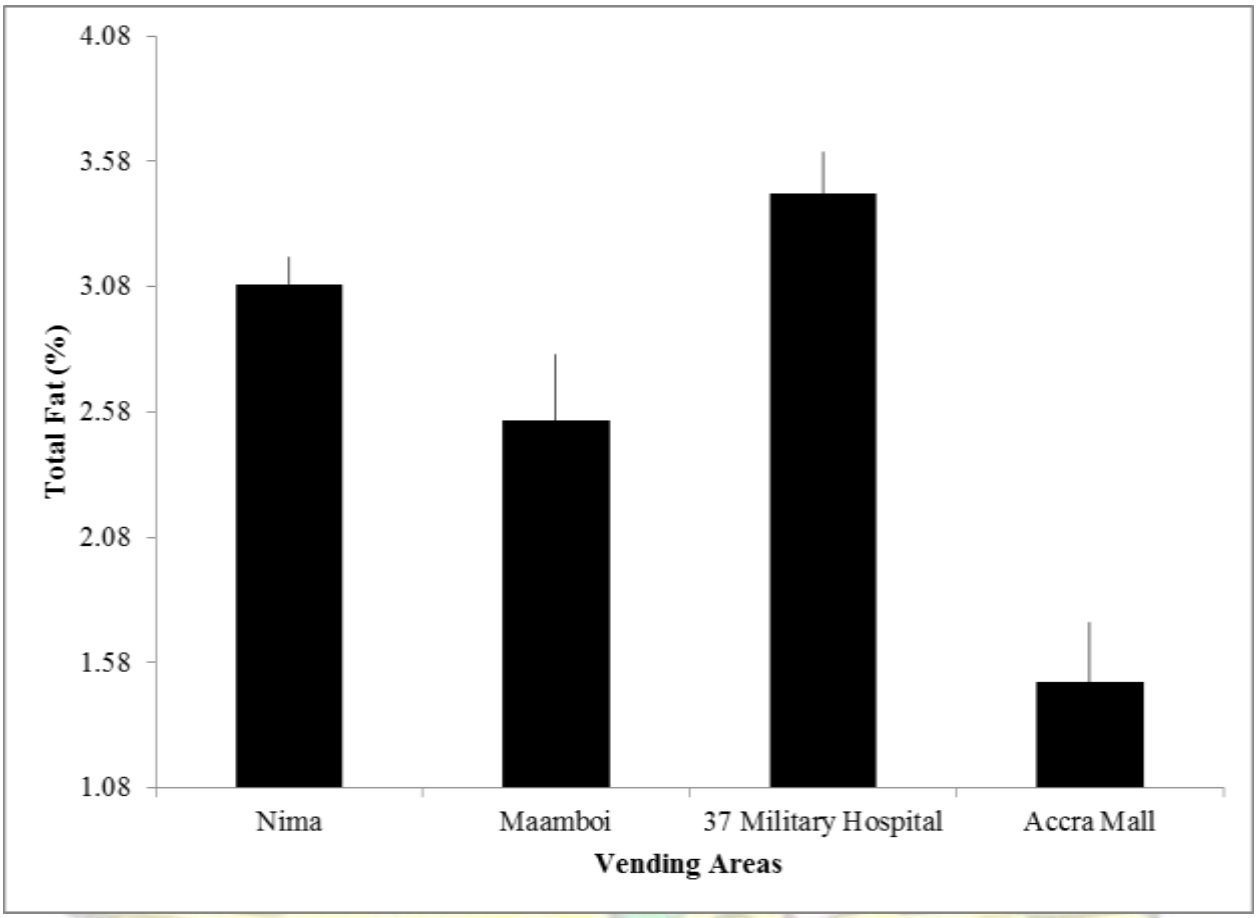


Figure 7: Fat content of "Burkina" drink in the four sampling areas

From Table 2, Maamobi vending area had the highest total Ash content of 0.47±0.04% with samples from the 37 Military Hospital vending area having the lowest value of 0.41±0.02%. In increasing order of magnitude, the total Ash content were as follows; 37 Military Hospital vending area had 0.41±0.02%, Accra Mall vending area had 0.42±0.00%, Nima vending area had 0.42±0.05%, whilst the Maamobi vending area had 0.47±0.04%. A One Way Analysis of Variance (ANOVA) was used to determine the significant difference with the following hypothesis;

The null hypothesis H_o: The mean of total Ash content in the ―Burkina‖ drink is the same across the four vending areas.

Alternative hypothesis H_1: At least two of the total Ash means differs.

From Table17, the test statistic F = 10.047 and the critical value (F crit) = 4.066 is less than the test statistic and so the test might call for the rejection of the null hypothesis.

The p-value is 0.004, which falls into the rejection region and so is quite significant and supports the rejection of the null hypothesis. 95% of the time, there appears to be enough evidence to infer that differences exist in the total ash content of the ―Burkina‖ drink across vending areas.

From (Table 18), at 95% confidence interval we can conclude that the total ash content in the ―Burkina‖ drink at Maamobi is significantly higher compared to those in Nima and 37 Military Hospital. Hence it can be inferred that from the (Table 18) that the ash content in the ―Burkina‖ drink at Maamobi is significantly more than at Accra Mall.

