UNIVERSITY OF CAPE COAST

COLLECTION, IDENTIFICATION, PHYSICO-CHEMICAL AND MICROBIAL QUALITY ANALYSIS OF NON-ALCOHOLIC DRINKS IN THE CAPE COAST METROPOLIS

THELMA NANA DASOBERI

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BY

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Thesis submitted to the Department of Vocational and Technical Education, Faculty of Science and Technology Education, College of Education Studies, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Home Economics

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate: Thelma Nana Dasoberi

Signature:..... Date:…….....

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor: Prof. (Mrs.) Sarah Darkwa

Signature:..... Date:….....

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Signature:..... Date:….....

ABSTRACT

Free-trade policies across countries have allowed an influx of diverse non-alcoholic drinks into Ghana. Frequent consumption of such drinks may contribute to non-communicable diseases (NCDs). The study evaluated the microbial quality and physico-chemical properties of selected non-alcoholic drinks in the Cape Coast Metropolis. Out of 122 non-alcoholic drinks catalogued, 22 were analysed. Aerobic mesophilic counts, total coliforms (TC), faecal coliforms (FC), yeasts and moulds (Y&M), *Salmonella* spp. and *Escherichia coli* were determined following standard procedures. The numbers of microorganisms present were calculated using the weighted mean from the dilution and expressed as colony forming unit per milliliter (cfu/ml). Total sugars and vitamin C concentrations were determined by the Anthrone method; the AOAC (1990) method was used for physico-chemical analyses. Concentrated fruit juices preserved by physical and chemical means represented the highest percentage (59%) of samples analyzed. Bacterial growth, yeasts and moulds were observed in two (33%) and only 1 (16.6%) drink samples. Bacteria, yeasts and moulds growth were found on 5 drink samples; whiles faecal coliforms and *Escherichia coli* were found in 2(40%) samples respectively; *Salmonella* spp. was not detected in any of the samples tested. The highest sugar concentration was 40.71±14.38%. The highest vitamin C concentration was 0.15±0.03 ml/100ml. The highest pH was 3.602±0.86 in the carbonated soft drinks and carbonated malt category. The highest titrable acidity was 14.72±3.11 with acid percentage of 94%. Although microbial growth was not widespread in the drinks sampled, numbers were above the Ghana Standards Authority's acceptable limits.

KEY WORDS

Microbiological Analysis

Non-Alcoholic Drinks

pH

Physico-chemical Analysis

Sugar

Vitamin C

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DEDICATION

To my parents, Deborah Davida Dadzie and Mr. George Boroh Dasoberi

(of blessed memory).

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CHAPTER ONE

INTRODUCTION

Economic policies have allowed free trade across countries and thus, there has been an influx of all kinds of non-alcoholic drinks on the Ghanaian market. These drinks form part of the daily diets of Ghanaians and so cannot be avoided. Parents give them to their children as snacks; they are served at parties and social gatherings. The recent craze over non-alcoholic drinks is becoming the norm in our fast-paced world, but many of these drinks can be hazardous to our health, if drunk in excess and standards governing the manufacturing and production of these drinks are overlooked by the authorities involved.

Background to the Study

Food is any nutritious substance that people or animals eat or drink or that plants absorb in order to maintain life and growth (English Oxford Dictionaries, 2015). Food and Agriculture Organization and the World Health Organization (2007), report that food is one of the basic needs for every living being and is composed of Carbohydrates, Water, Fats and Proteins, which can be eaten or drunk by animals, including humans for nutrition or pleasure. Food safety and factors affecting it have been major emerging areas within the food supply chain and have attracted a lot of attention from various governmental and regulatory bodies (Ayalew, Birhanu & Asrade, 2013). The United States Department of Agriculture (2013) defined food safety as conditions and practices that preserve the quality of food to prevent contamination and foodborne illnesses while the World Health Organization (2015) stated that food safety encompasses actions

aimed at ensuring that all food is as safe as possible and thus food safety policies and actions need to cover the entire food chain, from production to consumption.

Non- alcoholic drinks, which are liquids, form part of our daily diets. These include fruit juices, soft drinks/soda, carbonated drinks and Ghanaian local drinks. These drinks are often taken with snacks, meals or alone. They are usually presented in cans, glass bottles and plastic bottles (the latter in a variety of sizes ranging from small 0.5 litre bottles to large 1.5 litre containers). Demand for these drinks has increased their vulnerability to adulteration during processing to promote patronization by consumers and economic gains for producers and sellers (Jha, Jaiswala & Grewala, 2016).

Davenport (2005) reveals that the nutritional value of soft drinks is sometimes exaggerated by manufacturers who want consumers to perceive their products to be of special benefit. Their balance of sweetness and acidity, coupled with pleasant flavours, make them attractive to all ages of consumers. Products are specially formulated to meet the tastes, nutritional needs and physiological constraints of the whole population, from babies to geriatrics. Mercola (2015) reports that, drinking just one-eight (1/8oz, equivalent to 4mL) glass of orange juice will add about 25 grams of fructose to your body system which is more than required for the entire day. However, many people, especially kids and teenagers, drink more than 4mL glass of sugary drinks in a day.

According to Mercola (2015), fructose has been identified as one of the primary perpetrators in the rise of obesity and other related health problems. Fructose has also been discovered to cause non-liver disease in millions of children in the United States of America, which comes about by a build-up of fat

within liver cells (Mercola, 2015). Frequent consumption of these drinks which are often loaded with high concentrations of sugars and other substances may be contributing to the increase in diabetes, tooth decay in children and probably some type of cancers in Ghana as a whole and specifically in the Cape Coast Metropolis.

The small amounts of vitamins and antioxidants in the juice do not make up for the large amount of sugar (Bjarnadottir, 2015). However, though fruit juice contains vitamins, minerals and antioxidants, it lacks fibre. The regulations behind food labeling are complex, so the average consumer has a hard time understanding them. Consequently, manufacturers have also added components that are of questionable nutritional value. Hughes, Wellard, Lin, Suen and Chapman (2013) posit that the number of products carrying nutrition content claims that do not meet the nutrient profiling criteria suggests that comprehensive regulation is warranted and the promotion of unhealthy foods using claims is potentially misleading for consumers and hinders their ability to select healthier foods.

According to Tasnim, Hossain, Nusrath, Hossain, Lopa and Haque (2010), while the quality of fruit juices is strictly being maintained in the developed countries under several laws and regulations, unfortunately, in many developing countries including Ghana, manufacturers are not much concerned about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law. Thus the transmission of certain human diseases through juice and other drinks becomes a serious problem.

Most consumers follow the sweetness and tastes of these locally produced drinks and are only concerned about the relief, which is mainly to quench their thirst and also to delay hunger as they travel in vehicles or on foot from one area to another. As for the preparation of these drinks, no one can tell, as producers do not follow the same procedure or a standard in preparing them. Each has their own method (Africa Processing, 2016).

There is a growing health and wellness consciousness among consumers and an increasing importance given to fitness and healthy lifestyle choices. Changing work and lifestyle habits leave less time for home cooking and therefore spur demand for convenience and 'complete nutrition' from meal replacements. Consumers are still in need of further education on the use of ingredients, additives, and packaging materials in non-alcoholic products and their potential effects on human health. This study seeks to evaluate some non-alcoholic drinks in the Cape Coast Metropolis.

Statement of the Problem

In many developing countries including Ghana, millions of people are widely consuming non-alcoholic drinks in every season as it provides an affordable source of nutrients and refreshment to them (Ohiokpehai, 2003). There is a growing health and wellness consciousness among Ghanaians and an increasing importance given to fitness and healthy lifestyle choices. According to Frazier and Westhoff (1997), there have been serious health hazards due to the presence of pathogenic microbes in food and this can lead to food poisoning outbreaks. Thus, freshly extracted fruit juices, which have always been considered

as healthful drink, may not always be safe owing to the heavy load of microbes (Kumari, 1995).

Many non-alcoholic drinks companies are producing and marketing different drinks, but most of the companies have no concern about the quality of the drink products.Most of them think commercially and they are only concerned about the marketing (with colorful advertisement) of their products (Mortuza,2016). Though they might maintain a lot of hygiene in their factory to avoid contamination, but in most of the factories they use preservatives or harmful chemicals to lower the microbial growth in juice. In long time effect, these harmful preservatives and chemicals can cause disease that is a thousand times more powerful to human being than the microbes, or cause mutation inside our body that eventually kills people ten times faster than normal diseases (Mortuza,2016). In many developed countries, the quality of fruit juice is strictly maintained under several laws and regulations. Unfortunately, in Ghana, manufacturers are not much concerned about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law. Thus, the transmission of certain human diseases through juice and other drinks becomes a serious problem (Tasnim, 2010).

In recent times, because of economic policies that allow free trades across countries, there has been an influx of all kinds of sugary non-alcoholic drinks on the Ghanaian market and since these drinks form part of most Ghanaian diets, many people are patronizing and over-consuming them. These sugars in the non-alcoholic drinks are overloading consumers' livers and may have resulted in increased rates of Non-Communicable Diseases (NCDs) such as dental caries,

diabetes, cancer, obesity and high blood pressure among Ghanaians in general and the people of Cape Coast Metropolis specifically.

A study conducted to explore the prevalence of Diabetes mellitus and resources available for managing it in three hospitals and four health centres in the Cape Coast Metropolis by Darkwa (2011) revealed that, Diabetes mellitus and dental caries conditions have been increasing since 2005. Gradually, more Ghanaians are becoming diabetic and experiencing dental issues as a result of eating more sugary and fatty foods and exercising less. Data available at one of the major hospitals in Ghana, University of Cape Coast hospital reveals the total number of Diabetes mellitus and dental caries cases reported over the period 2010 to 2015. Table 1 presents the data available.

Table 1: Diabetes mellitus and Dental Caries cases

Year	Diabetes mellitus	Dental caries
2010	1080	501
2011	854	3070
2012	1115	2498
2013	507	4137
2014	702	4856
2015	4627	4627

University of Cape Coast Health services, (2016).

Although a direct link has not been established with over-consumption of these drinks and non-communicable diseases, there have been several studies on the relationship between sugary drinks and non-communicable diseases. Along these lines of evidence, a prompt assessment of non-alcoholic drinks in general

needed to be undertaken to assess the microbiological and chemical safety of non-alcoholic drinks for the sake of the better management of public health.

This study seeks to evaluate some non-alcoholic drinks in the Cape Coast Metropolis.

Purpose of the Study

The primary purpose of the study was to evaluate and analyse non-alcoholic drinks in the Cape Coast Metropolis. Specifically, the study tackled the following research objectives:

1. to catalogue and categorise non-alcoholic drinks in the Cape Coast Metropolis based on the standards from the Ghana Standards Authority.
2. to determine the microbial loads in the non-alcoholic drinks
3. to determine total sugars and vitamin C concentrations in the non-alcoholic drinks.
4. to determine if the products meet the standards available?
5. What differences exist in the microbial loads, the level of concentration of sugar and vitamin C of the selected non-alcoholic drinks?

Research Hypothesis

H_0: There is no significant difference in the microbial load of non-alcoholic drinks on the market

H_1: There is a significant difference in the microbial load of non-alcoholic drinks on the market

H_2: There is no significant difference in the chemical constituent of non-alcoholic drinks on the market

H_3: There is a significant difference in the chemical constituent of non-alcoholic drinks on the market.

Significance of the Study

Findings from this study would guide consumers in the Cape Coast Metropolis to be more vigilant in patronizing non-alcoholic drinks on the market bearing in mind their nutritional content, hygienic and safety conditions. The findings of the study would also raise awareness on possible consequences of non-communicable diseases in the Cape Coast Metropolis. Again, the findings of the study would also contribute to scanty existing knowledge on non-alcoholic drinks.

Delimitations

The study was delimited to the Cape Coast Metropolis in the Central Region of Ghana.Samples of fresh fruit juices, except blue skies juice were collected from only shops in and around the University of Cape Coast, Abura and Kotokuraba markets in the Cape Coast Metropolis.

The study did not include drinks such as water, flavoured water, non-alcoholic champagne, energy drinks, sports drinks, non-alcoholic beer, non-alcoholic sparkling wine, iced tea, sweet tea, fruit punch and milk based drinks.

The generalization of the findings was also limited to the samples of the study due to non-probability sampling procedure that was to be used.

Limitations

After the initial survey and documentation of non-alcoholic drinks available on the Cape Coast markets, some of them could no longer be found during the period that laboratory analyses were carried out. Also, due to high cost

of analyzing the microbial and chemical parameters and transportation, the sample size was reduced from 25 to 22. All the analyses could not be completed at the Centre for Scientific and Industrial Research (CSIR), Accra, because the outfit was preparing for assessment by external auditors and hence, the researcher had to complete the rest of the analyses at the University of Cape Coast Medical Sciences and the School of Agriculture Research Laboratories.

Definition of Terms

Adulterant: Adulterants are chemical substances which should not be contained within our food or drink.

Adulteration: Adulteration is the act of either adding extraneous substances (adulterants) into food items or products or reducing essential nutrients partly or wholly for financial gain or due to carelessness and lack of proper hygienic condition during processing, storing, transportation and marketing.

Carbonated drinks: Drinks which have carbon dioxide dissolved into them.

Carbonates: Sweetened, beverages with carbon dioxide, syrups for home dilution and out-of-home carbonated soft drinks.

Contamination: The action or state of making or being made impure by polluting or poisoning.

Fruit juice: A natural product that contains few or no additives

Food safety: The conditions and practices that preserve the quality of food to prevent contamination and food-borne illnesses.

Fruit powders: Non-ready-to-drink fruit products in powder form.

Juice: 100% pure fruit or vegetable juice without ingredients, except permitted minerals and vitamins, with sweetening agents (less than 2%).

Nectars: Diluted fruit/vegetable juice and pulp, with sweetening agents, minerals, and vitamins.

Non-alcoholic drink: A drink that contains no alcohol.

Packaging: The wrapping material around a consumer item that serves to contain, identify, describe, protect, display, promote and otherwise make the product marketable and keep it clean

Pathogens: A biological agent that causes disease or illness to its host.

Preservatives: A substance or a chemical that is added to products such as food, beverages to prevent decomposition by microbial growth or by undesirable chemical changes.

Soft drinks: Soft drinks are flavoured, frequently coloured drinks and often contain an amount of fruit juice, fruit pulp or other natural ingredients.

Squash/syrups: Non-ready-to-drink products, marketed as concentrates for home consumption including fruit and non-fruit-based products and flavours.

Still drinks: Flavoured ready-to-drink, non-carbonated beverages containing fruit or non-fruit flavours or juice content (to 25%).

Sugar: A white crystalline carbohydrate used as a sweetener and a preservative.

Titrable acidity: A measure of the amount of acid present in a solution. It is expressed as grams/liter (g/L) and is obtained by multiplying percentage TA by 10.

Vitamin C: It is known as Ascorbic acid. A micro-nutrient mostly found in fruits and vegetables.

Organization of the Study

The study was organized under five chapters; Chapter Two of the study covered a review of relevant related literature. This includes conceptual issues and empirical literature. The framework shows the origin of non-alcoholic drinks, categories, types, their production and how their characteristic differences can help to identify them on the Cape Coast Metropolis Markets. Empirical reviews included past studies on the subject. Chapter Three described the research methods which included the research design, study area, population, sampling procedure, data collection instruments, data collection procedures and data processing and analysis. Results from the study were presented and discussed in chapter four. Chapter Five was devoted to the summary, conclusions and recommendations. Finally, suggestions for further studies were provided.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

Introduction

This chapter presents a review of related literature on all the issues and variables that are relevant to the study as well as give a summary. The sub- topics that will be reviewed are as follows:

Definition of Non-Alcoholic Drinks, Categories of Non-Alcoholic Drinks, The Microbiological Quality of Fruit Juices, *Escherichia Coli* and *Salmonella* spp. The rest are Yeasts and Moulds, Coliforms, Nutritional Value of Fruit Juices, Pasteurization of Fruit Juices, Sugar in Non-Alcoholic Drinks, The Negative Effects of Sugars and Sweeteners in Non-Alcoholic Drinks, Acids in Non-Alcoholic Drinks and Vitamin C. I will also discuss Health Benefits and Deficiencies of Vitamin C in the Human Body, pH, Titratable Acidity (TTA), Temperature, Storage of Non-Alcoholic Drinks, Packaging of Non-Alcoholic Drinks, Food Safety, Contamination of Non-Alcoholic Drinks, Types of Food/Drink Contamination, Adulteration of Non-Alcoholic Drinks, Food Poisoning, Non-Alcoholic Drinks and Related Health Issues, Food Laws and Regulations in Ghana and Food and Drugs Authority.

Again, the discussion will include Ghana Standards Authority, International Quality Standards (IQS), International Organisation for Standardisation (ISO) and the chapter will conclude with a Chapter Summary.

Definition of Non-alcoholic Drinks

A non-alcoholic drink is one that contains little or no alcohol (Gutierrez, 2014). The non-alcoholic beverages industry encompasses liquid refreshment beverages (LRB) such as bottled water, carbonated soft drinks, energy drinks, fruit beverages, ready-to-drink coffee and tea, sports beverages and value-added water. It also includes; low-alcohol beer, non-alcoholic wine, and apple cider if they contain less than 0.5% alcohol by volume.

The term "soft drink" specifies the absence of alcohol in contrast to "hard drink" and "drink". Beverages such as soda pop, sparkling water, iced tea, lemonade, root beer, fruit punch, milk, hot chocolate, tea, coffee, milkshakes, and tap water and energy drinks are all soft drinks (Gutierrez, 2014). Fruit juice is a natural product that contains few or no additives. Citrus products such as orange juice and tangerine juice are familiar breakfast drinks, while grapefruit juice, pineapple, apple, grape, lime, and lemon juice are also common. Coconut water is a highly nutritious and refreshing juice. Many kinds of berries are crushed; their juices are mixed with water and sometimes sweetened. Raspberry, blackberry and currants are popular juice drinks but the percentage of water also determines their nutritive value (The Portugal News, 2012).

Bert (2011) defined a soft drink as any of a class of non-alcoholic beverages, usually but not necessarily carbonated, containing a natural or artificial sweetening agent, edible acids, natural or artificial flavours, and sometimes juice. The term soft-drinks has been used to distinguish these drinks from ‘hard-drinks’, alcoholic beverages such as hard liquors and spirits (though soft-drinks are allowed an alcoholic percentage of less than 0.5%). Soft drinks may also contain

fruit juice, but if the drink contains over 25% fruit, it is considered a juice. There are several categories within soft drinks, which distinguish the different flavours of soft-drinks available (Stiles, Battey, Duffy & Schaffner, 2001).

Categories of Non-alcoholic Drinks

Soft Drinks

According to Ashurst (2005), soft drinks have no one definition available but it is generally accepted that they are sweetened water-based beverages, usually with a balancing acidity. Soft drinks are flavoured, frequently coloured and often contain an amount of fruit juice, fruit pulp or other natural ingredients. Water is the chief ingredient which is often ignored and frequently maligned. It should therefore be remembered that the primary function of soft drinks is hydration. Soft drinks are often carbonated and commonly consumed while cold. The most common soft drinks are colas, flavoured water, sparkling water, iced tea, sweet tea, lemonade, squash and fruit punch (Steen & Ashurst, 2006); and excludes tea, coffee, milk beverages and until recently, alcohol (Ashurst, 2005).

However, in many countries, the production of 'soft' drinks containing alcohol is growing. Many see this as an undesirable trend because traditionally the taste of alcoholic beverages has been associated with adulthood. The sweetness and other characteristics of soft drinks are in some respects secondary and yet they do have importance in the provision of energy and some of the minor essential nutrients needed to meet daily requirements. The two basic types of soft drinks are the "Ready-To-Drink" (RTD) products that dominate the world market and the "concentrated" or "dilute-to-taste" products that are still important in some markets. These include syrups, squashes and cordials. Whether RTD or

dilutable, soft drinks characteristically contain water, a sweetener usually a carbohydrate, although artificial sweeteners are increasingly important, an acid; citric or malic are the most common, flavouring, colouring and preservatives (Ashurst, 2005).

Concentrated soft drinks

Concentrated soft drinks according to NIIR Board of Consultants and Engineers (2016) and Ashurst (2005), became very important during, and in the early years following, the Second World War. Many were based on concentrated orange juice, which was widely available as a nutritional supplement, and were packed in flat-walled medicine bottles. The products became universally known as 'squashes' or 'cordials' and became enshrined as such in UK legislation in the 1960s. Another very important development was the production of citrus comminutes. These were produced by mixing together, in appropriate proportions, the juice, peel components and essential oils of citrus fruits and comminuting the mixture in a stone mill. The resulting product delivered a more intense flavour and cloud than could be obtained from juice alone and allowed the creation of 'whole fruit drinks' (NIIR Board of Consultants and Engineers, 2016; Ashurst, 2005).

Carbonated soft drinks

According to Sivasankar (2002), carbonated drinks refer to drinks which have carbon dioxide dissolved into them. This can happen naturally through fermentation and in natural water spas or artificially by the dissolution of carbon dioxide under pressure. Cola, orange, root beer, ginger and lemon/lime are commonly used to create non-alcoholic carbonated drinks; sugars and

preservatives may be added later (Sivasankar, 2002). Carbonated non-alcoholic beverages are generally sweetened, flavoured, acidified, coloured, artificially carbonated and sometimes chemically preserved. This can include a number of different steps, some prior to transport, others immediately prior to consumption. The most consumed carbonated soft drinks are produced by three major global brands: Coca-Cola, Pepsi Cola and the Dr. Pepper Snapple Group (Soft Drink Industry, 2013). Many carbonated soft drinks are optionally available in versions sweetened with sugar or with low caloric sweeteners (Osborne & Voogt, 1990; Pomeranz & Practice, 1994).

Carbonated malt drinks

Non-alcoholic malt drinks are consumed in many countries. Many people avoid alcohol because of their health. Malt drinks are classified based on the alcohol content as alcoholic (more than 1.2%), low alcoholic (0.5-1.2%) and with no alcohol (less than 0.5%). Non-alcoholic malt beverages are produced as non-fermentative or fermentative. Non-alcoholic malt drinks market in all over the world particularly in Islamic countries has expanded (Kamil, 2003). Malt drinks are generally produced by dissolving wort granulates in water, filtered, and the addition of pure hop aroma, followed by carbonation (Kamil, 2003). The malt drink has a lot of health benefits such as protection against coronary heart diseases, cancers and ulcers (Bamforth, 2002). It is believed that in general, non-alcoholic malt drinks compared to alcoholic beers, have poor taste, mostly due to the lack of good mouth feel and imbalance of flavour elements, which usually happens in the absence of alcohol (Malfliet, Goiris, Aerts, & DeCooman, 2009).

Fruit Juices

Fruits are highly perishable so the ability to extract juices and store them is of significant value. Some fruits are highly acidic and mixing them with water and sugars or honey was often necessary to make them palatable (Bajpai, 2014). Juice is a liquid naturally contained in fruit or vegetable tissue. It is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvent, and it is also a natural product that contains few or no additives. Suaad & Eman (2008) state that fruit juices are nutritious drinks with great taste and health benefits; unfortunately, there are several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the globe (Mosupye & Holy, 2000; Muinde & Kuria, 2005; Chumber, Kaushik, & Savy, 2007).

Gunnars (2014) asserts that, consumers often perceive fruit juice as healthy because it is natural and has the word “fruit” in it. Also, because fruits easily spoil, the ability to extract juices and store them is of significant value. However, what many people fail to realize is that fruit juice is also loaded with sugar. In fact, fruit juice contains just as much sugar and calories as a sugary soft drink and sometimes even more. People who drink sugary beverages do not feel as full as if they had eaten the same calories from solid food, and studies show that people consuming sugary beverages do not compensate for their high caloric content by eating less food (Pan & Hu, 2011). Fruit juice is not a better option. Even though it has more nutrients, it contains as much sugar (though from naturally occurring fruit sugars rather than added sugar) and calories as soft drinks.

A study by Piernas, Mendez, Ng, Gordon-Larsen & Popkin, (2014) showed that, consumers drinking sweetened beverages; whether low calories or not, tend to have an overall lower dietary quality, and this is one of the reasons why sugary drinks are among the most fattening foods in existence. They do not contribute to fullness, making one eat more (Malik, Schulze & Hu, 2006).

Forms and Types of Fruit Juices

Juice may be marketed in concentrate form, sometimes frozen, requiring the user to add water to constitute the liquid back to its "original state" (Doyle, 1991). Other juices are reconstituted before packaging for retail sale (Brandon & Ferreiro, 1998). Fruit juice consists of 100% pure juices and generally has no added ingredients (USDA, 2000). Although some minor exceptions exist like in cases where salt is added to tomato juice to ensure the final product is of an acceptable taste (Tetra Pak, 2000).

Types of Fruit Based Drink

Bajpai (2014) identified 10 types of fruit based drinks. They are:-

1. Fruit juice: - It is largely regulated throughout the world. Juice is often protected to be used for only 100% fruit.
2. Fruit drink: - 10% fruit is liquefied and water is added
3. Fruit squash: - It is produced using strained 25% fruit juice, 45% sugar and preservatives.
4. Fruit cordial: - All 'suspended matter' is eliminated by filtration and clarification. This type of drink is described as 'flavoured' and has no fruit.
5. Fruit punch: - It is a mixture of 25% fruit juices. It contains around 65% sugar.

