UNIVERSITY OF GHANA

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

DEPARTMENT OF CHEMISTRY

NUTRITIONAL VALUE AND TRACE METAL CONTAMINATION OF SOFT DRINKS SOLD ON THE GHANAIAN MARKET

PRESENTED BY

BASHIRU RAFIU AKANDE

10553052

A DISSERTATION SUMBITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MPHIL CHEMISTRY DEGREE.

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DECLARATION

I declare that except for references to other people's works which I have duly cited, this dissertation is based on original research work conducted by me under the supervision of Prof. Augustine Donkor and Dr. Michael Kojo Ainooson both of the Department of Chemistry, University of Ghana, Legon.

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DEDICATION

This work is affectionately dedicated to my family for their unending love, support and encouragement throughout my academic life.

ACKNOWLEDGEMENT

My profound gratitude goes to my supervisors; Prof. Augustine Donkor and Dr. Michael Kojo Ainooson for their directions, time and making me confident. Above all for their great patience and guidance throughout this project.

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ABSTRACT

Soft drinks are consumed daily in Ghana on social occasions and for recreation. Generally, it is affordable and consumed widely across all age groups particularly due to their thirst quenching potential. These drinks are also important sources of nutrition as well as trace metal contamination. Thus, the study monitored for 4 months the nutritional content and trace metal contamination of forty-two different brands of new day soft drinks (totaling 672 samples), sampled from some markets in Accra Metropolis, Ghana. The metals were measured with atomic absorption spectrometer (AAS) after acid digestion. Physical parameters of the drinks, for example, pH, turbidity, conductivity and total dissolved solids were also determined. The results indicated most of the drinks were acidic (pH: 2.20-3.52) and very rich in total dissolved solids. The major nutrients Ca, Mg and Na were found in appreciable levels whereas potentially toxic elements (Pb, Cd and As) were below detection limit in all the drinks examined. There was also associated health risks with few soft drinks (about 7%) in relation to trace metal contamination. Therefore, there is the need for continuous monitoring of these class of drinks flooding the Ghanaian markets lately.

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CHAPTER ONE

1.1 INTRODUCTION

Owing to the change in lifestyle and eating trends, the rate of soft drink consumption is on the increase. In certain instances, it is believed that soft drinks have replaced the role of water in the diet of a person. Thus, one prefers to take a soft drink to quench his or her thirst instead of water. Beverages consumed globally, are either alcoholic or non-alcoholic. Some of the alcoholic beverages are wine, beers and cider whilst fruit juices, carbonated drinks, energy drinks, probiotic drinks and tea are non-alcoholic beverages (Rahman et al., 2019). Soft drinks are either carbonated or non-carbonated.

According to Ibiyemi (1986), a soft drink, usually carbonated contains water, sugar, carbon dioxide, natural or synthetic sweeteners, edible acids (citric acid, tartaric acid), flavors, preservatives (e.g.: benzoate, sulphur dioxide, sodium metabisulphite), vitamins, amino acids, fruit juice and a pH normally between 2-5. They come in different packages to suit the varied lifestyles and preferences of individuals. Coca-Cola, PepsiCo and Schweppes are among the top manufacturing companies worldwide. In Ghana, the Coca-Cola company products have dominated the soft drink market with Accra Brewery and Guinness following suit. Howbeit, recently new local soft drink producing companies have also sprung up and flooded the market with their relatively low-cost drinks as compared to products from the well- known traditional companies (eg: Coca-Cola and PepsiCo). Since the inception of soft drinks in the 17th century to present, this line of drink has undergone several modifications to improve its quality and increased its shelf life.

Soft drinks have some nutrients like minerals, vitamins, dietary fiber and phytochemicals, that are very important to human health (Rahaman et al., 2019, Owolade et al., 2017). In addition, WHO recommends that travelers, in particular, consume soft drinks where the quality of water in certain areas are questionable. This is as a result of its carbonation and the ability to provide hydration to the human body (Ryan, 2014) and are served during festive occasions and celebrations; weddings, funerals and naming ceremonies amongst others. Although soft drinks are considered innocuous, health experts have advised consumers to take them in moderation (Anastacio et al., 2018). Likewise, countries with high rates of consumption of soft drinks, have more cases of obesity, diabetes, dental corrosion and osteoporosis. In Ghana, soft drinks are mostly consumed nationwide with children and students identified as chief patrons. For instance, a study conducted among junior high schools in Tema indicated that, 56.4% of the students took at least 1 to 2 soft drinks per week (Vuvor & Harrison, 2017). Similarly, a survey conducted at the University of Ghana in 2012, revealed that students consume soft drinks on the average of three bottles per day with the ratio of 2:1 for ladies and gentlemen, respectively.

Nevertheless, soft drinks can be a probable source of toxic metal with some having a growing effect on consumers due to their high or low concentrations (Anastacio et al,. 2018). Likewise, some trace elements (e.g. Fe, Cu and Mn) are very necessary for man, because of their roles in many biochemical processes.

In contrast, metals such as Hg, Pb, As and Cd are non -essential and toxic and have no biological importance which causes severe adverse effects detrimental to the health of the individual. Studies have demonstrated that Cd (Buha et al., 2018) is carcinogenic, whilst Pb can be stored up in erythrocytes and interchange Zn thus inhibiting its function (Flora et al., 2012 & Matovic et al., 2015). On the other hand, the presence of heavy metals in the environment can be absorbed into human body by inhalation, dermal absorption and ingestion. These heavy metals are well thought-out as the key contaminants in packaged food, particularly beverages from current studies (Toni et al., 2017, Ahmed et al., 2017).

The core sources of beverage's contamination by toxic elements includes their high concentration in the processed fruits which could originate from the soil in which the plant was cultivated, the water used for irrigation, excessive use of fertilizers and pesticides in their cultivation. Equally, the contamination of the water used in the production of the beverages as well as other constituents such as sugar, colorants and flavors, packaging materials, storage conditions, processing technologies and deposition of metals in the atmosphere particularly from industrial activities and vehicular emissions.

Hence to sustain human well-being, the concentrations of both vital and non-vital elements need to be monitored continuously among the many soft drinks products on the market. However, not much has been done in the case of Ghana with regards to studies into the new day soft drinks overflowing the Ghanaian markets by the new players in the soft drink business unlike the traditional companies. (e.g.: Coca-Cola and PepsiCo.). Therefore, this study seeks to fill this void on the information concerning the nutritional value and trace metal contamination on soft drinks on the Ghanaian market and compare to the international acceptable values to safeguard the safety of the end users whilst serving as baseline information for further studies.

1.2 PROBLEM STATEMENT

The sale of soft drinks in Ghana is on the rise due to increased patronage. Lately, several manufacturing companies (e.g.; Special Ice Company Limited, Kasapreko Company Limited, Twellium Industries Limited etc.), have sprung up and flooded the market with a variety of soft drinks in different packages at a lower price as compared to the "traditional" soft drinks (e.g.; products from Coca-Cola, Accra Brewery and Guinness Ghana). Due to the large consumption rate of these soft drinks, determining the safety of these soft drinks is paramount particularly, the mineral content and its pattern as well as the metal concentrations.

However, there is a paucity of data on this area of research. Currently, there is only one publication on heavy metal investigation of soft drinks and fruit juice from retail markets located in Accra, Ghana (Ackah et al., 2014) and on the trends of soft drink consumption (Gyeduaah et al.,2018) and the knowledge, practice and perception of taking soft drinks with food and its metabolic effects on high school students in Ghana (Vuvor & Harrison, 2017). In all these none of the authors indicated whether the drinks examined were of the newly established companies products or the long existing traditional ones.

Consequently, the consumption of these soft drinks has been singled out as a global public health concern in the light of their contribution to total dietary sugar intake, high glycemic index, concentrations of heavy metals and their ostensible role in excess energy intake. Hence, for the protection of public health it becomes imperative to examine the contents of the newly branded soft drinks produced presently in Ghana.

1.3 AIMS AND OBJECTIVES

The sole purpose of this work is to evaluate the nutritional and trace metal contents of carbonated soft drinks on the market in the Accra Metropolis.

1.4 SPECIFIC OBJECTIVES

1. To sample different brands of soft drink samples mostly consumed in Ghana.
2. To determine the physiochemical parameters (e.g.; pH, total dissolved solids, conductivity, etc.) of sampled soft drinks on the Ghanaian market.
3. To evaluate the nutritional content and trace metal concentrations in the soft drinks and any possible associated health risk.
4. To compare the results obtained with permissible limits and other similar studies.

CHAPTER TWO

LITERATURE REVIEW

2.0 INTRODUCTION

The consumption of soft drinks and juice products is on the rise among the Ghanaian populace due to their roles in satisfying hunger, thirst or cravings and its availability. Hence the local market for new day soft drinks in Ghana progresses steadily.

Also, differences in climate and weather conditions enhance the demand for chilled soft drinks especially throughout the dry period. Thus, Ghana presents a rapid and growing atmosphere for the manufacturing companies and an avenue for prospective investment into the sale and production of soft drinks, evident in the number of new manufacturing companies, producing soft drinks and juices. However, the harmful effects associated with the consumption of carbonated soft drinks especially by children, teenagers and the vulnerable population like pregnant women is a matter of great concern to the medical and scientific community.

The ingredients used in the manufacture of these soft drinks though may have some nutritional benefits, may also contain toxic metals (Hg, Cd, As) which have significant health impacts on man.

Research in the western world has shown considerable evidence, that modern way of living has led to the upsurge in diet related diseases and excessive soft drink consumption plays a role in this.

It is therefore imperative that consumers are well-informed about the make-up of soft drinks particularly, new day soft drinks on the Ghanaian market, produced by the new players in the business and the adverse health impacts if consumed in excess.

2.1 TRENDS IN SOFT DRINKS CONSUMPTION.

The popularity of soft drinks has skyrocketed worldwide, with about 200 countries consuming these soft drinks. The first soft drinks, thus a mixture of lemon juice, sweetened with honey was introduced to the consumer market in the 17th century in the year 1676 by the Campagnie de Limonadiers in Paris as they were granted monopoly for the sale of its products. Carbonated beverages and water were developed in Europe in the 17th century and in 1886, John Pemberton, a pharmacist in Atlanta, Georgia, invented Coca Cola, the first cola drink (Pietka & Korab, 2017).

Per the Global Soft Drinks report in 2008, it was reported that only America represents 25% of the total intake of beverage with about 50 billion litres of soft drinks per annum (Zenith International, 2008). It was also discovered that each individual consumes approximately 82.5 litres of carbonated and or non-carbonated drinks on a yearly basis. Hence there have been aggressive swamping of customers by the soft drink industries using captivating advertisements. (Moreno et al, 2005).

In comparison with the 2009 global consumption rate of soft drinks, consumption rate increased by 4% in 2010 with a per capita consumption of 81 litres. The highest growth in the soft drinks market in the year 2010 was observed in Asia, with India and China reporting growth rates of 16% and 14%, respectively.

This trend is expected to rise, as the economic powerhouse of the world shifts from the western world to the eastern world. China and India have a rapidly expanding development rate, a rising population in the youth demographic category and with the resultant increase in consumer income and wages; the demand for non-essential luxury consumer products, including soft drink and beverages, will only rise. Mass consumption has also played a significant role in altering consumption patterns over the years, allowing for the consumption of products that were formerly out of the price range of ordinary middle to lower income citizens.

2.1.1 TRENDS IN CONSUMPTION OF SOFT DRINKS IN DEVELOPED COUNTRIES

According to data from the National Health surveys of America from 1977 to 1979 and 1994, there has been a continuous increase in rates of consumption of soft drink particularly among teenagers. The prevalence of soft drink intake was 82.5% in adolescents with daily soft drink consumption rate, increasing by 74% and 65% in adolescent boys and girls, respectively.

In 2010, it was reported that soft drinks accounted for 47 percent of all added sugars in the American diet. Statistics indicates that the global consumption of soft drinks has continually increased from the early 2000s to 2010 with simultaneous growth in population. However, following the new millennium and the surge of mass consumption, the rate of soft drink consumption has risen over and above the rate of population growth. A research conducted in Los Angeles on 707 female teenagers revealed high percentages of soft drinks intake (Kassem et al., 2003). The European Union in 2010, consumed 49807.26 million litres of soft drinks and this value remained unchanged at 49205.03 million litres in 2015. Considering the EU's soft drink consumption pattern in terms of percentage change from 2014 to 2105, there has been a 1.23% change. Thus, soft drink consumption though robust, tends to fluctuate due to consumers altering preferences. It was also noted that in 2010, approximately 210.6 litres of soft drinks were consumed per person in the UK. By 2015, this amount decreased to 203.6 litres per person owing to the increase in health consciousness among consumers. Nevertheless, despite this marginal drop, statistics discloses the consumption of soft drinks in the UK continues to remain robust and inelastic (UNESDA, 2015).

A survey conducted in Australia from 2011-2012, demonstrated that 42% of the 9 million Australians aged 2 years and above, consumed sweetened beverages on any given day. Also, a study carried out by Hafekost et al (2011), on 4834 Australian adolescents disclosed that the mean consumption rate of carbonated drinks among adolescents was 426ml/day whiles the mean consumption rate for fruit drinks was 175ml/day with a greater preference for carbonated beverages.