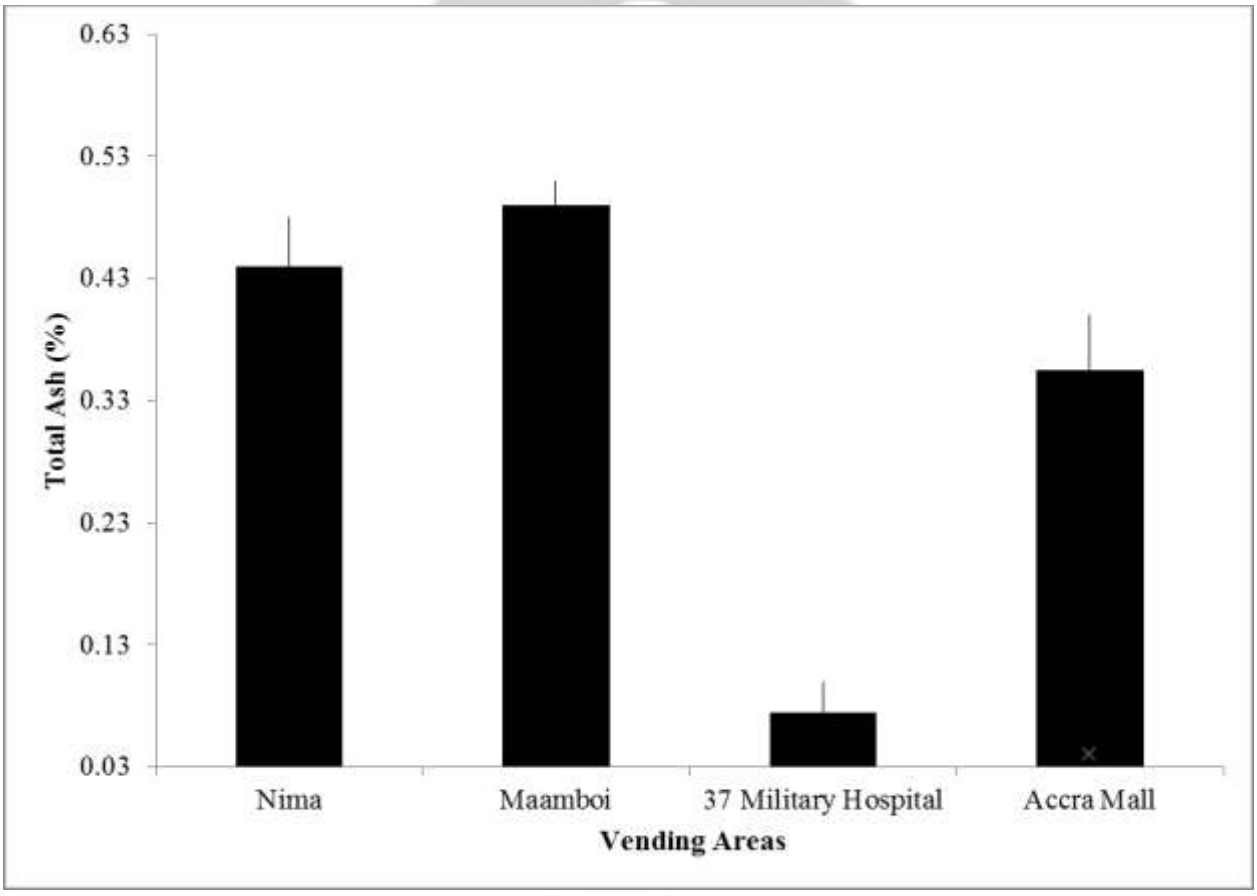


Figure 8: Total Ash content of "Burkina" drink in the four sampling areas

Table 2: Percentage proximate composition of ―Burkina‖ drink from the four sampling areas.

VENDING AREAS	VENDOR	MOISTURE	CARBOHYD-RATE	PROTEIN	TOTAL FAT	TOTAL ASH
Nima	A	81.47±0.25	11.97±0.35	2.99±0.04	3.2±0.07	0.40±0.01
	B	83.10±0.07	9.68±0.10	4.15±0.04	2.68±0.08	0.38±0.01
	C	82.62±0.08	10.11±0.11	3.16±0.00	2.98±0.04	0.48±0.00
	Average	**82.40 ±0.84**	**10.80±1.22**	**3.43±0.63**	**2.95±0.26**	**0.42±0.05**
Maamobi	A	81.26±0.12	11.26±0.02	4.21±0.08	2.81±0.09	0.43±0.02
	B	80.25±0.15	13.78±0.15	3.807±0.07	1.70±0.76	0.47±0.00
	C	80.78±0.29	12.30±0.29	2.96±0.04	2.28±0.09	0.51±0.01
	Average	**80.76±0.51**	**12.45± 1.27**	**3.66±0.64**	**2.25±0.53**	**0.47±0.04**
37 Military Hospital	A	79.95±0.21	11.53±0.20	4.82±0.04	3.28±0.05	0.43±0.01
	B	81.78±0.33	9.89±0.35	4.31±0.06	3.62±0.04	0.40±0.00
	C	82.21±0.09	9.18±0.23	5.36±0.07	2.83±0.10	0.40±0.00
	Average	**81.31± 1.20**	**10.20± 1.21**	**4.83±0.53**	**3.24±0.40**	**0.41±0.02**
Accra Mall	A	82.38±0.24	12.52±1.34	3.42±0.09	1.26±0.40	0.42±0.00
	B	81.81±1.05	12.75±1.198	3.28±0.05	1.74±0.04	0.42±0.00
	C	82.38±0.10	12.91±0.56	3.05±0.09	1.23±0.31	0.43±0.00
	Average	**82.19±0.76**	**12.73± 0.20**	**3.25±0.01**	**1.41±0.39**	**0.42±0.00**
% MEAN		**81.67± 0.77**	**11.55±1.24**	**3.79±-0.71**	**2.46± 0.82**	**0.43±0.03**

4.3. MICROBIOLOGICAL ANALYSIS

From Table 3, it is observed that, the total viable counts increased from 6.86±0.13 log cfu/ml in samples from the 37 Military Hospital vending area to 7.94± 0.21log cfu/ml in samples from the Nima vending area. There was no significant difference (p= 0.07) among the total viable counts of the drink from the four sampling areas (Table 19). The average log count of samples from the four sampling areas in decreasing order of magnitude were as follows; 7.94± 0.21 log cfu/ml in samples from the Nima vending area, 7.78± 0.10 log cfu/ml in sample from the Maamobi vending area, 7.38± 0.21log cfu/ml in samples from the Accra Mall vending area and 6.86± 0.13log cfu/ml in samples from the 37 Military Hospital vending area.

The loads obtained for *S. aureus* are indicated in Table 4. They ranged from 3.78±0.84 log cfu/ml in samples from the 37 Military Hospital vending area, 3.81±0.02 log cfu/ml in samples from the Accra Mall vending area, 4.11±1.31log cfu/ml in samples from the Nima vending area to 4.68±0.08 log cfu/ml in samples from the Maamobi vending area. There was no significant

difference (p = 0.74) among the loads of *S. aureus* in the drink from the four sampling areas (Table 20).

Table 3: Loads of Total Viable Bacteria in Samples from the four sampling areas.