6. Fruit syrups: - One fruit crushed into puree and left to ferment and then heated with sugar to create syrup.
7. Fruit juice concentrates: - Water removed from 100% fruit juice by heating or freezing.
8. Carbonated fruit beverages: - Carbon dioxide added to fruit drink.
9. Fruit nectars: - Mixture of 30% fruit pulp, sugar and water which is consumed as 'one shot'.
10. Fruit sherbets: - Cooled drink of sweetened diluted fruit juice.

Microbiological quality of fruit juices

Microbiological quality of drinks is established in order to ensure the safety of the consumer, and examining drinks microbiologically may assist in the assessment of hygienic precautions during production, the efficacy of a preservation process, allow prediction of the potential shelf-life, and also the identification of potential health hazards by the use of suitable indicators or by direct detection of pathogen. In recent years, the increasing consumer awareness has emphasized the need for microbiologically safe food.

Rashed *et al.,* (2012) investigated to resolve the microbiological attributes of the fruit juices collected from different areas around Dhaka city. To check the total bacterial load, coliforms and *staphylococci,* 26 vendor fruit juices and 15 packed juices were examined. Samples were found to harbor viable bacteria within the range between 10^2-10^7cfu/ ml. 30 samples exhibited the presence of *staphylococci.* Total coliforms were detected in 31 samples within the range of 10^2-10^6cfu/ ml which were further detected as *Escherichia coli* and *Klebsiella*

spp. Faecal coliformswere found in 4 vendor fruit juice samples (10^2cfu/ ml), while in the industrially packed samples, they were completely absent.

Drug resistance among the isolates was found against ampicillin, ciprofloxacin, amoxicillin, erythromycin, chloramphenicol, ceftriaxone, piperaciline, trimethoprime-sulfomethoxazole, nalidixic acid and vancomycin. Overall, the study demonstrates that the quality of both packed and fresh juices was unsatisfactory and hence the products needed to be microbiologically controlled in order to ensure the overall health safety. Tasmina *et al.,* (2010) conducted their study to assess the microbial quality of fresh and commercially packed available juices collected from different locations of Dhaka city. A total of six fresh juice and nine commercially packed juice samples were collected. Standard culture techniques were followed to assess total viable count (TVC), total Staphylococcal count (TSC), total Bacillus count (TBC) and total fungal count (TFC) on different culture media. The TVC varied from the range from 10^2 to 10^5cfu/ ml with the highest of 2.4 x 10^5cfu/ ml.

A large number of Staphylococci and Bacillus was also found from several samples. Total coliform and fecal coliform was found in six and five (out of fifteen) samples, respectively. Among total coliforms, *Klebsiella* spp., *Enterobacter* spp. along with *E. coli* was detected. From all the assessment it was determined that the microbial quality of commercially packed juice was fairer than that of fresh juice collected from local market.

Md. Munjur *et al.,* (2014) investigated to resolve the microbiological attributes of the fruit juices collected from different areas around Jessore city. Ten

fresh fruit juices and ten commercially packed fruit juices were collected. Standard plate count techniques were followed to assess total viable count (TVC), total coliform count (TCC) and total *Staphylococcal* count (TSC) on different culture media. Samples were found to harbor viable bacteria within the range between 10^3-10^8cfu/ ml. 19 samples exhibited the presence of *Staphylococci*.

Total coliforms were detected in 17 samples within the range of 10^3 - 10^6cfu/ ml which were further detected as *Escherichia coli, Klebsiella* spp. and *Enterobacter* spp. From the assessment, the study demonstrates that the quality of both packed and fresh juices was unsatisfactory and hence the products needed to be microbiologically controlled in order to ensure the overall health safety.

Joy *et al.,* (2006) aimed at examining the quality and safety of freshly squeezed fruit juices in a metropolitan city (Visakhapatnam) in south India, based on standard techniques (For example, culturing on selective media), showed that in most localities the street vended fruit juices remained hygienically poor since bacterial loads (Total viable counts and Total coliforms) on the whole are abnormally high (HVC 0.88-33.6×10^4cfu/ 100 ml; TC 0.8-22.2×10^4 CFUs/ 100 ml). Based on the presence of faecal coliforms (0.4-11.0 cfu/ 100 ml) and faecal Streptococci (0.0-6.6 cfu/ 100 ml), it is concluded that fruit juices in certain areas inside the city (for example, R.T.C., Complex, Fishermen's colony, Vegetable market) are highly impacted and unfit for human consumption. Overall, it was contended that contamination was mainly due to poor quality of water used for 11 dilutions, prevailing unhygienic conditions related to washing of utensils, maintenance of the premises, and location by the side of a busy road with heavy vehicular traffic or by the side of the waste disposal system and overcrowding.

The occurrence of pathogenic *E. coli*, Streptococcus faecal, *Salmonella typhi* and *Salmonella typhimurium* is alarming enough for an immediate action by the suitable agency. It was suggested that regular monitoring of the quality of fruit juices for human consumption be introduced to avoid any future pathogen outbreaks.

Another study was done to assess the microbial quality of fruit juices sold for immediate consumption in the markets of Kashmir valley. Twelve fruit juice samples (3 from each apple, orange, pineapple and mango juices) were procured from different markets and tested for their microbiological quality. Microbial quality was determined by enumerating the total viable count. About 25% of the samples (orange juice) did not comply with the standards of microbial quality as per the guidelines for microbiological quality of ready to eat foods while as apple, orange and pineapple juices complied with the standards. The microbial load in orange juice was comparatively higher than that in the apple, pineapple and mango juice which had the microbial load within acceptable limits (Gulzar *et al.*, 2013).

In developing nations like Ghana and Nigeria, it has not been possible to have control over the processing of hawked foods, because most of the vendors lack the adequate knowledge of food processing and handling practices (Essien, Monago & Edor, 2009). As such, there is likely to be a high risk of chemical and microbial contamination (Essien *et al.*, 2009). A large number of lactic acid bacteria, coliforms, Yeasts and Moulds, have been reportedly implicated in food spoilage as they use the carbohydrate content of the foods for undesirable

fermentation processes (Amusa, Ashaye, Aiyegbayo, Oladapo, Oni, & Afolabi, 2005; Essien *et al.,* 2009).

Freshly squeezed fruit and vegetable juices have little or no process steps that reduce pathogen levels if contaminated (Victorian Government Department of Human Services 2005; Durgesh, Ranjana, & Varsha, 2008). There are few published works on the health risk, especially in the Third World, that could arise from the consumption of soft drinks directly from the orifice of the opened bottles (Kigigha & Jonathan, 2012). Hoffmann *et al.,* as cited in Kigigha and Jonathan (2012), carried out the microbiological survey of non-alcoholic carbonated beverages, while Griffiths *et al.,* also cited in Kigigha and Jonathan (2012), carried out an analysis of the quality of the ingredients used in the soft-drink industries.

Amusa *et al*., (2005), carried out studies on the microbiological and nutritional quality of a hawked locally brewed soft drink called zobo in Nigeria while Oranusi *et al.,* cited in Kigigha and Jonathan (2012) studied the microbial contaminants of commercially bottled non-alcoholic drinks produced in Nigeria. Kigigha and Jonathan (2012) carried out microbiological assessment of opened soft drink bottles for pathogenic bacteria associated with drinking directly from the orifice in Nigeria. The aim of their study was to determine the microbiological quality of some packaged fruit juice sold in Port-Harcourt Metropolis, Nigeria. It also evaluated the sanitary quality of marketed packaged fruit juice. This would provide a background of microbiological data for development methods that would effectively reduce the microbial load of fruit juice including those

considered to constitute spoilage threat and potential health hazards to subsequent consumers.

According to the Ghana Standards Authority (2009), the Australia and New Zealand Standards (2002), there are requirements for food preparation in order to make it safe for consumption. Below is a table containing required microbiological standards for non-alcoholic drinks by the Ghana Standards Authority.

Table 2: Microbiological limits for non-alcoholic drinks from the Ghana Standard Authority

No.	Product	Test	n	c	m	M
i.	Carbonated soft drinks and carbonated malt drinks	APC	5	2	10^2	10^3
		Yeasts and Moulds	5	2	10^1	$5x10^2$
ii.	Fruit juices, squashes and cordials, fruit juices preserved by physical and chemical means	APC	5	2	10^2	10^3
		Coliforms	5	1	10^1	10^2
		Yeasts and Moulds	5	2	10^1	$5x10^2$
iii.	Concentrated fruit juice preserved exclusively by physical means	APC	5	2	10^1	10^2
		Coliforms	5	1	$5x10^1$	-
		Yeasts and Moulds	5	2	10^1	$5x10^1$
iv.	Fresh fruit juice with no physical or chemical preservatives	APC	5	2	10^2	10^3
		Escherichia coli	5	2	10^1	10^2
		Salmonella spp.	10	0	0	-

(GSA 7265, 2003)

n = the number of sample units which must be examined from a lot* of food. Most sampling plans specify taking five sample units. However, when the risk has been assessed as relatively high, a greater number of sample units is specified.

c = the maximum allowable number of defective sample units. This is the number of sample units, which may exceed the microbiological limit specified by **'m'**. These are considered marginal results, but are acceptable providing they do not exceed the limit specified by **'M'.** For example, the standard for carbonated soft drinks and carbonated malt drinks allows for two samples (c=2) to exceed the acceptable microbiological level of 10^2 ('m'=10^2), providing no sample exceeds a level of 10^3 ('M'=10^3). In many cases c=0 which means no sample may exceed the specified limit 'm'.

m = the acceptable microbiological level in a sample unit. Sampling plans in which m=0 and **c**=0 are equivalent to 'absent' or 'not detected' reporting for the stated analytical unit size. In most cases this is 25 g (e.g. not detected in 25 g).

M = the level which, when exceeded in one or more samples, would cause the lot to be rejected.

*A lot means a quantity of food, which is prepared or packed under essentially the same conditions.

Escherichia coli

E. coli is a gram negative bacterium, which lives in the digestive tracts of humans and animals. There are many types of *E. coli*, and most of them are harmless. *E. coli* infection occurs by coming into contact with the feces, or stool of humans or animals. By drinking water or eating food that has been contaminated by feces anyone can get infected by *E. coli*.

Salmonella spp

Salmonella spp. are infectious bacteria associated with food borne and gastrointestinal illnesses. *Salmonella* bacteria can be found in food products such as raw poultry, eggs, and beef, and sometimes on unwashed fruit. There are two main diseases caused by *Salmonella* spp. and they are Salmonellosis and typhoid fever. *Salmonella enteritidis* or *Salmonella typhimurium* causes Salmonellosis and Typhoid fever is caused by *Salmonella typhi*. People who eat food contaminated by *Salmonella* can become ill with salmonellosis.

Yeasts and Moulds

Yeasts and Moulds are large and diverse group of microscopic foodborne yeasts and Moulds (fungi) includes several hundred species (Lodder, 1970). The ability of these organisms to attack many foods is due in large part to their relatively versatile environmental requirements. Although the majority of yeasts and Moulds are obligate aerobes (require free oxygen for growth), their acid/alkaline requirement for growth is quite broad, ranging from pH 2 to above pH 9. Endrizzi, Pirretti, Calo, and Gasperi (2009), stated that both yeasts and Moulds cause various degrees of deterioration and decomposition of foods. They can invade and grow on virtually any type of food at any time; they invade crops such as fruits in fields before harvesting and during storage. They also grow on processed foods and food mixtures. Their detectability in or on foods depends on food type, organisms involved, and degree of invasion.

The contaminated food may be slightly blemished, severely blemished, or completely decomposed, with the actual growth manifested by rot spots of

various sizes and colours, unsightly scabs, slime, white cottony mycelium, or highly coloured sporulating moulds. Abnormal flavours and odours may also be produced. Occasionally, a food appears Moulds-free but is found upon mycological examination to be contaminated. Contamination of foods by yeasts and Moulds can result in substantial economic losses to producer, processor, and consumer (Endrizzi, Pirretti, Calo, & Gasperi, 2009).

Coliforms

The presence of coliforms on the surface of vegetables is indicative of faecal contamination (Reddy & Reddy, 2000). According to Salle (2000), cholera transmission was associated with most consumption of street vended beverages. The normal habitat of faecal coliforms is the intestinal tracts of man and animals and they are not known to be found in nature in the absence of faecal contamination from the man and animals. They are excluded out of animal body through excretion process, in the form of faeces. Some of them are pathogenic and cause diseases like typhoid, dysentery, enteric fever and many more. Thus, the presence of these organisms in water and fruit juices is dangerous for human consumption (Salle, 2000).

Nutritional Value of Fruit Juices

According to Ashurst (2016), fruit juice is important in human nutrition far beyond its use as a refreshing source of liquid. Many fruits contain a variety of minor ingredients, particularly vitamins and minerals, as well as carbohydrates, which are the predominant solid component. Although fruit contains small amounts of protein and fat, these are not important ingredients of juices. Nutrients

frequently consumed in sub-optimal concentrations by humans are proteins, calcium, iron, vitamin A, thiamin (vitamin B1), riboflavin (vitamin B2) and ascorbic acid (vitamin C). However, some of these nutrients occur in higher concentrations in fruit juices than in other foods (Ashurst, 2016).

Fruit juices are mostly consumed for their perceived health benefits. For example, orange juice is rich in vitamin C, folic acid, potassium, which are an excellent source of bioavailable antioxidant phytochemicals (Franke, 2005) and in people affected with hypercholesterolemia it significantly improves blood lipid profiles (Kurowska, Spence, Jordan, Wetmore, Freeman, Piché, & Serratore, 2000). Prune juice is associated with a digestive health benefit. Cranberry juice has long been known to help prevent or even treat bladder infections, and it is now known that a substance in cranberries prevents bacteria from binding to the bladder.

Pomegranate juice reduce dangerous LDL-cholesterol in blood, improve blood flow to the heart in patients with coronary artery disease, reduce thickening of the arteries that supply blood to the brain, lower the level of systolic blood pressure also an antioxidant-rich fruit. This fruit may also be able to help fight cancer which researchers have been looking for. Mango juices are perfect to replenish salts, vitamins and energy after physical exercise. In gall bladder cancer a protective effect of mangoes consumes has been proven. Mango juice also contains a lot of tryptophan, the precursors of serotonin. Litchi juice contains high amount of antioxidants which is effective to prevent early ageing also effective to protect from asthma and a rich source of nutrient that is required for the

production of blood. It provides manganese, magnesium, copper, iron and folate that are required for the formation of Red Blood Cells (RBC) (Mortuza, 2016).

The most predominant nutrient in grape juice is manganese. Drinking grape juice helps fight conditions associated with cardiovascular disease, including high blood pressure and plaque build-up. Jujube fruit contains high levels of Vitamin C (higher than in most citrus fruits like the orange), Vitamins B1 (thiamine), B2 (riboflavin), B3 (niacin) and B6 (which are the complex B vitamins), Vitamin A (in 2 forms), and minerals that include calcium, potassium, phosphorus and manganese. It also contains significant levels of copper, zinc, iron and sodium. Perhaps the most significant of all of the jujube benefits is the fact that the fruit contains 18 amino acids. Also, some vegetable juices provide the same health benefits as whole vegetables in terms of reducing risks of cardiovascular disease and cancer (Shenoy, Kazaks, Holta, & Keena, 2008).

Consumption of non-alcoholic beverages also contributes various kinds of nutrients to the diet. The microbiological safety and stability of this diverse group of products depend on their formulation (including the use of chemical preservatives, carbonation, low pH values, and pasteurization). Most microbiological problems arise because of poor quality of raw materials (fruit concentrates, sugars, and syrups) and poor processing hygiene, which lead to overcoming of the preservation system applied during manufacture by the spoilage organisms (Kregiel, 2015).

There is a need for routine sampling of raw materials, preventive maintenance of equipment, and monitoring of those parts of the process where

microbiological contamination can occur (during filling and in holding tanks). For the routine monitoring of raw materials, simple microbiological tests such as the use of the direct microscopic count, yeast and Moulds count, or a standard plate count (using a medium capable of supporting lactic acid bacteria) are most generally applicable (Kregiel, 2015).

Pasteurization of Fruit Juices

Pasteurization of fruit juice involves brief exposure to high temperature (80^0C at 3 seconds) in ultra-pasteurization, which will destroy all forms (Kisko & Roller, 2005). The main reason for the heat treatment of juice, nectar and still drinks are to make them safe for consumption and prolong shelf life. Pasteurization kills microorganisms that can grow during storage and inactivates enzymes that cause unwanted clarification (cloud loss). Since fruit beverages in general are high-acid products (pH 4.6 or less), they do not require ultra-high temperature treatment (UHT). This is because their high acidity inhibits the growth of bacteria, fungi and yeast. Their heat treatment should be safe, but still be able to deliver quality in terms of vitamins, colour and taste (Tetra Pak, 2000).

Fruit juices are watery mixtures of mostly unstable volatile organic compounds. They are heat sensitive and their colour and flavour deteriorate rapidly as processing temperatures are increased (Belibagli & Dalgic 2007; Yildiz, Bozkurt, & Icier, 2009). They are rendered practically sterile by brief pasteurization as the pH is low. Kisko and Roller (2005), reported that a simple heat treatment can be used to destroy all of the microorganisms present in a juice, which will render it sterile (the process of sterilization), or heat treatment can be used to destroy most of the microorganisms (the process of pasteurization). There

are various reports on the outbreaks of illness in humans associated with consumption of unpasteurized fresh fruit juices. In 1995, unpasteurized fresh orange juice contaminated with *Salmonella* was linked to an outbreak in Florida Theme Park, USA, where more than 60 visitors were affected (Schmidt, Sims, Parish, Pao & Ismail, 1997).

Sugars in Non-Alcoholic Drinks

Sugar is a white crystalline carbohydrate used as a sweetener and a preservative. Sugars are used extensively in food industries and at home as a sweetener and as a source of energy particularly in non-alcoholic beverages (Langer, 1995). Sugars are the most abundant and widely distributed food component in the form of carbohydrate. Sugar, one of the major contents of soft drinks is a paradox. In as much as it plays vital roles in food technology and medicine, it is also toxic to the body when it is above normal levels in human blood (4.5-5.5 g/dm^3). High blood sugar levels have been found to promote heart failure and stroke. High sugar levels can poison the body and destroy the organs unless kept in check by an agent called insulin (Agbazue, Ibezim & Ekere, 2014). Carbohydrates provide most of the energy in almost all human diets. In the diets of poor people, especially in the tropics, up to 90 percent of the energy may come from this source. On the other hand, in the diets of the rich in many countries the figure may be as low as 40 percent (Davidson, 1999).

Types of Sugars and Sweeteners

Sugars can be divided into three major groups: monosaccharides, oligosaccharides and polysaccharides. Simple sugars are sweet in taste and are broken down quickly in the body to release energy. Table sugar (sucrose) is

obtained commercially from only two plants, sugar cane and beet sugar, which provide 56% and 44%, respectively of the world's total. Cellulose and Sugar (especially sucrose) can be obtained from natural sources such as sugar cane, beet sugar, honey, and others. It is difficult to determine when sugar (saccharimofficinarum) first became known to mankind, but there is an indication that it came from New Guinea to India many centuries before Christ (ICUMSA, 1978; Birch & Parke, 1980; Brain & Allan, 1984; Gary, 1986).

With the exception of zero calorie products, soft drinks usually contain between 1% - 12% sugars (w/w) (Kregiel, 2015). Sucrose, glucose, or fructose in various forms is used as natural carbohydrate sweeteners. The most common natural sweeteners provide glucose, the primary source of energy. Sucrose (saccharose) is a disaccharide composed of glucose and fructose molecules bound by an α-1, 2 linkage. This sugar can preserve and enhance the flavour of a drink and gives a satisfying sensation. Other natural carbohydrate sweeteners are as follows: trehalose, isomaltulose (Palatinose), and D-tagatose (Ashurst & Hargitt, 2009). Trehalose is a disaccharide composed of two glucose molecules bound by an α-1, 1-linkage. This compound is characterized by high thermostability and a wide pH stability range. Its relative sweetness is around 45% that of sucrose. The metabolism of trehalose is similar to other disaccharides. Ingested trehalose is hydrolysed into glucose and absorbed in the small intestine (Mortensen, 2006). Isomaltulose, like saccharose, is a disaccharide of glucose and fructose, but in contrast to sucrose is joined by an α-1, 6 glycosidic bond. Isomaltulose is a tooth-friendly disaccharide with slow energy release, low glycemic index, and mild sweetness (Hausmann, 2009). Tagatose has a structure similar to that of fructose.

It is almost as sweet as sucrose and has flavour-enhancing properties. Most ingested tagatose is fermented by colon microflora, resulting in the production of short-chain fatty acids, which are then absorbed almost completely and metabolized.

Due to health; including dental health concerns, alternative sweeteners are commonly added to soft drinks which are produced and labelled as containing "no added sugar" (Serpen, 2012). According to Fitch & Keim (2012), most low-calorie beverages contain intense sweeteners, which have been approved for use within levels of Acceptable Daily Intake (ADI) and in accordance with the appropriate regulations. The most commonly used sweeteners (with maximum permitted dosage in the EU) are aspartame (600 mg/L), acesulfame K (350 mg/L), sucralose (300 mg/L), and saccharin (80 mg/L) (Fitch &Keim, 2012).

Aspartame (E951) consists of two amino acids: L-phenylalanine and L-aspartic acid, esterified to methyl alcohol. This compound is 200 times sweeter than sucrose and leaves no unpleasant aftertaste. However, it is unstable at high temperatures and therefore is unsuitable for use in pasteurized beverages. Aspartame is also unstable in aqueous solutions, where it is gradually converted into diketopiperazine (DKP). In the body, aspartame is broken down into phenylalanine (about 50% by weight), aspartic acid (40%), and methanol (10%). Soft drinks with aspartame must carry a label indicating that the product contains phenylalanine, which can be harmful to individuals with phenylketonuria, who must strictly limit the intake of this amino acid. Aspartame is permitted in more than 100 countries in the world. Authorities that have approved aspartame include the FDA, the Agence Française de Sécurité Sanitaire des Aliments (AFSSA), and

the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In 2013, the European Food Safety Authority (EFSA) approved aspartame for use in food and beverages (EFSA, 2013).

Saulo (2005), posits that, acesulfame K (E950) is 200 times sweeter than sucrose, thermo- and pH-stable, and freely soluble in water. This compound is neither metabolized nor stored in the body. The FDA, FAO/WHO, JECFA, and the Scientific Committee on Food of the European Union (SCF) have concluded that acesulfame is safe for use in foods and beverages. Sucralose (E955) is derived from sucrose through selective replacement by chlorine atoms of three hydroxyl groups. This compound is 600 times sweeter than sucrose but has no calories. Readily soluble in water and acid solutions, sucralose hydrolyses slowly to its monosaccharides. Sucralose has been determined safe by FAO/WHO, JECFA, and FDA and is permitted for use in beverages in more than 40 countries, including the United States, Canada, Australia, and Mexico (Saulo, 2005). In 2011, sucralose, along with other sugar substitutes, was cleared for use by the EFSA as a sweetener in food and beverages (EFSA, 2011). On the other hand saccharin (E954) is 300 times sweeter than sucrose but leaves a bitter/metallic aftertaste. The use of saccharin in foods dates back to 1907. This sweetener is permitted in more than 100 countries around the world (Stratford & James, 2003; Mortensen, 2006).

Other less common sweeteners include thaumatin (E957) and stevioside (E960). Thaumatin is a mixture of proteins isolated from the katemfe fruit (Thaumatococcusdaniellii Benth) from West Africa. It is the most powerful natural sweetener, 2000 times sweeter than sugar. It is used in food as a safe

sweetener and flavour modifier (Ashurst & Hargitt, 2009). Stevioside is another intense sweetener, 200 times sweeter than sucrose, extracted from the leaves of the stevia plant (*Stevia rebaudiana* Bertoni). It has a long history of use in several countries, including Japan and Paraguay (Mortensen, 2006). Stevioside is permitted for use in many countries, including the USA, France, Mexico, Korea, Taiwan, China, Russia, Australia, Argentina, New Zealand, Colombia, Peru, Uruguay, Brazil, Switzerland, and Malaysia. In Canada, stevia extract is sold as a natural health product. In Europe (except France), stevia is permitted as a dietary supplement but is not yet permitted for use as a sweetener in food and beverages (EFSA, 2010). In France, stevia extract (rebaudioside A) is permitted for use as a sweetener in foods and beverages (McQuate, 2011). Other sweeteners used more rarely in soft drinks include cyclamate, erythritol, and neotame (Fitch & Keim, 2012). Over the years, the number of available sweeteners has steadily increased.

Negative Health Effects of Sugars and Sweeteners in Non-Alcoholic Drinks

As is well known, overconsumption of sugars can cause negative health effects, such as obesity, diabetes mellitus, or non-alcoholic fatty liver disease. Natural sweeteners provide 0.4 calories per gram and have been linked with weight gain (Fitch & Keim, 2012). Fructose contributes to the formation of advanced glycation end products, which may be factors in the onset of diabetes, hasten aging processes, and cause thickening of artery walls (Serpen, 2012). Since tagatose is absorbed slowly and only incompletely in the intestine, consuming excessive amounts may lead to flatulence and/or laxation (Stratford & James, 2003).