A study by Lew &Barlow (2005) conducted among 100 Singaporean between the ages of 11 and 21 disclosed that 97.6% consumed sweet drinks with 49.4% of them consuming soft drinks 2 to 5 times a week. Also, 32.5% consumed soft drinks daily with about 25.3% consuming soft drinks weekly.

Garriguet (2008) stated that beverages provided 30% of the daily average calories among adolescents resident in Canada. The survey also showed that on an average, the daily intake of soft drinks ranged from 68 grams to 376 grams in males and quite lower among females.

In Greece, Linardakis et al (2008) revealed that 59.8% of the 856 Greek children within ages 4 to 7 years, all consumed soft drinks daily, having males as the majority of soft drinks consumers.

2.1.2 TRENDS IN CONSUMPTION OF SOFT DRINKS IN DEVELOPING COUNTRIES

In 2005, Norwegan investigations conducted among 2,870 students in the 9th and 10th grade disclosed 63% of the participants to consume soft drinks regularly whilst 27% took the diet forms at least twice a week (Bere, Glomnes, Velde & Klepp, 2007). Sayegh et al., (2002) established that snacking was fairly higher among children in lower grades living in Amman, Jordan having 50% of the children consuming carbonated soft drinks of which 60.7% were boys.

In Sri Lanka, about 2841.54 rupees was spent on soft drinks in the year 2010 per household amounting to approximately 1,081,992.50 rupees spent on beverages throughout the island. The rural population in Sri Lanka constituted majority of the expense on soft drink consumption costing 2,168 rupees whiles the urban population consumed 673 rupees worth of soft drinks in 2010. According to data from the Department of Census and statistics on food items consumption in the years 2012 and 2013 in Sri Lanka, it was stated that a total of 487.11 rupees was spent on non-alcoholic beverages per person in that time frame with monthly expenditure per household for soft drinks only amounting to Rs. 26.21. While soft drink consumption per household in a given month is less than tea consumption said to be Rs. 281.92, it is higher than milk coffee, coffee and bottled water consumption combined. Likewise, soft drink consumption in Sri Lanka on a national basis is also higher than the national consumption of fruit drinks and cordials. Fruit drink and cordial expenditure per household per month was Rs. 10.53 which is considerably lower than the soft drink consumption expenditure of Rs. 26.21 during the same period. These trends in Sri Lanka demonstrate the high demand or strong consumption pattern of soft drinks within the country.

The study on the frequency and amount of soft drink consumption in Dar-EsSalaam, Tanzania in 2010 among 400 adolescents between the ages of 8 and 17 years was carried out. This showed that 61.5% of the adolescents consumed soft drinks out of which the consumption of carbonated drinks was 61.3%. Carbonated drink consumption was observed to be more predominant among the government school students while students from private schools enjoyed more fruit juices (Dhirani, 2010). A study from Nigeria, revealed that nearly 16% of children in Ibadan aged 6-18 months were fed with soft drinks at least once per day for weaning purposes (Bankole, Aderinokum, Odenloye & Adeyemi, 2006). On a daily basis, it was reported that 24-37% of school children in an urban areas of Uganda, took in soft drinks (Kiwanuka, Astrom & Trovik, 2006).

In South Africa, the intake of carbonated drinks was more in the urban areas (33%) as compared to that of the rural areas (Steyn et al, 2003). It was reported by Theron et al (2007) that carbonated drinks were the most frequently consumed drink among South African urban kids. Also, consumption was also to observed to be high in the remote areas of South Africa (MacKeown and Faber, 2005), where children below 4 years consumed soft drinks 2-3 times a week.

In Ghana, a study conducted at the Sunyani Technical University comprising of 159 students revealed that all the students consume non-alcoholic beverages comprising of smoothies, carbonated soft drinks, fruit juices, tea, coffee and cocktails. Out of this, 11.2% of the students consumed carbonated soft drinks and 12.8% consumed fruit juices. Tea recorded the highest percentage of preference with 13.4 % (Gyeduaah et al., 2018). Vuvor and Harrison (2017) conducted a study on a group of students on their knowledge and perception of soft drinks. Per their results, majority of the students had an idea on soft drinks but not much about the nutrients contained in soft drinks. A greater number of them also had perceptions about the health effects of soft drinks (Vuvor & Harrison, 2017).

Kusi (2013) also conducted studies on the benzoic acid and benzene in soft drinks. He found out that soft drinks contributed immensely to the consumption of benzoic acid and benzoates in mostly children and some adults.

2.2 INGREDIENTS

The ingredients listed on the packages of soft drinks are normally in the order of importance along with any other information concerning the product (Long, 1959). The basic ingredients of these drinks are water, sugar/sweetener, preservatives, flavouring, acid regulators, carbon dioxide and colourings. Each and every constituent has an important role of enhancing the taste of a particular drink. Water serves as the primary component of every soft drink; thus about 86-90% of soft drinks contain water (Saikia & Kumar, 2006).

Water used in soft drink production should be one of high quality thus, it should be devoid of all forms of bacteria and chemical pollutants (Long, 1959). Following the EU Water Directive for Drinking Water, the water should lack chlorine and hardness, as these may react with the flavour and affect the acidity of the soft drink. Nitrate levels are to be considered if consumer is an infant. Ashurst & Hargitt, (2009) stated calcium in water will lead to an unpleasant end product.

Sugar or sweeteners are added to drinks to make them sweet. Refined sugar (sucrose) is the standard sweetener used in most soft drinks production. Sucrose, a common natural sweetener added to soft drinks, energy drinks and other processed food beverages, is obtained from sugar beet or sugarcane (Steen, 2006). Sugar is accessible as commercially granulated or a concentrated syrup. In some instances when sugar content is to be limited, artificial sweeteners or sugar substitutes are used in place of sugar (Ashurst, 2005). Sucralose and aspartame are the common artificial sweeteners used in diet soft drinks. In other cases, fruit extracts are employed to play the role of sugar, yet adding some nutritional value.

The fizzy nature of soft drinks is attributed to the presence of soda which forms the basis of carbonation in most soft drinks. It is chemically sodium hydrogen carbonate, a white crystalline powder used to produce carbon dioxide. Dissolved carbon dioxide results in the formation of bubbles and adds a feeling of lightness when consumed. Also, the expected formation of the bicarbonic acid gives the drink a tangy taste. However, in the past, carbonation was achieved by bubbling carbon dioxide in water. A carbonated soft drink would typically comprise of 3–4 volumes (6–8 g/L) CO_2 (Ashurst & Hargitt, 2009).

Soft drinks are likely to support microbial growth which may result in spoilage. Nevertheless, yeasts, moulds and bacteria are unlikely to be supported since the pH of these products is almost always below 4.0. Preservatives widely employed in soft drink companies include sorbic acid and benzoic acid (Steen, 2006). They are used in their potassium or sodium salts before being acidified. Sulphur dioxide is also used in glucose containing drinks.

Acidulants are added to soft drinks to control the pH and the overall acidity of the drink. The sharpness of a soft drink is essential to the taste and arises from the acid content of the drink. The Brix Acid Ratio measures the balance between sweetness and sharpness of a drink (Steen, 2006). This ratio is a key consideration in the formulation of most drinks. Carbonic acid, citric acid, malic acid, phosphoric acid and tartaric acid are the commonly used acidulants.

Flavouring is added in soft drink production to attain a certain identifiable taste and smell. Flavourings are chemical substances with flavouring properties, usually from food essences, with a known chemical structure. Frequently used flavours in soft drinks are those of vanilla, orange, kola, cherry, strawberry and pineapple.

Food additives have over the years been added to soft drinks in quite minute quantities. The purpose of such additives is to give an additional value. Additives may be in the form of macro and micro nutrients, mineral vitamins or even functional chemicals. Common food additives include vitamins A, C and E, caffeine and morphine.

Several other ingredients are added to drinks which help characterize the brand of drinks.

2.3 HEALTH IMPLICATIONS OF CONSUMING SOFT DRINKS

Soft drinks are made up of nutrients such as vitamins (particularly vitamin C), minerals, antioxidants, phytochemicals, carotenoids and dietary fiber, which are essential for human well-being (Rahaman et al., 2019, Owolade et al., 2017). Nevertheless, soft drinks can be a probable source of toxic elements with some having a cumulative effect or leading to nutritional problems due to high or low concentrations (Anastacio et al,. 2018). Similarly, some trace elements including Fe, Cu and Mn are of great importance for human life, as they serve as naturally vital substances that play significant roles in many biochemical processes in the human body.

2.3.1 ESSENTIAL ELEMENTS

Copper in the body is essentially known for its physiological effects necessary for brain development and function, foetal/infant development and growth, immune function, bone and collagen strength, iron metabolism, myocardial contractility and maintenance of hair and skin. Cu also participates in both iron and energy metabolism. Nonetheless, adverse health effects due to copper excess are concentrated on the liver which is prone to copper-related toxicity. With a damaged liver (cirrhosis); a high amount of zinc may produce adverse nutrient interactions with Cu and reduce immune function and the levels of high-density lipoproteins (FDA, 2001).

Iron (Fe), is fundamental in oxygen transport and forms a significant part of the haeme moiety of haemoglobin. Inside the cell, iron is complexed in the haeme or found in iron-sulphur clusters. Symptoms of Fe deficiency greatly results in anaemia but is prevalent when the Fe stored is very little. Fe deficiency may include weakness, hair loss, depression and a decreased ability to concentrate. Chronic Fe intoxication may frequently occur and it is associated with genetic and metabolic diseases, repeated blood transfusions, or with excessive Fe intake (Fraga and Oteiza, 2002). Liver failure, loss of appetite, fatigue, weight loss, headache, vomiting, nausea and dizziness are however associated with large Fe intake.

Zinc (Zn), another essential element, is highly needed by humans to promote biochemical functioning of cells and tissues. Zn possesses antioxidant properties and it is a recommended dietary supplement. Zn is mainly transported by ceruloplasmin and its activity affects about 100 enzymes, e.g. RNA polymerase, carbonic anhydrase, Cu–Zn superoxide dismutase, angiotensin I converting enzyme and it is present in Zn-fingers associated with DNA (Fraga, 2005). Zn deficiency results in diseases such as diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in intercurrent infections. Again, symptoms of moderate zinc deficiency include: growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing (US EPA, 2005). However, Zn toxicity can disrupt Cu and Fe absorption and create large amounts of toxic free radicals.

Manganese (Mn), is found in the tissues of kidney, pancreas, liver and bones. Mn promotes normal brain functioning and activity of the nervous system in the body (Organic Facts, 2015). High blood pressure, heart ailments, muscular contraction, bone malformation, high cholesterol, poor eyesight, hearing trouble, severe memory loss, shivers and tremors are some deficiency associated with low Mn intake. Even though some medical experts argue that Mn deficiency is quite rare, more than

35% of the world population is thought to be deficient (Organic Facts, 2015). Nevertheless, excessive exposure may cause a condition known as manganism, a neurodegenerative disorder that causes dopaminergic neuronal death and parkinsonian- like symptoms (Avila et al., 2013; Emsley, 2001).

2.3.2 NON-ESSENTIAL ELEMENTS

Non-essential elements are toxic to the human body even in minute quantities and have no biological importance. These non-essential elements also known as heavy metals include Pb, Hg, As, Cd, Co etc. The presence of toxic metals in soft drinks have been linked to the quality of ingredients in their preparation. (Anastacio et al,. 2018).

Buha (2018), indicated that Cd, is notable in inducing carcinogenic diseases such as thyroid and pancreatic cancers. Cadmium has no known beneficial effects in humans and its accumulation disrupt cell mechanisms which may lead to death. The average daily intake for Cd is low and range between 0.2 to 0.7 µg/kg for an adult. When Cd is ingested by humans, it is first transported to the liver through the blood. There, it binds to proteins to form complexes that are transported to the kidneys. Cd then accumulates in the kidneys and damage filtering mechanisms. This causes the excretion of essential proteins and sugars from the body, aside the kidney damage. This effect is strengthened by the fact that Cd stored in the kidneys takes a very long time to be excreted from the human body (LENNTECH, 2015) as its biological half-life is about 10–35 years (WHO, 2008). Cd poisoning leads to diarrhoea, hypertension, lung damage, damaged central nervous system, psychological disorders, and infertility in humans.

Pb on the other hand can be accumulated in erythrocytes and mostly replace Zn in δ-aminolevulinic acid dehydratase hence inhibiting its function (Flora et al., 2012 & Matovic et al., 2015). Generally, Pb toxicity leads to high blood pressure, cardiovascular diseases, kidney damage, nervous system damage (brain damage), and psychological disorder and subsequently to death.

Arsenic causes various health effects such as irritation of the stomach and intestines, decreased production of red and white blood cells, skin colorings and lung irritation, its high exposure renders damage in both men and women. It is further suggested that, the uptake of significant amounts of inorganic arsenic can intensify the chances of cancer development, especially the development of skin cancer, lung cancer, liver cancer, and lymphatic cancer. Lastly, inorganic arsenic can damage DNA. However, organic arsenic exposures may only render nerve injury and stomach aches (LENNTECH, 2015).