VENDING AREA	A (cfu/ml)	B (cfu/ml)	C (cfu/ml)	Average (log cfu/ml)	Average (logcfu/ml±SD)
Nima	1.47×10^{8} 8.17	7.3×10^{7} 7.86	6.0×10^{7} 7.78	7.94	7.94± 0.21
Maamobi	6.6×10^{7} 7.8	7.2×10^{7} 7.86	4.7×10^{7} 7.67	7.78	7.78± 0.10
37 Military Hospital	8.0×10^{8} 6.90	5.1×10^{6} 6.71	9.3×10^{6} 6.97	6.86	6.86± 0.13
Accra Mall	1.8×10^{7} 7.26	3.9×10^{6} 6.59	2.0×10^{8} 8.30	7.38	7.38± 0.21

Table 4: Loads of *Staphylococcus aureus* in samples from the four sampling areas

VENDING AREA	A (cfu/ml)	B (cfu/ml)	C (cfu/ml)	Average (log cfu/ml)	Average (log cfu/ml ±SD)
Nima	**2.5×10^{4} 4.40**	**1.1×10^{3} 3.04**	**7.8×10^{4} 4.89**	**4.11**	**4.11 ±1.31**
Maamobi	**3.3×10^{4} 4.52**	**3.9×10^{4} 4.59**	**4.8×10^{4} 3.81**	**4.60**	**4.60 ±0.08**
37 Military Hospital	**5.3×10^{4} 4.72**	**1.3×10^{3} 3.11**	**3.2×10^{3} 3.51**	**3.78**	**3.78 ±0.84**
Accra Mall	**6.8×10^{3} 3.83**	**6.2×10^{3} 3.79**	**6.4×10^{3} 3.81**	**3.81**	**3.81 ±0.02**

From Table 5, yeast and mould counts increased from 2.65± 0.12 log cfu/ml in samples from the Maamobi vending area, 3.37 ±0.65log cfu/ml in samples from the 37 Military Hospital vending area, 3.68 ±0.06log cfu/ml in samples from the Accra Mall vending area to 4.31 ± 0.57 log cfu/ml in samples from the Nima vending area. There were significant differences (p= 0.045) among the yeast and mould counts in the four sampling areas. Differences were between

Nima, Maamobi and 37 Military Hospital as shown in the post Hoc (Table 22) for yeasts and moulds.

Table 5: Loads of Yeasts and Moulds in Samples from the four Sampling areas

VENDING AREA	A (cfu/ml)	B (cfu/ml)	C (cfu/ml)	Average log cfu/ml)	Average (log cfu/ml ±SD)
Nima	9.0×10^4 4.95	1.3×10^4 4.11	7.2×10^3 3.86	4.31	4.31 ±0.57
Maamobi	4.5×10^3 2.65	8.5×10^3 3.93	6.2×10^2 2.79	2.65	2.65 ±0.12
37 Military Hospital	3.8×10^4 2.58	3.7×10^2 2.57	3.3×10^3 3.52	3.37	3.37 ±0.65
Accra Mall	4.2×10^4 3.62	4.8×10^3 3.68	5.4×10^3 3.73	3.68	3.68 ±0.06

Loads of *E. coli* in samples from the 37 Military Hospital vending area ranged from 21 to 110 MPN/ml, 21 to >110 MPN/ml in samples from the Nima vending area, 46 to>110 MPN/ml in samples from the Maamobi vending area 12 to >110MPN/ml in samples from the Accra Mall vending area (Table 6).

Table 6: Loads of *E. coli* in Samples from the four sampling areas

VENDING AREA	A(MPN/ml)	B(MPN/ml)	C(MPN/ml)
Nima	>110	>110	21
Maamobi	>110	46	>110
37 Military Hospital	110	21	46
Accra Mall	12	>110	15

Salmonella typhi was not detected in any of the samples from the four sampling areas

CHAPTER FIVE

5.0. DISCUSSION

The pHs of the ―Burkinaǁ drinks were all within the acidic range. There were significant differences (p=0.00) among the pH values from the four sampling areas. The findings of this study compare with results obtained by El-Bakri and El-Zubeir, (2009) for plain yoghurt. It also

compares with the range obtained by Okonkow (2011) for a traditional fermented product ―Nono‖ in Nigeria. When compared, however, with report from Adesokan *et al.,* (2011), the values from this study were lower than the pH values for their plain yoghurt which ranged from 5.51 to 6.29. According to Marriot and Gravani, (2006), acidophilic (acidloving) bacteria thrive on food or debris with a pH of close to 5.2 whilst yeasts thrive both in acid environment and intermediate acids (4 .0 - 4.5) environments with mould tolerating a wider pH range (2.0–8.0). The low pH of fermented foods makes generally them safe against foodborne diseases (Gadaga *et al.,* 2004). El -Bakri and El-Zubeir, (2009) reported that such results are expected by virtue of the effect of inappropriate storage conditions emanating mostly from high temperatures encountered in tropical areas that may ferment milk products such as yoghurt. The pH value differ significant might be as a result of the fermentation processes.

Moisture content ranged from 80.76±0.51% to 82.40±0.84% and increasing order from the Maamobi vending area, the 37 Military Hospital vending area, the Accra Mall vending area to the Nima vending area (Table 2). The results showed no significant differences (p= 0.11) among the moisture content of ―Burkina‖ drinks from the four sampling areas. Milk is a main supply of protein, minerals, vitamins and fats in human foods which approximately comprises of 87% of water, 3.7% of protein, 4.9% of lactose, 0.7% of ash and 3.6% of fat (Ramesh *et al.,* 2008). Comparing the results from this study to the findings of Uaboi-Egbenni *et al.,* (2010), the moisture content for a fermented milk product like cheese was 64% whilst the value of the moisture content of the ―Burkina‖ drink in this study was higher with an average mean percentage of 81.67± 0.77%. Water activity is a critical parameter in finding the shelf- life and textual properties of a product (Aqua Lab, 2015). Microorganisms need water in any small form to thrive in food products and therefore the regulating of the moisture content in foods helps in its preservation. The required amount of water in food or environment for microbial activity is termed as the water activity (Aw) of (FDA, 2012). It can also be defined as the ratio of the