It does not matter whether the sugars you drink come from fruit juice, smoothie or fizzy drinks; the liquid fructose sugar is dangerous for our health, irrespective of the source. These sugars are overloading our livers and leading to health issues such as heart problems, cancers, diabetes, dental caries and obesity, which affect our quality of life. Hodgekiss (2013), reports that using fizzy drinks and fruit juices by human beings as a way to get their five a day is high in fructose, which affects blood sugar levels. Researchers Zelber-Sagi, Nitzan-Kaluski, Goldsmith *et al*., (2007) and Ouyang, Cirillo, Sautin, *et al.*, (2008), further explain that, any sugar that the body does not need is converted to fat which makes people become fat and also when the liver takes in more fructose than it can handle, some of it gets turned into fat. Some of the fat can be stored in the liver and contribute to fat build-up and insulin resistance.

Although small amounts of fruit juice (or soda) are unlikely to cause major problems for healthy lean and active people, this can be a complete disaster for people who are overweight or have diet-related metabolic problems (Stanhope & Havel, 2008). Controlled metabolic studies show that liquid sugar can cause insulin resistance, raise triglycerides and small, dense LDL cholesterol, elevate oxidized LDL cholesterol and cause belly fat accumulation in as little as 10 weeks (Stanhope, Schwarz, Keim, Griffen, Bremer & Graham *et al*., 2009).

Gunnars, (2014) explains that a single serving of the so-called healthy fruit juice has been found to contain the same amount of sugar as three-and-a-half doughnuts or 13 hobnob biscuits. Until recently, consumers perceived Coke and Pepsi as the ‘bad’ drinks, while the fruit juices were the easy way to get the ‘five a day’, but the goal posts have shifted because many more experts are warning that

sugary drinks in any form are largely to blame for the expanding waistlines of consumers (Gunnars, 2014).

According to Hollis, Houchins, Blumberg and Mattes (2009), although most of the studies are on sugar-sweetened or fructose-sweetened drinks, there is no reason to assume that 100% fruit juice would be any different. The sugar molecules are identical and the liver will not be able to tell the difference. They report that, some studies did use actual fruit juice and in one of them, 480 ml (16 ounces) of grape juice per day for 3 months caused insulin resistance and increased waist circumference in overweight individuals. In another study, consuming 2 or more servings of fruit juice per day was associated with more than a doubled risk of gout in women (Choi, Willett & Curhan, 2010). Most people are already eating way too much sugar and reducing sugar intake is much more important than getting the small amount of nutrients found in fruit juice.

Acids in Non-alcoholic Drinks

Sorbic acid and the sorbates are the preservatives mostly used in the soft drinks industry. The sorbic acid acts efficiently against the growth of yeasts and Moulds and of some bacteria, acting at a low pH, however, it continues to be efficient at a pH of 6.5. The microorganisms' growth inhibition is achieved through the interaction of the system of the two conjugated double bound in the aliphatic chain with cellular dehydrogenases which most of the yeasts and Moulds cannot metabolize. In fruit beverages preservation, the sodium salt of the benzoic acid is frequently used, as it is more soluble than the free acid; however, the un-dissociated acid, formed from the salt dissolved in solution, is responsible for the

antimicrobial activity, which is optimum at a pH value ranging from 2.5 to 4 (Varnam & Sutherland, 1999; Akpan & Kovo, 2005).

Although there are other acids present in fruit juices (for example, oxalic, iso-citric, tartaric), it is usual to record acidity in terms of citric acid, both for citrus fruit juices and for the majority of soft fruit juices. Where apple and other pome fruit juices are concerned, the major organic acids are malic and citric, although usually malic predominates. In some varieties of pears, the two acids can occur in approximately equal proportions. Both acids are usually measured in % w/w terms in their anhydrous form, although it is sometimes convenient to determine titratable acidity for citric acid in terms of its monohydrate form, as this form of the acid may be used in the formulation of certain beverages.

As a general rule, the acidity of juices will decrease with increasing maturity of the fruit source, or with increasing levels of sugars in the resulting juice. Hence the ratio of soluble solids (Brix values) to acidity is an important value in the assessment of juice quality. The Brix/acid ratio is frequently used to establish standard sensory, or taste, qualities for incoming juice supplies and to minimise the effect of seasonal variation. The higher the Brix value in relation to the acid content of the juice, the higher the ratio and the 'sweeter' the taste.

Vitamin C (Ascorbic Acid)

Many juices contain ascorbic acid or vitamin C, which is quantitatively the most important vitamin in soft fruits, ranging from a negligible level in some whortleberries to around 200 mg/100 g in blackcurrants. Ascorbic acid performs a valuable function as an antioxidant in minimising degradation of certain flavour principles, and it is often important for it to be included in the processed juice or

in a soft drink formulation. Levels in the range 200–400 mg/kg are typical. It should be noted that ascorbic acid can be added to natural strength juice only if it is intended for direct use; otherwise, it should be added to the juice concentrate. Addition of vitamin C to natural strength juice before its concentration will result in its own degradation during the heating process and ultimate spoilage of the product when an intense browning reaction takes place (Steen & Ashurst, 2006).

Health Benefits and Deficiencies of Vitamin C in the Human Body

Vitamin C's immuno-protective, anti-inflammatory, antiviral, and antibacterial roles are well-known. It works as the co-factor for a number of enzymes (including collagen synthesis) and as a water-soluble antioxidant. Researchers prize vitamin C because it benefits numerous conditions including cancer and neurodegenerative diseases. Vitamin C plays multiple roles to support and maintain health (Maxliving Health Expert, 2018).

Immunity

That vitamin C could successfully treat or prevent the common cold. Subsequent studies have yielded mixed conclusions, and today many critics regard Pauling as either brilliant or controversial. Several cells within the immune system, such as phagocytes and T-cells, can accumulate vitamin C. Conversely, vitamin C deficiencies result in a reduced resistance against certain pathogens; more of this vitamin can support the immune system. Oxidative stress (when free radicals overpower your body's antioxidant defenses) and inflammation are drivers in many illnesses including the common cold. Vitamin C can help reduce both. As a powerful antioxidant, vitamin C contributes to immune defense by supporting the immune system. Vitamin C deficiencies result in impaired

immunity and higher susceptibility to infections, including respiratory infections and whole-body infections. In turn, infections significantly affect vitamin C levels due to increased inflammation (Maxliving Health Expert, 2018).

Detoxification

Over 80,000 chemicals exist in furniture, cosmetics, cleaners, and food. Most have not been adequately tested for their impact on human health. Detoxification is not something that happens a few times a year (although a professionally designed plan can help elevate your body's ability to detoxify). Your cells constantly detoxify so providing them optimal amounts of nutrients becomes crucial. Among them includes vitamin C. One animal study found that high doses accelerated the excretion of lead, one of the most toxic heavy metals, compared with low doses of vitamin C (Maxliving Health Expert, 2018).

As an antioxidant, vitamin C can neutralize and remove environmental pollutants, the damage that occurs due to ultraviolet (UV) radiation and other pollutants. One study found healthy young adults low in vitamin C had significantly increased levels of oxidative stress and reduced antioxidant capacity. Vitamin C is only one player in the antioxidant arsenal. Others include vitamin E, glutathione, and carotenoids (vitamin A). For instance, vitamin C can help reduce oxidative damage to the skin combined with vitamin E (Maxliving Health Expert, 2018).

Inflammation

Vitamin C also has the power to reduce chronic inflammation, which can increase with high levels of toxicity in some people. One study found that supplementing with 500 mg of vitamin C twice daily could alleviate inflammatory

markers in hypertensive and/or diabetic obese patients. Vitamin C also plays a role in glutathione. This master antioxidant protects against oxidative stress, mercury, other toxic metals, alcohol, and persistent organic pollutants (POPs). The amount of glutathione within your cells is highly associated with health and longevity (Maxliving Health Expert, 2018).

Glutathione helps detoxify potentially dangerous compounds, excreting them or directly neutralizing them. Researchers associate low levels of glutathione with numerous complications including macular degeneration, Parkinson's disease, and other neurodegenerative disorders. Glutathione and vitamin C work as a team. Glutathione recycles vitamins C and E. In turn, vitamin C's free radical-fighting abilities can spare glutathione levels, thereby increasing glutathione levels and leaving this master antioxidant to protect against other free radicals. Vitamin C can also perform as an antioxidant in its own right, even with sufficient amounts of glutathione. One study found just a 500-mg vitamin C supplement daily maintains glutathione concentrations and improves overall antioxidant protection (Maxliving Health Expert, 2018).

Collagen Production

Collagen is an abundant structural protein, comprising of about one-third of the total protein in humans. If you want glowing skin, healthy hair and nails, supple joints, and strong bones and muscles, you want optimal amounts of this ubiquitous protein. To support collagen production, you need optimal vitamin C, which contributes to two key enzymes in collagen synthesis. Vitamin C interacts with amino acids within collagen cells to provide hydrogen and oxygen so those amino acids can make collagen. Without optimal vitamin C levels, collagen

production slows down making your skin more susceptible to wrinkles and bruising. Several reports find lower vitamin C levels in aged or photo damaged skin (Maxliving Health Expert, 2018).

Skin-related aging occurs from many factors, including excessive sun exposure, smoking, and environmental stress. Some research shows that consuming significant amounts of vitamin C can improve elasticity, wrinkling, and other skin benefits. These studies included a high intake of fruit and vegetables, which contribute vitamin C, as well as other nutrients (Maxliving Health Expert, 2018).

Vitamin C also plays a role in joint health. After controlling for confounding variables, researchers found that vitamin C supplementation could help prevent knee osteoarthritis, the most common form of arthritis that includes joint pain and swelling. Vitamin C can also benefit rheumatoid arthritis and provide joint protection due to its antioxidant potential, its role as a cofactor in collagen synthesis, and its ability to fight infection via its anti-inflammatory protection (Maxliving Health Expert, 2018).

pH

pH is a measure of the acidity or alkalinity of a food. Acid content (% w/w) of a drink is determined using a pH meter by direct titration against standardised alkali solution (for example, 0.1 M sodium hydroxide) to an end-point at pH 8.2. When the juice is naturally clear, or has been clarified, and is of low colour intensity, the end-point may be accurately found using phenolphthalein as indicator. The effects of acids and alkalis depend on the

strength of the acid or alkali and the concentration. Strong concentrated acids or alkalis are corrosive, whereas dilute and weak acids and alkalis are not corrosive.

pH alone is not the primary determinant of adverse effects, and in water, acids and alkalis are normally extremely dilute. The pH of stomach fluid, which contains hydrochloric acid, is between 1.0 and 3.5, with a mean of approximately 2.0, and there is a range of commonly encountered foods that are also of low pH. These include lemon juice, with a pH of 2.4, and vinegar, with a pH of 2.8. Moreover, because pH can affect the degree of corrosion of metals as well as disinfection efficiency, any effect on health is likely to be indirect and due to increased ingestion of metals from plumbing and pipes or inadequate disinfection (FAO/WHO, 2007). pH has a profound effect on the growth of microorganisms. Most bacteria grow best at about pH 7 and grow poorly or not at all below pH 4. Yeasts and molds, therefore, predominate in low pH foods where bacteria cannot compete. The lactic acid bacteria are exceptions; they can grow in high acid foods and actually produce acid to give us sour milk, pickles, fermented meats, and similar products. Some strains, called Leuconostoc contribute off-flavours to orange juice (Aggie Horticulture, 2016). Chung and Goepfer, (1970) report that the minimal pH for growth of principal foodborne disease organism such as *Salmonella* spp. is 4.0

The acidity or alkalinity of a food affects the ability of bacteria to survive and grow. While most bacteria prefer a pH near neutral (pH = 7.0), the bacteria associated with foodborne illness from juice and cider have been known to grow in a pH of as low as 3.5 (acidic). The pH of most fruit juices falls between 2.3 and 3.5, while the pH of apple cider can range from around 3.4-4.0. The pH of tomato

juice ranges from 4.1 – 4.2; carrot juice around 6.4; pineapple juice from 3.3-3.5; cranberry juice from 2.3-2.5; orange juice from 3.6-4.3 and lemon juice from 2.2-2.6. pH alone is not the primary determinant of adverse effects, and in water, acids and alkalis are normally extremely dilute. The pH of stomach fluid, which contains hydrochloric acid, is between 1.0 and 3.5, with a mean of approximately 2.0, and there is a range of commonly encountered foods that are also of low pH. These include lemon juice, with a pH of 2.4, and vinegar, with a pH of 2.8. Hence, because these are weak acids, they pose no threat to health from their consumption (WHO, 2007). Igyor et al. (2006) reported that the pH value of traditional Nigerian beer (Burukutu) ranged from 3.36-4.86.

Titratable Acidity (TTA)

TTA is a measure of the amount of acid present in a solution. The evaluation of fruit juice will as a rule, require an expert appraisal of the entire analysis. In practice there will be key parameters to be noted and adhered to during the manufacture of fruit juices and related products. Of these, the soluble solids content and titratable acidity are the major indicators to be taken into account when identifying the status and suitability of a juice product for use in an application. The acidic character of a juice contributes to its flavour type and is taken into consideration when assessing the value of the juice for inclusion into new beverage product formulations (Ashurst, 2016).

Temperature

According to Aggie Horticulture (2016), temperature is the most efficient means to control microbial growth. Based on their tolerance of broad temperature ranges, microorganisms are roughly classified as follows: i. Psychrophilies grow

only at refrigeration temperatures. Ii. Psychrotrophs grow well at refrigeration temperatures, but better at room temperature. iii. Mesophilies grow best at or near human body temperature, but grow well at room temperature. Thermophiles grow only at temperatures about as hot as the human hand can endure, and usually not at all at or below body temperature (Aggie Horticulture, 2016).

Storage of Non-Alcoholic Drinks

Preservation of fruit juice by pasteurization, refrigeration and sterilization are popular methods used to attain microbiological stability by destroying pathogenic microorganisms and to preserve the colour, aroma and taste of fresh juice (Elmahmood & Doughari, 2007). In a situation where storage of juice is indispensable, may be for a short duration, then the use of simple processing techniques soon after its extraction is essential for improving the keeping quality to ensure its safety, nutritive quality and acceptability for consumption.

Packaging of Non-Alcoholic Drinks

According to Business Dictionary (2017), packaging is the process such as cleaning, drying, preserving and materials such as glass, metal, paper or paperboard, plastic employed to contain, handle, protect, and/or transport an article. The role of packaging is broadening and may include functions such as to attract attention, assist in promotion, provide machine identification (barcodes and others), impart essential or additional information, and help in utilization. Packaging has a very important role in protecting and extending the shelf life of non-alcoholic beverages, and it is widely categorized in different package types such as bottles, cans, cartons, pouches, and so on. By choosing the right packaging types, it can definitely help preserve the taste of non-alcoholic

drinks and prevent unwanted chemical reactions that can possibly endanger the health of the consumers. Thus, using efficient packaging is crucial for every type of drink (Admin, 2015).

According to Ashurst (1995), traditionally, most beverages are packed in glass and this has many attractive features, not least that it is an excellent protective medium, but its overriding disadvantages are its weight and brittleness. Despite this, high volumes of soft drinks and juices are still packaged in glass, some of it multi-trip packaging. The development of the board–polymer–aluminium package used to form in-line boxes, which are packed aseptically, has been perhaps the outstanding packaging development for beverages. The pack provides an almost ideal combination of protection, minimal weight and economic size. Another important packaging development area is plastic. Various plastics have been and continue to be used: high- and low-density polyethylene (HDPE, LDPE), polyvinyl chloride (PVC), polystyrene (PS) and various barrier plastics. These can be formed into bottles of conventional shape or fed into machines producing form–fill–seal packages, typically cups. By far the most important plastic is polyethylene terephthalate (PET) (Ashurst, 1995).

Drinkopaedia (2016), also states that non-alcoholic beverages are packaged in a variety of formats: Glass, cans, cartons and plastic bottles known as PET. They are also available in a wide variety of sizes. Packaging protects the quality of beverages and also provides a platform to communicate with consumers by providing information on pack such as details of ingredients or calories.

Types of Packaging Materials

Drinkopaedia (2016) identified four different types of packaging materials for non-alcoholic drinks:

1. ***Cans:*** In Europe, aluminium and steel are used as materials for beverage cans. Cans have undergone considerable light weighting programmes in recent years and today are more than 40% lighter than they were in 1970. Forty years ago a can weighed around 80 grams, today a 330 ml steel can weighs around 21 grams and an aluminium can may weigh as little as 10 grams. As a result the industry can produce almost three times as many cans using the same amount of metal as 30 years ago. Lighter cans also mean that many more can be transported on one truck, making for less energy use and reduced emissions. Beverage cans are the most recycled beverage containers globally. In the EU 15, the recycling rate of steel packaging is over 70% and the recycling and use of recycled content in cans saves us to 95% of the energy used for the production of virgin materials.
2. ***PET (Polyethyleneterephthalate):*** Plastic bottles known as PET containers are an increasingly popular packaging format. They are re-sealable and so ideal for people who need to stay hydrated, and they are transparent, enabling consumers to see the drink that they are buying. PET containers are 100% recyclable and the average bottle has reduced in weight by some 50% over the past 10 years.
3. ***Glass:*** Glass bottles are particularly used to package non-alcoholic beverages sold in cafes, bars and restaurants (known as the on trade). There are two types

of bottles – those for single use – which are light in weight, and those which are reused and refilled and so are heavier and more robust as a result.

4. ***Cartons:*** Beverage cartons are made out of paper and have an aluminium lining. They are used to package both single serve and multi-serve drinks. They are relatively light and increasingly recyclable with some 35% of beverage cartons now being recycled in the EU.

In addition, Admin (2015) also identified the seven different types of packaging materials:

The Sixer: This is an innovative, portable, and lightweight package that has gained the attention of consumers. Introduced by Coke, the 6-pack of 12 ounce cans in a slim cardboard package can be displayed vertically or horizontally in stores or retail shops. This compact packaging offers opportunity for convenience and retail stores to boost their sales.

1. ***Aseptic Cartons:*** Aseptic packaging is seen to stand out in the future because it replaces round bottles, and it offers benefits such as shelf impact since it has a flat front panel. It can also withstand in non-refrigerated trucks; it is space efficient and safe because its caps are sealed well after filling and offers extended shelf life that minimize waste for customers to consume the product before its spoilage
2. ***Foil-Wrapped Packs:*** This packaging was introduced by Lipton, and instead of using the individually white tea bags, consumers can opt for the foil packs that resemble gold bullion bars. This stay-fresh type of packaging connects with the label's famous tagline "Brighten your day with Lipton tea."

3. ***Pure-Pak Carton:*** These are redesigned cartons that integrate environmental profile and convenience. The materials of these types of packaging utilize bio-based low-density polyethylene (LDPE). Tetra Pak is one company that is known for this packaging type, and it aspires to make the first carton that is purely plant-based and will be commercially available. On the other hand, another company, Elopak, features certified renewable polyethylene (PE) beverage cartons that have renewable coating.
4. ***XanCan:*** This patent-pending aluminium beverage packaging has patterns that are customizable. XanCan can be recycled and ideal for any type of beverages from soft drinks, energy drinks, and iced teas.
5. ***Thermally Insulated Packaging:*** This packaging type is perfect for all-natural organics, vegetables, fruits and herbal juices. The concept of its label design is clean and simple with bottle specifications that are 17oz PET juice bottle. The clearness of the bottle aids consumers in identifying the type of juice they are drinking.
6. ***Mocktails:*** These packaging forms have polypropylene (PP) dispensing lids. Its package can be sealed in colourful full-body shrink label. These Mocktails are packed in 21-ounce cocktail shakers that contain 18 ounce beverage or four servings. To use this, open the bottle, add ice, apply the shaker top, and shake to make it cool then serve.

Chen, Zhang and Wang (2010), Guillotin, Sanoner and Renard (2009), Petrisor, Radu and Cimpeanu (2010) stated that fruits undergo tremendous chemical changes once separated from the parent plant, until finally spoilage sets

in as a result of attack from bacteria, yeasts and fungi. Typical changes may show in texture, colour, flavour and respiratory activity which affect the processed fruits. Among the organisms specified as agents of bacterial food intoxication are *Clostridium botulinum, Clostridium perfringens,* Staphylococci and with restriction, *Bacillus cereus* (Arnesen, Fagerlund & Granum, 2008; Hunter & Poxton 2002; Pavic, Brett, Petric, Lastre, Smoljanovic & Atkinson *et al.,* 2005; and Rodriguez & Vargas, 2002).

Sachet packed fruit juices are free from a considerable quantity of tin, or iron salts that may be formed or contribute a distinct metallic flavour as in fruits processed or stored in metal containers. The containers are useful for packaging liquid foodstuffs, for example fruit juices which are liable to deteriorate in the presence of air. Aside from packaging materials, additional contamination may come from equipment coming in contact with the juice and from production personnel. Various workers suggest that human beings shed from 10^3 to 10^4 viable organisms per minute. The numbers and type of organism shed is closely related to the subjects working environment.

Food Safety

Steen and Ashurst (2006), explain that although the primary function of food for humans is survival, it now has the additional associations with health, enjoyment and acceptability. Consumers look to suppliers and manufacturers for a product with which there is no associated risk in consumption and which is marketed in accordance with strict observance of the laws governing food safety. Although microbial contamination must always be a point of concern for food, it becomes much less of a potential hazard for beverages where the lower pH

conditions make the survival of pathogenic species virtually impossible and the likelihood of food poisoning equally unlikely. Most fruits contain bacterial counts up to 1.0x10.5cm^2 on their surfaces. Improper washing of fruits add these bacteria to extracts leading to contamination (Lewis *et al.*, 2006).

Microbial population in food samples vary enormously, depending on the characteristics of the sample and the processing conditions. For instance, unprocessed foods are likely to contain a very wide variety of species, whereas heat-processed products may contain heat-resistant spores and heat-resistant vegetative cells. A total viable cell count can only provide an estimate of the microbial population based on those cells that are recoverable under the test conditions. It is worthy to note that, a conventional total viable cell count as the quality indicator is determined using an aerobic plate count method.

In addition, any attack from yeasts and moulds will manifest itself as spoilage and be easily detectable either visually or organoleptically well before any lasting danger can be done.

To be assured of complete safety, however it is necessary to look further into the actual ingredient makeup of the drink itself. With a current world population of over six billion, the challenge facing the soft drinks industry remains largely unchanged in essence, but it is perhaps increasingly more complex. Steen and Ashurst (2006), state that provision of products that are wholesome, durable and acceptable in character and of interest to an ever-growing market has been the objective for many years, but with the increasing realisation that almost anything that is consumed can have detrimental as well as beneficial

effects, the focus is very much on additives, processing aids and functional ingredients.

Contamination of Non-Alcoholic Drinks

The factors which influence the microbial alteration of the food include the number and type of contaminated microorganisms, humidity (and water activity), the pH (the level of acidity and alkalinity), the presence or absence of oxygen, the type and availability of nutrients, the temperature, the food physical condition (Morris, Barnett & Burrows, 2004).

Sources of Contamination of Fruit Juices

Pathogenic Contamination

Contamination of juices with pathogenic microorganisms such as *Escherichia coli* and *Salmonella* spp. has caused numerous illnesses and even some fatalities (Cody, Glynn, Farrar, Cairns, Griffin, Kobayashi *et al.*, 1999). Sulphites inhibit yeasts, moulds and bacteria and are most effective as inhibitors of browning in foods. They also reduce the number of growth of microbes and increase the shelf life of juice products (Prescott, Harley & Kleen, 2002).

Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts and splits that occur during growing or harvesting (Durgesh, Ranjana & Varsha, 2008).

Yeasts and moulds are typical contaminants in soft drinks. They are common in the brewery environment and in the ingredients (Stratford 2006). Yeasts are considered as the primary spoilage microbes in carbonated products mainly due to their ability to withstand carbonation levels exceeding 3.0 vol. They also tolerate acidic conditions well. Most species grow in the pH range 1.5–

8.5 (Sperber, 2009), and have their growth optimum in the pH range 3.0–6.5 (Lawlor, Schuman, Simpson, & Taormina, 2009). Yeasts that form heat-resistant ascospores are also the principal spoilers in thermally processed carbonated soft drinks (Lawlor *et al.*, 2009).

Yeasts and moulds are more regarded as spoilage agents of fruit juices compared to bacteria because of the physical and chemical properties of the fruit juices. Some of these properties include the low pH of fruit juices, the positive oxidation-reduction potential of the fruit juices and the rich nutrient composition of the juice (Obire, Ramesh, Dick, & Okigbo, 2008; Okigbo & Obire, 2009).