2.3.3 SOME DISEASES RESULTING FROM SOFT DRINK CONSUMPTION

Non-communicable diseases are mostly contracted from the food one consumes. Cardiovascular diseases, cancer, respiratory diseases, and diabetics are among the four major non communicable diseases. The main reasons for these chronic diseases are tobacco and alcohol consumption, physical inactivity, unhealthy diets and drinks, and environmental conditions. Soft drink consumption has a direct relationship to cardio vascular diseases and diabetics due to its added flavors and sugar content. In addition, soft drinks have other negative impacts which include gum and teeth related diseases and obesity. The economic costs of such consumption vary from country to country. Loss of income is one of the main impacts to household level. This loss of income may be from expenditure for such products as well as medical bills and costs once the consumer suffers from long term implications of soft drink consumption. Furthermore, there is an unmeasured psychological cost and emotional cost once a consumer contracts such chronic diseases.

Currently, soft drinks are viewed by many as a major contributor to health problems and have consequently been fingered for the rising prevalence of obesity, particularly among children. The continuous intake of soft drinks over long period can erode the tooth enamel. (Bassiouny &Yang, 2005).

Gulati (2014) argued that, considering the sugar intake, obesity and diabetes in India, there is a strong relationship between calorie intake (especially from soft drinks) and obesity. In India, the occurrence of obesity is on the rise at a rapid speed due to upsurge in energy drinks consumption. Mostly, owing to the purchasing power and availability of high fat, energy-rich foods, together with a decrease in the energy disbursement consequent to mechanization and urbanization.

Studies conducted by the American Society for Clinical Nutrition demonstrated that many adult diseases have their sources from childhood, whilst excessive weight gain is a forerunner to a wide variety of physiologic irregularities that ultimately affect morbidity and mortality. Obesity and overweight in children is of much worry because of the related developmental abnormalities (Heymsfield et al., 2003). More than half of the adults in advanced economies are overweight or obese and the numbers are growing. This epidemic of obesity causes 2.8 million deaths a year and cost $147 billion in the United States alone in 2008 (Finkelstein, 2009).

One of the major concerns associated with soft drink consumption is dental health. Jandt (2006) states that a greater number of the standard soft drinks cause dental erosion. Over the last decade, soft drinks and sweets induced demineralization of dental enamel has seen a sharp increase and replaced dental carries as the prior reason for tooth decay in young people (Jandt, 2006). The continuous intake of acidic, sugar-rich soft drink puts children in inherent danger of developing dental caries (Tahmassebi et al., 2006). Most research, currently conducted on the problems of soft drinks and dental health all agree on the relation of dental erosion and caries as an effect of continuous soft drink consumption. Studies by Prati et al (2003) have also revealed that enamel, which is composed of cementum and denim, protect the teeth. Dentine absorbency is increased by acidic drinks by opening dentinal tubules, completely exposing and removing the smear layer (Prati et al., 2003). However, studies carried out in the 1940's compared the prevalence of soft drinks with consumption of soft drinks. Data collation was carried out using reports of consumption of soft drinks by states in the USA and that of dental caries and no relationship could be found (Tahmassebi et al., 2006). Soft drink production and consumption has however increased by a large scale and since that time. Currently, research conducted makes it safe to say that dental erosion and caries directly relate to the consumption of soft drink beverages (Panich & Poolthong, 2009).

Higher percentages of sugar in drinks can result in non-alcoholic fatty liver disease (Medilexicon Medical Dictionary, 2012). People consuming more soft drinks a day have a possibility of this diseased condition leading to cirrhosis of the liver (Mercola, 2009). Also the presence ioof synthetic sweeteners may also be critical in the determination of most liver diseases (Byme, 2011).

2.4 ANALYTICAL METHODS FOR HEAVY METAL ANALYSES

Elements in soft drinks can be determined in the laboratory using the following laboratory assays: the Atomic Absorption Spectroscopy (AAS), Atomic Fluorescence Spectroscopy (AFS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Hydride Generation Atomic Absorption Spectroscopy (HGAAS), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-

AES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), X−ray fluorescence (XRF), Electron Microprobe (EM) Flame Photometer (FP) and Instrumental Neutron Activation Analysis (INAA). These instruments accurately measure elements in environmental sample to parts per billion (ppb) concentrations i.e. µg/L and µg/kg samples or mg/kg (ppm). Before any element is determined with any of these instruments, pre-treatment with acidic extraction or acidic oxidation digestion of the sample is very necessary. The significance of pre-treatment is that all elemental species are solubilized for easier detection and measurement. `

Accordingly, the goal of this work was to investigate the nutritional content and heavy metal contamination of new day soft drinks produced by several sprung-up modern day companies in Ghana. This could form a basis of developing a database to support soft drink research in Ghana. This dissertation is structured as follows:

- ✓ **Chapter 3** describes the methodology of the study, captures the site of study, the digestion process, the nutritional and heavy metal analysis and the assessment tools used for risk analysis.

- ✓ **Chapter 4** presents result of analysis; evaluates results using the statistical tools and discusses these results to expose the effects that may be posed to the consumer whiles presenting a long-term data for comparative and further studies.

- ✓ **Chapter 5** provides a general conclusion and emphasizes the major findings of this study, as well as recommendations for further studies.

CHAPTER THREE

METHODOLOGY

3.0 MATERIALS AND METHODS

3.1 REAGENTS AND CHEMICALS

Ultra-pure water was used for the preparation of reagents and standards. All chemicals were trace metal grade. Nitric acid (HNO_3) was obtained from Sigma (St. Louis, MO, USA), Hydrochloric acid (HCl) from Fisher Co. (USA). Nutritional and Trace Metal standard solutions were prepared by dilution of respective stock solutions from Merck (Kenilworth, NJ, USA) conserved in 1000µg m/L conserved in 1% Hydrochloric acid solution.

3.2 GLASSWARE TREATMENT

Glass wares were rinsed with de-ionized water and immersed in warm liquid soap bath for two days, then rinsed with de-ionized water and placed in 10% Nitric acid at room temperature for another three days. Glass wares were rinsed again three times with de-ionized water and then immersed in 50% nitric acid bath at 90°C for 24 hours. Glass wares were further rinsed with deionized water contained 1% Hydrochloric acid solution. They were then placed in a clean oven at 60°C for 12 hours. The glass wares were then removed from the oven allowed to cool down and bagged.

3.3 MAP OF STUDY AREA

The map shows the sampling area of the various retails shops dispersed across the Accra metropolis, Ghana where the samples were obtained.

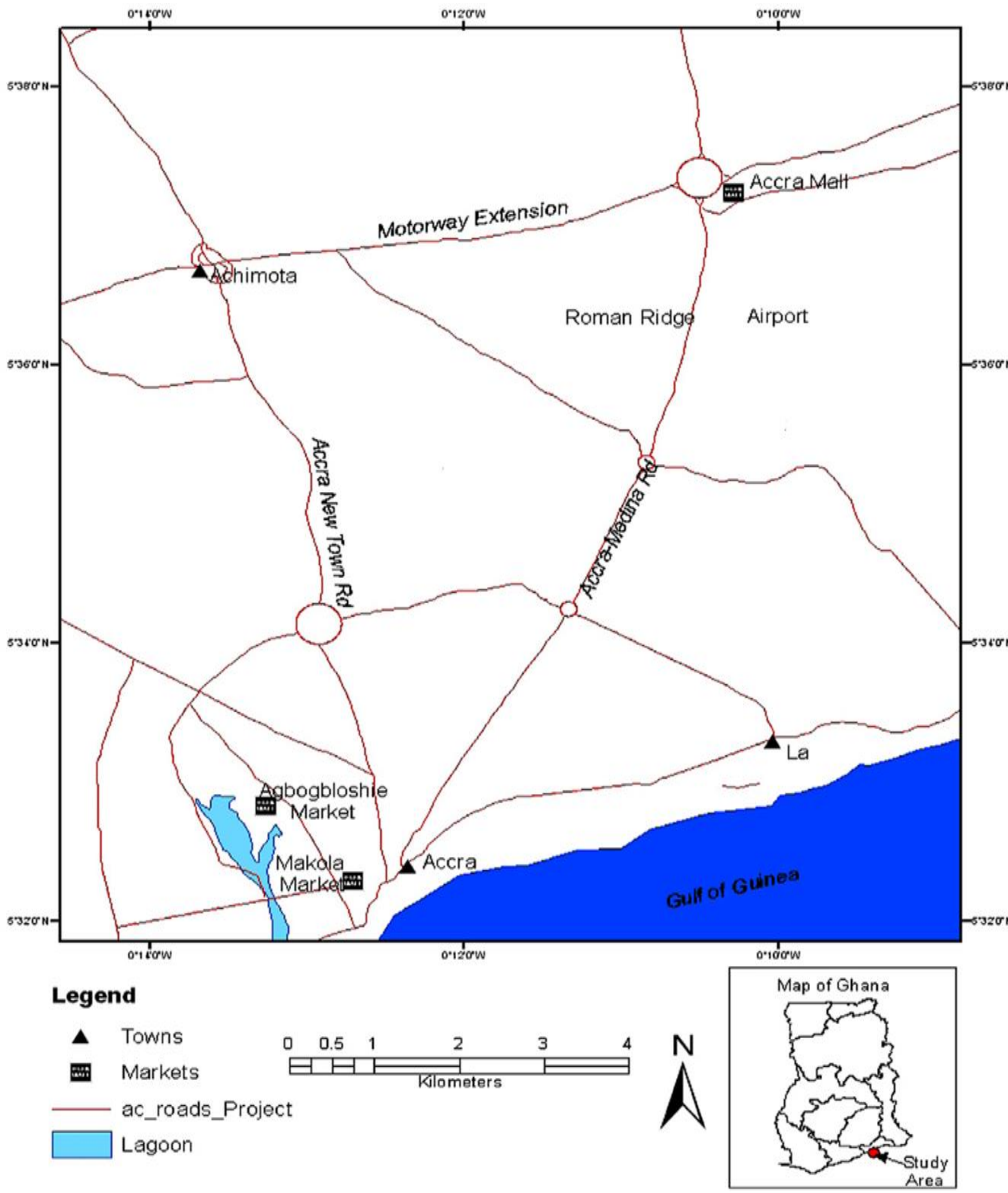

Figure 1 Map of sampling area within the Accra Metropolis, Ghana

3.4 SAMPLING

Random sampling of 42 different brands of new day Carbonated Soft Drinks from 8 different Companies were purchased from different markets and retail shops across the Accra Metropolis, Ghana in four (4) replicates for 4 months, (totaling 672 samples) running from mid-June to mid-October 2018. These were bagged and placed in ice-chest with ice pak and transported to the Department of Chemistry Laboratory; these were kept in fridge till analysis. Samples studied, purchased were the brands of the following companies: Special Ice Company Limited, Kasapreko Company Limited, Twellium Industries Limited, Blow Chem Industries Ltd, Multi-Pac Ltd, Calomaana Limited, Sailo Food and Drink Co. Ghana Ltd and Tri-Star Food Processing Ltd

3.5 SAMPLE PREPARATION

3.5.1 PHYSICOCHEMICAL ANALYSIS

pH, Turbidity and Electrical Conductivity of each sample (about 100 ml) were measured using portable field type instrument (Hanna digital pH meter, Hanna HI 98703 Turbidity portable meter and Hanna H19032 micro conductivity meter). All instruments were calibrated before usage.

3.5.2 NUTRITIVE AND TRACE METAL ANALYSIS

All samples were wet-digested according to Wallace et al (2013). 20ml of concentrated nitric acid was added to 20 mL of the sample and the mixture heated on a hot plate until the brown fumes disappeared in a fume cupboard. Each sample was then topped up with de-ionized water to 100 mL and filtered. The concentrations of Fe, Cu, Cr, Co, Mn, Zn, Na, Mg, Ca, Pb, Cd, As and Hg were quantified using the Atomic Absorption Spectrophotometer/Flame Photometer.

3.6 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

QA/QC criteria were met by running reagent blanks, standard solutions and replicate analysis.

3.7 DATA ANALYSIS

Results were subjected to analysis using Microsoft Excel (2010) to determine mean, standard deviation and variance. The results were the average of four experiments ($p \leq 0.05$).

3.7.2 HAZARD INDEX

The hazard index of the trace metals that exceeded the WHO limits were evaluated by the equations;

$$D_i = \frac{C_w * IR * EF * ED}{B_w * AT} \qquad (1)$$

$$HQ = \frac{D_i}{RfD} \qquad (2)$$

Where $\mathbf{D_i}$ is the daily dose of trace metals to which consumers might be exposed.

$\mathbf{C_w}$ is the concentration of the trace metal

IR is the absorption rate of trace metals

EF is the exposure frequency

ED is the total duration of exposure

$\mathbf{B_w}$ is the body weight (in this case 70kg for average adult, Ghana Health Service, was used)

RfD is the reference dose of each trace metal

HQ is the hazard quotient of trace metals.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 SAMPLING

A total of 168 samples from 42 different brands of soft drinks were collected from 8 different companies each month for this study within the Accra Metropolis, Ghana. An average of 12% of the soft drinks were purchased from companies (A-E) for the study with the largest company having 19% of the total samples collected and analyzed (Fig 2). The quantities of different brands of soft drinks produced by each company greatly affected the number of samples they contributed for the study. Fig 2 summarizes the contributions from each company for four (4) month period.