pressure of water vapour of the food substrate to the pressure of vapour of pure water at the same temperature (Jay, 2000). According to FDA, (2012), microorganisms act in response to A_W in diverse ways depending on a number of factors. Alterations in water activity affect microbial growth and the production of microbial metabolites. In addition, they stated that microorganisms normally have maximum and minimum levels of A_W for growth in accordance with other growth factors within their environments. According to Edema *et al.,* (2001), water is a critical factor influencing the thriving of microorganisms in foods and other microbial environments, nevertheless, many bacterial pathogens are regulated at water activities well above 0.86. However, *S. aureus* can thrive and produce toxin below A_W 0.90. In the case of the carbohydrate content, Accra Mall vending area had the highest carbohydrate content (12.73±0.20%) with 37 Military Hospital vending area having the lowest value (10.20±1.21%). Significant difference (P = 0.04) existed among samples from the four sampling areas and this might be probable from the quantities of the various ingredients used in its preparation. The carbohydrate content of the drink from different manufacturers could be attributed to the composition of the ingredients used (milk, millet and sugar). Significantly, carbohydrates provide energy for the body. Carbohydrate accounted averagely for 11.25±1.24% of the whole product analyzed, which is the second highest value to percentage mean moisture of 81.67±0.77% (Table 2). Carbohydrates such as Millet, fermented milk and sugar are the major components of the ―Burkina‖ drink. Lorenzen *et al.,* (2002), stated that the main source of carbohydrate in milk and milk products is lactose. The high carbohydrate of yoghurt increases its total solids which consequently improves texture and viscosity while decreasing syneresis.

Protein content of the drink did differ significantly (p= 0.02) from the various sampling areas, and ranged from 3.25±0.01% (Accra Mall) to 4.83±0.53% (37 Military Hospital). All the four sampling areas had protein contents higher than the minimum acceptable limit of 2.7% for yoghurt according to the Codex Standards (Codex Standards 243, 2003). The results also

compares with the findings of Miller, *et al.,* (2007), high quality protein is obtained from milk. Cow's milk, for instance, consist of about 3.5% protein by weight, and of this overall protein, casein accounts for 80% and 20% whey. The presence of millet is also accounting for the high value because according to Devi *et al.,* (2014) millets are comparatively unique to other cereals due to its rich source of protein, calcium, polyphenols and dietary fiber. According to Taylor *et al.,* (2010) a whole grain of pearl millet has 14.8% of protein whilst according to Issoufou *et al.,* (2013), distinctively millet protein contains rich quality essential amino acids mostly the sulphur containing amino acids (methionine and cysteine). However, milling millet during processing removes fibre and phytochemicals that are found in high quantities in the bran and germ layers thus causing significant loss.

The total Fat content of the ―Burkina‖ drink from the various vendors increased from 1.41±0.39% to 3.24±0.40% resulting from the fermented milk and millet in the product. Statistically there was significant difference (p= 0.002) among the total fat content of all the four sampling areas. The fat content of the samples may be as a result of the quantity and quality of ingredients (yoghurt and millet) used. Fat is known to promote good mouth feel (Onweluzo and Nwakalor, (2009). Significantly, it is used by cells of organs and glands to provide energy and in the synthesis of some of their secretions. The total fat content of the products analyzed were comparable to the value of 3.18±0.01% for plain yoghurt and 2.0 ±0.62 % for fruit yoghurt reported by Younus *et al.,* (2002). The results of this study is also comparable to fat content of low fat yoghurt(1%) and fat free yoghurt (0.2%) and drinking yoghurt. (The Dairy Council, 2015).

From Table 2, it is observed that, Maamobi vending area had the highest total Ash content (0.47±0.04%) with the 37 Military Hospital vending area having the lowest (0.41±0.02%). Significant difference (p= 0.004) existed among the drinks. The results comparable to the total

Ash content in plain yoghurt samples were lower than that reported by El Zubeir *et al.*,(2005), who reported that ash content of yoghurt had an average of 0.81 ± 0.29% and while Younus *et al.*, (2002), reported 0.66±0.09% by for fruit yoghurt.

The total count of bacteria in milk has a decisive effect on the quality and safety of dairy products (Szteyn *et al.*, 2005). Total Viable Count (TVC) is a key factor in determining the conditions of the raw material, efficiency of procedures (such as heat treatment) and hygienic surroundings during processing, uncontaminated conditions of equipment and utensils, and time and temperature duration during storage and distribution. From Table 3, the total viable counts increased from 6.86 ±0.13 log cfu/ml (37 Military Hospital vending area) to 7.94±0.21 log cfu/ml (Nima vending area). There were no significant differences among the total viable counts of the drink (p= 0.07). The least mean of 6.86±0.02 $\log_{10}$ cfu/ml of bacteria counts in this study were not comparable to the least count of 3.19±0.02 $\log_{10}$ cfu/ml for beverages reported by Gitahi (2012). The results may be due to the beverages being subjected to heat treatment as seen in the preparation of ―Burkina‖ drink. According to Kenya Bureau of Standards (KBS) (2003) and Gilbert *et al.*, (2000) all cooked food should not have more than 6.00 $\log_{10}$ cfu/g of viable total counts. The manufacturers and the vendors recruited for this study were observed to practice minimal personal hygiene. They did not put on aprons or caps and handled the drink with bare hands. Abdalla *et al.*, (2009), noted that during food processing, foodborne microbes can be brought in from contaminated personals who handle the food, or by cross contamination from some raw materials and/or the working environment. Their study revealed that the hands are the main source of transfer for microorganisms from the faces, face, skin or other sites to vendors. In addition, vendors should not handle money because, according to Haileselassie *et al.*, (2012), dirty money can contaminate safe food. The large surface area of paper currency serves as a breeding site for pathogens (Podhajny, 2004). Pope *et al*., (2002), revealed in his study carried out in Western Ohio that bacteria were able to survive on currency

notes. Observations made at the site of the manufacture was that, some of the vendors especially the ones who mixed the ingredients on site, handled the money and at the same time handled the food with their bare hands. According to Micheal (2002), money on which pathogenic microorganisms might survive, represents an often overlooked reservoir for enteric disease. In most parts of the developed world, there is evidence that simultaneous handling of food and money contributes to the incidence of food-related public health incidents (FSA, 2000). Most of the manufacturers were not producing the drink in exclusive area and therefore exposing the drink to all forms of contamination. In addition, the use of previously fermented starter culture of the yoghurt could also be a possible source of high count of Total Viable Bacteria in the samples. Proper food hygiene should be ensured by handling, storing, preparing and serving in such a way and under such conditions so as to prevent, as best as possible, the contamination of the food. In addition, food should be prepared properly on a clean and sanitized place to prevent foodborne diseases (Kidiku, 2001). Understanding the key role temperature plays in keeping food safe is vital. When the temperature at which food has been processed is known, then the question, ―Is it safe? Can be answered (USDA, 2011). Observation at the site of preparation showed that, most of the street vendors of the product did not refrigerate or chill the drink from their source of supply. They commute long distances from their suppliers or their home to the street with the products in bulk packed in polypropylene sacks or Styrofoam containers. It is at their vending sites that these drinks are packed into plastic selling containers and chilled with ice. Some of the vendors mix the ingredients on site depending on the amount purchased by the consumer. For such vendors, virtually all the ingredients were not covered, contributing to the high counts of total viable bacteria observed. Most Federal agencies recommend that foods derived from animal products should not be held unrefrigerated for more than two (2) hours (Anonymous, http:// probioticyoghurtinfo. blogspot.com/2011/01/ideal-storage-temperature-for-probiotic.html, accessed on 2nd February, 2015). According to WFLO