Unhygienic conditions

Fruit juices can be contaminated through untidy instruments and utensils, unhygienic water for dilution, dressing with contaminated ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies, airborne dust, raw materials and equipment, additional processing conditions and improper handling (Victorian Government Department of Human Services (2005); Oliveira, Seixas, Sousa & Souza, (2006); Nicolas, Razack, Yollande, Aly, Tidiane, Philippe, De Souza & Sababénédjo, (2007) & Durgesh *et al.*, 2008). Prevalence of unhygienic conditions contributes substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables. Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, species of *Salmonella*, *Shigella*, and *staphylococcus aureus* (Buchamann, Edelson, Miller & Sapers, 1999; Sandeep, Waker & Abhijit, 2001; Barro, Bello, Aly, Ouattara, Ilboudo, & Traoré, 2006; Lewis, Thompson, Rao, Kalavati & Rjanna, 2006).

Lelieveld (2003), explains further that foods can be contaminated:

a. during growth and harvesting of raw materials

b. storage and transport to the factory

c. processing into finished products

The final product may then be (re)contaminated during subsequent storage and transport to shops, and during storage and preparation by the consumer. The main sources of contamination are the environment, animals and people. The main transmission routes (vectors) of contamination are contaminated surfaces (Lelieveld, 2003).

Infection by fungus

According to Al-Hindi *et al*., (2011), fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decay (Singh & Sharma, 2007; Al-Hindi *et al*., 2011).

Tetra pak (2000), states that although the processing of fruit juice has been maintained at a considerable hygienic standard, a variety of yeasts and moulds, and some bacteria are still able to find their way into industrially produced juices; it may therefore seem clearly that these potential spoilage organisms originate from the raw fruits used for processing or from the processing equipment.

Types of Food/Drink Contamination

There are three main types of food contaminants. These are microbiological, chemical and physical.

Microbiological Contamination of food/drinks

Since the human food supply consists of plants and animals or products derived from them, it is understandable that our food supply can contain microorganisms in interaction with the foods (Panda, 2009). When the microorganisms involved are pathogenic, their association with our food is critical from a public health point of view. Serious health hazards due to the presence of pathogenic microbes in food can lead to food poisoning outbreaks (Frazier & Westhoff, 1997). Freshly extracted fruit juices, which have always been considered as healthful drink, may not always be safe owing to the heavy load of microbes (Kumari, 1995).

Jayalakshmi, Krishnamoorthy, Ramesh Kumar and Sivamani (2011), explain that microbes play a central role in the spoilage of foods and beverage, mainly those with high acidity and reduced water activity. They observed that spoilage of soft drinks depends on the composition and the quality of ice used to cool drinks. They reported that 9% of the rink contained *Escherichia coli* and 1% Enterococci, and the microbiological quality of ice depend on the type of use, the type of premises, and the type and place of production. In the last 5 years, a number of high profile cases of food-borne illness have been linked to the consumption of fruit juices, particularly apple and orange juices. In most of these cases, the juice was consumed in a fresh (non-pasteurized) form.

Coliform is the principal indicator of the suitability of a particular drink for consumption (Derlet 2008). Large numbers of coliforms may cause gastroenteritis, and streptococci may be a better indicator (Kay, Fleisher, Salmon, Wyer, Godfree, Zelenauch-Jacquott & Shore, 1994; Fleisher, Kay, Salmon, Jones,

Wyer & Godfree, 1996). Indicator organism in food serves as a tool to evaluate the microbial quality of the product. The aerobic plate count, with its inherent nutritional limitations that are due to the heterogeneous distribution of bacteria in food, gives a useful measure of the quality of a product.

The factors which influence the microbial alteration of the foods include the number and type of contaminated microorganisms, humidity (and water activity), the pH (the level of acidity and alkalinity), the presence or absence of oxygen, the type and availability of nutrients, the temperature and the food physical condition (Morris, Barnett & Burrows, 2004).

Due to their low pH, soft drinks constitute a hostile environment in which the great majority of microbes die, although *Escherichia coli* O157 and *Salmonella* species can persist for weeks in chilled, fruit juices (Barnett, Payne, Yarrow & Davenport, 2000). A limited number of yeasts, moulds and acid tolerant bacteria cause spoilage of soft drinks. Spoilage effects include formation of clouds, particulates, taints and excessive gas (Back, Bohak, Ehrmann, Ludwig, Pot, Kersters & Schleifer, 1999). Infection of soft drinks commonly occurs via raw materials, returned bottles or aerial vectors (Byrne, 1994).

Insects are increasingly recognized as a vector for yeasts. Although many of the 800 or so yeasts discovered hitherto have been found in soft drinks or fruit juices (James, Collins & Roberts, 1994), relatively few species can grow in this environment or cause spoilage (Lachance, Gilbert & Starmer, 1995).

Chemical Contamination of food/drinks

Chemical contamination causing poisoning or burns/corrosion occurs most commonly when chemicals are stored or used incorrectly and mistaken for

food or drink. Chemical contamination is a potential food hazard and the sources and routes of contamination include naturally occurring chemicals in food, mycotoxins produced by some fungi, residues of veterinary drugs and pesticide, food additives, contact reactions from food containers and cleaning chemicals; that is either the incorrect type or usage (Croner-i, 2019).

Chemical contamination from pesticides can also be introduced on fruit, vegetables and salad items. Procedures for the safe handling and use of chemicals must be provided for staff, with specific training and monitoring in the use of cleaning chemicals (Croner-i, 2019). According to Tasnim, Hossain, Nusrath, Hossain, Lopa and Haque, (2010), manufacturers commonly use Sulphur Dioxide (SO_2) and benzoate as preservatives in processed fruit juices which can significantly damage the vegetative cells. There is therefore an emphasis in food premises on provision for the safe storage of chemicals (Croner-i, 2019).

Physical Contamination

Physical contaminants are those that can be seen, touched or felt and this type of contamination is fairly common. Physical contamination may cause offence (a hair in food), harm (glass in food) or introduce bacteria (a fly in food). Contamination may be in the food itself (intrinsic), for example, fish bones, or from an external source (extrinsic), for example, a metal screw (Croner-i, 2019). UNL Food (2019), explains that a physical hazard is any extraneous object or foreign matter in a food item which may cause illness or injury to a person consuming the product. These foreign objects include, but are not limited to bone or bone chips, metal flakes or fragments, injection needles, BB's or shotgun pellets, pieces of product packaging, stones, glass or wood fragments, insects or

other filth, personal items, or any other foreign material not normally found in food products. Stier (2014), also contributes that foreign material such as glass, wood, metal, fruit pits, bone or stones have the potential to cause injury.

According to Croner-i (2019), sources of physical contamination include people, packaging, environment, equipment and the food itself. UNL Food (2019), states that sources of contaminants include raw materials, badly maintained facilities and equipment, improper production procedures and poor employee practices.

Food safety law requires controls to be in place to prevent physical contamination or to reduce the risk of it occurringand the food safety management system based on the principles of HACCP will cover this. The controls of physical contaminants are based on: removing the source, for example, no jewellery worn by staff; reducing the risk, for example, covering hair; covering food; cleaning (equipment, premises and some foods); visual checks and maintenance (Croner-i, 2019).

Adulteration of Non-Alcoholic Drinks

Adulteration is the act of either adding extraneous substances (adulterants) into food items or products or reducing essential nutrients partly or wholly for financial gain or due to carelessness and lack of proper hygienic condition during processing, storing, transportation and marketing. The consumer is either cheated or often become victim of diseases and because of this, it is important for the consumer to know the common adulterants and their effect on health since the increasing number of food producers and the outstanding number of foodstuffs

imported enable the producers to mislead and cheat consumers (Anita & Neetu, 2013).

Arthey and Ashurst (2001), state that adulteration of fruit juice is widespread in the world. As with any commodity, juice manufacturers, blenders and users can secure considerable financial benefit from adulterating fruit juice. It should be emphasized that food safety issues are not normally an issue in fruit juice adulteration. An adulterated fruit juice sold as pure fruit juice is not as it has been labeled. Although adulteration is becoming increasingly sophisticated, it is normally seen as falling into one of three types: over dilution of juices with water; use of cheaper solid ingredients (particularly sugars) and blending of cheaper with more expensive juices (Arthey & Ashurst, 2001).

Consumers are more health-conscious than ever, so food manufacturers use misleading tricks to convince people to buy their products. They often do this even when the food is highly processed and unhealthy (Campos, Doxey & Hammond, 2011; Hughes, Wellard, Lin, Suen & Chapman, 2013 & Helfer & Shultz, 2014). Food and drink products are often a target of adulteration, intentional or unintentional; while supply chains usually deal with perishable products that could be harmful to consumers if they are not managed properly (SGS, 2013).

Adulterants are chemical substances which should not be contained within our food or beverage, and may be intentionally added to more expensive substances to increase visible quantities and reduce manufacturing costs, or for some other deceptive or malicious purpose (Anita & Neetu, 2013). Lakshmi, LABS, Guntur and Pradesh (2012), posits that, the usage of adulterants has been

common in societies from very ancient time with few legal controls on food quality due to poor or nonexistent monitoring by authorities; sometimes this usage has even extended to exceedingly dangerous chemicals and poisons. More recently, adulterant use for example, in the People's Republic of China (Chinese milk scandal case with melamine) in which some children were killed and thousands also harmed has inspired much public attention in China and the world at large (Lakshmi, LABS, Guntur & Pradesh, 2012).

Arthey and Ashurst (2001) also add that, another category of adulteration is by far the most common. For example, apple juice will normally contain around 11% by weight of solids. At least 90% of these solids are carbohydrates which are; sucrose, dextrose and fructose predominating. Considerably cheaper sources of carbohydrates can be found, and the simple addition of a mixture of carbohydrates in roughly the same proportion as those found naturally in apple juice can be used to 'stretch' apple juice by a considerable proportion. In more sophisticated forms of adulteration the added components can be made to carry a similar 'signature' to the juice. In the third category a cheaper juice can be used to adulterate a more expensive one; for example, elderberry juice can be used to extend strawberry or raspberry juice (Ashurst & Dennis, 1996).

Again they argue that, detection of adulteration and its quantification have induced some elegant scientific techniques, some borrowed from other fields and some developed specifically for use in fruit juice work. Detection of over-dilution and the presence of sugars of other origin is now carried out largely by the method of measuring key isotope ratios such as carbon 13:12 ratios, deuterium; that is, hydrogen ratios and oxygen, 18:16 ratios; and comparing them with both

those found naturally in fruit and agreed international standards. An important part of the fight against adulteration has been the development of databases examining fruit of different origin and season. Another elegant method of detecting sugar addition in particular has been the use of high performance liquid chromatography (HPLC) to determine the presence of oligosaccharides that are characteristic of the added sugars but not the fruit. The use of enzymic methods for determining the presence of specific components (D-malic acid, which does not occur naturally) is also helpful (Arthey & Ashurst, 2001).

Food Poisoning

According to Kisko and Roller (2005), food poisons are substances that in small amounts are capable of producing serious injury or death. It is a commonplace for flavours and colour additives of unchallenged benefit in appropriate doses may become seriously poisonous where such doses are exceeded. Food poisoning is a painful stomach disorder caused by eating food that contains harmful bacteria or poisonous substances. Nzeako & Al- Hashimi (2006) explain the danger of infection in juice manufactured and objectionable filling into containers. Common food poisoning bacteria are *Salmonella enteritidis, Clostridium welchi, Clostridium botulinum, Vibrio parahaemolyticus* and *Bacillus cereus*.

Acid forming microaerophilic bacteria such as the lactobacilli tolerate lower pH values down to 3.5 in the case of some strains. Weenk, Corry, Curtis and Baird (1995), reported that the enumeration of microorganisms in food is by pour plate, surface spread plate, surface drop, agar droplet and micro dilution methods. It is important to recognize that much of what happens to food has

nothing to do with the materials with which it comes into contact, whether these are plastics, bottle, sachets and thin layers or not (Matanda, 1996). Marquenie, Geeraerd, Lammertyn, Soontjens, Van, Michiels, & Nicolai, 2003; Fiori, Fadda, Giobbe, Berardi, & Migheli, 2008), emphasized that *Monilia* spp. and *Botrytis cinerea* are the most troublesome contaminants of fruit juices.

Foods and beverages prepared and sold by street vendors have contributed to transmission of cholera and enteric diseases. Improperly prepared fresh fruits and vegetable juices are recognized as an emerging cause of food borne illness (Sandeep Mudgil, Aggarwal & Ganguli, 2004). Food borne disease outbreaks from enter pathogenic bacteria, such as *Salmonella* spp., *Vibrio cholera*, *Vibrio parahaemolyticus* and *Staphylococcus aureus* are common cases of food borne infection through the world (Chomvarin, Kotimanusvani & Rhompruk, 1993).

Non-Alcoholic Drinks and Related Health Issues

When the human body becomes dehydrated it experiences the sensation of thirst. This craving of fluids results in an instinctive need to drink. Thirst is regulated by the hypothalamus in response to subtle changes in the body's electrolyte levels, and also as a result of changes in the volume of blood circulation. As water is essential for life, it has also been the carrier of many diseases. As mankind evolved, new techniques were discovered to create drinks from the plants that were native to their areas (Burnett (1999). The ugly truth about fruit juices is that, most types contain a similar amount of sugar as a sugar-sweetened beverage, sometimes with even more total calories. Fruit juices contain some nutrients, but less compared with many plant foods. It contains no fiber and is just as high in sugar and calories as most sugar-sweetened beverages. A large

part of the sugar found in fruit juice is fructose. The liver is the only organ that can metabolize fructose in meaningful amounts (Bray, 2007).

From 1989 to 2008, calories consumed in the form of sugary beverages increased by 60% in children ages 6 to 11, and the percentage of children consuming them rose from 79% to 91% in the United States of America (Lasater, Piernas & Popkin, 2011). Beverage companies spend billions of dollars marketing carbonated beverages, with a significant portion of marketing aimed directly at youth ages 2–17 (Wilks, 2009). Each year, the youth see hundreds of television adverts for sugar-containing drinks. The beverage industry, however, generally rebuffs suggestions that its products and marketing tactics play any role in the obesity epidemic (Coca-Cola, 2012). Studies funded by the beverage industry are four to eight times more likely to show a finding favourable to industry than independently-funded studies (Lesser, Ebbeling, Goozner&Wypij, 2007).

According to Pan, Malik, Hao, Willett, Mozaffarian& Hu (2013), the average can of sugar-sweetened soda or fruit punch provides about 150 calories, almost all of them from sugar, usually high-fructose corn syrup. That is the equivalent of 10 teaspoons of table sugar. They explain that, drinking just one can of a sugar-sweetened soft drink every day, and not cutting back on calories elsewhere, could make the consumer gain up to 5 pounds in a year. Moreover, drinking water in place of fruit juices is associated with lower long-term weight gain.

In a recent study by Bray and Popkin (2013), they found that, consumption of calorie-sweetened beverages has continued to increase and may play a role in the obesity epidemic, metabolic syndrome and fatty liver disease, whereas

reducing intake of soft drinks is associated with less weight gain and metabolic improvement. Dozens of studies have explored possible links between soft drinks and weight, and they consistently show that increased consumption of soft drinks is associated with increased energy (caloric) intake. One meta-analysis of 88 studies showed that the effect appeared to be stronger in women (Vartanian, Schwartz & Brownell, 2007). Moreover, studies in children and adults have found that reducing sugary drink consumption can lead to better weight control among those who are initially overweight (Ebbeling, Feldman, Osganian, Chomitz, Ellenbogen & Ludwig, 2006). According to de Ruyter, Olthof, Seidell & Katan (2012), replacement of sugar-containing beverages with non-caloric beverages reduces weight gain and fat accumulation in the normal-weight of children. Similarly, people who increase their sugary drink consumption by one 12-ounce serving per day gain more weight over time on average, an extra pound every 4 years than people who did not change their intake (Mozaffarian, Hao, Rimm, Willett & Hu, 2011).

The World Health Organisation (2002) notably sparked the obesity debate in 2002 when it released a draft report recommending the restriction of sugar-rich items, such as soft drinks, particularly those consumed by children. The WHO report stated that, food and food products have become commodities produced and traded in a market that has expanded from an essentially local base to an increasingly global one. Changes in the world food economy are reflected in shifting dietary patterns. For example, increased consumption of energy-dense diets high in fat, particularly saturated fat, and low in unrefined carbohydrates.

These patterns are combined with a decline in energy expenditure that is associated with a sedentary lifestyle. Sugar converts into fat.

Overabundance of dextrose is transformed to fatty acids, later to triglycerides, and finally stored in the body as adipose tissue. The percentage of obese people increase almost every year. Heart disease is also connected to the effect of consuming too much sugar. A study published in the Journal of the American Medical Association suggests that, persons with 25% or more calories in-take from added sugar have more than three times higher probability of contracting heart disease, heart attack or stroke, than persons with less than 5% of calories intake from sugars. According to the research presented at the American Heart Association's 63rd High Blood Pressure Research Conference, a diet high in fructose elevates the blood pressure. Soft drinks have many potential health problems. The inherent acids and sugars have both acidogenic and cariogenic potential, resulting in dental caries and potential enamel erosion (Cheng, Yang, Shao, Hu & Zhou, 2009).

According to Ashurst and Hargitt (2009), soft drinks have been associated with dental issues in two ways. Firstly, sugar-sweetened drinks are cariogenic, that is, it can cause dental caries, due to acid formation caused by bacterial action on sugar in the mouth. Secondly, dental enamel will soften and dissolve slowly at a pH less than 5 and continual exposure to acid foods or drinks, having a pH less than 5, will tend to slowly remove tooth enamel. This process is known as `erosion'. Acid foods and drinks include pickles, fruits, fruit juices and soft drinks. However, there is also a reverse process known as remineralisation by which, at higher pH levels in the presence of minerals such as calcium and

fluoride, the enamel can reform. Saliva also assists neutralisation of the acidity in the mouth and helps to promote remineralisation. Drinks can be designed with the pH buffered as high as possible, whilst reformulating to minimise the impact on flavour.

The pH and total acidity are key characteristics of a drink's flavour. The presence of calcium in the drink also reduces the dissolution of enamel. Such drinks can be consumed with minimal dissolution of tooth enamel. Unfortunately the relatively high pH and high calcium content do tend to impact significantly on the character of the drink. `Tooth-friendly' drinks were developed and marketed in the UK by Glaxo Smith Kline (GSK) who holds many patents on the subject. Some early work was also carried out by Procter & Gamble (P&G) who also hold patents on formulations (Ashurst & Hargitt, 2009).

Food Laws and Regulations in Ghana

The wholesomeness of food is a matter of interest to both government and the citizenry of every country. On the part of government, this concern is evidenced by the enactment of laws to regulate the activities of food handlers. The legal requirements for food safety and food quality have been established by many national governments, with the objective of protecting consumers and ensuring that foods are fit for human consumption. These requirements are contained in food laws and regulations, the scope of which varies across countries. In Ghana, the pursuance of the 1981 Provisional National Defence Council Establishment Proclamation (PNDCL 3035) of the Food and Drugs Law of 1992 was promulgated. The Law prohibits the sale of unwholesome, poisonous

and adulterated food. It also takes into consideration the sale of food under insanitary conditions, which makes the food vulnerable to contamination.

According to the Law, food must be manufactured under supervision and it is also an offence punishable by a fine, imprisonment or at worse closure of premises for selling unwholesome food. To ensure food quality and safety in Ghana, food is controlled through licensing and regular inspection (Ntiforo, cited in Tawiah, 2013). The only deviation may be the food safety training and assessment of prospective food handlers. Personnel of the controlling authority from hygienic point of view conduct initial inspection and once license is issued, food vendors are obliged to meet mandatory local authority by-laws. In Accra, there exists Street Market By-law under the Accra Town Council Ordinance of 1943, which has provision for enhancing the sale of safe foods to the public. Ntiforo (2001) stressed that the enforcement of the laws was not effective due to inadequate trained staff properly equipped for that task. FAO (2005) indicated that any ideal food control system should include effective enforcement of mandatory requirements achieved through regular inspection programmes.

FAO (2005) continued that the implementation of any food law requires a qualified, trained and efficient food inspection service, since inspectors are the main functionaries who have daily contact with the food industry. It is worthwhile to have a reactive and enforcement-oriented programme rather than a preventive and holistic approach to reducing the risk of food-borne illness.

Food and Drugs Authority

Food and Drugs Authority (FDA) formerly the Food and Drugs Board (FDB) was established in August 1997 under the Food and Drugs Law, 1992

(PNDCL 305B). It is the National Regulatory Authority mandated by the public Health Act, 2012 (Act 851) to regulate food, drugs, food supplements, herbal and homeopathic medicines, veterinary medicines, cosmetics, medical devices, household chemical substances, tobacco and tobacco products. The FDA Ghana's legal mandate is found in part 6 (Tobacco Control Measures), part 7 (Food and Drugs), and part 8 (Clinical trials) of the Pulic Health Act, Act 851 of 2012. The FDA is an Agency under the Ministry of Health with an eleven-member Governing Board inclusive of the Chief Executive Officer who is responsible for the day to day administration of the FDA.

The objective of the Authority is to provide and enforce standards for the sale of food, herbal medicinal products, cosmetics, drugs, medical devices and household chemical substances.

Functions of the Authority

- Ensure adequate and effective standards for food, drugs, cosmetics, household chemicals and medical devices;
- Monitor through the District Assemblies and any other agency of State compliance with the provisions of Part 6,7 and 8 of the Public Health Act, 2012 (ACT 851);
- Advise the Minister on measures for the protection of the health of consumers;
- Advise the Minister on the preparation of effective Regulations for the implementation of Part 6,7 and 8 of the Public Health Act, 2012 (ACT 851);
- Approve the initiation and conduct of clinical trials in the country;
- Perform any other functions that are ancillary to attaining the objects of the Authority;

Ghana Standards Authority (GSA)

Ghana Standards Authority is an Agency of Government of Ghana that promulgates Standards, promotes Standardization and undertakes Conformity Assessment activities in the country. These activities ensure that products or goods and services produced in Ghana, whether for local consumption or for export are safe, reliable and are of good quality.

Its mission is to promote standardization for the improvement of the quality of goods, services and sound management practices in industries and public institutions in Ghana. The Mandate of the Authority is:

1. Establishing and promulgating standards to ensure high quality of goods produced in Ghana, whether for local consumption or for export.
2. Providing quality assurance through inspection, testing and metrology.
3. Assisting operators in both the manufacturing and service sectors to improve their competitiveness by establishing effective Quality Management Systems along ISO/IEC 9001: 2008 and 22000: 2005.
4. Promoting standardization in industry and commerce
5. Promoting standards in public and industrial welfare, health and safety (ISO, n.d)

Food standards establish requirements for the safety and quality of food. However, unless a food standard is part of food regulations, it is not a legal requirement. Penalties for offenders usually include fines, imprisonment, and closure of premises where there is risk of contamination (International Organization for Standardization, 2001).

International Quality Standards (IQS)

To meet the food safety and quality concerns of consumers all over the world, different organizations in many countries implement quality control programmes and establish quality standards for all food items including animal products. Standards are specifications for processes, procedures and product composition. They are normally put together by National Bureau of Standards for specification products and processes in the United States of America.

International Organization for Standardization (ISO)

ISO is an independent, non-governmental international organization with a membership of 161 national standards bodies. Through its members, it brings together experts to share knowledge and develop voluntary, consensus-based, market relevant International Standards that support innovation and provide solutions to global challenges. International Standards make things work. They give world-class specifications for products, services and systems, to ensure quality, safety and efficiency. They are instrumental in facilitating international trade. ISO has published 22042 International Standards and related documents, covering almost every industry, from technology, to food safety, to agriculture and healthcare. ISO International Standards impact everyone and everywhere (International Organization for Standardization, 2001).

ISO International Standards ensure that products and services are safe, reliable and of good quality. For business, they are strategic tools that reduce costs by minimizing waste and errors and increasing productivity. They help companies to access new markets, level the playing field for developing countries and facilitate free and fair global trade (ISO, 2001).International Organization for

Standardization (ISO, 2001) of food quality management series include ISO 9000: 2000 and ISO 22000: 2005. ISO 9000 is set up as a collection of guidelines that help company establish, maintain and improve a quality management system. It is important to stress that ISO 9000 is not a rigid set of requirements and the organisations have flexibility in implementing their quality system. The ISO 9000 family comprises the following standards: ISO 9000, which embraces the basis of the quality management systems and terminology (ISO 9000:2000), ISO 9001, which specifies requirements concerning the quality management system. It is precisely this standard that is implemented in enterprises (ISO 9001: 2000).

One major important aspect of ISO 9000 is its proceess-oriented approach. It places emphasis on integrating the important aspects of product and services and not individual processes. It also simplifies purchase and supplier qualification procedures and, at the same time, reduces costs associated with these operations. The quality management system based on the ISO 9000 standard covers the following areas: management of the organization, management of resources, process of product realization as well as measurements, analyses and improvement (ISO, 2001).

Chapter Summary

The soft drinks industry is a rapidly growing aspect of the fast food phenomenon. In line with healthy eating attitudes prevalent in today's societies the consumption of non-alcoholic drinks plays significant part in this expansion. More so it is not only the responsibility of the food industry to provide safe and nutritious food to the public, but also the duty of government to see that industry is meeting its responsibility.