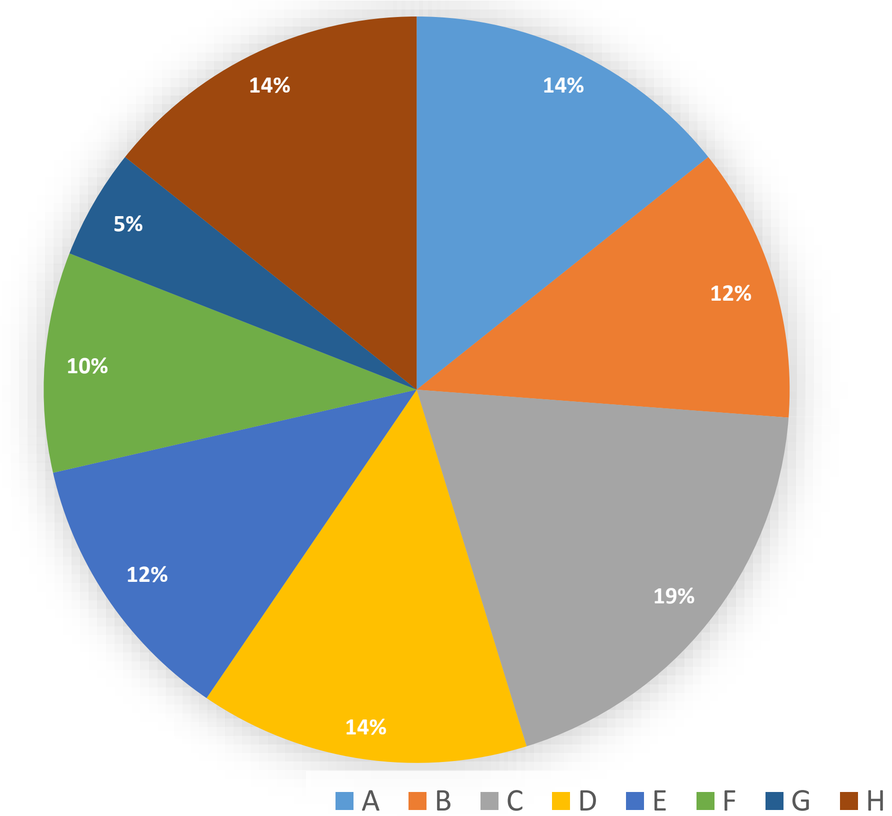

Table 4. 1 A Summary of Companies' Contribution in the Study

Company	Number of Brands	Total number of Brands sampled
A	6	24
B	5	20
C	8	32
D	6	24
E	5	20
F	4	16
G	2	8
H	6	24
Total	**42**	**168**

Figure 2 Percentage Total Samples collected from companies

4.2 PHYSICOCHEMICAL ANALYSES

Table 4.2 represents the overall average of the physicochemical parameters measured in this study (Appendix I). The pH ranged from 2.2 - 3.5 across all the soft drinks brands examined, revealing how acidic these drinks were. The level of acidity observed from the study was found to be similar to the findings of Patil et al. (2015). The acidity could be attributed to the presence of phosphoric and citric acids which acts as preservatives and the possible formation of carbonic acid from the reaction between CO_2 and H_2O as some of the CO_2 present exist as free and unbound; hence the long storage of the drinks could be predicted to produce low pH in most soft drinks. In all, drinks from company D had the highest pH whiles drinks from companies B and H were of lower pH. Although acidity could be of great value in killing gastrointestinal bacteria in the body, low pH could also be responsible for teeth enamel erosion (Panich & Poolthong, 2009). The effect of low pH has become evident in many studies to be a causative factor of tooth decay especially from soft drink.

In the case of total dissolved solids (TDS), the samples analyzed ranged from 120.30 mg/L to 2546.00 mg/L which showed that the samples had much dissolved solids in them which corroborates with high levels of nutritional metals determined. Conductivity which measures how well the water in the sample can transmit electrical current appeared to follow a similar trend with range of about 179.50 µS to 3800.00 µS. This observation also suggested that the sample again had more solids which were not soluble and was confirmed by the turbidity measurement of the drinks (0.05 NTU - 500 NTU). This result was expected as this soft drinks included flavoring and coloring agents most of which are insoluble with water. The high turbidity and TDS values showed that the samples contain many constituents which may serve as pockets, interfaces and hiding places for various metals both nutritional and trace metals.

Table 4. 2 Overall Mean Values of Physicochemical Parameters Analyzed in the Soft Drinks for the 4-month Study (Mid-June to Mid-October) 2018

Company	Brand ID	pH	Conductivity (µS)	Total Dissolved Solid (mg/L)	Turbidity (NTU)
A	**SD 1**	2.37	619.00	414.70	0.70
	SD 2	2.20	1201.00	804.70	1.60
	SD 3	2.28	810.00	542.70	120.00
	SD 4	2.40	632.00	423.40	0.60
	SD 5	3.12	533.00	357.10	270.00
	SD 6	2.60	444.00	297.50	500.00
B	**SD 7**	2.86	952.00	637.80	1.00
	SD 8	3.17	565.00	378.60	0.35
	SD 9	3.52	476.00	318.90	180.00
	SD 10	2.72	805.00	539.40	45.00
	SD 11	2.80	378.00	253.30	0.25
C	**SD 12**	2.50	586.00	392.60	0.45
	SD 13	2.35	760.00	509.20	450.00

	SD 14	2.63	539.00	361.10	0.30
	SD 15	2.85	568.00	380.60	2.30
	SD 16	3.20	1598.00	1070.70	2.00
	SD 17	3.16	518.00	347.10	0.45
	SD 18	3.15	3380.00	2264.00	120.00
	SD 19	2.47	798.00	534.66	230.00
D	**SD 20**	2.87	904.00	605.60	3.60
	SD 21	2.66	1024.00	686.10	4.80
	SD 22	2.55	701.00	469.67	2.50
	SD 23	2.56	633.00	424.10	220.00
	SD 24	2.70	558.00	373.89	140.00
	SD 25	2.60	735.00	492.20	1.00
E	**SD 26**	3.12	480.00	320.00	0.10
	SD 27	3.25	1590.00	1065.30	0.40
	SD 28	2.87	366.00	245.20	0.10
	SD 29	2.42	1030.00	690.10	2.31

	SD 30	2.52	3800.00	2546.00	85.00
F	**SD 31**	2.67	587.00	393.30	350.00
	SD 32	2.73	538.00	360.50	120.00
	SD 33	3.31	396.00	265.30	0.45
	SD 34	2.70	574.00	384.60	320.00
G	**SD 35**	2.88	580.00	388.60	1.70
	SD 36	3.12	652.00	436.84	0.05
H	**SD 37**	2.92	557.00	373.20	1.00
	SD 38	3.10	521.00	349.10	2.20
	SD 39	3.00	415.00	278.10	0.40
	SD 40	3.08	761.00	509.90	370.00
	SD 41	2.86	776.00	519.92	0.85
	SD 42	3.41	179.50	120.30	2.20

4.3 NUTRITIONAL LEVEL AND TRACE METAL CONTENT

From the study, all the metals analyzed were present in most of the samples except for Pb, Hg, As, Cd, and Cu which were found to be below detection limit. Metals like Zn, Mn, and Co were present in fewer samples with Fe, Na, Mg, Ca present in all samples while Cr was detected in most samples. The variations in metals concentrations under study could be attributed to the different source of raw materials for the production of these drinks by the different companies as well as the manufacturing procedures employed by each company.

In Table 4.3, (Appendix II) the nutritional metals measured, showed higher concentrations ranging from 26.00 - 195.70 mg/L for Na, 1.23 - 55.00 mg/L for Ca and 0.048 - 0.612 mg/L for Mg indicating that the soft drinks measured had much nutritional benefits as these metals are known in helping to regulate muscle and nerve functioning in humans, aid in high blood pressure lowering and general body growth (Wolfson Institute of Preventive Medicine, 2015). Hence their presence in new day soft drinks being produced in Ghana by many companies is of much importance.

On the other hand, trace metals like Fe, Cr, Co whose nutritional benefits are only essential in small quantities were found in moderate amounts with Fe and Zn have concentrations ranging from 0.031 - 0.494 mg/L and 0.012 - 0.028 mg/L respectively. However, Co and Cr were found to be present in much higher concentrations than needed in the human body with concentrations between 0.008 - 0.062 mg/L and 0.10 - 0.380 mg/L respectively which pose a serious health concern as Cr is known to cause various forms of lung and skin cancers (Feng et al., 2015), although the health defects of Co have not been established it is believed to cause cardiomyopathy, a condition which affects the heart muscles and can demonstrate to be high chronic and hereditary effects (Packer, 2016). The higher concentrations of this essential but toxic metals as well as the nutritional metals can be attributed to the sources of water for the production of these soft drinks which exist as the

Table 4. 3 Overall Average Concentrations of Nutritional and trace metal measured in soft drinks

Company	Sample ID	Fe	Zn	Cu	Mn	Co	Cr	As	Pb	Hg	Cd	Na	Ca	Mg
	MRL	**2.000**	**3.000**	**2.000**	**0.400**	**0.020**	**0.050**					**-**	**-**	**-**
A	SD 1	0.038	-	-	-	-	0.048	-	-	-	-	53.900	54.540	0.394
	SD 2	0.056	-	-	0.012	-	0.054	-	-	-	-	52.800	55.000	0.288
	SD 3	-	-	-	0.010	-	0.018	-	-	-	-	74.000	1.600	0.362
	SD 4	0.050	-	-	0.012	0.036	-	-	-	-	-	39.800	53.600	0.398
	SD 5	0.046	-	-	-	-	0.020					64.300	6.880	0.326
	SD 6	-	-	-	-	-	0.044	-	-	-	-	78.800	6.480	0.484
B	SD 7	0.038	-	-	-	-	0.034	-	-	-	-	26.900	5.240	0.238
	SD 8	0.108	-	-	-	-	-	-	-	-	-	70.100	50.230	0.420
	SD 9	0.264	-	-	-	0.380	0.010	-	-	-	-	119.700	7.340	0.278
	SD 10	0.224	-	-	-	0.226	0.018	-	-	-	-	105.900	1.230	0.170
	SD 11	0.180	-	-	-	0.210	0.048	-	-	-	-	35.900	10.440	0.232
C	SD 12	-	-	-	-	-	0.042	-	-	-	-	107.100	6.530	0.054
	SD 13	-	-	-	-	-	-	-	-	-	-	69.200	6.430	0.058

	SD 14	-	-	-	-	-	0.022	-	-	-	-	86.400	16.200	0.198
	SD 15	-	-	-	-	0.020	0.008	-	-	-	-	115.500	15.800	0.530
	SD 16	-	-	-	-	-	-	-	-	-	-	131.400	16.530	0.056
	SD 17	-	-	-	-	-	0.018	-	-	-	-	33.300	16.380	0.048
	SD 18	-	-	-	-	-	0.034	-	-	-	-	143.100	6.360	0.090
	SD 19	-	-	-	-	-	0.030	-	-	-	-	57.400	7.200	0.066
D	SD 20	0.422	-	-	-	0.010	0.058	-	-	-	-	195.700	1.830	0.224
	SD 21	0.268	-	-	-	-	-	-	-	-	-	120.000	5.940	0.468
	SD 22	-	-	-	-	0.016	0.048	-	-	-	-	30.000	6.030	0.376
	SD 23	0.266	0.014	-	-	-	-	-	-	-	-	101.000	53.480	0.258
	SD 24	0.410	0.016	-	-	-	-	-	-	-	-	89.800	6.920	0.244
	SD 25	0.372	0.012	-	-	-	-	-	-	-	-	78.800	4.440	0.202
E	SD 26	0.400	0.014	-	-	-	0.054	-	-	-	-	101.400	7.830	0.148
	SD 27	0.292	0.012	-	-	-	-	-	-	-	-	93.800	16.200	0.204
	SD 28	0.320	0.022	-	-	-	0.008	-	-	-	-	84.000	6.520	0.210
	SD 29	0.494	0.012	-	-	-	0.026	-	-	-	-	37.600	1.730	0.132

	SD 30	-	0.028	-	-	-	-	-	-	-	-	33.500	6.120	0.160
F	SD 31	0.440	0.022	-	-	-	0.062	-	-	-	-	79.400	16.320	0.608
	SD 32	0.038	0.026	-	-	-	0.046	-	-	-	-	120.300	6.740	0.276
	SD 33	0.072	-	-	-	-	0.012	-	-	-	-	86.300	17.380	0.462
	SD 34	0.031	-	-	-	-	-	-	-	-	-	41.000	6.950	0.612
G	SD 35	0.408	-	-	-	-	0.012	-	-	-	-	94.600	2.380	0.606
	SD 36	0.404	-	-	-	-	0.024	-	-	-	-	82.900	17.580	0.530
H	SD 37	0.320	-	-	-	-	-	-	-	-	-	58.700	17.550	0.068
	SD 38	0.054	-	-	-	-	0.062	-	-	-	-	112.300	11.670	0.308
	SD 39	0.420	-	-	-	-	-	-	-	-	-	52.800	10.430	0.060
	SD 40	0.420	-	-	-	-	0.040	-	-	-	-	61.600	52.580	0.498
	SD 41	0.388	-	-	-	-	-	-	-	-	-	60.100	1.840	0.346
	SD 42	0.428	-	-	-	-	0.040	-	-	-	-	141.200	7.370	0.266

main constituent of these soft drinks as well as from the equipment used for their production and human anthropogenic activities closer to the manufacturing plant. Also, the efficiency in the process of purification could influence the concentration and the occurrence of toxic metals in the water used in the production.