(2008), stated that it is safe to assume that storage conditions for yoghurt-based products are equivalent to the products they resemble. Thus, for example, yoghurt and liquid yoghurt should be handled and stored in a manner similar to milk. According to FDA (2012), the minimum temperature for the growth of *E. coli, S. aureus* (growth), *S. aureus* (toxins) and *Salmonella* are 7°C, 7°C,10°C and 5°C, respectively.

In a similar work carried out on ―kunun-zaki‖, an African fermented cereal -based food drink, refrigerated samples stored longer than at room temperature, which had high counts of bacteria (Olasupo *et al.*, 2000). Dairy Authority of South Australia (DASA) (2011), reported that storing yoghurt at low temperature slows the growth of bacteria, even those that could cause illness. They also stated that, if there is a problem with the acidity, storing at the appropriate temperature helps to keep the yoghurt fresh during its storage life and protect it against the growth of acid-tolerant micro-organisms. Acid-tolerant microbes hardly cause illness, but can cause the yoghurt to give off-flavour or go mouldy and spoil. The best temperature to prevent the development of harmful bacteria, is that, the food or beverages should be stored at temperature below 4°C. (Anonymous, probioticyohgurtinfo. BlogSpot.com).

As stated earlier, no standard exist for the product, however, Ghana Standard (GS734:2003) for millet based product was used as a guide. The Maximum acceptable limit for TVC should be 1.0×10^{6}cfu/ml. However; all the samples exceeded this acceptable maximum limit. The result justifies the assertion that, milk is a medium for the transmission of lots of bacteria of both human and animal source (Angulo *et al.,* 2009). Most of the time, pathogens that have been noted for foodborne outbreaks when milk is consumed include *Salmonella, Listeria monocytogenes, Campylobacter, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum* and *Escherichia coli*. Aerial contamination from dust, cross contamination from personnel and inadequately cleaned containers are likely sources of contamination into Ready-to-eat (RTE) foods and drinks (Food Standards Agency, 2011).

In all the samples, bacterial load was very high than permitted by standard. The presence of these high counts may be related to environmental conditions under which the products were prepared. It was observed that, most of these products were prepared in unkempt environmental conditions and the food left exposed when packaging into the plastic bottles that were not sterilized. Furthermore, the storage conditions under which these plastic bottles were might have accounted for the high TVC. Some are even sold near unkempt gutters and open drains right along the street. All these practices might have added to the high levels of bacterial load noticed in the product. Additionally, unhygienic surroundings such as improper waste disposal system among others further increase food contamination (Chumber *et al.,* 2007). Streets vended products were not sold in appropriate storage conditions and this may proliferate the activities of microbes under the temperature in which they were sold. Most vendors kept open the containers in which the products were sold in order to be able to quickly collect and sell to patrons in moving vehicles. The ice blocks placed on the products thus melt easily leading to the products being sold at inappropriate temperatures for yoghurt based products. Apart from the containers not being appropriate, their deplorable state also affect the storage condition.

Naturally, *S. aureus* is found on human body in areas such as skin, hair and nose and their presence in large numbers in food indicates the potential presence of enterotoxin and/or improper sanitary or production practices. In most studies, *S. aureus* are one of the major known pathogens found on hands (Shojoei *et al.*, 2006; Rabbi *et al.*, 2011). It has also been documented that majority of street-vended food handlers are agents of *S. aureus* (Rabbi *et al.*, 2011). Results obtained in this study, indicated that the level of *S. aureus* ranged from 3.78±0.84 log cfu/ml obtained in samples from the 37 Military Hospital vending area, 3.81±0.02 log cfu/ml for Accra Mall vending area, 4.11±1.31 log cfu/ml for the Nima vending area to 4.68±0.08 log cfu/ml in samples from the Maamobi vending area in increasing order of magnitude (Table 4) with no significant difference (0.74). Ghana Standard (GS 955, 2013)

states that, the maximum acceptable limit for *S. aureus* must be 3.0 log cfu/ml. All the samples analyzed in this study did exceed the acceptable maximum limit; however, values from the Accra Mall vending area and 37 Military Hospital vending area were close to meeting the acceptable limit of Ghana Standards (GS 955, 2013). Mensah *et al.* (2002), revealed that street foods in Ghana were particularly heavily contaminated with *S. aureus* due probably to the excessive handling and cross contamination after cooking.

Staphylococcus spp. produces toxins that survive high temperatures and have endospores which grow and give out enterotoxins. Eating foods containing toxins can result in vomiting, nausea, abdominal pains and diarrhoea. Food poisoning caused by *Staphylococcus aureus* affects many people annually (Rabbi *et al.*, 2011). All the samples analyzed tested positive for the presence of *Staphylococcus aureus* probably due to post-production contamination. Often, the packaging of the drink was done manually using a cup held by their bare hands. All the samples analyzed in this study had high amounts of *S. aureus*. What probably might be accounting for the high presence of *S. aureus* as reported by Bhaskar *et al.*, (2004), is that, poor personal hygiene can speed up the transmission of disease- causing bacteria existing in the environment and on the hands of personals handling food to humans. Personal observations made revealed that, in packaging the drink into the bottles, lots of helping hands were involved amid chatting and with no protective garment or nose masks worn. According to Muinde and Kuria (2005), personals handling food with bare hands might lead to cross contamination, by introducing microbes on safe foods. The pH values of most of the ―Burkina‖ drink that were collected were suitable for the growth of the some common microorganisms such as *S. aureus* implicated in food borne diseases and which grows at a pH of about 4.5 to 9 (FDA, 2012).