Microbiological and chemical tests may assist in the assessment of non-alcoholic drinks for wholesomeness. Non-alcoholic drinks, especially fruit juices can be contaminated microbiologically with untidy instruments and utensils, unhygienic water for dilution, dressing with contaminated ice, prolonged preservation without refrigeration, and unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust. Some pathogenic bacteria have been found to be able to survive in soft drinks at (pH 3.5) for 3 days at 30°C. In addition, concentrates used for soft drink production may provide a good environment for pathogenic bacteria to survive. Many exotic juices used in beverage formulations have low acidity (pH 4.8–6.2) and these juices provide conditions suitable not only for the survival but also for the growth of pathogenic bacteria, and such bacteria are able to survive in acidic juices long enough to transmit diseases.

The consumption of calorie-sweetened beverages has continued to increase and may play a role in the obesity epidemic, type II diabetes, dental caries, metabolic syndrome and fatty liver disease, whereas reducing intake of soft drinks is associated with less weight gain and metabolic improvement. On the other hand, vitamin C which is quantitatively the most important vitamin in non-alcoholic drinks are sometimes absent from the drinks or in negligible quantities. To ensure that non-alcoholic drinks are wholesome for human consumption, manufacturers must apply stipulated standards by the Ghana Standards Authority (GSA) and the International Organisation for Standards (ISO), and the Food and Drugs Authority (FDA) must ensure their compliance at all levels of production.

CHAPTER THREE

RESEARCH METHODS

Introduction

This section of the study presents the research design, study area, population, sampling procedure, data collection instruments, data collection procedures as well as the data processing and analysis and chapter summary.

Research Design

The study was conducted using the mixed methods approach. Mixed method research focuses on collecting, analyzing, and mixing both quantitative and qualitative data in a single study or series of studies. Its central premise is that the use of quantitative and qualitative approaches, in combination, provides a better understanding of research problems (Creswell & Plano Clark, 2011). Mixed research method takes an eclectic, pragmatic, and common sense approach, suggesting that the researcher mixes quantitative and qualitative methods in a way that works best for the given research questions being studied or investigated in a particular context without any interference.

Mixed method research obtains both quantitative and qualitative data, attempts to corroborate and complement findings, and takes a balanced approach to research. According to Harvey (1990), and Walliman (2004), a mixed method research strategy is sometimes appropriate in collecting data in descriptive studies. Based on the strength of this reference, both quantitative and qualitative methods were used in carrying out the study.

Qualitative method was used to assist me catalogue and categorise non-alcoholic drinks in the Cape Coast Metropolis based on the standards from the

Ghana Standards Authority, develop an observation checklist for observing the conditions of the drinks and recording the information provided on their packages and interpret the findings of the research in the results and discussion section.

I adopted descriptive research design to help me describe the differences that exits in the microbial loads, the level of sugar and vitamin C concentrations of the selected non-alcoholic drinks with respect to the parameters being investigated and draw conclusions (Grix, 2001; Babbie, 2005).

Quantitative method was employed to find answers to the volume of microbial contamination in the non-alcoholic drinks, total sugars and vitamin C concentrations as well as to compare the figures obtained to already existing standards to know if the products met the standards available.

Study Area

The study was limited to the Cape Coast Metropolis. The study was conducted in three major markets in the Cape Coast Metropolis which were University of Cape Coast, Abura and Kotokuraba markets comprising of various grocery shops and supermarkets. These markets were selected because a high volume of shoppers from a wide range of socio demographic backgrounds in Cape Coast shop there.

Study Samples

The study samples consisted of some non-alcoholic drinks sold on the various markets in the Cape Coast Metropolis. The population was estimated to be 120 different types of non-alcoholic drinks based on observation and recording from the survey conducted earlier. Non-alcoholic drinks were collected, identified and recorded. The brand names of the drinks, country of origin, manufacturer's

name, expiry date, Food and Drugs Authority (FDA) number, type of packaging and weight were recorded. The drinks were grouped into four based on the standards from the Ghana Standards Authority; namely, carbonated malt drinks; carbonated soft drinks; fruit juices prepared from concentrates; and locally manufactured fresh fruit juices.

Population

The estimated population was 120 different non-alcoholic drinks comprising 22 carbonated malt drinks, 12 carbonated soft drinks, 74 fruit juices prepared from concentrates and 12 locally manufactured fresh fruit juices obtained from the three major markets in the Cape Coast Metropolis, which were University of Cape Coast, Abura and Kotokuraba markets.

Sampling Procedure

Creswell's (2003) research design states that, for a population of 120, 20% of it is enough for a study. The initial sample size for the analyses was 25. The specific sampling techniques used were purposive and proportionate sampling to select the sample size for the analyses. Bearing in mind this method, the sampled drinks were grouped into four (4) categories using standards from the Ghana Standards Authority:

1. Category "A" - carbonated soft drinks and carbonated malts;
2. Category "B"- concentrated fruit juices preserved exclusively by physical means
3. Category "C" – Fresh Fruit Juices with no physical or chemical preservatives

4. Category "D" - fruit juices, squashes and cordials preserved by physical and chemical means.

The selected sample of drinks were purchased from the UCC, Kotokuraba and Abura markets in the Cape Coast Metropolis and transported in batches of five to the laboratories. All the samples were bought with their security seals intact and ensured were in their required storage temperatures with their expiry dates checked. All the samples had shelf life of one year. Three malt drinks and three soft drinks were selected from category 'A' namely; Malta Guiness, Magic Malt and Beta Malt; Club Cola, Original American Cola and 5Star Kolah Cool, respectively. In category 'B', six drinks were selected comprising Alvaro Malt drink (pear flavour), FruTelli all natural juice tropical mix drink, Don Simon Multi Frutas, Pure Heaven tropical fruit juice, Tampico citrus punch and Patapata Bissap Ice. Category 'C' was made up of six fresh fruit juices, namely; Anidak's Bissap, Farm Fresh Pinecarrot, Farm Fresh Pinemelon, Farm Fresh Pine Citrus, and Blue Skies Mango, Orange and Banana Smoothie.

There were also five drinks selected for category 'D' which were; Juvita Flavoured drink, Fruity Refreshing Drink, Squiz Orange drink, Kalypo natural juice drink and Juicee Pineapple drink. These drinks produced in Ghana, South Africa, Great Britain, Nigeria and other countries, were purchased from street vendors and supermarkets between the months of July and September, 2016 in the Cape Coast Metropolis and taken immediately to the Laboratories for the various analyses. The samples chosen for the tests represented the types of non-alcoholic drinks that are often consumed in homes, schools, offices, hospitals, and during occasions in the Cape Coast Metropolis. The sample size was deemed

appropriate because adequate samples could be obtained for the microbial and physico-chemical analyses and as Fraenkel *et al.,* (2012), suggests the sample size should be representative of the population.

Data Collection Instruments

The main data collection instrument was laboratory analysis, which gave way for other instruments to be employed. A non-participatory observation (direct observation), which took place at the various supermarkets, shops and street vendors. In addition, a survey of two major hospitals in the Cape Coast Metropolis was also carried out. Drinks Standards were obtained from the Ghana Standards Authority and selected samples of drinks were purchased for the microbiological and physico-chemical analyses from supermarkets between the months of July and September, 2016

Data Collection Procedures

Data were collected in four phases. An observation checklist was developed to serve as a guide for observing the conditions of the drinks and recording the information provided on their packages. The checklist was adapted from Oranusi, Braide and Nezianya (2012) who conducted similar studies on microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria. The checklist covered information on the labels which was recorded to include the Name of the drink, Type of packaging, Volume, Nutrition Information, Food and Drug Authority (FDA) number, and the Expiry date. Samples of drinks were purchased and sent to the Centre for Scientific and Industrial Research (CSIR) in Accra to identify possible microbiological contamination in the non-alcoholic drinks. Those sent to University of Cape

Coast College of Agriculture Research Laboratory had their chemical analysis determined, especially the level of sugar and vitamin C present in the drinks available on the markets of the Cape Coast Metropolis respectively.

A survey of two major hospitals in the Cape Coast Metropolis, thus University of Cape Coast Hospital and Cape Coast District Health Centre, were contacted for secondary data on cases of Diabetes mellitus and dental caries reported at the centres between the years 2010 to 2016. This was to give the researcher an insight on the prevalence of Diabetes mellitus and dental caries conditions in the metropolis. Secondary data available at the two hospitals were collected for 10 days from 17th to 27th May 2016 and observation took about 6 weeks starting from 18th June 2016 to 28th July 2016.

An introductory letter was obtained from the Vocational and Technical Education Department of University of Cape Coast, which was presented to relevant authorities like the Head of Microbiological Unit of the Food Research Institute, Accra, the Medical Director of the University of Cape Coast Hospital, as well as the Director of the District Hospital Directorate. After the various authorities granted permission, data collection started.

What differences exist in the microbiological loads of non-alcoholic drinks available on the Cape Coast Metropolis markets?

The objective was to identify possible microbial contamination in the non-alcoholic drink samples. To achieve this, samples of non-alcoholic drinks obtained from the three major markets in the Cape Coast Metropolis were taken to the laboratory for analyses. Microbiological analyses were based on the standards available at the Ghana Standards Authority. The samples were examined to

enumerate aerobic mesophilic bacteria (viable plate count method), enumerate yeast and moulds, detect *Salmonella* species, enumerate *Escherichia coli*, and to enumerate and isolate Total coliforms. The mean numbers of colonies counted were expressed as log colony forming units (cfu)/100 ml.

Microbiological Analyses

In order to identify the possible microbial hazards in the drinks, the following microbiological tests were conducted at the Centre for Scientific and Industrial Research (CSIR) in Accra using ISO and NMKL Analytical Standards.

1. Enumeration of mesophiles using the Plate Count Agar (PCA);
2. Enumeration of yeasts and moulds;
3. Enumeration and isolation of total coliforms;
4. Confirmation of faecal coliform;
5. Enumeration and isolation of *Escherichia coli* (*E. coli*)
6. Enumeration and isolation of *Salmonella* spp.

To ensure accurate results free from contamination, various control measures were put in place. For instance, before testing the samples, the whole laboratory was disinfected with methylated spirit and allowed to air dry. The floor and walls of the inoculation room were also sterilized with disinfectant solution made up of 70% alcohol prepared in the laboratory. All petri dishes and pipettes were sterilized by autoclaving at 121^{o}C and left to cool at ambient temperature in a desiccator before use. Ethanol was used to clean all work and sachet surfaces and a pair of scissors used for cutting also sterilized. The forceps and loops were flame-sterilized by Bunsen burner flame before every use. The methanol ignition produces formaldehyde, which sterilizes the unit. The laboratory was closed for

15 minutes for effective sterilization to take place. Gloves and clean laboratory coats were worn throughout the process. Talking, sneezing and coughing were avoided during inoculation and conscious efforts were made not to pass hands over sterilized equipment and opened media.

Preparation of Media used for the tests

Plate Count Agar (PCA) OXOID CM – 0325 ENGLAND

Tryptone Glucose Yeast Agar

This medium is used for the enumeration of viable organisms in food, animal feed and water samples. This is a quantitative test because the number of bacteria in the sample is counted after incubation for 24 hours.

Procedure

Exactly 17.5g of Tryptone Glucose Yeast Agar was suspended in 1 litre of distilled water. It was then dissolved by bringing to the boil with frequent stirring. It was mixed and distributed into final containers. The media was sterilized by autoclaving at 121^{o}C for 15 minutes. The plates were also incubated for 3-5 days at 20°C. The quality control of Plate Count Agar includes testing in accordance with ISO 11133:2014 (International Organization for Standardization, 2014). The dehydrated medium was stored at 10-30°C and used before the expiry date on the label. The prepared plates were also stored at 2-8°C. The appearance of the dehydrated medium was straw coloured, free-flowing powder and the prepared medium, a straw coloured gel.

Dichloran Rose-Bengal Chloramphenicol Agar (DRBC)

Agar Base – CM-0727 ENGLAND

Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) is a selective medium for yeasts and moulds associated with food spoilage.

Procedure

Exactly 15.75g of Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) was suspended in 500 mL (31.5g/L) of distilled water and heated to dissolve completely. Approximately 1 vial of chloramphenicol supplement (SR0078E) was dehydrated as directed and was added to the DRBC Agar Base and sterilized by autoclaving at 121^0C for 15 minutes. It was then cooled to 50^oC; mixed well and poured into sterile petri dishes. Approximately 40 mL of the drink sample was added to 200 mL of 0.1% peptone water and processed in a Seward `Stomacher' for 30 seconds. Exactly 0.1 mL of the prepared drink sample was inoculated on the medium surface. The plates were incubated at 25°C. The plates were examined after 3, 4 and 5 days. The enumerated colonies were reported as the number of colonies per gram of food.

Violet Red Bile Agar (VRBA) (OXOID CM 0107)

Violet Red Bile Lactose Agar (VRBA) is a selective medium for the detection and enumeration of coliform organisms. The medium is been used for the determination of the coli-aerogenes content of water, milk and other dairy products, dairy equipment, and food products at 37^oC for 24 hours (American Public Health Association, 1978; American Public Health Association, 1992).

Procedure

Exactly 38.5g of Violet Red Bile Agar (VRBA) was suspended in 1 litre of distilled water. It was brought to the boil and continued to boil for 2 minutes to dissolve completely and ensured that there was no remaining flecks of un-melted agar. No further sterilisation was necessary. It was then mixed well before pouring. Pour-plates containing 1.0, 0.1 and 0.01mL of the sample in Violet Red Bile Lactose Agar were incubated for 20-24 hours at 37°C and 44 ±1°C for coliforms and *Escherichia coli* respectively. Four pour-plates of Violet Red Bile Lacto Agar were employed. Approximately, 10mL of the VRBA was poured into each of the 3 plates and 1mL on a single plate, and incubated for 20-24 hours at 30°C. Coliform organisms form purple-pink colonies which are 1mm in diameter, and surrounded by a purple zone. Growth of red, non-mucoid colonies of Gram-negative rods indicate possible presence of *E. coli*. This is confirmed by suitable biochemical tests, such as indole production. The product passes the test if such colonies are not seen or if the confirmatory biochemical tests are negative.

Sodium Polyanethol Sulfonate (SPS) Media

A combination of 8.5g/L sodium chloride and 1g/L Bacteriological peptone.

***Bacteriological Peptone* (*OXOID LP 0037*)**

As a mixed pancreatic and papaic digest of selected animal proteins this peptone contains a wide molecular weight distribution of media for the investigation of numerous organisms including *Escherichia coli*, *Brucella*, *Lactobacillus* and *Pseudomona* species.

First, the samples were prepared through homogenization and serial dilutions. Data collection of the experimental analysis started with preparation of samples, which were homogenization and serial dilutions of the samples collected. Using a sterile pipette, 1 mL of the 10^{-1} dilution was pipetted into 9mL of sterile salt peptone (SPS) water to obtain 10^{-2} dilution. This procedure was repeated for 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. Further, from appropriate tenfold serial dilution, 1 mL aliquot of each dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) was inoculated into sterile Petri dish plates and the appropriate media added for enumeration and isolation.

After appropriate incubation of 3-5 days at 20ºC, dilutions with 30–300 colonies were selected and counted. The number of colony-forming units per millilitre (cfu/mL) of each sample was calculated by multiplying the number of bacteria counted by the dilution level. All analysis was done in triplicate to improve reliability of results. The specific microbial tests conducted in the laboratory were based on standards from the Ghana Standards Authority and as follows:

1. Carbonated soft drinks and Carbonated Malts
    i. enumeration of yeasts and moulds
    ii. enumeration of aerobic mesophiles (viable plate count)
2. Concentrated fruit juices preserved exclusively by physical means
    i. enumeration of aerobic mesophiles (viable plate count)
    ii. enumeration and isolation of total coliform
    iii. enumeration of yeasts and moulds

3. Fruit Juice with no physical or chemical preservatives

i. enumeration of aerobic mesophiles (viable plate count)

ii. enumeration of *Escherichia coli*

iii. biochemical confirmation

iv. enumeration and isolation of total coliform

v. biochemical confirmation of faecal coliform

vi. enumeration of yeasts and moulds:

vii. detection of *Salmonella* species

viii. biochemical confirmation

ix. triple sugar iron confirmatory (TSI) test

4. Fruit juices, Squashes and cordials, fruit juices preserved by physical and chemical means.

i. enumeration of aerobic mesophiles (viable plate count)

ii. enumeration and isolation of total coliforms

iii. biochemical confirmation of total coliforms

iv. enumeration of yeast and moulds

Enumeration of Aerobic Mesophilic Count (Viable Plate Count)

Aerobic mesophiles were cultured on Plate Count Agar (PCA) by the pour plate (Balows *et al.*, 1991) (OXOID CM 325). The plates were incubated at 30°C for 48 hours [NMKL no. 86, 2006].

Enumeration and Isolation of Total Coliforms

Coliforms bacteria were counted by the pour plate method using Tryptone Soya Agar medium (OXOID CM131) and adjusted to pH 7.3 and overlaid with Violet Red Bile agar (VRBA) (OXOID CM 0107) with pH adjusted to 7.4 and

incubated at 37°C for 24 hours. Purplish red colonies surrounded by reddish zone of precipitated bile were counted for coliforms. Colonies were confirmed using Brilliant Green Bile broth (OXOID CM 31) at pH of 7.4 and incubated at 37°C for 24 hours [NMKL no.44, 2004].

Confirmation of faecal coliforms (EC Medium)

Procedure

Dehydrated ingredients were added to water and mixed thoroughly and heated to dissolve at pH 6.9 ± 0.2 after sterilization. Before sterilization, fermentation tubes, each with an inverted vial were dispensed with sufficient medium to cover the inverted vial at least partially after sterilization. The tubes were closed with heat-resistant plastic caps.

Results

Two presumptive fermentation tubes showed an amount of growth within 48 hours of incubation to the faecal coliform test. The tubes were gently shaken. A sterile 3.5mm-diam loop was used to transfer growth from each presumptive tube to EC broth. Inoculated EC broth tubes were incubated in a water bath at 44.5 ± 0.2^{o}C for 24 ± 2 hours. The EC tubes were placed in water bath within 30 minutes after inoculation. Sufficient water depth was maintained in water incubator to immerse tubes to upper level of the medium. There was gas production with growth in the EC broth culture within 24 ± 2 hours and thus, it was considered a positive faecal coliform reaction. MPN was calculated from the two positive EC broth tubes used.

Enumeration of Escherichia coli

Escherichia coli bacteria were enumerated by the pour plate method using Trypsin Soya agar medium (OXOID CM131) adjusted to the pH 7.3 and overlaid with Violet Red Bile agar (OXOID CM 0107) with pH adjusted to 7.4 and incubated at 44°C for 24 hours and colonies counted. Colonies were confirmed using E.C. broth (OXOID CM 853) with pH adjusted to 6.9. Colonies that produced gas were confirmed for Indole production. This was done by sub-culturing into Tryptone water and incubated at 44°C for 24 hours. Indole testwas done by putting a drop of Kovac reagent into the culture. Red ring colouration at the surface of Tryptone indicated Indole positive [NMKL no.125, 2005].

Biochemical confirmation of Escherichia coli

Five typical and suspected colonies of *Escherichia coli* were sub cultured in tubes of E.C. broth containing inverted durham tubes. They were incubated at 44°C for 24 hours. Positive tubes turned cloudy and their durham tubes trapped gas as a result of the fermentation of lactose by the suspected *Escherichia coli.* Approximately,0.1mL of the broth was sub cultured in tryptone broth and incubated at 44°C for 24 hours. About 2 to 3 drops of Kovac's reagent was added to each of the tubes and left to stand for about 10 seconds. A violet/pinkish ring-like structure that appeared on top of the broth indicated the presence of *Escherichia coli* in the sample. It was then recorded based on the number of positive tubes over the total number of tubes which were five. Two out of the five tubes were positive while three were negative.

Enumeration of Yeasts and Moulds

Yeasts and Moulds were enumerated by the Pour Plate method on Dichloran Rose Bengal Chloramphinicol (DRBC) medium, (Oxoid CM0727; Oxoid Ltd., Basingstoke, Hampshire, UK) to which 1% chloramphinicol in absolute ethanol was added as supplement to suppress bacteria growth. The pH was adjusted to 5.6 and incubated at 25°C for 3-5 days in accordance with ISO 7954 (1987). Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as moulds.

Detection of Salmonella species

Approximately 25g of the sample was weighed using the Ohaus Explorer Pro Balance (Model EP613C, Switzerland) aseptically into sterile Stomacher bag and 225mL buffered peptone water added. This was homogenized thoroughly and incubated at 37°C for 16-21 hours. Following incubation, 0.1mL of broth (buffered peptone water) was transferred into 10 mL of Rappaport-Vasilliadis (RVS) broth and incubated in water bath at 42°C ± 0.5°C for 24 ± 3 hours. Following enrichment, a loopful of the millilitre of Rappaport-Vasilliadis (RVS) broth culture was streaked onto XLD Agar plates using a sterile inoculation loop, and the plates were incubated at 37°C ± 0.5°C for 24hours ± 3 hours. Presumptive colonies (lightly transparent zone of reddish colour and black centre) were maintained on non-selective TSA agar slants for further biochemical tests.

Biochemical confirmation of Salmonella species

Suspected colonies were confirmed on Triple Sugar Iron (TSI) medium, Urea Agar Base (UAB) medium and Lysine Decarboxylase Broth medium and incubated at 37°C for 24 hours.

Serological confirmation

Suspected colonies were streaked onto Tryptone Soya Agar medium and incubated at 37°C for 24 hours. A colony was picked and put onto a clean slide. One drop of anti-serum O was added onto the colony on the slide. A clean cover slip was used to mix the colony and the anti-sera. If no clotting/ agglutination is indicated, then *Salmonella* species were absent from the sample.

Results of Triple Sugar Iron Confirmatory (TSI) Test of salmonella species

Using aseptic techniques, a colony of the test organism was picked with a sterile inoculating loop, and the slant of the media streaked. Using a sterile inoculating needle, the butt of the medial was then stabbed. Tubes were then inoculated at 37°C for 24 hours after which they were observed. A yellow butt and a red slant indicated fermentation of lactose/ or sucrose. Blackening of agar indicated the production of Hydrogen Sulphide (H_2S) gas. A red butt and a slant indicated that none of the sugars were fermented and neither gas nor H_2S were produced. Blackening and gas formation in TSI tube showed no blackening and gas formation. This indicates the absence of *Salmonella* spp.

Bio-Chemical Analyses

Chemical properties of the drinks were also examined to include; the level of concentration of sugar and vitamin C present in the drinks, acidity level, pH ranges and temperatures that are acceptable for human consumption. Acidity

level, pH and Temperature were determined using the pH metre and thermometre. In addition, the Anthrone colorimetric method was used to determine the concentration of Total Sugars and vitamin C in the drinks (Rutkowski *et al.*, 1999).

Determination of Titrable Acidity, pH and Temperature

Prior to the analysis, the samples were thoroughly shaken. The pH and temperatures of the samples were determined using pH meter (pH 4222 microprocessor, Hanna instruments) and Greisinger Electronic Thermometre (Germany). Titrable acidity in g/100mL was determined by taking 10 mL of the sample into a 150 mL conical flask after which distilled water was added to make the volume up to 20 mL. Approximately, 10 mL of each sample was titrated with 0.1mL Sodium Hydroxide (NaOH) solution to an end point of 8.2 (measured with pH meter) and the milliliters (mL) of NaOH used were recorded. The readings were taken of each sample to give a mean measurement for that sample (AOAC, 1990).

TA= MNaOH x mL NaOH x 0.09 x 100mL juice sample

Where, TA = Titratable acidity; MNaOH = Molarity of NaOH used;

mL NaOH = amount (in mL) of NaOH used; 0.09 = equivalent weight of the acid.

Calculation of titratable acidity using the formula below:

$$\% \text{ acid} = \frac{[\text{mls NaOH used}] \times [0.1 \text{ N NaOH}] \times [\text{milliequivalent factor}] \times [100]}{\text{Grams of sample}}$$

Table 3: Predominant Acids in Fruits and their Milliequivalent Factors

Commodity	Predominant acid	Milliequivalent factor
Stone fruit, apples, kiwifruit	Malic Acid	0.067
Citrus	Citric Acid	0.064
Grapes	Tartaric Acid	0.075

Source: AOAC (1990)

Determination of Soluble Sugars

The concentration of sugar in the drinks was determined according to Rutkowski *et al.* (1999) method.

Preparation of Reagent (glucose solution)

Stock solution: (1 mL is equivalent to 0.25mg glucose), 0.250g D-glucose (dried in a vacuum oven at 70^{o}C oven P_2O_5) was dissolved in water and diluted to 1 litre. Working standards: a range from 0 – 20 mL stock solution was pipetted into 50 mL flasks such that 2 mL of each standard gives a range from 0- 0.20 mg glucose and diluted to volume.

Anthrone Reagent

Exactly 760 mL concentration of $H_2SO_4$was added carefully to 330 mLwater in a boiling flask and was kept cool while mixing. 1g anthrone, 1g of thiourea were added and dissolved using a magnetic stirrer. The mixture was transferred into a dark bottle and left for 2 hours before use. It was then Stored at + 1^{o}C.

Procedure: Extraction

The non-alcoholic drinks were labeled from 1 to 22. The beakers and conical flasks were labeled accordingly to correspond to the numbers on the drink

samples. A reasonable quantity of the drinks was poured into the beakers. One millilitre of aliquots was pipetted into a 50 mL conical flask. Distilled water was added to increase the volume to 50 mL. One millilitre of the concentration was pipetted into a 100 mL conical flask and distilled water was added to increase the volume to 100 mL. A blank was prepared by using the same procedure.