4.4 COMPARISON WITH RECOMMENDED DRINKING WATER STANDARDS

With the prevalence of human diseases such as lung cancers, anemia and heart failure, metal contamination stands out as being a potential cause, hence sources of metal contamination in the diet required to be well monitored. As such, the safety of new day soft drinks have come under much discussions and concerns as it may represent a hidden source of human exposure to toxic metal, therefore, the levels of the metals study were compared to World Health Organization Maximum Recommended Limit for drinking water, WHO MRL (Pérez & Brown, 2017). Most trace metals analyzed were found to be below their MRL except for Cr and Co whose average concentrations predicted contaminations in some of the drinks. Although Co was present in few samples it showed the highest exceedances of about 3000. Table 4.4 and Figure 3 and 4 summarizes the exceedances of Cr and Co samples based on their concentrations and WHO MRLs on company basis. It can be observed that soft drinks manufactured by companies A, D, E, F and H had samples which exceeded the WHO MRL with company F having majority of its samples exceeding their MRLs which suggested that either the mode of production or the sources of raw materials for these companies could be responsible for their exceedances. On the other hand most companies did not have Co either present or exceeding the MRL which was a plus to the companies. The high exceedances for Co compared to the standard limits suggests that about 60% of the product from company B were not safe for consumption and are variable for cobalt poisoning. Hence seems more threatening to consumers' health.

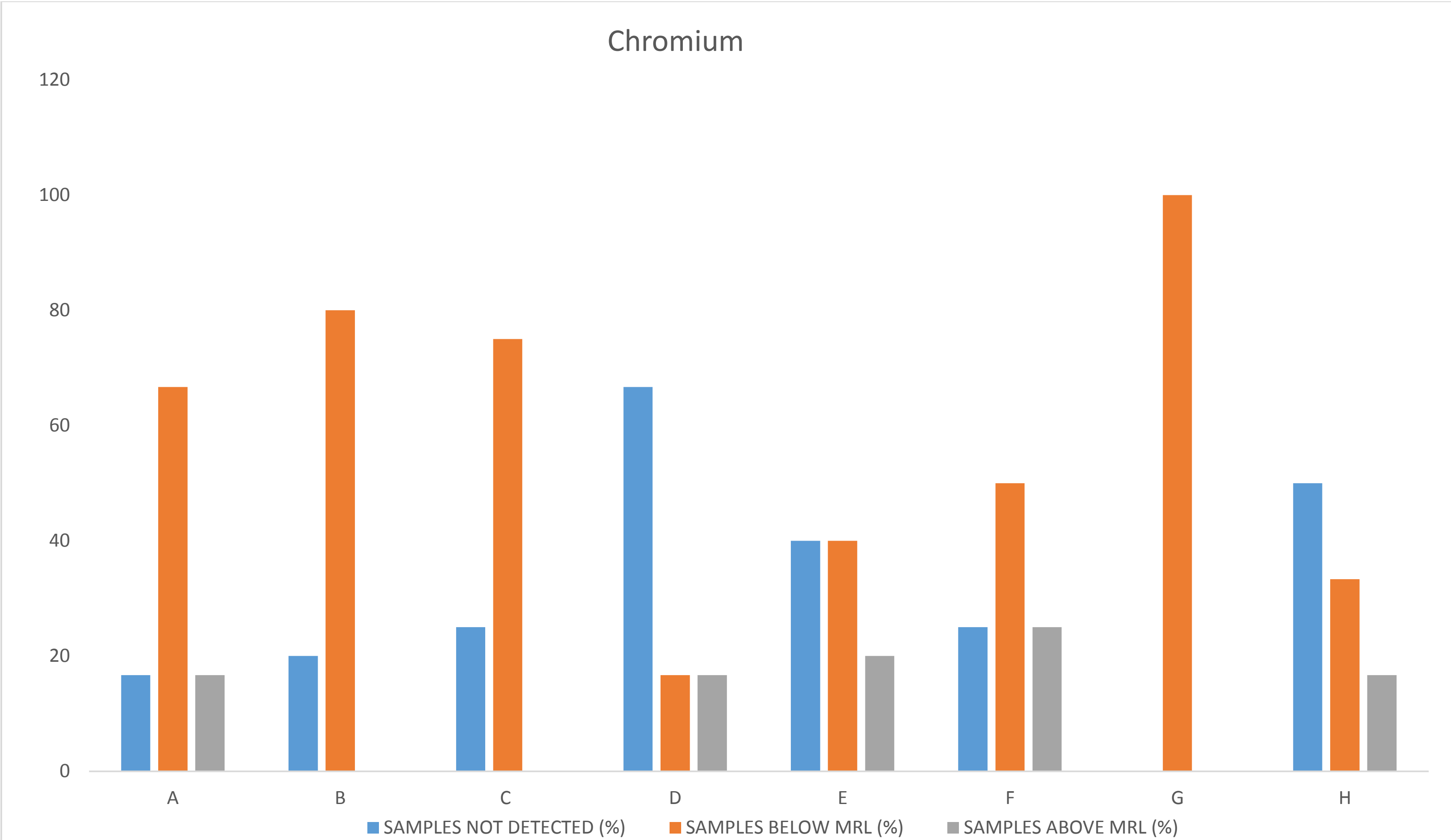

Figure 3 A graph showing the percentage exceedances of Cr in the study

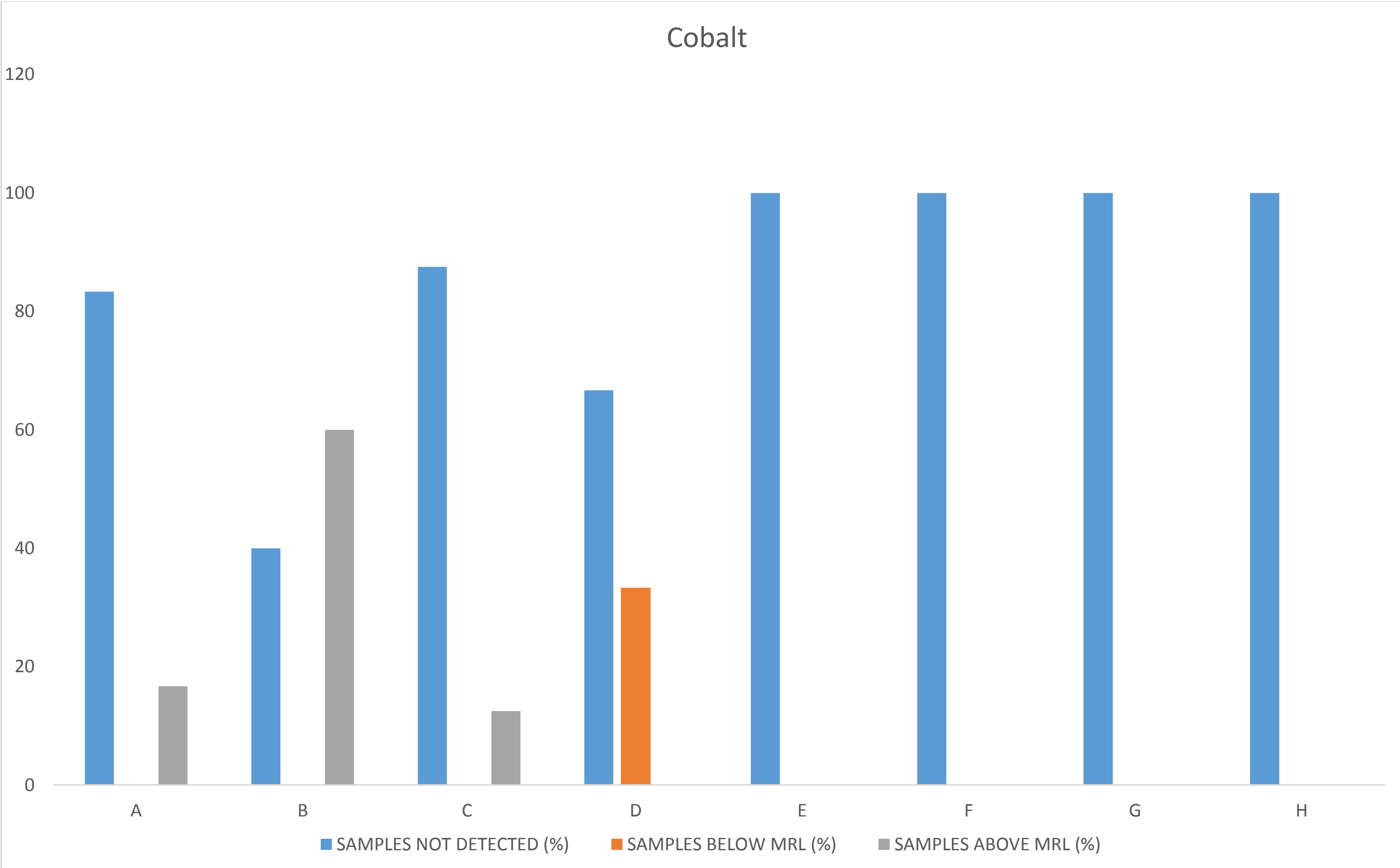

Figure 4 A graph showing the percentage exceedances of Co in the study

Table 4. 4 Percentage Exceedance of Chromium (Cr) and Cobalt (Co) in this study

Chromium

COMPANY	SAMPLES NOT DETECTED (%)	SAMPLES BELOW MRL (%)	SAMPLES ABOVE MRL (%)
A	16.67	66.67	16.67
B	20.00	80.00	0.00
C	25.00	75.00	0.00
D	66.67	16.67	16.67
E	40.00	40.00	20.00
F	25.00	50.00	25.00
G	0.00	100.00	0.00
H	50.00	33.33	16.67

Cobalt

COMPANY	SAMPLES NOT DETECTED (%)	SAMPLES BELOW MRL (%)	SAMPLES ABOVE MRL (%)
A	83.33	0.00	16.67
B	40.00	0.00	60.00
C	87.50	0.00	12.50
D	66.67	33.33	0.00
E	100.00	0.00	0.00
F	100.00	0.00	0.00
G	100.00	0.00	0.00
H	100.00	0.00	0.00

4.5 COMPARISON WITH OTHER STUDIES

Much studies have been carried out into the various component of soft drinks in Ghana and beyond. However, there is a paucity of data on Ghana (Ackah et al., 2014) unlike the European countries (Briggs et al., 2017; Diogo et al., 2013; Fallico et al., 2011; Hendriksen et al., 2011) and a few African countries, for example; Nigeria (Godwill et al., 2015; Nkono & Asubiojo, 1997; Onianwa, Adetola, Iwegbue, Ojo, & Tella, 1999) and Egypt (Abdel-Rahman et al., 2019). All these studies did confirm the presence of some trace metals notable are Cu and Fe. All the same, most of the findings were similar to this study. Investigations in and around Egypt using Inducted Coupled Plasma Optical Emission Spectrometry (ICP-OES) revealed the absence of Cd and Pb in 100 percent of the samples examined which was in agreement with this study (Abdel-Rahman et al., 2019). Likewise, Mn was also found below detection limits in most of the samples analyzed.

On the other hand, metals like Fe and Co differed between both studies with opposite observations in each case. Cr was detected in the samples with about 20% exceeding their MRLs while Cr was not detected at all in their sample. Also, Cu and Fe were not detected and below their MRL respectively from the studies however it was reported that the presence of Fe increased across the different containers of the soft drinks from plastics to cans. Such differences could be due to the time of study, different handling procedures, method of analyses and instrumentation employed. Another point in case is the findings of Oniawa et al (1999) from Nigeria on metals like Pb, Cu, Cd, Fe and As which were all present but within recommended limits following analysis with Atomic Absorption Spectroscopy (AAS). This observation disagreed with the results of this study which could stem from some of the reasons outline earlier. Also the difference in manufacturing processes for soft drinks across countries and the geographic could impact the levels and distribution.

In general, different countries are expected to show different levels of metal concentrations due to the independent nature of production of these soft drinks and the different environmental conditions each country finds itself.

4.6 HEALTH RISK ASSESSMENT

In the determination of the health risk, Hazard quotients (HQ) which were found to be less than one were termed as risk free while those greater than one were noted to cause health risk. Assessment of the health risk of the trace metals indicated that, metals like Pb, Hg, Cd and As, which are known to be harmful did not stand to pose any health risk as they were not detected in any sample. Equally, metals Fe, Mn and Zn which were below their recommended WHO MRLs also showed less health risk. However, metals Co and Cr which exceeded their recommended Limit were computed for their average exposure intake and corresponding Hazard Quotient as shown in Table 4.5.