From Table 5 it is observed that, yeast and mould counts increased from 2.65±0.12 log cfu/ml in samples from the Maamobi vending area to 4.31±0.57 log cfu/ml in samples from the Nima

vending area. Significant differences (p= 0.045) were observed among the yeast and mould count of the four sampling areas. This significant difference might be as a result of the processing of the millet. Ghana Standards (GS337: 2003) and (GS734:2003) limits for yeasts and moulds in yoghurt-based products and that for millet products, 3.0 log cfu/ml and 4.0 log cfu/ml respectively. Ghana Standards (GS337: 2003) and (GS734:2003) limits for yeasts and moulds in yoghurt-based products and that for millet products, are respectively 3.0 log cfu/ml and 4.0 log cfu/ml. Comparable to Codex Standard (2003) for yeasts and moulds in yoghurt, the samples did not meet the requirement which must be 0.0 cfu/g. Different surveys of retail yoghurt revealed that samples could contain counts more than 5.0 logcfu/g (Al-Tahiri, 2005). The presence of yeasts and moulds may be attributed to the production practices of the manufacturers, because most of the producers observed did not adhere to Good Manufacturing Practices (GMPs) and good personal hygiene practices. Mould growing on foods has its characteristic fussy or cottony appearance, among others. Generally, mouldy or ―mildewed‖ food is considered unfit to eat. Yeasts on the other hand are a major cause of spoilage of fermented milk (Vetier *et al.*, 2003). Although moulds are involved in the spoilage of many kinds of foods, some moulds are useful in the manufacture of certain foods or as ingredients of food (Carroll, 2003). The growth of yeasts and moulds could be as a result of the high moisture content of the product because yeasts and moulds are able to form cyst and germinate under favourable and other suitable environmental conditions. Findings of Oyeleke (2009) in Nigeria, indicated that the primary contaminant of yoghurt in Nigeria is yeasts and moulds. The yeasts and moulds utilize acid in the yoghurt thereby producing a corresponding low acidity which may favour the multiplication of putrefactive bacteria. Of the various sites visited during this study, most of the processors did not wash the grain and neither did they pick foreign materials such as stones but rather poured the grains straight into water for steeping. The pH of the ―Burkina‖ drink ranged from 3.78 - 4.85. The pH for maximum growth of many

microorganisms is close to neutral (7.0). However, yeasts can survive in an acidic medium, with best growth in an intermediate acid range (4.0-4.5) Marriot *et al.,* (2006). In the production of ―Burkina‖ drink, the fermented milk is blended with other major ingredients such as millet, sugar and optionally roasted groundnuts which may probably account for the presence of yeasts and moulds in the drink. Since yeasts and moulds are considered as natural contaminant in yoghurt, there are inconsistencies in their acceptable maximum limits. It has, however, been reported by Al-Tahiri, (2005) as 5.0 log cfu/g.

In this study, all the samples tested negative for *Salmonella typh*i. According to Barro *et al.,* (2002), *Salmonella* spp causes the most frequently occurring bacterial food infections. In addition to the typical food-poisoning salmonellosis, typhoid fever and paratyphoid fever can also occur following the consumption of Salmonella-contaminated foods (Bhan *et al.,* 2005). Contamination of street foods with *Salmonella* spp. has been reported in other countries with incidences of 5% in Mexico (Estrada-Garcia *et al*., 2004) and 6% in Addis Ababa, Ethiopia (Muleta and Ashenafi, 2001). As indicated by Tambekar *et al.,* (2009), the ingestion of street vended foods cannot be halted on hygienic grounds. Hygiene practices can instead be improved to ensure more safety. According to Ghana Standards (GS337:2003) for milk and milk product, specification for yoghurt and sweetened yoghurt products, *Salmonella typhi* should not be present. All the samples analyzed in this study were negative for the presence of *Salmonella*, thus, attaining the acceptable limit of zero according to the Ghana Standards (GS337:2003).

Levels of *E. coli* in samples from the 37 Military Hospital vending area ranged from 21 to 110 MPN/ml, Nima ranged from 21 to >110 MPN/ml, Maamobi ranged from 46 to >110 MPN/ml with those from Accra Mall vending area ranging from 12 to >110 MPN/ml. In respect to this observation, samples from Maamobi area had the highest compared to the others. This is not surprising due to the low level of hygiene in these places. Relatively higher levels of *E.*

coli was obtained in samples from the 37 Military hospital vending area and the Nima vending area compared to the Accra Mall vending area. Thus, samples from Maamobi area may be more faecally contaminated than the other areas, however, all the samples analyzed did not meet the required Ghana Standard (GS337:2003) that, *E. coli* should be absent in all the yoghurt products. The results of this study is similar to findings by Kolawole *et al.*, (2007) who noted the presence of *E. coli* and *Staphylococcus aureus* from a number of traditional beverages such as ―Pito‖ and ―Burukutu‖ produced in Ghana.

Most *E. coli* strains are harmlessly in the digestive tract of humans and animals (Bhunia, 2008). Although *E. coli* is known to be part of the flora of the human digestive tract, many adapted *E. coli* clones have evolved and have the potential to cause disease in many part of the human body (Eslava *et al*., 2003). It is been reported by Gelsomino *et al*., (2002) that, the presence in food of *E. coli* and other Enterobacteriaceae such as *Enterococcus faecalis* is indicative of faecal contamination and this suggests poor hygiene during preparation, handling and storage, as well as lack of reheating and improper vending temperatures. Food contaminated with *E. coli* connotes a possible presence one or more of enteric pathogens in the food (Tambekar *et al*., 2011). The high *E. coli* counts observed in this ―Burkina‖ product which is a ready-to-eat food sample is similar to other studies by Badrie *et al.*, (2002) and Kumar *et al.*, (2006). *E. coli* count is an important criterion for evaluating the sanitary quality of various foods (Gelsomino *et al.*, 2002). Additionally, in most parts of the world, foodborne disease incidences are more commonly associated with *Salmonella* serotype *enteritidis*, *Vibrio cholera, Escherichia coli* serotype 0157:H7, *Listeria monocytogenes* and foodborne trematodes (Mensah *et al*., 2002; Wawa *et al.*, 2009; Tambekar *et al*., 2011; Annan-Prah *et al*., 2011). It presence might also be attributed to the source of water because investigations by Gitahi *et al.*, (2012) found that, street vended foods with the presence of *E. coli* might be as a result of the water used in cooking and serving and that the main cause of contamination of these street foods may be the washing and