Colour Development

Two millilitres of each standard was pipetted into a set of boiling tubes and 2 mL of the extract and water blank were also pipetted into boiling tubes. Standards and samples were treated the same way. Ten millilitres of anthrone solution was added rapidly to mix and the tubes immersed in running tap water or ice bath. The tubes were placed in a beaker of boiling water in a dark fume cupboard and boiled for 10 minutes. The tubes were then placed in cold water and allowed to cool in the dark. The optical density was measured at 625nm or with a red filter using water as a reference. A calibration graph was drawn from the standards and used to obtain milligram glucose in the sample aliquot. The blank determination was treated in the same way and subtractions done where necessary.

Determination of Vitamin C

The vitamin C (ascorbic acid) concentration in the drink samples was determined according to Rutkowski *et al.* (1999) method.

Materials: Comestible or biological liquid, homogenate (or extract) of a food sample or tissue.

Equipment: A centrifuge, test-tubes shaker, a water bath, an UV lamp 250-300 nm, a VIS or UV spectrophotometer, glass or quartz cuvettes (1-1.5 mL).

Reagents: Phosphotungstate reagent (PR) prepared periodically was used. One hundred and fifty grams sodium tungstate molybdenum-free and 60 g sodium hydrogen phosphate anhydrous were suspended in 240 mL deionized (DI) water. It was then mixed with heating to dissolve and added slowly to 145 ml 3.7 M sulphuric acid (VI); the solution was heated for 2 hours with reflux condenser without allowing it to boil; after cooling the solution down, the pH was adjusted to 1.0 while adding drop-wise concentrated sulphuric acid (VI). After this, the reagent became light greenish-yellow, which is the required colour. 56.8 µM vitamin C (L-ascorbic acid) standard solution was made with 50 mM solution of oxalic acid as a solvent. Fresh samples were analysed by working on the stand protected against the direct raining light.

Exactly, 1 mL of the analysed liquid was measured into the centrifugal test-tube, 1 ml of the phosphotungstate reagent (PR) was added, mixed thoroughly and left at a room temperature for 30 minutes. The tube (7000×g, 10 minutes), was centrifuged and the whole of the separated supernatant collected with a pipette – the supernatant was a test sample for Spectrophotometric measurements. The standard sample was prepared as above (using 1 mL of the standard solution instead of the analysed liquid), without centrifugation

The absorbance of the test sample was measured Ax and of the standard sample as at 700nm against the mixture PR: 50 mM solution of oxalic acid = 1:1 (v/v) as a reference sample.Concentration Cx of vitamin C (µM) in the analysed liquid was calculated using the formula:

$$C_X = \frac{A_X}{A_S} . C_S$$

Where: Cs = Concentration of the standard solution.

Determining Samples of drinks for the study

	Concentrated fruit juice preserved by physical and chemical means	Carbonated malt drinks	Fresh fruit juice	Carbonated soft drinks	Total
	73	17	15	15	120
20%	15	3	4	3	25

(Creswell, 2003)

Data Processing and Analysis

The completed data for the observation was captured in Graphs (Pie chart) help answer research question 1. The results from the laboratory tests were also captured in Microsoft excel spread sheet and analyzed using a Statistical Product for Service Solutions (SPSS version 21.0) at 95% confidence interval. A $\log_{10}$ transformation of bacterial count was done before the data analysis. The analysis was done using the means of the laboratory results presented in tables and figures. Means of bacterial counts in the tested samples were compared to the microbiological limits provided by the Ghana Standards Authority to answer questions 2 to 4. The significant differences between means were calculated by One-way Analysis of Variance (ANOVA) using the Duncan test where p= 0.05 to respond to the hypotheses.

Chapter Summary

The research methods employed for the study were the mixed methods approach, comprising survey, observation and experimental designs. The researcher encountered the following limitations during the study. After the initial survey and documentation of non-alcoholic drinks available on the Cape Coast markets, some of them could no longer be found during the period that laboratory

analyses were carried out. In addition, due to high cost of analyzing the microbial loads, chemical parameters and transportation, the sample size was reduced to 22 instead of 25. Not all the analyses could be completed at the Centre for Scientific and Industrial Research (CSIR), Accra, because the outfit was preparing for assessment by external auditors and hence, the researcher had to complete the rest of the analyses at the University of Cape Coast Medical Sciences and the School of Agriculture research laboratories.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

This chapter presents the results and discussions from the laboratory analysis of non-alcoholic drink samples bought from UCC, Kotokuraba and Abura markets in the Cape Coast Metropolis. The findings were presented according to the specific research questions raised. This chapter contains detailed results and discussions reflecting on the research questions and gives a detailed explanation to the findings in the study. It also reveals the extent to which gathered data was supported by existing empirical research findings reviewed to complement the study. The analyses and discussions were done under the following thematic areas:

1. What categories of non-alcoholic drinks are found on the Cape Coast Metropolis markets?
2. What differences exist in the microbiological loads of non-alcoholic drinks available on the Cape Coast Metropolis markets?
3. What differences exist in the chemical constituents of non-alcoholic drinks available on the Cape Coast Metropolis markets?
4. Do the products meet standards?
5. Discussions
6. Research hypothesis

What categories of non-alcoholic drinks are found on the Cape Coast Metropolis markets?

The objective of this research question was to categorise the non-alcoholic drinks that were found on the UCC, Kotokuraba and Abura markets. This was done to bring to the fore the different types of non-alcoholic drinks available on the markets both locally manufactured and imported; and taking into accounts their brand names, types of packaging, volume, whether nutritional information was provided or not, FDA numbers and expiry dates.

The data collected from the three major markets in the Cape Coast Metropolis are presented in Tables 4 to 7:

Table 4: Carbonated Malt Drinks

Name of Drink	Type of Packaging	Volume	Nutrition Information Provided	FDA Number	Expiry Date
Malta Guinness	Canned	330ml	Yes	Nigerian mark of quality FT - 174	20/06/16
Imported magic Malt	Canned	330ml	Yes	CP - 081587/G3Wa25A	29/01/17
Beta malt	Plastic bottle	330ml	Yes	FDA/DK 10-033	05/06/16
BB malt tonic	Canned	33cl	Yes	None	07/02/16
Mighty malt	Canned	330ml	Yes	G4VJ29A	05/10/16
Lion malt	Canned	330ml	Yes	H2WA14E	09/01/16
Vita malt	Canned	330ml	Yes	REXAM 2W51F26D	22/02/17
Vicco malt	Canned	330ml	Yes	P5CA21B	19/08/16
ULTI malt	Canned	330ml	Yes	P5CD23E	11/11/16
Giant malt	Canned	330ml	Yes	HWA14/D	12/08/16
Akwaaba malt	Canned	330ml	Yes	B2VD26B	1/11/15 (Expired)
Solo malt	Canned	330ml	Yes	RITE14D	25/12/15
Pure heaven royal malt	Canned	330ml	Yes	C330057/1B	04/ 16
Hyper malt	Canned	330ml	Yes	B2VK13/B	18/05/16
Supermalt	Canned	330ml	Yes	9F53D15C	22/10/16
Karamalz	Canned	330ml	Yes	None	02/06/17
Dr. malt	Plastic bottle	300ml	Yes	None	04/05/17
Uncle T premium malt	Canned	330ml	Yes	N61B17A	13/08/17
Euro malt	Canned	330ml	Yes	REXAM TL64BZ6C	04/09/17
Choco malt	Plastic bottle	500ml	Yes	None	28/11/16
Schweppes malt	Glass bottle	300ml	Yes	None	13/11/16

Data source: Dasoberi, (2016)

Table 5: Carbonated Soft Drinks

Name of Drink	Type of Packaging	Volume	Nutrition Information Provided	FDA Number	Expiry Date
Coca cola	Canned	330ml	Yes	C1AO4F15D	06/09/16
Fanta	Canned	330ml	Yes	C1AO6J15N	17/09/16
Sprite	Canned	330ml	Yes	C1AO6H15N	08/07/16
Club cola	Canned	350ml	Yes	FDB/DK 04-078	03/03/16
DJ redmix	Canned	330ml	Yes	C52B05B	20/03/17
Solo coca café	Canned	330ml	Yes	R2VB15A	29/01/17
Bubble up	Plastic bottle	350ml	Yes	FDB/DK 14-183	11/11/16
Original American Cola	Plastic bottle	350ml	No	FDB/DK 14-133	13/10/16
5 star vanda cool	Plastic bottle	350ml	Yes	FDB/DK 12-136	29/09/16
King cola	Canned	325ml	No	M52D24C	28/04/17
Cuba cola	Plastic bottle	2Litres	Yes	None	None
5 Star kolah cool	Plastic bottle	350ml	Yes	FDB/DK 12-136	29/09/16

Data Source: Dasoberi, (2016)

Table 6: Concentrated Fruit Juices

Name of Drink	Type of Packaging	Volume	Nutrition Information Provided	FDA Number	Expiry Date
Oye flavoured drink	Plastic bottle	350ml	Yes	FDB/DK 13-435	02/ 17
langers apple orange pineapple 100% pure juice	Plastic bottle	1.89Litres	Yes	None	11/08/16
Tika flavoured drink	Canned	330ml	Yes	7L53E29B	25/12/16
BB cocktail de fruits juice	Canned	33cl	Yes	C2WD27A	30/07/16
MACCAW 100% orange juice	Glass bottle	250ml	Yes	None	05/11/16
MACCAW exotic fruits and fibers	Glass bottle	250ml	Yes	None	19/06/16
POP orange sacs	Canned	238ml	Yes	None	17/10/16
Ceres 100% juice blend	Paper pack	1Litre	Yes	None	05/10/16
Fruity refreshng drink	Paper pack	125ml	Yes	None	15/10/16
Healthi life go fruit juice drink	Paper pack	125ml	Yes	NAFDAC REG. NO. 81-6338	13/10/16
Happy delight juice with milk	Paper pack	125ml	Yes	None	10/08/16
Top up strawberry drink	Paper pack	100ml	Yes	None	28/10/16
Pure joy 100% juice blend	Paper pack	1Litre	Yes	None	07/02/16
Don simon multifrutas	Paper pack	1Litre	Yes	None	08/12/16
Pure heaven pineapple and coconut juice	Paper pack	1Litre	Yes	FSC CO14047	09/16
Fru telli all natural Juice tropical mix	Paper pack	1Litre	Yes	None	27/11/16
Juicee fruit drink	Paper pack	200ml	Yes	None	11/06/16
Kalypo natural juice drink	Paper pack	250ml	Yes	None	06/11/16
Hollandia fruit drink	Paper pack	1Litre	Yes	NCS 0000119	13/01/16
Bebe pineapple drink	Plastic bottle	200ml	Yes	FDB/Da 11-003	28/01/16
Squiz orange drink	Plastic cup	180ml	Yes	None	08/08/16
Shezan all pure grape nectar	Paper pack	1000ml	Yes	None	15/05/16
Sunrise organic Unsweetened Soya drink	Paper pack	1Litre	Yes	None	08/06/16

Table 6: continued

Ocean spray cranberry classic juice drink	Paper pack	1Litre	Yes	None	14/01/16
Halma ginger drink	Paper pack	350ml	Yes	None	None
PATAPATA bissap ice	Plastic bottle	350ml	Yes	None	29/04/16
Alvaro malt (taste of pear)	Plastic bottle	330ml	Yes	FDB/DK/08/158	15/02/17
Vintage pineapple 100% natural drink	Glass bottle	330ml	Yes	FDB/DK 13-025	18/06/16
Jazzy juice	Canned	310ml	Yes	None	12/05/16
Pino star drink	Canned	330ml	Yes	None	06/04/16
Tamek pineapple nectar	Canned	330ml	Yes	None	06/04/16
Quench lime cordial drink	Plastic bottle	1Litre	Yes	FDB/DK GS 168	03/09/16
Zhivchik natural apple juice	Canned	500ml	Yes	None	08/04/16
Spec sparkling lemon juice	Canned	325ml	Yes	None	22/05/17
Fruittis pineapple-coconut pure fruit juice	Paper pack	1Litre	Yes	None	19/05/17
Fruittis pineapple pure fruit juice	Canned	330ml	Yes	KR6PCFT2	24/12/16
O lemon drink	Canned	300ml	Yes	U142JO2A	17/06/16
Mountain dew drink	Canned	355ml	Yes	P34740	05/07/16
Sagiko soursoup juice drink	Canned	320ml	Yes	AS15A15C1	27/03/2018
Cheers flavoured carbonated drink	Canned	325ml	Yes	1004-03/2004	22/04/17
Grace ginger beer	Canned	330ml	Yes	B5VI13A	18/08/16
Royalty ginger beer	Canned	330ml	Yes	305764	02/ 2016
Elisha flavoured drink	Plastic canned	330ml	Yes	7842050086	30/06/16
Tampico citrus punch	Plastic bottle	500ml	Yes	None	20/03/16
Superman banaiz	Plastic bottle	220ml	Yes	NAFDAC REG. NO. B1-2981	13/01/16
Planet drink	Plastic bottle	350ml	Yes	FDB/DK 14-182	11/07/16
5 Star drink	Plastic bottle	300ml	Yes	FDB/DK 11-097	28/08/16
Pure heaven tropical fruit juice	Paper pack	200ml	Yes	None	None
San Anton multifrutas	Paper pack	1Litre	Yes	2530-071	08/12/16
Dimes premium fruit juice	Paper pack	1Litre	Yes	1PIHR0685	24/01/17
Purity soy	Paper pack	1Litre	Yes	FSC0CO14047	06/02/16
Easy mouzuoooo	Paper pack	1Litre	Yes	None	23/05/16

Table 6: continued

Minutemaid tropical drink	Paper pack	1Litre	Yes	None	17/03/16
Juver angry birds drink	Paper bottle	330ml	Yes	None	15/03/16
Juvita flavoured drink	Paper pack	180ml	Yes	None	10/06/16
Squiz drink	Plastic bottle	139ml	Yes	None	15/6/16
Premium fresh 100% juice	Paper pack	1Litre	Yes	C/PAP/PE/ALU	22/07/16
Freezy drink	Plastic strip	100ml	Yes	None	07/10/16
Pran frooto mango juice	Plastic bottle	500ml	Yes	BDS 1581	02/08/16
Black star fruit juice	Glass bottle	330ml	Yes	FDB/DK 13-117	05/02/16
Apple and eve 100% juice	Paper pack	200ml	Yes	None	26/05/16
Viva fresh fruit juice	Paper pack	1Litre	Yes	None	21/06/16
Chabaa juice	Paper pack	1000ml	Yes	None	08/02/16
Darina instant drink	Sachet	759g	Yes	None	15/9/16
Caprisum all natural 100% juice	Sachet	None	None	None	13/02/16
Frujus 100% fruit juice	Paper pack	1Litre	Yes	None	04/02/16
Peach nectar	Paper pack	1Litre	Yes	None	09/01/16
Beyti for Tropicana	Paper pack	1Litre	Yes	None	24/7/16
So fresh	Paper pack	1Litre	Yes	None	02/10/16
OKF aloevera king drink	Canned	240ml	Yes	None	26/2/17
Cuba exotic	Canned	2litres	Yes	TD 01-1995/05	None
Easy drink	Plastic bottle	350ml	Yes	None	10/10/16
Robinsons fruit shoot	Plastic bottle	275ml	Yes	None	04/ 16
Royal apple drink	Plastic bottle	500ml	Yes	FDA/DK 16-393	02/08/16

Data Source: Dasoberi, (2016)

Table 7: Fresh Fruit Juices with no physical or chemical preservatives

Name of Drink	Type of Packaging	Volume	Nutrition Information Provided	FDA Number	Expiry Date
Blue skies mango, orange and banana smoothie	Plastic bottle	500ml	Yes	FDB/DK05-179	15/ 04/016
Farm fresh pinemelon	Plastic bottle	500ml	No	FDB/DK/14-208	None
Farm fresh pinecitrus	Plastic bottle	500ml	No	FDB/DK/14-208	None
Farm fresh pinecarrot	Plastic bottle	500ml	No	FDB/DK/14-208	None
Anidak's bissap	Plastic bottle	350ml	Yes	None	None
Anidak's fruit juice	Plastic bottle	350ml	Yes	None	29/ 04/ 16
Noble's pineapple juice	Glass bottle	350ml	No	None	15/02/2017
Anidak's fresh pineapple juice	Plastic bottle	350ml	No	None	None
Marven fresh juice	Plastic bottle	500ml	Yes	None	09/02/16
Tasty terfty sugarcane juice	Plastic bottle	2.25litres	No	None	None
Hossana sobolo	Plastic bottle	500ml	Yes	None	20/ 04/16
Fruity juice (mixed fruits)	Glass bottle	330ml	Yes	FDB/DK 07-020	20/02/17

Data source**:** Dasoberi, (2016)

Table 7: continued

NAME OF DRINK	TYPE OF PACKAGING	VOLUME	NUTRITION INFORMATION PROVIDED	FDA NUMBER	EXPIRY DATE
Blue Skies Mango, Orange and Banana Smoothie	Plastic Bottle	500ml	Yes	FDB/DK05-179	9th April - 15th April 2016
Farm fresh pinemelon	Plastic Bottle	500ml	No	FDB/DK/14-208	None
Farm fresh pinecitrus	Plastic Bottle	500ml	No	FDB/DK/14-208	None
Farm Fresh pinecarrot	Plastic Bottle	500ml	No	FDB/DK/14-208	None
Anidak's Bissap	Plastic Bottle	350ml	Yes	None	None
Anidak's fresh mixed fruit juice	Plastic Bottle	350ml	Yes	None	29th April 2016
Noble's pineapple juice	Glass Bottle	350ml	No	None	15th February 2017
Anidak's fresh pineapple juice	Plastic Bottle	350ml	No	None	None
Marven fresh juice	Plastic Bottle	500ml	2Yes	None	9th February 2016
Marven fresh pineapple juice	Plastic Bottle	500ml	Yes	None	9th February 2016
Hossana Sobolo	Plastic Bottle	500ml	Yes	None	20th April 2016
Hossana orange juice	Plastic Bottle	500ml	Yes	None	20th April 2016

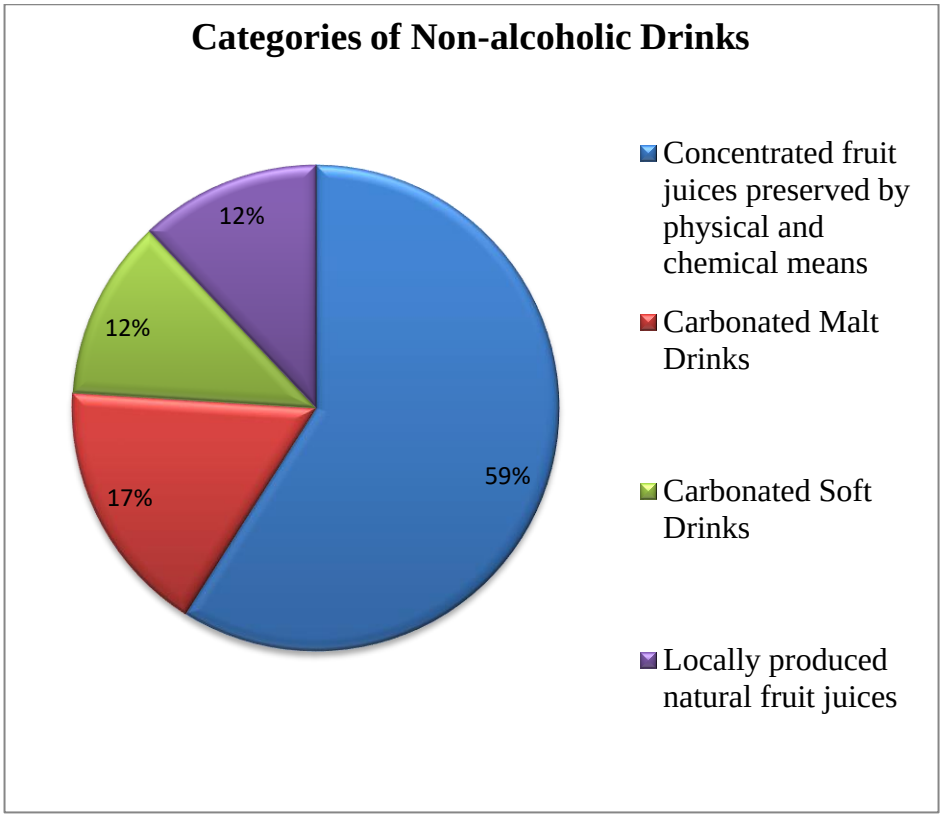

Figure 1: Categories of non-alcoholic drinks

Source: Dasoberi, (2016)

Figure 1 shows the data that was collected from the various markets in the Cape Coast Metropolis. The figure shows that concentrated fruit juices preserved by physical and chemical means represent 59% while carbonated malt had 17%. Comparatively, carbonated soft drinks and locally produced fruit juices with no preservatives were 12%. This implies that concentrated fruit juices preserved by physical and chemical means were more on the markets than any other drink. It is worth noting that, the soft drinks industry has witnessed large-scale adoption of non-alcoholic drinks market in the Cape Coast Metropolis.

Results of Microbiological Analyses

Category A: Carbonated Soft Drinks and Carbonated Malt Drinks

Table 8: Mean Microbial counts (Log cfu/mL) for carbonated soft drinks and carbonated malt drinks

Sample(s)	Aerobic Plate Count	Yeast and Moulds
MM	1.84±0.004	0.00
MG	0.00	0.00
BM	0.00	0.00
CC	0.00	0.00
OAC	0.00	0.00
KC	2.71±0.005	1.12±0.11

MM= Magic Malt MG=Malta Guiness BM= Beta Malt CC=Club Cola OAC= Original American Cola KC= 5 Star Koolah Cool

Source: Dasoberi, (2016).

From Table 8, 5Star Koolah Cool (KC) drink showed aerobic mesophilic counts (APC) 2.71±0.005, and yeasts and moulds, 1.12±0.11. The results of Aerobic Plate Count (APC), and yeasts and moulds for Magic Malt (MM) were 1.84±0.004 and 0.00, respectively. However, there was no growth for the rest of the drinks.

Category B: Concentrated fruit juices preserved exclusively by physical means

Table 9: Mean microbial counts (Log cfu/ml) for concentrated fruit juices preserved exclusively by physical means

From table 9, results obtained from the analysis of concentrated fruit juices preserved exclusively by physical means showed no growth for all the samples examined.

Category C: Fresh fruit juices with no physical or chemical preservatives

Table 10: Mean Microbial counts (Log cfu/mL) for fresh fruit juices with no physical or chemical preservatives

Sample(s)	Aerobic Plate Count	Total Coliforms	Faecal coliforms	*E. coli*	Yeasts and Moulds	*Salmonella* sp.
AD	2.696±0.004	0.0	0.0	0.0	6.368±0.009	Not Detected
PR	5.271±0.033	2.427±0.010	0.0	0.0	4.902±0.038	Not Detected
PM	5.689±0.008	3.054±0.001	3.057±0.022	3.285±0.016	3.728±0.017	Not Detected
PC	5.151±0.043	2.528±0.003	2.429±0.044	0.0	4.134±0.067	Not Detected
BS	5.631±0.072	3.971±0.010	0.0	2.434±0.034	2.812±0.036	Not Detected

AD= Anidak bissap PR= Pinecarrot PM= Pinemelon PC= Pinecitrus BS= Blue skies

Source: Dasoberi, (2016).

From Table 10, mean APCs in all fresh fruit samples ranged between 2.696±0.004 Log cfu/mL and 5.689±0.008 Log cfu/mL which were greater than the permissible limits. Mean coliform counts in all fresh fruit juices ranged between 0.00 Log cfu/mL and 3.971±0.010 Log cfu/mL for PR and BS, respectively, also did not meet the acceptable permissible limit for total coliforms(<1 log cfu/mL). In the case of faecal coliforms, it was discovered that mean counts ranged between 2.429±0.044 Log cfu/mL and 3.057±0.022 Log cfu/mL for PC and PM, respectively, which were also above the acceptable limits (<1 log cfu/ml). With significant values, *Escherichia coli* ranged from 0.00 Log cfu/ml - 3.285±0.016 Log cfu/mL for BS and PM, respectively. Moreover, yeasts and Moulds ranged from 0.00 Log cfu/mL - 6.368±0.009 Log cfu/mL for BS and AD, respectively.

Category D: Fruit juice, squashes and cordials preserved by physical means

Table 11: Mean Microbial counts (Log cfu/ml) for Fruit juice, squashes and cordials preserved by physical means

Results for Table 11 showed that, there was no growth for all the parameters examined in the drink samples.

What differences exist in the physico-chemical constituents of non-alcoholic drinks available on the Cape Coast Metropolis markets?

The objective was to determine levels of concentration of sugars and vitamin C, pH ranges and acidity levels in the non-alcoholic drinks. To achieve this, samples of non-alcoholic drinks obtained from the three major markets in the Cape Coast Metropolis were sent to the laboratory for analyses. Chemical

analyses were performed to determine the concentrations of soluble sugars and vitamin C, using the Anthrone and the Rutkowski *et al.*, (1999) methods respectively. Determination of pH, temperature and Titrable acidity were also done using the AOAC (1990) method.