The daily exposure intake of Co ranged from (0.004 - 0.140) mg/Kg/day for samples from companies A, B, C, D which was alarming due to the known toxicity of Co to consumers and the possible prevalence of Co in the ecosystem. With a reference dose (RfD) of 20 µg/kg/day from USEPA (2004), it could be noted that samples from company B showed very high health risk and therefore the excessive consumption of these products may be detrimental to consumers health.

Whiles companies A, C, and D displayed no associated health risk, nonetheless consumers stand to face a chance of bioaccumulation for long term consumption. In contrast, Cr did not pose any health risk in its detected samples, though it also stand a possibility of bioaccumulation.

It had an exposure intake of (0.003 - 0.023) mg/Kg/day with a reference dose of 140 µg/kg/day, (USEPA, 2004). In general with the majority of the hazard quotient less than 1 except in samples from company B, it indicates that a critical review be done on the water quality for these new day soft drinks as well as other components of these soft drink.

Table 4. 5 Health Risk Assessment of Co and Cr from the study

Company	Sample ID	Co (RfD = 20 µg/Kg/day)			Cr (RfD = 140 µg/Kg/day)		
		Exposure intake	HQ	Risk	Exposure intake	HQ	Risk
A	SD 1	-	-	-	0.018	0.126	No
	SD 2	-	-	-	0.020	0.142	No
	SD 3	-	-	-	0.007	0.047	No
	SD 4	0.013	0.661	No	-	-	-
	SD 5	-	-	-	0.007	0.052	No
	SD 6	-	-	-	0.016	0.115	No
B	SD 7	-	-	-	0.012	0.089	No
	SD 8	-	-	-	-	-	-
	SD 9	0.140	6.980	Yes	0.004	0.026	No
	SD 10	0.083	4.151	Yes	0.007	0.047	No
	SD 11	0.077	3.858	Yes	0.018	0.126	No
C	SD 12	-	-	-	0.015	0.110	No
	SD 13	-	-	-	-	-	-

	SD 14	-	-	-	0.008	0.058	No
	SD 15	0.007	0.367	No	0.003	0.021	No
	SD 16	-	-	-	-	-	-
	SD 17	-	-	-	0.007	0.047	No
	SD 18	-	-	-	0.012	0.089	No
	SD 19	-	-	-	0.011	0.079	No
D	SD 20	0.004	0.184	No	0.021	0.152	No
	SD 21	-	-	-	-	-	No
	SD 22	0.006	0.294	No	0.018	0.126	No
	SD 23	-	-	-	-	-	-
	SD 24	-	-	-	-	-	-
	SD 25	-	-	-	-	-	-
E	SD 26	-	-	-	0.020	0.142	No
	SD 27	-	-	-	-	-	-
	SD 28	-	-	-	0.003	0.021	No
	SD 29	-	-	-	0.010	0.068	No

	SD 30	-	-	-	-	-	-
F	SD 31	-	-	-	0.023	0.163	No
	SD 32	-	-	-	0.017	0.121	No
	SD 33	-	-	-	0.004	0.031	No
	SD 34	-	-	-	-	-	-
G	SD 35	-	-	-	0.004	0.031	No
	SD 36	-	-	-	0.009	0.063	No
H	SD 37	-	-	-	-	-	-
	SD 38	-	-	-	0.023	0.163	No
	SD 39	-	-	-	-	-	-
	SD 40	-	-	-	0.015	0.105	No
	SD 41	-	-	-	-	-	-
	SD 42	-	-	-	0.015	0.105	No

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.0 CONCLUSION

The following conclusions could be drawn from the studies into the physicochemical properties, nutritional and trace metal concentrations of soft drinks:

1. The drinks analysed contained high levels of nutritional metals hence safer for consumption.
2. Most soft drinks contained essential trace metals with the concentration of few products exceeding their WHO MRLs.
3. All new day soft drinks were free from potentially toxic trace metals.
4. There are also associated health risks with few soft drinks in relation to trace metal contamination

5.1 RECOMMENDATIONS

1. The state of some soft drinks in the country is quite alarming and hence continuous monitoring is recommended to ensure the safety of the consumer.
2. Legal and Statutory actions should be taken by the various food agencies on companies producing drinks below the accepted reference dose.
3. Further studies are also recommended into other macro-constituent of soft drinks and their effects such as sugar and flavouring agents.

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APPENDIX I

Table showing the average values of the physicochemical parameters measured for the study.

Company	Brand ID	Description	pH	Conductivity (µS)	Total Dissolved Solid (mg/L)	Turbidity (NTU)
A	SD 1	Apple Drink	2.34	614	411	0.68
			2.41	623	417	0.72
			2.38	618	414	0.71
			2.35	621	416	0.69
		Mean	**2.37±0.03**	**619±4**	**415±3**	**0.70±0.02**
	SD 2	Cola Drink	2.18	1177	789	1.57
			2.19	1213	813	1.62
			2.23	1225	821	1.63
			2.21	1189	797	1.58
		Mean	**2.20±0.02**	**1201±23**	**805±15**	**1.60±0.03**
	SD 3	Lemon Drink	2.27	794	532	118
			2.31	826	554	122
			2.26	818	548	121
			2.29	802	537	119

		Mean	**2.28±0.02**	**810±15**	**543±10**	**120±2**
	SD 4	**Apple Drink**	2.36	626	419	0.59
			2.39	638	428	0.61
			2.42	645	432	0.61
			2.43	626	419	0.59
		Mean	**2.40±0.03**	**632±9**	**423±6**	**0.60±0.01**
	SD 5	**Cocktail**	3.14	538	361	273
			3.11	544	364	275
			3.13	528	354	267
			3.10	522	350	265
		Mean	**3.12±0.02**	**533±10**	**357±7**	**270±5**
	SD 6	**Coconut Drink**	2.59	451	302	508
			2.60	448	300	505
			2.59	437	293	493
			2.61	440	295	495
		Mean	**2.60±0.01**	**444±7**	**298±4**	**500±7**
B	**SD 7**	**Cola Drink**	2.90	966	647	1.02
			2.89	962	644	1.01

		2.83	942	631	0.99
		2.82	938	628	0.99
	Mean	**2.86±0.04**	**952±14**	**638±9**	**1.00±0.01**
	Apple Drink	3.15	562	377	0.348
		3.14	559	375	0.347
SD 8		3.19	568	380	0.352
		3.20	571	382	0.354
	Mean	**3.17±0.03**	**565±5**	**379±3**	**0.350±0.003**
	Passion Fruit Flavored	3.51	475	318	179.6
		3.53	477	320	180.5
SD 9		3.53	477	320	180.4
		3.51	475	318	179.5
	Mean	**3.52±0.01**	**476±1**	**319±1**	**180.0±0.5**
	Tamarind Fruit Flavored	2.71	802	537	44.8
		2.72	804	539	45.0
SD 10		2.73	808	542	45.2
		2.72	806	540	45.0
	Mean	**2.72±0.01**	**805±3**	**540±2**	**45.0±0.2**

		Berry-Grape Drink	2.79	377	253	0.250
			2.81	379	254	0.251
	SD 11		2.79	377	253	0.249
			2.81	379	254	0.251
		Mean	**2.80±0.01**	**378±1**	**253±1**	**0.250±0.001**
		Apple-Lime Drink	2.50	586	392	0.450
			2.48	580	389	0.446
	SD 12		2.50	586	393	0.450
			2.53	592	397	0.455
		Mean	**2.50±0.02**	**586±5**	**392.6±3**	**0.450±0.004**
		Apple,Orange, Pineaple Mix	2.34	758	508	449
C			2.34	757	507	448
	SD 13		2.36	762	511	451
			2.36	763	511	452
		Mean	**2.35±0.01**	**760±3**	**509±2**	**450±2**
			2.60	534	357	0.30
	SD 14	**Pear-Malt**	2.58	528	354	0.29
			2.66	544	365	0.30

		2.68	550	368	0.31
	Mean	**2.63±0.05**	**539±9**	**361.1±6**	**0.30±0.01**
SD 15	**Pineapple-Malt**	2.82	562	377	2.28
		2.79	557	373	2.25
		2.88	573	384	2.32
		2.91	579	388	2.35
	Mean	**2.85±0.05**	**568±10**	**381±7**	**2.30±0.04**
SD 16	**Energy Drink**	3.17	1582	1060	1.98
		3.14	1566	1049	1.96
		3.23	1613	1081	2.02
		3.26	1630	1092	2.04
	Mean	**3.20±0.06**	**1598±29**	**1071±19**	**2.00±0.04**
SD 17	**Apple Drink**	3.13	513	344	0.446
		3.10	508	340	0.441
		3.19	523	350	0.454
		3.22	528	354	0.459
	Mean	**3.16±0.06**	**518±9**	**347±6**	**0.450±0.008**
SD 18	**Orange Drink**	3.12	3346	2241	119

			3.09	3312	2219	118
			3.18	3412	2286	121
			3.21	3448	2309	122
		Mean	**3.15±0.06**	**3380±61**	**2264±41**	**120±2**
	SD 19	**Cocktail**	2.45	790	529	228
			2.42	782	524	225
			2.49	806	540	232
			2.52	814	545	235
		Mean	**2.47±0.04**	**798±14**	**535±10**	**230±4**
D	**SD 20**	**Apple Drink**	2.86	899	603	3.58
			2.86	902	604	3.59
			2.88	909	609	3.62
			2.88	907	607	3.61
		Mean	**2.87±0.01**	**904±4**	**606±3**	**3.60±0.02**
	SD 21	**Passion Fruit Flavored**	2.65	1019	683	4.78
			2.65	1022	685	4.79
			2.67	1029	690	4.82
			2.67	1027	688	4.81

	Mean	**2.66±0.01**	**1024±5**	**686±3**	**4.80±0.02**
SD 22	**Orange Drink**	2.54	697	467	2.49
		2.54	700	469	2.50
		2.56	705	472	2.51
		2.56	703	471	2.51
	Mean	**2.55±0.01**	**701±3**	**469±2**	**2.50±0.01**
SD 23	**Cola Drink**	2.55	630	422	219
		2.55	632	423	220
		2.57	636	426	221
		2.57	635	425	221
	Mean	**2.56±0.01**	**633±3**	**424±2**	**220±1**
SD 24	**Lemon-Lime Drink**	2.69	555	372	139
		2.69	557	373	140
		2.71	561	376	141
		2.71	560	375	140
	Mean	**2.70±0.01**	**558±3**	**374±2**	**140±1**
SD 25	**Energy Drink**	2.59	731	490	0.995
		2.59	734	491	0.998

			2.61	739	495	1.005
			2.61	737	494	1.003
		Mean	**2.60±0.01**	**735±3**	**492±2**	**1.000±0.005**
		Lemon Drink	3.10	478	318	0.100
			3.11	479	319	0.100
	SD 26		3.14	482	322	0.101
			3.13	481	321	0.100
		Mean	**3.12±0.01**	**480±2**	**320±1**	**0.100±0.001**
			3.23	1582	1060	0.398
			3.24	1587	1063	0.399
E	**SD 27**	**Cola Drink**	3.27	1598	1071	0.402
			3.26	1595	1068	0.401
		Mean	**3.25±0.02**	**1590±7**	**1065±5**	**0.400±0.002**
		Orange Drink	2.86	364	244	0.0995
			2.86	365	245	0.0998
	SD 28		2.88	368	246	0.1005
			2.88	367	246	0.1003
		Mean	**2.87±0.01**	**366±2**	**245±1**	**0.1000±0.0005**

		Cocktail	2.41	1025	687	2.30
			2.42	1028	689	2.31
	SD 29		2.43	1035	694	2.32
			2.43	1033	692	2.32
		Mean	**2.42±0.01**	**1030±5**	**690±3**	**2.31±0.01**
		Orange Cocktail	2.51	3781	2533	84.6
			2.51	3792	2541	84.8
	SD 30		2.53	3819	2559	85.4
			2.53	3811	2554	85.3
		Mean	**2.52±0.01**	**3800±17**	**2546±12**	**85.0±0.4**
		Apple Cocktail	2.66	584	391	348
			2.66	586	393	349
	SD 31		2.68	590	395	352
			2.68	589	394	351
F		**Mean**	**2.67±0.01**	**587±3**	**393±2**	**350±2**
			2.72	535	359	119.4
	SD 32	**Orange Drink**	2.72	537	360	119.8
			2.74	541	362	120.6

			2.74	540	362	120.4
		Mean	**2.73±0.01**	**538±2**	**361±2**	**120.0±0.5**
	SD 33	**Cola Drink**	3.29	394	264	0.448
			3.30	395	265	0.449
			3.33	398	267	0.452
			3.32	397	266	0.451
		Mean	**3.31±0.02**	**396±2**	**265±1**	**0.450±0.002**
	SD 34	**Apple Drink**	2.69	571	383	318
			2.69	573	384	319
			2.71	577	387	322
			2.71	576	386	321
		Mean	**2.70±0.01**	**574±3**	**385±2**	**320±2**
G	**SD 35**	**Herbs and Multi frink extract drink**	2.87	577	387	1.69
			2.87	579	388	1.70
			2.89	583	391	1.71
			2.89	582	390	1.71
		Mean	**2.88±0.01**	**580±3**	**389±2**	**1.70±0.01**
	SD 36	**Malt Extract**	3.10	649	435	0.0498