the processing water. All the products analyzed in this study tested positive to the presence of *E. coli* (Table 6). The positive results might be as a result of the use of contaminated ingredients, aerial contamination from dust, cross contamination from personnel. Inadequately cleaned containers are also likely sources of contamination to ready-to-eat (RTE) foods and drinks (Food Standards Agency, 2011). According to Ghana Standards (GS337:2003) for yoghurt-based products, *E. coli* should not be present. Ready-to-eat foods or drinks are considered to be of high risk to consumers (Wawa *et al*., 2009), especially if pathogenic strains of the organism such as verotoxigenic *E. coli* 0157H and enterotoxigenic *E. coli* are involved (Woodward *et al.*, 2002).Variation in *E. coli* counts among the different street foods under the present study may largely be attributed to the differences in the methods of preparation and use of raw materials (Estrada-Garcia *et al*., 2004). Although pasteurization kills most pathogens in food products (Allen *et al*., 2004), contamination can still occur during packaging and are the most likely explanations for the presence of *E. coli.* The Nima community is often reported as Accra's biggest slum with the largest number of poor people in Accra who are mostly illiterate. The Nima community is poorly planned and densely populated. There are no proper drainage systems in these areas and most of the households do not have running water and those who have often suffer from shortage of water (Developing unity, through nurturing knowledge (DUNK), 2013). According to AMA (2012), for decades, the Nima-Maamobi area has had trouble with access to safe and readily reliable water and the limited water network in Nima may be due in part to the rising and falling relief of its terrain in relationship to Maamobi, Kanda and Accra New Town and this may probably account for the high levels of *E. coli* encountered. Good personal hygiene in handling foods has the potential to reduce contamination. Individuals preparing the product should be educated on the pathogenic microorganisms that can be introduced into the food under improper conditions.

That laws and regulations should be put in place in order to check the product to meet standard before selling on the market. Bhaskar *et al.,* (2004) reported that poor personal hygiene can speed up the transmission of pathogenic bacteria existing in the environment and on people's hands through food to humans. Hence, training for _Burkina‘ producers and seller on dairy product handling, processing and packaging by local health workers is recommended.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

The present study as part of its objectives was to determine the pH, the proximate composition, microbial load and the types of microbes associated with street vended

―Burkina‖ drink.

All the test samples were within the acidic range with significant differences observed among the pH values from the four sampling areas with a p = 0.00.

Results obtained for the percentage composition showed that, the drink is nutritious and can provide a source of energy for its consumers. Significant differences existed in its carbohydrate, protein, total fat and total ash contents but not the moisture content among the

―Burkina‖ drink from the four sampling areas. These differences are probably as a result of the lack of standardized method for its preparation. Its high moisture content implies high water activity which supports microbial growth and consequently reducing the shelf life of the product. Hence, it requires that, the product be stored in appropriate cold storage temperature.

The study showed a high microbial load with counts of aerobic bacteria, *Staphylococcus aureus*, yeasts and moulds, and *E. coli* being higher than the limits set by the Ghana standards Authority. *Salmonella typhi* was not detected in any of the samples analyzed and therefore met the limit set by the Ghana Standards Authority. There was no significant difference in Total Viable Bacteria counts and *Staphylococcus aureus* probably as a result of the same personnel hygiene, environmental conditions, water used for processing as well as the equipment used during processing which might have harbour numerous microorganisms and the exposure of the drink during selling.

The results indicate that, although the ―Burkina‖ drinks had high nutritious composition, their microbiological qualities were compromised. Hence, there may be significant public health hazards linked to the consumption of ―Burkina‖ drinks in the vending areas investigated.

6.2. RECOMMENDATIONS

It is recommended that further research be done on; the shelf life of the product, the microbial loads from the raw material through to the production site to the consumer and the appropriate storage temperature of the product.

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APPENDICES

Appendix 1: MPN index and 95% confidence limits for various combinations of positive results when various numbers of tubes are used. (Inocula of 1, 0.1 and 0.10g)

3 Tubes per dilution			
Combination of positives	MPN Index per g	95% Confidence Limits	
		Lower	Upper
0-0-0	<0.3	<0.05	<0.9
0-0-1	0.3	<0.05	0.9
0-2-0	--b	--	--
1-0-0	0.4	<0.05	2
1-0-1	0.7	0.1	2.0
1-1-0	0.7	0.1	2.3
1-1-1	1.1	0.3	3.6
1-2-0	1.1	0.3	3.6
2-0-0	0.9	0.1	3.6
2-0-1	1.4	0.3	3.7
2-1-0	1.5	0.3	4.4
2-1-1	2.0	0.7	8.9
2-2-0	2.1	0.4	4.7
2-2-1	2.8	1.0	15
2-3-0	--	--	--
3-0-0	2.3	0.4	12
3-0-1	3.9	0.7	13
3-0-2	6.4	1.5	38
3-1-0	4.3	0.7	21
3-1-1	7.5	1.4	23
3-1-2	12	3	38
3-2-0	9.3	1.5	38
3-2-1	15	3	44
3-2-2	21	3.5	47
3-3-0	24	3.6	130
3-3-1	46	7.1	240
3-3-2	110	15	480
3-3-3	>110	>15	>480

Appendix 2: Table 7: Load of *Salmonella typhi* in samples from the four sampling areas

VENDING AREA	Vendor1(cfu/ml)	Vendor2(cfu/ml)	Vendor3(cfu/ml)

Nima	None Detected	None Detected	None Detected
Maamobi	None Detected	None Detected	None Detected
37 Military Hospital	None Detected	None Detected	None Detected
Accra Mall	None Detected	None Detected	None Detected

Appendix 3: Statistical Analysis of Data on "Burkina" Drink

Table 8: ANOVA: Single Factor for pH of the samples from the four locations.

SUMMARY

Groups	Count	Sum	Average	Variance
Nima	3	11.530	3.843	0.003
Maamobi	3	11.490	3.830	0.003
37 Military Hospital	3	14.450	4.817	0.000
Accra Mall	3	11.380	3.793	0.000

Source of Variation	SS	df	MS	F	P-Value	F crit
Between Groups	2.229	3.000	0.743	521.425	0.000	4.066
Within Groups	0.011	8.000	0.001			
Total	2.240	11				

Table 9: One Way ANOVA Post Hoc Tests on pH of the samples from the four locations.