Results of the Chemical Analyses

The results of the chemical analyses are presented in tables 4.9 to 4.12 according to their categories.

Category A: carbonated soft drinks and carbonated malt drinks

Table 12: Mean Sugars and Vitamin C concentrations of carbonated soft drinks and malt drinks

Sample(s)	Sugars (%)	Vitamin C(mL)
MM	20.59±1.07	0.115±0.007
MG	21.38±2.67	0.095±0.007
BM	21.04±0.30	0.115±0.007
CC	23.13±0.65	0.04±0.00
OAC	23.18±3.43	0.02±0.00
KC	19.20±1.84	0.02±0.00

MM= Magic Malt MG=Malta Guiness BM= Beta Malt CC= Club Cola OAC= Original American Cola KC= 5 Star Koolah Cool

Source: Dasoberi, (2016)

The mean ± SD sugar concentrations recorded for all the samples in (Table 12) ranged between 19.20±1.84 to 23.18±3.43 and vitamin C concentrations also ranged from 0.02±0.00- 0.115±0.007

Category B: Concentrated fruit juices preserved exclusively by physical means

Table 13: Mean Sugars and Vitamin C concentrations of concentrated fruit juices preserved exclusively by physical means

Sample(s)	Sugars (%)	Vitamin C (mL)
AV	17.53±0.18	0.04±0.03
FT	28.49±8.81	0.14±0.01
DS	17.32±1.65	0.015±0.01
PH	6.643±0.015	0.01±0.00
TP	30.96±3.43	0.15±0.03
PPB	6.983±0.0	0.05±0.0

AV= Alvaro FT= Frutelli DS= Don Simon PH= Pure Heaven TP= Tampico PPB= Patapata bissap ice

Source: Dasoberi, (2016).

Results from Table 13 recorded mean sugars and Vitamin C concentrations for all the drink samples. The concentrations ranged from 6.983±0.0 - 30.96±3.43 and 0.01±0.00 - 0.15±0.03 for sugar and vitamin C respectively.

Category C: fresh fruit juices with no physical or chemical preservatives means

Table 14: Mean Sugars and Vitamin C concentration of Fresh Fruit Juices with no physical or chemical preservatives

Sample(s)	Sugars (%)	Vitamin C(mL)
AD	7.001±0.00	0.045±0.0
PR	6.718±0.01	0.055±0.001
PM	6.739±0.03	0.03±0.003
PC	6.726±0.01	0.0625±0.001
BS	5.863±0.01	0.0165±0.004

AD= Anidak Bissap PR= PineCarrot PM= PineMelon PC= PineCitrus BS= Blue Skies

Source: Dasoberi, (2016)

Table 14 presents results of the mean sugars and vitamin C concentrations of fresh fruit juices with no physical or chemical preservatives. The frequencies indicate that the highest mean of sugar in a single fresh fruit juice sample was 7.00 ± 0.00 having a vitamin C content of 0.045 ± 0.0. Blue Skies (BS) had a mean of 5.863±0.01 and vitamin C content of 0.0165±0.004 being the lowest.

Category D: Fruit juice, squashes and cordials preserved by physical and chemical means

Table 15: Mean Sugars and Vitamin c concentration of Fruit juice, squashes and cordials preserved by physical and chemical means

Sample(s)	Sugars (%)	Vitamin C (mL)
JU	26.40±1.95	0.00
FR	25.77±2.01	0.1±0.00
SQ	40.71±14.38	0.115±0.01
KA	27.28±7.57	0.015±0.01
JP	7.37±0.005	0.0095±0.002

JU= Juvita FR= Fruity SQ= Squiz KA= Kalypo JP=Juicee Pineapple

Source: Dasoberi, (2016)

Analysis of the data (Table 15) revealed that the mean sugar content and vitamin C content in a single sample of fruit juice, squashes and cordials preserved by physical and chemical means were higher than expected. Fruity juice (FR) (25.77 ± 2.01) value was higher than what humans are expected to consume. Another 40.71±14.38, Squiz (SQ) was also above the quantity expected.

Table 16: Physico-chemical parameters of Drinks and Juices

Drink and Juices categories	pH	Temp (O^C)	Acid %	Titrable acidity
Concentrated Fruit Juices	3.243±0.26	30.78±2.85	0.42±0.21	7.13±1.37
Fruit Juice, Squashes and Cordials	3.17±0.26	32.12±1.04	0.292±0.12	4.39±1.90
Fresh Fruit Juices	3.45±0.33	23.98±1.37	0.94±0.19	14.72±3.11
Carbonated Soft Drinks and Malt	3.602±0.86	26.82±2.58	0.18±0.03	2.52±0.35

Source**:** Dasoberi, (2016).

Table 16 shows the physico-chemical properties of all categories of Non-alcoholic drinks. It presents the mean ± SD of pH, Temperature, acid percentage and the Titrable acidity of all the drink samples. Results from Table 16 showed that, fruit juices, squashes and cordials recorded the lowest pH 3.17±0.026 and carbonated soft drinks and carbonated malt drinks recorded the highest pH 3.602±0.86.

Temperature Fresh fruit juices without preservatives recorded the lowest pH value 23.98±1.37, while fruit juice, squashes and cordials recorded the highest value 32.12±1.04. For Percentage Acid (Acid %), Carbonated soft drinks and Carbonated Malt drinks had the lowest acid percentage 0.18±0.03 and Fresh fruit juices without preservatives had the highest 0.94±0.19. Results for Titrable Acidity showed carbonated soft drinks and carbonated malt drinks recorded the lowest value of 2.52±0.35 and fresh fruit juices without preservatives recording the highest value 14.72±3.11.

Discussion of Results

In many developing countries including Ghana, millions of people are widely consuming non-alcoholic drinks in every season as it provides an affordable source of nutrients and refreshment to them. Fruit juices being good in taste are available at low price and liked by the consumers (Ohiokpehai, 2003). Non-alcoholic drinks are loaded with sugars and microbes. The increase in the overconsumption of these drinks may be contributing to the rise in non-communicable diseases (NCD) such as dental caries, diabetes, obesity, high blood pressure and some cancers among Ghanaians in general. There is therefore a growing health and wellness consciousness among consumers and an increasing importance given to fitness and healthy lifestyle choices. Changing work and lifestyle habits of consumers leave less time for home cooking and therefore non-alcoholic drinks have become convenient replacement for real meals.

The quality of fruit juices is strictly maintained in many developed countries under several laws and regulations. Unfortunately, in many developing countries including Ghana, manufacturers are not much concerned about the microbiological safety and hygiene of fruit juices because of lack of enforcement of the law, inadequate or lack of knowledge by some of the manufacturers. Thus, the transmission of certain human diseases through juice and other drinks becomes a serious problem (Tasnim, 2010). This study seeks to evaluate some non-alcoholic drinks in the Cape Coast Metropolis.

The Types of Non-Alcoholic Drinks Found on the Markets

From Tables 4 to 7, all drink samples were in appropriate packages and this corresponds to the literature reviewed in chapter two. Most drinks had nutrition information on their labels with the exception of a few which did not. At the time of data collection, almost all the drinks under review had not expired, except one (Akwaaba Malt).

Concerning the Food and Drug Authority (FDA) number, out of the one hundred and twenty drinks that were recorded, only twenty had FDA numbers on them. These drinks are: "Beta malt", "Club cola soft drink", "Bubble up soft drink" "Original American cola soft drink" and "5 star vanda cool soft drink". The rest were "5 star kolah cool soft drink", "Oye flavoured drink", "Bebe pineapple drink", "Alvaro malt soft drink", "Vintage pineapple natural drink", "Quench lime cordial drink", "Planet drink", "5 star drink", "Black star fruit juice", "Royal apple drink", "Blue skies mango, orange and banana smoothie", "Farm fresh pinemelon", "Farm fresh pinecitrus", "Farm fresh pinecarrot", and "Fruity mixed juice".

Other drink samples also had different numbers that were not recognizable FDA numbers in Ghana. Moreover, most of the drinks did not have FDA numbers at all. This is evidently a great concern since consumers cannot be sure of the authenticity of the drinks they are consuming. Health and wellness are known to significantly pose positive impact on the food and beverages industry. There are definitely more varieties of drinks that are non-alcoholic, at least in terms of their nutritional variation.

The Microbiological Quality of the Non-Alcoholic Drinks

The laboratory analysis of the microbial safety of the non-alcoholic drinks showed that some drink samples were above the acceptable limits specified in the GSA Standards, (GSA 7265, 2003). The APC counts showed growth of microorganisms in some brands of non-alcoholic drinks. Out of the 6 drink samples from category 'A', Carbonated Soft Drinks and Carbonated Malt Drinks analysed, bacteria growth was observed in only two (33%) of the samples tested thus Magic malt (MM) and 5 Star Koolah cool (KC). The Aerobic plate Count ranged from 1.84±0.004 to 2.71±0.005 cfu/ml. This was within the acceptable limits (10^2 – 10^3 cells/ml) specified in the Ghana Standards (ISO 4833-1:2013).

Moreover, bacteria growth was observed on all 5 drink samples from category 'C', fresh fruit juices with no physical or chemical preservatives that were tested (Table 10). These drink samples had Aerobic Plate Count ranging from 2.696±0.004 to 5.689±0.008 cfu/ml. These counts were not within the acceptable limits (10^2 – 10^2 cells/ml) specified in the Ghana Standards (GSA 1002:2009). Yeasts and moulds count for drink sample (KC) in category 'A' was 1.12±0.11 that was within the accepted limits (10^1 - $5x10^2$cells/ml) specified in the Ghana Standards (ISO 21527:2008).

However, in category 'C' yeasts and moulds was observed on all 5 (100%) drink samples, with counts ranging from 2.812±0.036 to 6.368±0.009 cfu/ml. The coliform counts ranged from 0.0 to 3.971±0.010 cfu/ml. Moreover, only two (40%) drink samples out of the 5 samples recorded count for *Escherichia coli*. The counts ranged from 0.0 to 3.285±0.016 cfu/ml. some of the drink samples were not within the acceptable limits (10^1 – 10^2 cells/ml) by the Ghana Standards

(GSA, ISO 7251:2005). However, *Salmonella* spp was not enumerated and this was within the acceptable limits (0cells/ml) by the Ghana Standards (GSA, ISO 6579:2002).

Results obtained from tables 9 and 11, category 'B', concentrated fruit juices preserved exclusively by physical means and category 'D', fruit juices, squashes and cordials preserved by physical means respectively, showed no growth for all the parameters examined in the drink samples.

Drink samples from tables 9 and 11 is sterile (Carter, Charley & Bristol, 2007). This is because of the presence of sulphite that inhibits the growth of bacteria, yeast and Moulds (Doughari & Elmahmood, 2007). This result is consistent with a study carried out by Carter, Charley & Bristol (2007), who reported that sterility of many products could be maintained safely by pasteurization with the addition of sodium metabisulphite. The results presented also corroborates with Stratford (2006) who indicated that, spoilage will require a certain critical cell number (10^5 –10^6 cells/mL) and therefore microbial growth. Again, the study is in agreement with other studies (Back, 2005; Stratford, 2006; Lawlor, 2009; Tribst, Sant'Ana & deMassaguer, 2009), that a range of microbes can be associated with soft drink manufacture, but only a few are able to cause spoilage. Microbiological spoilage leads to deterioration of the sensory quality of the drink and typically appears as off-flavours, odours and visual changes in the product. As microbes differ in the growth requirements, different beverages support different spoilage microbes.

Results presented in Table 10 for drinks in category 'C' showed high counts of APC, as well as yeasts and moulds. *Escherichia coli* and faecal

coliforms recorded (40%) count of samples tested. This represents the lowest count for drink samples tested. The presence of coliforms and *Escherichia coli* in food is usually an indication of fecal contamination (Anderson *et al.,* 2005; Amusa *et al.,* 2005). The presence of the coliforms is consistent with some studies that have shown that coliforms (for example, Klebsiella, Citrobacter and Enterobacter) and other 35 members of Enterobacteriaceae can also proliferate in fruit juices and drinks due to their acid tolerant nature and ability to thrive in mediums with pH values below 4.3 (Lawlor *et al.,* 2009).

Most of the fresh fruit juices in this study were found to be unwholesome for human consumption because of the presence of bacteria that were above the limits. Moreover, the presence of coliforms in high numbers is a health hazard causing spoilage of fruit juices and food borne diseases (Gulf Standards, 2000).

A number of factors may have contributed to the relatively high microbial loads. This could have been due to poor fruit quality and improper washing of fruits leading to contamination, non-hygienic containers or peeling of the fruits (Haghighat-Afshar *et al.,* 2014). Lack of appreciation of basic safety issues by vendors may also have contributed to augmentation of the microbial loads in the fruit juice samples (Gulf standards, 2000).

In addition, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust may also have contributed to the high levels of contamination of the drinks (Lewis *et al.,* 2006). The results corroborate with (Harrigan, 1998; Al-Jedah *et al.,* 2002) that a number of factors are responsible for contamination of freshly squeezed fruit juices. The study revealed that both total coliforms and fecal coliform loads were seen to be higher

in some of the drinks sampled. These findings about the bacterial load deviate from the findings of GSA 7265, (2003) and Tasnim *et al.*, (2010); who reported the bacterial counts of fruit juices within the standard limits (<1 log cfu/ml).

The study again supports Nagaraja *et al.*, (2015), research on microbes in fruit juices, in kims-amalapuram. They reported that the analysed samples of fruit juices were found to be contaminated with different bacteria, *Escherichia coli* (30%) *Klebsiella pneumoniae* (10%) *Staphylococcus aureus* (20%) *Enterococcus faecalis* (4%) *Pseudomonas aeruginosa* (10%) Aerobic Spore Bearers (ASB) (04%), Micrococci (2%) *Proteus* (20%) *Salmonella* spp. However, *Shigella* and *Vibrios* were not isolated.

Yeasts and moulds were detected in only (KC) in category 'A', Table 8, representing carbonated soft drinks and carbonated malt drinks. The results obtained may have been due to carbonation that may have inhibited the growth of spoilage microbes by inhibition of cell division, inhibition of amino acid uptake, perturbation of cytoplasmic buffering, induction of sporulation, and lowering cytoplasmic pH (Stratford, 2006).

This also corroborates with the findings of Back (2005) that carbonated soft drinks are generally less prone to microbial spoilage than non-carbonated soft drinks. However, yeasts and moulds were detected in all the drink samples in category 'C' (Table 10) representing fresh fruit juices with no physical or chemical preservatives. High yeast and moulds count present in the various fresh fruit juices may have been due to the presence of fermentative organisms as cited by Odu & Adeniji (2013). The results corroborates with the findings of Obire *et al*., (2008) and Okigbo *et al*., (2009), that yeasts and moulds are more regarded as

spoilage agents of fruit juices compared to bacteria because of the physical and chemical properties of the fruit juices. Moreover, some of these properties include the low pH of fruit juices, the positive oxidation reduction potential of the fruit juices and the rich nutrient composition of the juice. Endrizzi, Pirretti, Calo, and Gasperi (2009), stated that both yeasts and moulds cause various degrees of deterioration and decomposition of foods.

Furthermore, Tetra pak (2000) agrees that, although the processing of fruit juice has been maintained at a considerable hygienic standard, a variety of yeasts and moulds and some bacteria are still able to find their way into industrially produced juices. It may therefore seem clearly that these potential spoilage organisms originate from the raw fruits used for processing or from the processing equipment. Again, fruit juices contain essential nutrients that support the growth of acid tolerant bacteria, yeasts and moulds (Kamal *et al.,* 2014). However, *Salmonella* spp. was not enumerated in any of the drink samples. Thus, meeting the acceptable standard (0.0 log cfu/mL) by the Ghana Standards Authority.

The results of the microbiological analysis suggest bacterial contamination of the packaged non-alcoholic drinks, especially the fresh fruit juices with no physical or chemical preservatives. The presence of bacteria and fungi could be an indication of spoilage. This could have happened during handling since they are liquid, which might have contributed to the development as well as multiplication of these contaminants. Moreover, additional sources of contamination might be the packaging materials, which are not usually or properly sterilized (Amusa *et al.,* 2005). These microbial contaminations of

drinks normally occur during the production process, due to the raw materials, factory environment, cleanliness of the equipment and packages, and lack of hygiene (Stratford & James, 2003).

Manufacturers of non-alcoholic drinks must maintain proper hygienic conditions and use good quality fruits and water in the preparation of these drinks. These will certainly improve the microbiological quality of juices, and make them acceptable to quality conscious consumers both locally and internationally.

There is also the need to educate the drink makers and retailers on the hazards associated with the cultivation of nonchalant attitudes to hygienic processing, display and packaging of these drinks. The lack of knowledge on safe fruit juice preparation as well as the contamination sources can contribute to the elevation of pathogens in prepared juices. It is therefore, essential for the people who handle and prepare juices, to be properly trained on safe fruit handling and processing technique.

Regular monitoring of the quality of fruit and vegetable juices for human consumption must also be enforced by the Ghana Standards Authority and the Food and Drugs Authority.

Physico-chemical Quality of the Non-Alcoholic Drinks

The objective was to determine sugar and Vitamin C concentrations and also the levels of pH, temperature and Titrable acidity in the non-alcoholic drinks. To achieve this, samples of non-alcoholic drinks obtained from the three major markets in the Cape Coast Metropolis were sent to the laboratory for analyses. Chemical analyses were performed to determine the concentrations of soluble sugars and vitamin C, using the Anthrone and the Rutkowski *et al.*, (1999) methods respectively. Determination of pH, temperature and Titrable acidity were also done using the AOAC (1990) method.

Sugar Levels in the Non-Alcoholic Drinks

Out of the 5 samples tested for sugar levels in Table 12, representing carbonated soft drinks and carbonated malt drinks, none of them was within the standard (1% - 12%) specified by Kregiel (2015). The sugar concentrations in the samples tested ranged from 19.20±1.84 to 23.18±3.43. Drink sample Original American Cola (OAC) recorded the highest sugar percentage (23.18%).

Table (13) recorded sugar levels for concentrated fruit juices preserved exclusively by physical means. Out of the 6 drink samples tested, only two (33%) were within the acceptable limit. The concentrations ranged from 6.643±0.015–30.96±3.43. Drink sample Pure Heaven (PH) recorded the lowest percentage (6.643%) of sugar concentration.

Table (14) also represents the mean sugars and concentrations of fresh fruit juices with no physical or chemical preservatives. All the 5 drink samples in this category recorded values that were within the acceptable standard (1% - 12)

stipulated by Kregiel (2015). The levels ranged from 5.863±0.01 to 7.001±0.00. The drink sample Anidak Bissap (AD) obtained the highest sugar concentration (7%) in this category and Blue Skies (BS) had the lowest (5.863%).

Table (15) revealed a higher percentage of sugar concentrations in the drink samples of fruit juice, squashes and cordials preserved by physical and chemical means than expected. Drink sample Squiz (SQ) obtained the highest value (40.71±14.38) of sugar concentration, which is higher than the recommended sugar level for human consumption in a day. These ranges are significant values of great concern.

Data available presented (Table 2) reveals that Diabetes mellitus and dental caries conditions have been gradually increasing since 2010. This means that more Ghanaians are becoming diabetic and experiencing dental issues because of eating more sugary. To prevent or minimize these diseases, non-alcoholic drinks be avoided if possible or be consumed sparingly.

Vitamin C concentrations in the Non-Alcoholic Drinks

All the 22 drink samples from the different categories (Tables 12 to 15) tested for vitamin C concentration failed to meet the standard (10ml/100ml) specified by the Malaysian standards (1999) for Vitamin C. From the tables, it is obvious that the results were very low and therefore, did not meet the standard set. According to Steen & Ashurst, (2006), ascorbic acid, better known as vitamin C, is used not only as a contributory acidulant but also as a stabiliser within the soft drinks system, and its antioxidant properties help to improve the shelf-life and

stability of flavour components. Many of the ingredients used in flavourings are susceptible to oxidation, particularly aldehydes, ketones and ketoesters. Ascorbic acid shields these from attack by becoming preferentially oxidised and lost, leaving the flavour component unaffected. It is worth noting that although ascorbic acid acts well as a browning inhibitor in unprocessed fruit juices, its effect can be destroyed if the juice is subsequently pasteurised or heat-treated. These drinks could however be fortified with ascorbic acid (Vitamin C).

pH, Temperature, Percentage Acid and Titrable acidity of the non- alcoholic drinks

Table 16 shows the physico-chemical properties of all categories of non-alcoholic drinks. It presents the mean ± SD of pH, temperature, percentage acid and the titrable acidity of all the drink samples. The results indicate that the pH of the various categories of drinks ranged from 3.17±0.026 to 3.602±0.86. Fruit juices, squashes and cordials recorded the lowest pH and carbonated soft drinks and carbonated malt drinks recorded the highest. The temperature of the drinks ranged from 23.98±1.37 to 32.12±1.04. Fruit juices, squashes and cordials recorded the highest temperature, while Fresh Fruit Juices with no physical or chemical preservatives recorded the lowest temperature.

For Percentage Acid (Acid %), the ranges were 18% - 94%. Carbonated soft drinks and carbonated malt drinks had the lowest acid percentage while Fresh Fruit Juices with no physical or chemical preservatives had the highest. Results for titrable acidity ranged between 2.52±0.35 and 14.72±3.11. Carbonated soft

drinks and carbonated malt drinks recorded the lowest value while Fresh Fruit Juices with no physical or chemical preservatives recorded the highest value.

pH has a profound effect on the growth of microorganisms. Most bacteria grow best at about pH 7.0 and grow poorly or not at all below pH 4.0. In the present study there was no growth recorded for all parameters tested for categories B and D, which are concentrated fruit juice preserved exclusively by physical means; and fruit juice, squashes and cordials preserved by physical means respectively; since their pH were below 4.0 (Aggie Horticulture 2016). The acidity of the samples show that fresh fruit juices with no physical or chemical preservatives had a higher acidity but pH was within the acceptable range. However, carbonated soft drinks and carbonated malt drinks had the lowest acidity level but the highest pH value. The results also indicate no growth for *Salmonella* spp in all the fresh fruit samples tested and this could be because of the low pH. This result agrees with the findings of Chung & Goepfer (1970), that the minimal pH for growth of principal foodborne disease organism such as *Salmonella* spp is 4.0.

In this study, the lowest temperature value was 75.16°F (23.98±1.37) and the highest value was 89.82°F (32.12±1.04). The results are in agreement with Wagner (2008), that generally bacteria grow and multiply best within the temperature danger zone, 41°-140°F, though there are some bacteria, including Listeria monocytogenes that grow at refrigerator temperatures. This could account for the growth of certain bacteria such as coliforms, *Escherichia coli* and yeasts and moulds in the drinks tested.

Do the products meet standards?

The objective was to compare the test results obtained after microbiological and physico-chemical analyses were performed, with the standards available. This was to know if drinks on the Cape Coast Metropolis were wholesome for human consumption. The details are presented in tables 17 and 18 below.

Table 17: Summary of results of Mean Microbial Counts (Log cfu/mL) found in the Non-Alcoholic drinks tested compared with the GSA Standard

Category	Aerobic Plate Count	Total Coliforms	Faecal Coliforms	*E. coli*	Yeast & Moulds	*Salmonella*
Carbonated soft drinks and Carbonated Malt Drinks	1.84 – 2.71	*	*	*	0.00 – 1.12	*
Concentrated fruit juices preserved exclusively by physical means	0.00	0.00	*	*	0.00	*
Fresh fruit juice with no physical or chemical preservatives	2.69 – 5.69	2.43 – 3.97	2.43 – 3.06	2.43 – 3.29	2.81 – 6.37	Not Detected
Squashes & Cordials preserved by Physical means	0.00	0.00	*	*	0.00	*

*Test is not applicable

Ghana Standards Authority Mean Microbial Counts (Log cfu/mL) (GS 7265, 2003): Aerobic Plate Counts (<2 log cfu /mL), Total Coliforms (<1 log cfu/mL), Faecal Coliforms (<1 log cfu/mL), *E. coli* (<2.0 log cfu/mL), Yeasts & Moulds (< 2.0 log cfu /mL) and *Salmonella* spp. (<0.0 log cfu/mL).

Table 18: Summary of Mean Sugars and Vitamin C of non-alcoholic drinks

Category	Mean Sugars (%)	Average Mean Sugar (Kregiel, 2005) (%)	Vitamin C Concentrations (ml)	Average Mean vitamin C (Malaysia Standard s, 1999) (ml)
Carbonated malt Drinks	19.20 – 23.18	1 – 12	0.02 - 0.15	10ml/100ml
Concentrated Fruit Juices preserved exclusively by physical means	6.983 – 30.96	1 – 12	0.01 – 0.15	10ml/100ml
Fresh Fruit Juices with no physical or chemical preservatives	0.03 – 7.00	1 – 12	0.016 – 0.06	10ml/100ml
Fruit juice, Squashes & Cordials preserved by Physical means	25.77 – 40.71	1 – 12	0.00 – 0.12	10ml/100ml

As evidenced in table 18, all drink samples tested had high sugar content except fresh fruit juices with no physical or chemical preservatives which met the

standards specified by Kregiel (2015). This, therefore, means that there was more sugar in those samples.