			3.11	651	436	0.0499
			3.14	655	439	0.0503
			3.13	654	438	0.0502
		Mean	**3.12±0.01**	**652±3**	**437±2**	**0.0500±0.0002**
H	SD 37	Apple Drink	2.91	554	371	0.995
			2.91	556	372	0.998
			2.93	560	375	1.005
			2.93	559	374	1.003
		Mean	**2.92±0.01**	**557±3**	**373±2**	**1.000±0.001**
	SD 38	Strawberry Drink	3.08	518	347	2.19
			3.09	520	348	2.20
			3.12	524	351	2.21
			3.11	523	350	2.21
		Mean	**3.10±0.01**	**521±2**	**349±2**	**2.20±0.01**
	SD 39	Grape Drink	2.99	413	277	0.398
			2.99	414	278	0.399
			3.02	417	279	0.402
			3.01	416	279	0.401

	Mean	**3.00±0.01**	**415±2**	**278±1**	**0.400±0.002**
SD 40	**Orange Drink**	3.05	753	505	366
		3.04	751	503	365
		3.11	769	515	374
		3.12	771	517	375
	Mean	**3.08±0.04**	**761±10**	**510±7**	**370±5**
SD 41	**Co la Drink**	2.83	768	515	0.84
		2.82	766	513	0.84
		2.89	784	525	0.86
		2.90	786	527	0.86
	Mean	**2.86±0.04**	**776±10**	**520±7**	**0.85±0.01**
SD 42	**Apple Drink**	3.38	178	119	2.18
		3.37	177	119	2.17
		3.44	181	122	2.22
		3.45	182	122	2.23
	Mean	**3.41±0.05**	**180±2**	**120±2**	**2.20±0.03**

APPENDIX II

Table showing the average concentration of nutritional and trace metals measured for the study

Company	Brand ID	Description	Fe	Zn	Cu	Mn	Co	Cr	As	Pb	Hg	Cd	Na	Ca	Mg
			2	**3**	**2**	**0.4**	**0.02**	**0.05**					**-**	**-**	**-**
A	SD 1	Apple Drink	0.0379	-	-	-	-	0.0479	-	-	-	-	53.7	54.4	0.392818
			0.0378	-	-	-	-	0.0478	-	-	-	-	53.6	54.3	0.39203
			0.0381	-	-	-	-	0.0481	-	-	-	-	54.1	54.7	0.395182
			0.0382	-	-	-	-	0.0482	-	-	-	-	54.2	54.8	0.39597
		Mean	**0.0380±0.0002**	-	-	-	-	**0.0480±0.0002**	-	-	-	-	**53.9±0.3**	**54.5±0.3**	**0.394±0.002**
	SD 2	Cola Drink	0.0554	-	-	0.0119	-	0.0535	-	-	-	-	52.3	54.5	0.28512
			0.0557	-	-	0.0119	-	0.0537	-	-	-	-	52.5	54.7	0.28656
			0.0566	-	-	0.0121	-	0.0545	-	-	-	-	53.3	55.6	0.29088
			0.0563	-	-	0.0121	-	0.0543	-	-	-	-	53.1	55.3	0.28944
		Mean	**0.0560±0.0005**	-	-	**0.0120±0.0001**	-	**0.0540±0.0005**	-	-	-	-	**52.8±0.5**	**55.0±0.5**	**0.288±0.003**
	SD 3	Lemon Drink	-	-	-	0.0101	-	0.0182	-	-	-	-	74.8	1.6	0.365982
			-	-	-	0.0099	-	0.0179	-	-	-	-	73.5	1.6	0.359466
			-	-	-	0.0099	-	0.0178	-	-	-	-	73.2	1.6	0.358018
			-	-	-	0.0101	-	0.0181	-	-	-	-	74.5	1.6	0.364534
		Mean	-	-	-	**0.0100±0.0001**	-	**0.0180±0.0002**	-	-	-	-	**74.0±0.8**	**1.60±0.02**	**0.362±0.4**
	SD 4	Apple Drink	0.050	-	-	0.0119	0.036	-	-	-	-	-	39	53	0.39402
			0.049	-	-	0.0116	0.035	-	-	-	-	-	39	52	0.38606
			0.052	-	-	0.0124	0.037	-	-	-	-	-	41	55	0.40994
			0.051	-	-	0.0121	0.036	-	-	-	-	-	40	54	0.40198

		Mean	**0.050±0.001**	-	-	**0.0120±0.0003**	**0.036±0.001**	-	-	-	-	-	**40±1**	**54±1**	**0.40±0.01**
	SD 5	**Cocktail**	0.046	-	-	-	-	0.020	-	-	-	-	64	6.8	0.32274
			0.045	-	-	-	-	0.019	-	-	-	-	62	6.7	0.31622
			0.047	-	-	-	-	0.021	-	-	-	-	66	7.1	0.33578
			0.046	-	-	-	-	0.020	-	-	-	-	65	6.9	0.32926
		Mean	**0.046±0.001**	-	-	-	-	**0.020±0.001**	-	-	-	-	**64±2**	**6.9±0.2**	**0.326±0.008**
	SD 6	**Coconut Drink**	-	-	-	-	-	0.044	-	-	-	-	78	6.4	0.47916
			-	-	-	-	-	0.043	-	-	-	-	76	6.3	0.46948
			-	-	-	-	-	0.045	-	-	-	-	81	6.7	0.49852
			-	-	-	-	-	0.044	-	-	-	-	80	6.5	0.48884
		Mean	-	-	-	-	-	**0.044±0.001**	-	-	-	-	**79±2**	**6.5±0.2**	**0.48±0.01**
	SD 7	**Cola Drink**	0.038	-	-	-	-	0.034	-	-	-	-	26.6	5.2	0.23562
			0.037	-	-	-	-	0.033	-	-	-	-	26.1	5.1	0.23086
			0.039	-	-	-	-	0.035	-	-	-	-	27.7	5.4	0.24514
			0.038	-	-	-	-	0.034	-	-	-	-	27.2	5.3	0.24038
		Mean	**0.038±0.001**	-	-	-	-	**0.034±0.001**	-	-	-	-	**26.9±0.7**	**5.2±0.1**	**0.238±0.006**
B	SD 8	**Apple Drink**	0.107	-	-	-	-	-	-	-	-	-	69	50	0.4158
			0.105	-	-	-	-	-	-	-	-	-	68	49	0.4074
			0.111	-	-	-	-	-	-	-	-	-	72	52	0.4326
			0.109	-	-	-	-	-	-	-	-	-	71	51	0.4242
		Mean	**0.108±0.003**	-	-	-	-	-	-	-	-	-	**70±2**	**50±2**	**0.42±0.01**
	SD 9	**Passion Fruit Flavored**	0.261	-	-	-	0.38	0.0099	-	-	-	-	119	7.3	0.27522
			0.256	-	-	-	0.37	0.0097	-	-	-	-	116	7.1	0.26966
			0.272	-	-	-	0.39	0.0103	-	-	-	-	123	7.6	0.28634
			0.267	-	-	-	0.38	0.0101	-	-	-	-	121	7.4	0.28078

		Mean	**0.264±0.007**	-	-	-	**0.38±0.01**	**0.0100±0003**	-	-	-	-	**120±3**	**7.3±0.2**	**0.278±0.007**
		Tamarind Fruit Flavored	0.222	-	-	-	0.224	0.0178	-	-	-	-	105	1.22	0.1683
			0.217	-	-	-	0.219	0.0175	-	-	-	-	103	1.19	0.1649
	SD 10		0.231	-	-	-	0.233	0.0185	-	-	-	-	109	1.27	0.1751
			0.226	-	-	-	0.228	0.0182	-	-	-	-	107	1.24	0.1717
		Mean	**0.224±0.006**	-	-	-	**0.226±0.006**	**0.0180±0.0005**	-	-	-	-	**106±3**	**1.23±0.03**	**0.170±0.004**
		Berry-Grape Drink	0.178	-	-	-	0.208	0.048	-	-	-	-	35.5	10.34	0.22968
			0.175	-	-	-	0.204	0.047	-	-	-	-	34.8	10.13	0.22504
	SD 11		0.185	-	-	-	0.216	0.049	-	-	-	-	37.0	10.75	0.23896
			0.182	-	-	-	0.212	0.048	-	-	-	-	36.3	10.54	0.23432
		Mean	**0.180±0.005**	-	-	-	**0.210±0.005**	**0.048±0.001**	-	-	-	-	**35.9±0.9**	**10.4±0.3**	**0.232±0.006**
		Apple-Lime Drink	-	-	-	-	-	0.042	-	-	-	-	106	6.46	0.05346
			-	-	-	-	-	0.041	-	-	-	-	104	6.33	0.05238
	SD 12		-	-	-	-	-	0.043	-	-	-	-	110	6.73	0.05562
			-	-	-	-	-	0.042	-	-	-	-	108	6.60	0.05454
		Mean	-	-	-	-	-	**0.042±0.001**	-	-	-	-	**107±3**	**6.5±0.2**	**0.054±0.001**
		Apple, Orange, Pineapple Mix	-	-	-	-	-	-	-	-	-	-	69	6.37	0.05742
C			-	-	-	-	-	-	-	-	-	-	67	6.24	0.05626
	SD 13		-	-	-	-	-	-	-	-	-	-	71	6.62	0.05974
			-	-	-	-	-	-	-	-	-	-	70	6.49	0.05858
		Mean	-	-	-	-	-	-	-	-	-	-	69±2	6.4±0.2	0.058±0.002
			-	-	-	-	-	0.0218	-	-	-	-	86	16.0	0.19602
			-	-	-	-	-	0.0216	-	-	-	-	85	15.9	0.19404
	SD 14	**Pear-Malt**	-	-	-	-	-	0.0222	-	-	-	-	87	16.4	0.1999008
			-	-	-	-	-	0.0224	-	-	-	-	88	16.5	0.20195901

	Mean	-	-	-	-	-	**0.0220±0.0004**	-	-	-	-	**86±2**	**16.2±0.3**	**0.198±0.004**
SD 15	**Pineapple-Malt**	-	-	-	-	0.0198	0.0079	-	-	-	-	114	15.6	0.5247
		-	-	-	-	0.0196	0.0078	-	-	-	-	113	15.5	0.5194
		-	-	-	-	0.0202	0.0081	-	-	-	-	117	16.0	0.535088
		-	-	-	-	0.0204	0.0082	-	-	-	-	118	16.1	0.54059735
	Mean	-	-	-	-	**0.0200±0.0004**	**0.0080±0.0001**	-	-	-	-	**116±2**	**15.8±0.3**	**0.53±0.01**
SD 16	**Energy Drink**	-	-	-	-	-	-	-	-	-	-	130	16.4	0.05544
		-	-	-	-	-	-	-	-	-	-	129	16.2	0.05488
		-	-	-	-	-	-	-	-	-	-	133	16.7	0.0565376
		-	-	-	-	-	-	-	-	-	-	134	16.9	0.05711972
	Mean	-	-	-	-	-	-	-	-	-	-	**131±2**	**16.5±0.3**	**0.056±0.001**
SD 17	**Apple Drink**	-	-	-	-	-	0.0178	-	-	-	-	33.0	16.2	0.04752
		-	-	-	-	-	0.0176	-	-	-	-	32.6	16.1	0.04704
		-	-	-	-	-	0.0182	-	-	-	-	33.6	16.5	0.0484608
		-	-	-	-	-	0.0184	-	-	-	-	34.0	16.7	0.04895976
	Mean	-	-	-	-	-	**0.0180±0.0003**	-	-	-	-	**33.3±0.6**	**16.4±0.3**	**0.048±0.001**
SD 18	**Orange Drink**	-	-	-	-	-	0.0337	-	-	-	-	142	6.3	0.0891
		-	-	-	-	-	0.0333	-	-	-	-	140	6.2	0.0882
		-	-	-	-	-	0.0343	-	-	-	-	144	6.4	0.090864
		-	-	-	-	-	0.0347	-	-	-	-	146	6.5	0.09179955
	Mean	-	-	-	-	-	**0.0340±0.0006**	-	-	-	-	**143±2**	**6.4±0.1**	**0.090±0.002**
SD 19	**Cocktail**	-	-	-	-	-	0.030	-	-	-	-	57	7.1	0.06534
		-	-	-	-	-	0.029	-	-	-	-	56	7.1	0.06468
		-	-	-	-	-	0.030	-	-	-	-	58	7.3	0.0666336
		-	-	-	-	-	0.031	-	-	-	-	59	7.3	0.06731967