Scheffe	0.088		
Post Hoc	Nima	Maamobi	37 Military Hospital
Maamobi	0.013		
37 Military Hospital	**0.973**	**0.987**	
Accra Mall	0.050	0.037	**1.023**

Colored cells have significant mean differences

Table 10: ANOVA: Single Factor for Moisture content of the samples from the four locations.

SUMMARY

Groups	Count	Sum	Average	Variance
Nima	3	247.190	82.397	0.702
Maamobi	3	242.290	80.763	0.255
37 Military Hospital	3	243.940	81.313	1.440
Accra Mall	3	246.570	82.190	0.108

Source of Variation	SS	df	MS	F	P-Value	F crit
Between Groups	5.243	3.000	1.748	2.790	0.109	4.066
Within Groups	5.011	8.000	0.626			

Table 11: ANOVA: Single Factor for Carbohydrate content of the samples from the four locations.

SUMMARY

Groups	Count	Sum	Average	Variance
Nima	3.000	31.760	10.587	1.481
Maamboi	3.000	37.340	12.447	1.604
37 Military Hospital	3.000	30.600	10.200	1.453
Accra Mall	3.000	38.180	12.727	0.038

Source of Variation	SS	df	MS	F	P-Value	F crit
Between Groups	14.774	3.000	4.925	4.304	0.044	4.066
Within Groups	9.153	8.000	1.144			

Table 12: One Way ANOVA Post Hoc Tests for Carbohydrate Content of the samples from the four locations.

LSD	2.014		
Scheffe	2.491		
Post Hoc	Nima	Maamboi	37 Military Hospital
Maamboi	1.860		
37 Military Hospital	0.387	2.24 7	
Accra Mall	2.140	0.280	**2.527**

Colored cells have significant mean differences

Table 13: ANOVA: single factor for Protein Content of the samples from the four locations.

SUMMARY

Groups	Count	Sum	Average	Variance
Nima	3.000	10.300	3.433	0.392
Maamboi	3.000	10.977	3.659	0.407
37 Military Hospital	3.000	14.490	4.830	0.276
Accra Mall	3.000	9.750	3.250	0.035

Source of Variation	SS	Df	MS	F	P-Value	F crit
Between Groups	4.553	3.000	1.518	5.468	0.024	4.066
Within Groups	2.220	8.000	0.278			

Table 14: One Way ANOVA Post Hoc Tests for Protein Content of samples from the four Locations.

LSD	0.992

Scheffe	1.227		
Post Hoc	Nima	Maamobi	37 Military Hospital
Maamobi	0.226		
37 Military Hospital	**1.397**	1.171	
Accra Mall	0.183	0.409	**1.580**

Colored cells have significant mean differences

Table 15: ANOVA: single factor for Fat Content of the samples from the fourlocations.

SUMMARY

Groups	Count	Sum	Average	Variance
Nima	3.000	8.860	2.953	0.068
Maamobi	3.000	6.790	2.263	0.308
37 Military Hospital	3.000	9.730	3.243	0.157
Accra Mall	3.000	4.230	1.410	0.082

Source of Variation	**SS**	**Df**		**MS**	**F**	**P-Value**	**F crit**
Between Groups	5.994	.000	1.998		12.988	0.002	4.066
		.000	0.154				
Within Groups	1.231						

Table 16: One Way ANOVA Post Hoc Tests for Total Fat Content in Burkina Drink from the four Locations.

LSD	0.738		
Scheffe	0.913		
Post Hoc	Nima	Maamboi	37 Military Hospital
Maamobi	0.690		
37 Military Hospital	0.290		
Accra Mall		**0.980**	
	1.543	0.853	**1.833**

Colored cells have significant mean differences

Table 17: ANOVA: single factor for Total Ash Content of the samples from the four locations.

SUMMARY

Groups	Count	Sum	Average	Variance
Nima	3.000	1.260	0.420	0.003
Maamobi	3.000	1.410	0.470	0.002

37 Military Hospital	3.000	0.190	0.063	0.001
Accra Mall	3.000	0.750	0.250	0.035

Source of Variation	SS	Df	MS	F	P-Value	F crit
Between Groups	0.305	3.000	0.102	10.047	0.004	4.066
Within Groups	0.081	8.000	0.010			

Table 18: One Way ANOVA Post Hoc Tests for Total Ash Content in samples from the four Locations.

LSD	0.190		
HSD	0.263		
Scheffe	0.234		
Post Hoc	Nima	Maamobi	37 Military Hospital
Maamobi	0.050		
37 Military Hospital	**0.357**	**0.407**	
Accra Mall	0.170	0.220	0.187

Colored cells have significant mean differences

Table 19: ANOVA: single factor for loads of Total Viable Bacteria in the samples from the four locations.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Nima	3.00	23.81	7.937	0.042433
Maamobi	3.00	23.33	7.777	0.009433
37 Military Hospital	3.00	20.58	6.86	0.0181
Accra Mall	3.00	22.15	7.383	0.742433

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*
Between Groups	2.069892	3	0.689964	3.397163	0.074	4.066180551
Within Groups	1.6248	8	0.2031			
Total	3.694692	11				

Table 20: ANOVA: single factor for loads of *Staphylococcus aureus* in the samples from the four locations.

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Nima	3.00	12.33	4.11	0.9187
Maamobi	3.00	12.92	4.31	0.186233
37 Military hospital	3.00	11.34	3.78	0.7027
Accra Mall	3.00	11.43	3.81	0.0004

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*
Between Groups	0.5719	3	0.190633	0.421747	0.743	4.066181
Within Groups	3.616067	8	0.452008			
Total	4.187967	11				

Table 21: ANOVA: single factor for loads of Yeasts and Moulds in the samples from the four locations.

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Nima	3.00	12.92	4.306667	0.326033
Maamobi	3.00	9.37	3.123333	0.492933
37 Military hospital	3.00	8.67	2.89	0.2977
Accra Mall	3.00	11.03	3.676667	0.003033

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-Value*	*F crit*
Between Groups	3.587692	3	1.195897	4.272206	0.045	4.066181
Within Groups	2.2394	8	0.279925			
Total	5.827092	11				

Table 22: One Way ANOVA Post Hoc Tests for Mould and Yeast in samples from the Four locations.

LSD	0.996174
HSD	1.383447
Scheffe	1.231923

Post Hoc	Nima	Maamobi	37 Military hospital
Maamobi	1.183333		
37 Military hospital	**1.416667**	0.233333	

Accra Mall	0.63	0.553333	0.786667

Colored cells have significant mean differences