What differences exist in the microbial loads, the concentrations of sugar and vitamin C levels of the non-alcoholic drinks tested?

To assess the differences that exist in the microbial loads, level of concentrations of sugar and vitamin C in the selected non-alcoholic drinks, the mean counts were compared statistically using analysis of variance at $p \leq 0.05$ presented in tables. The details are presented in tables 19 and 20 below.

Looking at the descriptive for the categories of non-alcoholic drinks, there were no microbial counts found in some of the drink categories. Mean scores recorded 0.00 and therefore comparing them will not draw meaningful conclusions. These results may have occurred due to carbonation that might have inhibited the growth of spoilage microbes by inhibition of cell division, inhibition of amino acid uptake and lowering pH (Stratford, 2006). The statistical analysis showed that there was a relationship between sugar content and juice content. Excess sugar can affect diabetic individual and can cause tooth decay in children. Table 19 showed that drink samples had very low vitamin C concentrations. The results presented in table 20 shows a statistical significant difference between sugar groups as determined by $p<.008$ level for the 4 conditions [$F (3, 18) = 5.43$, $p = .008$].

In table 20, the mean difference is significant at the 0.05 level. The significant difference between sugar groups as determined by $p< .008$ level was explained by the Tukey Post-hoc test. The Tukey Post-hoc test revealed a statistically significant mean difference (MD= 14.81, $p=.028$) in the sugar levels

between category 'A' and 'B' non-alcoholic drinks. Also, there was a statistical significant mean difference (MD= 18.87, *p*= .006 in the sugar levels between category 'D' and 'C' non-alcoholic drinks.

ANOVA RESULTS

ANOVA FOR VITAMIN C

Table 19: Mean Differences of Vitamin C Levels between the Categories of Non-Alcoholic Drinks.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.003	3	.001	.402	.753
Within Groups	.043	18	.002		
Total	.046	21			

ANOVA FOR SUGAR

Table 20: Mean Differences of Sugar Levels between the Categories of Non-Alcoholic Drinks

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1000.561	3	333.520	5.430	.008
Within Groups	1105.670	18	61.426		
Total	2106.230	21			

Conclusion

Although the microbial growth was found less frequently among the non-alcoholic drinks sold on Cape Coast Metropolis markets, the microbial loads found in most cases were still above the standard limit for consumption stipulated by the Ghana Standard Authority. Out of the five fresh fruit juice samples, fecal coliform was detected in two drink samples (PM and PC). *E. coli* was also found in only two (PM and BS) of the five drink samples used for the study. Moreover, yeast and Moulds were detected in all the fresh fruit juice samples and one also in only one drink sample (KC) from carbonated soft drinks and carbonated malt category. However, *salmonella* spp. was absent from all the drinks samples.

Moreover, some of the drinks were found to harbour certain bacteria and, therefore, poor quality products. This could be attributed to unhygienic conditions under which the drinks were processed, stored, served and or sold.

The measure of the amount of acids present in the non-alcoholic drinks examined were too high (2.5% to 14.7%) as compared to the standards provided by the Ghana Standards Authority (1995) standards, acidity in non-alcoholic beverages should have (0.5 - 1.9%). According to Tenuta *et al.*, (2015), a low pH and a high titratable acidity of juices and cola-based beverages are relevant factors that contribute to dental erosion. Dental erosion often is associated with frequent consumption of acidic beverages (Dugmore *et al.*, 2004; Li *et al.*, 2012 and Lussi *et al.*, 2012). In addition, drink samples presented for the analyses showed high concentrations of sugar in them and therefore failed to meet stipulated standards. Again, vitamin C levels in the drinks were also low and thus, failed to meet recommended standards.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Introduction

The chapter focuses on the summary, conclusions and recommendation on the evaluation of some non-alcoholic drinks in the Cape Coast Metropolis that may help other researchers.

Summary of the Results

Non-alcoholic drinks, which are liquids, form part of our daily diets. These include fruit juices, soft drinks/soda, carbonated drinks and Ghanaian local drinks. These drinks are often taken with snacks, meals or alone. The value of non-alcoholic drinks must not be understated, because they are an essential means for hydration. Though fruit juice contains vitamins, minerals and antioxidants; it also lacks fibre. Consumers all over the world of which Ghana is no exception perceive non-alcoholic drinks especially the fruit juices to be healthier.

Furthermore, there has been increasing consumer awareness in recent times about the proliferation of so many non-alcoholic drinks on the various markets in Ghana. This has emphasized the need for microbiologically safe food and physico-chemical properties of non-alcoholic drinks.

The purpose of the study was to evaluate some non-alcoholic drinks in the Cape Coast Metropolis.

The objectives formulated for the study were to catalogue and categorise non-alcoholic drinks in the Cape Coast Metropolis based on the standards from the Ghana Standards Authority and the International Standard Organization

(ISO); determine possible microorganisms contaminations in the non-alcoholic drinks; determine total sugars and vitamin C concentrations in the drinks; and physico-chemical properties such as; pH, temperature, percentage acid and the titrable acidity in the non-alcoholic drinks.

The study was conducted using an experimental design, which made way for other designs such as, survey and observation to be employed. To achieve the stated objectives, the simple random and purposive sampling techniques were used for sampling. 120 names of some non-alcoholic drinks were purposively recorded from the three major markets. Carbonated malt drinks, carbonated soft drinks, concentrated fruit juices and squashes; and fresh fruit juices manufactured locally were recorded and grouped into four main categories. The simple random sampling was used to select 22 non-alcoholic drinks from the four categories and purchased from the markets for the experiment. All the samples purchased at every sampling period were transported to the Food Research Institute and the University of Cape Coast Agriculture Research Laboratory where they were subjected to laboratory analyses at different periods.

The research instrument used for the study were survey and observation checklist. The microbial analysis was done using ISO and NMKL Analytical Standard procedures, sugars and vitamin C concentrations were analysed using Rutkowski *et al.*, (1991) method and the physico-chemical analysis were performed using the AOAC (1990) method.

Key Findings

The key findings of the study have been presented in four sections, each relating to the research questions that underpinned the study.

Table 21: Categories of Non-Alcoholic Drinks in the Cape Coast Metropolis Market.

No.	Drink Categories	Percentage share
1	Concentrated fruit juices preserved by physical and chemical means	59%
2	Carbonated malts drinks	17%
3	Carbonated soft drinks	12%.
4	Fresh fruit juices with no physical or chemical Preservatives	12%
	Total	100%

Table 22: Results of the microbiological Analysis

Drink sample	Percentage and Type of Contaminants present in the Drinks Tested				
	Microbes	Yeasts & Moulds	Total coliforms	Faecal coliforms	*E.coli*
Carbonated Soft Drinks and Malts	**(**33%)				
5Star Koolah Cool (KC)	16.6%	16.6%	-	-	-
Fresh Fruit Juices with no physical or chemical preservatives					
Pinecarrot(PR), Pinemelon(PM), Pine citrus (PC) and Blueskies (BS)	100%	-	80%	-	-
Anidak Bissap (AD), Pinecarrot (PR), Pinemelon (PM), Pine citrus (PC) and Blue skies (BS)		100%	-	-	-
Pinemelon(PM)and Pinecitrus(PC)		-	-	40%	-
Pinemelon (PM) and Blue Skies(BS)		-	-	-	40%

Table 23: Drinks that did not meet the Ghana Standards Authority standards

Name of drink	Type of microbiological Analysis				
	APC	TC	FC	E.coli	Y& M
5Star Koolah Cool	-	-	-	-	16.6%
Anidak Bissap, Pinecarrot, Pinemelon, Pine citrus and Blue skies	100%	-	-	40%	-
Pinecarrot, Pinemelon, Pine citrus and Blue skies	-	80%	-	-	-
Pinemelon and Pinecitrus	-	-	40%	-	-
Pinemelon and Blue skies	-	-	-	-	-
Anidak Bissap, Pinecarrot, Pinemelon, Pine citrus and Blue skies	-	-	-	-	100%

Table 24: Percentage of drinks that did not meet Kregiel (2015) and Malaysia (1999) standards for sugars and Vitamin C concentrations respectively

Sugars	**63% (14)**
Vitamin C	**100% (22)**

Results of the Physico-chemical analysis

The pH, Temperature and Titrable acidity of the samples collected from category - D had the lowest mean pH (3.17±0.26), while the highest mean pH (3.602±0.86) was observed with samples from category - A. There were variations in the TTA of the samples examined and this could be attributed to the differences in chemical constituents in the drinks.

Conclusions

Although the microbial growth was found less frequently among the non-alcoholic drinks sold in the markets of Cape Coast Metropolis, the microbial loads found in most cases were above the standard limit for consumption stipulated by the Ghana Standard Authority. From the data presented in the current study, it can be concluded that the microbiological quality of most of the fresh fruit juices with no physical or chemical preservatives collected from the different markets in the metropolis were not satisfactory as total coliforms were detected in all the samples except one sample (AD). Out of the five fresh fruit juice samples, faecal coliform was detected in two drink samples (PM and PC). *E.coli* was also found in only two (PM and BS) of the five drink samples used for the study. Moreover, yeast and moulds were detected in all the fresh fruit juice samples and in only one drink sample (KC) from carbonated soft drinks and carbonated malt category. However, *Salmonella* spp was absent from all the drinks samples.

Recommendations

On the basis of the findings, it is recommended that:

1. People should also be encouraged to prepare their own fruit juices and drinks to ensure safety and proper hygiene.
2. Regular monitoring of the quality of fruit juices for human consumption by officers from the Food and Drug Administration is recommended to avoid any future bacterial pathogen outbreak from locally manufactured fresh fruit juices.

3. The Ghana Standards Authority should establish a standard for the level of sugars and fortificants; such as vitamin C for all non-alcoholic drinks for locally manufactured non-alcoholic drinks and also ensure that imported drinks coming into the country meet the required sugar and vitamin C standards and approved before they are distributed on the various markets.
4. More quality control units/laboratories must be established to detect contamination of either the raw materials or the products early enough.
5. There are no official regulations that apply to packaging and labeling of drinks at the local level; and where regulations do exist, they are hardly enforced. The government should impose stricter legislative bounds and international codes of practice to provide guidelines and hence strengthen the regulations available to ensure the best practice.
6. Besides the provision of education for the manufacturers of non-alcoholic drinks, FDA certification should not be limited to only certain products but should be mandatory for all foods especially non-alcoholic drinks to ensure food safety and absolute compliance by the manufacturers.
7. There must be regular checks by the FDA on small-scale producers in various homes and street corners, as many people are becoming entrepreneurs in this line of juice and drinks production.
8. Since the practice of consuming fruit and vegetable juices cannot be stopped on unhygienic grounds, and juice makers cannot be prohibited from selling such items because it is a source of their livelihood, government health agencies must adopt measures to educate the vendors on food safety and hygienic practices.

9. In addition to this, not only government authorized institution like the FDA, GSA and the Environmental Protection agencies but also some strongly active administrative organization like health officials should be given more authorization to undertake pre-emptive investigations to check the microbial and chemical quality of non-alcoholic drinks.
10. Government and non-government institutions should create public awareness about the contamination and adulteration of fruit juices more intensely with the help of the electronic media.
11. Health education training should be organized regularly for the producers, especially those at the local level by health workers on the importance of cleanness of themselves, equipment and their environment, and the use of sterilized packaging materials since fresh fruit juices are highly consumed by both the young and adults.

Suggestions for Future Research

Further studies should be conducted to investigate the acceptable packaging materials for non-alcoholic drinks and the acceptable storage temperatures for non-alcoholic drinks.

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APPENDICES

APPENDIX A

MICROBIOLOGICAL LIMITS FOR NON-ALCOHOLIC DRINKS

No.	Product	Test	Test method	Case	Plan clas	Sample unit	Microbiological Limit/Gram			
							n	C	m	M
i.	Carbonated soft drinks and Carbonated Malt Drinks	APC	ISO 4833-1:2013		2	3	5	2	10^2	10^3
		Yeasts and Moulds	ISO 21527:2008		2	3	5	2	10^1	$5x10^2$
ii.	Fruit juices, Squashes and Cordials, fruit juices preserved by physical and chemical means	APC	ISO 4833:2003		2		5	2	10^2	10^3
		Coliforms	GS ISO 4831:2006		3	3		1	10^1	10^2
		Yeast and Moulds	ISO 21527:2008		2	3	5	2	10^1	$5x10^2$
iii.	Fruit Nectars	APC	ISO 4833:2003		2	3	5	2	10^1	10^4
		Coliforms	GS ISO 4831:2006		3	3	5	1	$5x10^1$	-
		Yeast and Moulds	ISO 21527:2008		2	3	5	2	10^1	$5x10^2$
iv.	Concentrated fruit juices preserved exclusively by physical means	APC	ISO 4833:2003		2	3	5	2	10^1	10^2
		Coliforms	GSISO 4831:2006		3	3		1	$5x10^1$	-
		Yeast and Moulds	ISO 21527:2008		2	3	5	2	10^1	$5x10^1$
		S. aureus	GSISO 6888:1999							
v.	Cocoa Drinks	APC	ISO 4833:2003		2	3	5	2	10^2	10^3
		Coliforms	GS ISO 4831:2006		3	3		1	10^1	10^2
		Yeast and Moulds	ISO 21527:2008		2	3	5	2	10^1	$5x10^1$
		S. aureus	GS ISO 6888:1999		9	3	19	1	10^2	10^3
vi.	Fruit and Milk Based Beverages	APC	ISO 4833:2003		2	3	5	2	10^2	10^3
vii.	Fruit Drinks powdered fruit drinks for rehydration	APC	ISO 4833:2003		2	3	5	2	10^3	10^4
		Yeast and Moulds	ISO 21527:2008	2		3	5	2	10^1	$5x10^1$
viii.	Fresh fruit juice with no physical or chemical preservatives	APC	GS 1002:2009		3	3	5	2	10^2	10^3
		E. coli	GS ISO 7251:2005		5	2	5	2	10^1	10^2
		S. aureus	GS ISO 6888:1999	5			5	2	10^1	10^2
		Salmonella	GS ISO 6579:2002	11			10	0	0	-

Source: GS 7265: 2003

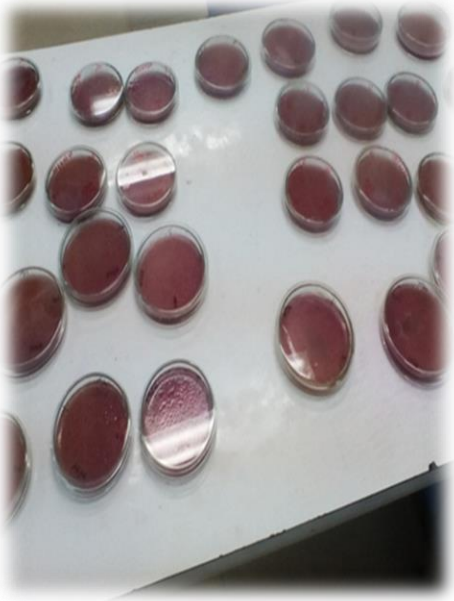
APPENDIX B

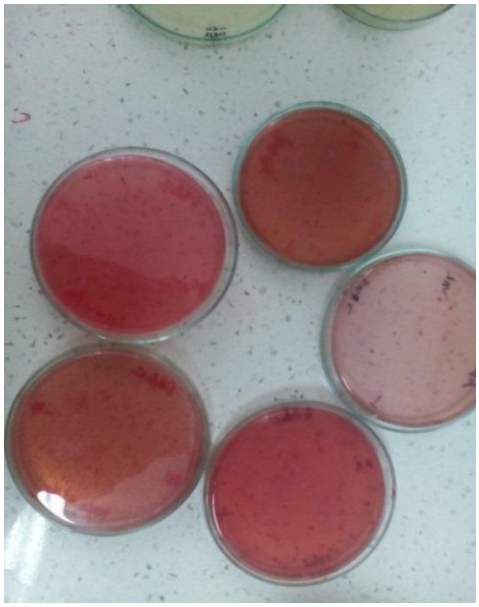
DRINK SAMPLES AND THEIR CODE

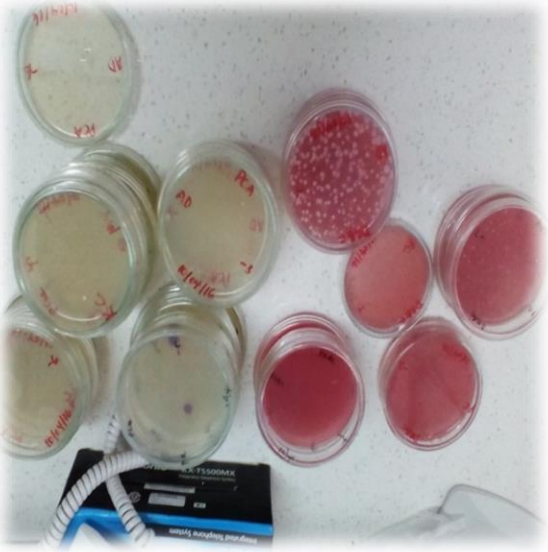
Name of Drink	Code
Magic Malt	MM
Malta Guiness	AD
Beta Malt	PR
Club Cola	PM
Original American Cola	OAC
5 Star Kolah Cool	KC
Alvaro (Pear flavour)	AV
Fru Telli	FT
Don Simon	DS
Pure Heaven 5 star citrus	PH
Tampico	TP
PATA PATA Bissap Ice	PPB
Anidak Bissap Drink	AD
Farm Fresh Pine Carrot	PR
Farm Fresh Pine Melon	PM
Farm Fresh Pine Citrus	PC
Blue Skies smoothie	BS
Juvita	JU
Fruity	FR
Squiz Pineapple	SQ
Kalypo Apple	KA
Juicee Pineapple	JP

APPENDIX C

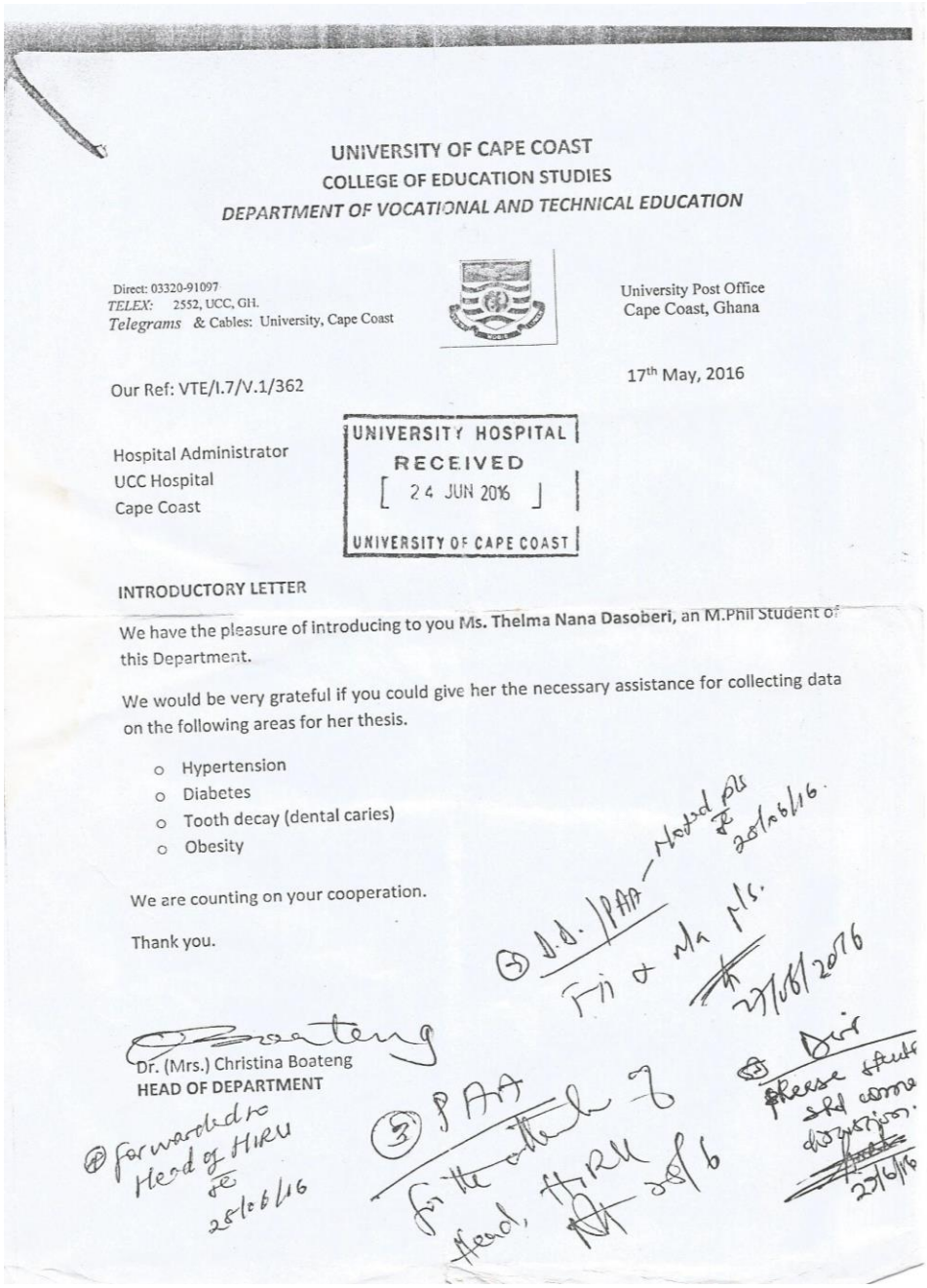
LABORATORY PICTURES







APPENDIX D
INTRODUCTORY LETTERS



UNIVERSITY OF CAPE COAST
COLLEGE OF EDUCATION STUDIES
DEPARTMENT OF VOCATIONAL AND TECHNICAL EDUCATION

Direct: 03320-91097
TELEX: 2552, UCC, GH.
Telegrams & Cables: University, Cape Coast

University Post Office
Cape Coast, Ghana

Our Ref: VTE/I.7/V.1/362

17th May, 2016

Hospital Administrator
UCC Hospital
Cape Coast

UNIVERSITY HOSPITAL
RECEIVED
[24 JUN 2016]
UNIVERSITY OF CAPE COAST

INTRODUCTORY LETTER

We have the pleasure of introducing to you **Ms. Thelma Nana Dasoberi,** an M.Phil Student of this Department.

We would be very grateful if you could give her the necessary assistance for collecting data on the following areas for her thesis.

- Hypertension
- Diabetes
- Tooth decay (dental caries)
- Obesity

We are counting on your cooperation.

Thank you.

Dr. (Mrs.) Christina Boateng
HEAD OF DEPARTMENT

UNIVERSITY OF CAPE COAST
COLLEGE OF EDUCATION STUDIES
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17th May, 2016

Hospital Administrator
Regional Health Directorate
Cape Coast

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- Diabetes
- Tooth decay (dental caries)
- Obesity

We are counting on your cooperation.

Thank you.

Dr. (Mrs.) Christina Boateng
HEAD OF DEPARTMENT

In case of reply the reference number and the date of this Letter should be quoted

Our Ref.: CCTH/MD—G/16-59
Your Ref.: VTE/1.7/V.1/362

P. O. Box CT.1363
Cape Coast
Tel: 03321-34010-14
Fax: 03321-34016
Website: www.ccthghana.com
www.ccthghana.org
email: info@ccthghana.org

12th July, 2016

THE HEAD
DEPARTMENT OF VOCATIONAL & TECHNICAL EDUCATION
COLLEGE OF EDUCATION STUDIES
UNIVERSITY OF CAPE COAST

Dear Madam,

RE: INTRODUCTORY LETTER
MS. THHELMA NANA DASOBERI

With reference to your letter number VTE/1.7/V.1/362, dated 17th May, 2016 on the above subject, I write to inform you that the Cape Coast Teaching Hospital (CCTH) has granted approval for the above named student to conduct her research.

The student will be required to pay an amount of Two Hundred Ghana Cedis (GH¢200.00) to enable the hospital support the Ethical Review Committee to monitor the research to make sure it aligns to the ethics regarding patient confidentiality and other ethical consideration.

The hospital would also appreciate to have a copy of any relevant findings.

Thank you.

Yours faithfully,

DR. ERIC K. NGYEDU
(MEDICAL DIRECTOR)
FOR: CHIEF EXECUTIVE OFFICER

Cc: Ms. Thelma Nana Dasoberi

APPENDIX E

POST-HOC TEST

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.
Category A	Category B	3.43233	4.52497	.872
	Category C	14.81060*	4.74583	.028
	Category D	-4.08600	4.74583	.825
Category B	Category A	-3.43233	4.52497	.872
	Category C	11.37827	4.74583	.113
	Category D	-7.51833	4.74583	.412
Category C	Category A	-14.81060*	4.74583	.028
	Category B	-11.37827	4.74583	.113
	Category D	-18.89660*	4.95686	.006
Category D	Category A	4.08600	4.74583	.825
	Category B	7.51833	4.74583	.412
	Category C	18.89660*	4.95686	.006