		Mean	-	-	-	-	-	**0.030±0.001**	-	-	-	-	**57±1**	**7.2±0.1**	**0.066±0.001**
D	SD 20	**Apple Drink**	0.420	-	-	-	0.0100	0.0577	-	-	-	-	194.7	1.821	0.22288
			0.421	-	-	-	0.0100	0.0579	-	-	-	-	195.3	1.826	0.223552
			0.424	-	-	-	0.0101	0.0583	-	-	-	-	196.7	1.839	0.22512
			0.423	-	-	-	0.0100	0.0582	-	-	-	-	196.3	1.835	0.224672
		Mean	**0.422±0.002**	-	-	-	**0.0100±0.0001**	**0.0580±0.0003**	-	-	-	-	**195.7±0.9**	**1.830±0.008**	**0.224±0.001**
	SD 21	**Passion Fruit Flavored**	0.267	-	-	-	-	-	-	-	-	-	119.4	5.91	0.46566
			0.267	-	-	-	-	-	-	-	-	-	119.8	5.93	0.467064
			0.269	-	-	-	-	-	-	-	-	-	120.6	5.97	0.47034
			0.269	-	-	-	-	-	-	-	-	-	120.4	5.96	0.469404
		Mean	**0.268±0.001**	-	-	-	-	-	-	-	-	-	**120.0±0.5**	**5.94±0.03**	**0.468±0.002**
	SD 22	**Orange Drink**	-	-	-	-	0.0159	0.0478	-	-	-	-	29.9	6.00	0.37412
			-	-	-	-	0.0160	0.0479	-	-	-	-	29.9	6.02	0.375248
			-	-	-	-	0.0161	0.0482	-	-	-	-	30.2	6.06	0.37788
			-	-	-	-	0.0160	0.0481	-	-	-	-	30.1	6.05	0.377128
		Mean	-	-	-	-	**0.0160±0.0001**	**0.0480±0.0002**	-	-	-	-	**30.0±0.1**	**6.03±0.03**	**0.376±0.002**
	SD 23	**Cola Drink**	0.265	0.01393	-	-	-	-	-	-	-	-	100.5	53.2	0.25671
			0.265	0.013972	-	-	-	-	-	-	-	-	100.8	53.4	0.257484
			0.267	0.01407	-	-	-	-	-	-	-	-	101.5	53.7	0.25929
			0.267	0.014042	-	-	-	-	-	-	-	-	101.3	53.6	0.258774
		Mean	**0.266±0.001**	**0.0140±0.0001**	-	-	-	-	-	-	-	-	**101.0±0.5**	**53.5±0.3**	**0.258±0.001**
	SD 24	**Lemon-Lime Drink**	0.408	0.01592	-	-	-	-	-	-	-	-	89.4	6.89	0.24278
			0.409	0.015968	-	-	-	-	-	-	-	-	89.6	6.91	0.243512
			0.412	0.01608	-	-	-	-	-	-	-	-	90.2	6.95	0.24522
			0.411	0.016048	-	-	-	-	-	-	-	-	90.1	6.94	0.244732

		Mean	**0.410±0.002**	**0.0160±0.0001**	-	-	-	-	-	-	-	-	**89.8±0.4**	**6.92±0.03**	**0.244±0.001**
		Energy Drink	0.370	0.01194	-	-	-	-	-	-	-	-	78.4	4.42	0.20099
			0.371	0.011976	-	-	-	-	-	-	-	-	78.6	4.43	0.201596
	SD 25		0.374	0.01206	-	-	-	-	-	-	-	-	79.2	4.46	0.20301
			0.373	0.012036	-	-	-	-	-	-	-	-	79.0	4.45	0.202606
		Mean	**0.372±0.002**	**0.0120±0.0001**	-	-	-	-	-	-	-	-	**78.8±0.4**	**4.44±0.02**	**0.202±0.001**
		Lemon Drink	0.398	0.01393	-	-	-	0.0537	-	-	-	-	100.9	7.79	0.14726
			0.399	0.013972	-	-	-	0.0539	-	-	-	-	101.2	7.81	0.147704
	SD 26		0.402	0.01407	-	-	-	0.0543	-	-	-	-	101.9	7.87	0.14874
			0.401	0.014042	-	-	-	0.0542	-	-	-	-	101.7	7.85	0.148444
		Mean	**0.400±0.002**	**0.0140±0.0001**	-	-	-	**0.0540±0.0002**	-	-	-	-	**101.4±0.5**	**7.83±0.04**	**0.148±0.001**
		Cola Drink	0.291	0.01194	-	-	-	-	-	-	-	-	93.3	16.12	0.20298
			0.291	0.011976	-	-	-	-	-	-	-	-	93.6	16.17	0.203592
	SD 27		0.293	0.01206	-	-	-	-	-	-	-	-	94.3	16.28	0.20502
			0.293	0.012036	-	-	-	-	-	-	-	-	94.1	16.25	0.204612
E		**Mean**	**0.292±0.001**	**0.0120±0.0001**	-	-	-	-	-	-	-	-	**93.8±0.4**	**16.20±0.07**	**0.204±0.001**
		Orange Drink	0.318	0.02189	-	-	-	0.00796	-	-	-	-	83.6	6.49	0.20895
			0.319	0.02196	-	-	-	0.00798	-	-	-	-	83.8	6.51	0.20958
	SD 28		0.322	0.02211	-	-	-	0.00804	-	-	-	-	84.4	6.55	0.21105
			0.321	0.02207	-	-	-	0.00802	-	-	-	-	84.3	6.54	0.21063
		Mean	**0.320±0.002**	**0.0220±0.0001**	-	-	-	**0.00800±0.0004**	-	-	-	-	**84.0±0.4**	**6.52±0.03**	**0.210±0.001**
			0.492	0.01194	-	-	-	0.0259	-	-	-	-	37.4	1.72	0.13134
			0.493	0.011976	-	-	-	0.0259	-	-	-	-	37.5	1.73	0.131736
	SD 29	**Cocktail**	0.496	0.01206	-	-	-	0.0261	-	-	-	-	37.8	1.74	0.13266
			0.495	0.012036	-	-	-	0.0261	-	-	-	-	37.7	1.74	0.132396

		Mean	**0.494±0.002**	**0.0120±0.0001**	-	-	-	**0.0260±0.0001**	-	-	-	-	**37.6±0.2**	**1.73±0.01**	**0.132±0.001**
	SD 30	**Orange Cocktail**	-	0.02786	-	-	-	-	-	-	-	-	33.3	6.09	0.1592
			-	0.027944	-	-	-	-	-	-	-	-	33.4	6.11	0.15968
			-	0.02814	-	-	-	-	-	-	-	-	33.7	6.15	0.1608
			-	0.028084	-	-	-	-	-	-	-	-	33.6	6.14	0.16048
		Mean	-	**0.0280±0.0001**	-	-	-	-	-	-	-	-	**33.5±0.2**	**6.120.03**	**0.160±0.001**
	SD 31	**Apple Cocktail**	0.438	0.02189	-	-	-	0.0617	-	-	-	-	79.0	16.24	0.60496
			0.439	0.021956	-	-	-	0.0619	-	-	-	-	79.2	16.29	0.606784
			0.442	0.02211	-	-	-	0.0623	-	-	-	-	79.8	16.40	0.61104
			0.441	0.022066	-	-	-	0.0622	-	-	-	-	79.6	16.37	0.609824
		Mean	**0.440±0.002**	**0.0220±0.0001**	-	-	-	**0.0620±0.0003**	-	-	-	-	**79.4±0.4**	**16.32±0.07**	**0.608±0.003**
	SD 32	**Orange Drink**	0.0378	0.02587	-	-	-	0.0458	-	-	-	-	119.7	6.71	0.27462
			0.0379	0.025948	-	-	-	0.0459	-	-	-	-	120.1	6.73	0.275448
			0.0382	0.02613	-	-	-	0.0462	-	-	-	-	120.9	6.77	0.27738
			0.0381	0.026078	-	-	-	0.0461	-	-	-	-	120.7	6.76	0.276828
F		**Mean**	**0.0380±0.0002**	**0.0260±0.0001**	-	-	-	**0.0460±0.0002**	-	-	-	-	**120.3±0.6**	**6.74±0.03**	**0.276±0.001**
	SD 33	**Cola Drink**	0.0716	-	-	-	-	0.0119	-	-	-	-	85.9	17.29	0.45969
			0.0719	-	-	-	-	0.0120	-	-	-	-	86.1	17.35	0.461076
			0.0724	-	-	-	-	0.0121	-	-	-	-	86.7	17.47	0.46431
			0.0722	-	-	-	-	0.0120	-	-	-	-	86.6	17.43	0.463386
		Mean	**0.0720±0.0003**	-	-	-	-	**0.0120±0.0001**	-	-	-	-	**86.3±0.4**	**17.38±0.08**	**0.462±0.002**
	SD 34	**Apple Drink**	0.0308	-	-	-	-	-	-	-	-	-	40.8	6.92	0.60894
			0.0309	-	-	-	-	-	-	-	-	-	40.9	6.94	0.610776
			0.0312	-	-	-	-	-	-	-	-	-	41.2	6.98	0.61506
			0.0311	-	-	-	-	-	-	-	-	-	41.1	6.97	0.613836

		Mean	0.0310±0.0001	-	-	-	-	-	-	-	-	-	41.0±0.2	6.95±0.03	0.612±0.003
G	SD 35	**Herbs and Multi frink extract drink**	0.406	-	-	-	-	0.0119	-	-	-	-	94.1	2.37	0.60297
			0.407	-	-	-	-	0.0120	-	-	-	-	94.4	2.38	0.604788
			0.410	-	-	-	-	0.0121	-	-	-	-	95.1	2.39	0.60903
			0.409	-	-	-	-	0.0120	-	-	-	-	94.9	2.39	0.607818
		Mean	**0.408±0.002**	-	-	-	-	**0.0120±0.0001**	-	-	-	-	**94.6±0.4**	**2.38±0.01**	**0.606±0.003**
	SD 36	**Malt Extract**	0.402	-	-	-	-	0.0239	-	-	-	-	82.5	17.49	0.52735
			0.403	-	-	-	-	0.0240	-	-	-	-	82.7	17.54	0.52894
			0.406	-	-	-	-	0.0241	-	-	-	-	83.3	17.67	0.53265
			0.405	-	-	-	-	0.0241	-	-	-	-	83.1	17.63	0.53159
		Mean	**0.404±0.002**	-	-	-	-	**0.0240±0.0001**	-	-	-	-	**82.9±0.4**	**17.58±0.08**	**0.530±0.002**
H	SD 37	**Apple Drink**	0.318	-	-	-	-	-	-	-	-	-	58.4	17.46	0.06766
			0.319	-	-	-	-	-	-	-	-	-	58.6	17.51	0.067864
			0.322	-	-	-	-	-	-	-	-	-	59.0	17.64	0.06834
			0.321	-	-	-	-	-	-	-	-	-	58.9	17.60	0.068204
		Mean	**0.320±0.001**	-	-	-	-	-	-	-	-	-	**58.7±0.3**	**17.55±0.08**	**0.0680±0.0003**
	SD 38	**Strawberry Drink**	0.054	-	-	-	-	0.0617	-	-	-	-	111.7	11.61	0.30646
			0.054	-	-	-	-	0.0619	-	-	-	-	112.1	11.65	0.307384
			0.054	-	-	-	-	0.0623	-	-	-	-	112.9	11.73	0.30954
			0.054	-	-	-	-	0.0622	-	-	-	-	112.6	11.71	0.308924
		Mean	**0.0540±0.0002**	-	-	-	-	**0.0620±0.0003**	-	-	-	-	**112.3±0.5**	**11.67±0.05**	**0.308±0.001**
	SD 39	**Grape Drink**	0.418	-	-	-	-	-	-	-	-	-	197.3	10.38	0.0597
			0.419	-	-	-	-	-	-	-	-	-	197.9	10.41	0.05988
			0.422	-	-	-	-	-	-	-	-	-	198.5	10.48	0.0603
			0.421	-	-	-	-	-	-	-	-	-	199.1	10.46	0.06018

	Mean	**0.420±0.002**	-	-	-	-	-	-	-	-	-	**198.2±0.8**	**10.43±0.05**	**0.0600±0.0003**
SD 40	**Orange Drink**	0.416	-	-	-	-	0.040	-	-	-	-	197.3	52.05	0.49302
		0.415	-	-	-	-	0.039	-	-	-	-	197.9	51.90	0.491526
		0.424	-	-	-	-	0.040	-	-	-	-	198.5	53.11	0.50298
		0.425	-	-	-	-	0.041	-	-	-	-	199.1	53.26	0.504474
	Mean	**0.420±0.006**	-	-	-	-	**0.040±0.001**	-	-	-	-	**195.7±0.10**	**52.58±0.05**	**0.4980±0.0003**
SD 41	**Cola Drink**	0.384	-	-	-	-	-	-	-	-	-	121.0	1.82	0.34254
		0.383	-	-	-	-	-	-	-	-	-	121.3	1.82	0.341502
		0.392	-	-	-	-	-	-	-	-	-	121.7	1.86	0.34946
		0.393	-	-	-	-	-	-	-	-	-	122.1	1.86	0.350498
	Mean	**0.388±0.005**	-	-	-	-	-	-	-	-	-	**120.0±0.6**	**1.84±0.03**	**0.346±0.005**
SD 42	**Apple Drink**	0.424	-	-	-	-	0.040	-	-	-	-	140	7.3	0.26334
		0.422	-	-	-	-	0.039	-	-	-	-	139	7.3	0.262542
		0.432	-	-	-	-	0.040	-	-	-	-	143	7.4	0.26866
		0.434	-	-	-	-	0.041	-	-	-	-	143	7.5	0.269458
	Mean	**0.428±0.006**	-	-	-	-	**0.040±0.001**	-	-	-	-	**141±2**	**7.4±0.1**	**0.266±0.004**