EVALUATION OF SOME CHEMICAL CONSTITUENTS OF SELECTED ENERGY DRINKS

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A THESIS SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ANALYTICAL CHEMISTRY

DEPARTMENT OF CHEMISTRY,

FACULTY OF SCIENCE

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NIGERIA

AUGUST, 2014

Declaration

I hereby declared that the work in this thesis entitled "**Evaluation of some chemical constituents of selected energy drinks**" has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____ _____

Abbas Nurudeen Sulaiman Date

Certification

This thesis entitled **EVALUATION OF SOME CHEMICAL CONSTITUENTS OF SELECTED ENERGY DRINKS** by Nurudeen Sulaiman ABBAS meets the regulations governing the award of the degree of Master of Science in Analytical Chemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This thesis is dedicated to Almighty Allah and to my family who has supported me throughout the course of this work.

Acknowledgement

All praises and thanks are due to Allah “the Exalted” Who in His Infinite mercies, granted me protection and ability to do this work.

I would like to express my deepest gratitude to my supervisors Prof. C.E Gimba and Dr. S.E Abechi, whose constructive criticisms and encouragement made it possible for the successful completion of this project. Their patience and invaluable contribution guided me throughout the research period for which I am extremely grateful.

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Abstract

This research work examined and compares the physicochemical properties and some chemical constituents of selected energy drinks. Fourteen (14) brands of energy drinks samples consisting eleven (11) liquid and three (3) powdered forms were randomly purchased. All samples were analyzed for their physicochemical properties (pH, turbidity, conductivity and total dissolved solids), trace and heavy metals, aspartame, sugar and caffeine contents. Results showed that the physicochemical properties (i.e. pH, turbidity, conductivity and total dissolved solids) ranged from 4.47 ± 0.012 - 5.96 ± 0.012, 8 ± 0.577 – 592 ± 1.155 NTU, 2.21 ± 0.006 – 1975 ± 1.732 µs/cm, and 243 ± 0.577 – 1064 ± 0.577 mg/L respectively. Energy drinks analyzed all fell within the FDA recommended range for the physicochemical properties analyzed. Iron, calcium, zinc and potassium were found in all the energy drinks and their concentration ranged from 1.961 ± 0.0003 - 0.294 ± 0.0005 mg/L, 2.763 ± 0.0009 - 19.310 ± 0.0015 mg/L, 0.045 ± 0.0001 - 13.887 ± 0.0037 mg/L, and 2.0 to 2500 mg/L respectively. The copper, lead and manganese concentration of energy drinks ranged from 0.002 ± 0.0002 - 0.102 ± 0.0003 mg/L, 0.028 ± 0.0006 - 0.209 ± 0.0009 mg/L and 0.003 ± 0.0001 - 0.024 ± 0.0002 mg/L respectively. The concentration of copper and manganese were below the MCL of 1.0 mg/L and 0.05 mg/L respectively while lead had a concentration above the MCL of 0.01 mg/L. Cadmium was not detected in all energy drinks except for sample EJ which had a concentration of 0.102 ± 0.0003 mg/L and exceeded the MCL of 0.005 mg/L. The caffeine, aspartame and sugar concentrations ranged from 1.11 mg/L – 2487.13 mg/L, 6.51 mg/L – 1491.19 mg/L, and 16.98 – 1686.73 mg/L respectively. Caffeine and aspartame concentrations in all the energy drink samples were below the FDA set standard of 400 mg/L and 3000 mg/L respectively except for sample AL which had a concentration above the set standard for

caffeine. Though the analyzed parameters were mostly below the set standards, especially caffeine, aspartame and sugar, it is important that the pattern of consumption of these drinks must be monitored to minimize ingestion of excess doses of harmful substances to prevent the reported adverse effects.

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List of Abbreviations

AAS	Atomic Absorption Spectroscopy
ATP	Adenosine Triphosphate
CYP1A2	Cytochrome P450 1A2
CYP2A6	Cytochrome P450 2A6
DKP	5-benzyl-3, 6-dioxo-2-piperazine acetic acid
DNA	Deoxyribonucleic Acid
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drinks Administration
GC–MS	Gas Chromatography–Mass Spectrometry
HDL	High Density Lipoprotein Cholesterol
HPLC	High Performance Liquid Chromatographic
NAT2	*N*-acetyl transferase 2
NAG	N-acetyl-beta-glucosamynidase
NNS	National Nutrition Survey
RDA	Recommended Daily Allowance
RNA	Ribonucleic Acid
SCF	Scientific Committee for Food
SSB	Sugar Sweetened Beverages
USA	United States of America
UV	Ultraviolet Visible Spectroscopy
WHO	World Health Organization
XO	

CHAPTER ONE

1.0 INTRODUCTION

Energy drinks refer to beverages that contain large doses of caffeine and other legal stimulants such as taurine, carbohydrates, glucuronolactone, inositol, niacin, panthenol, and β-complex vitamins which are considered as source of energy (Attila and Çakir, 2009). The consumption of readily available energy drinks has increased significantly with young adults forming the largest part of the consumers. History of energy drink dates back to 1987 when Red Bull was introduced in Austria. It became more popular in the 1990s following its introduction to the United States. Since then the sale of this drink has increased exponentially. In 2006, the energy drink market grew by 80% (Foran *et al.,* 2011). This is because manufactures claim the drinks can boost energy levels as well as physical endurance, improve concentration and reaction speed (Van den Eynde *et al.,* 2008).

In recent years, a number of different energy drinks have been introduced in the Nigerian market to provide an energy boost or as dietary supplements. These drinks are marketed specifically to children and young adults. These products have been used for various reasons. A survey conducted among college students shows that 67% of students admitted using energy drinks to cope with insufficient sleep, 65% mentioned increasing energy and 54% use it for fun at parties; 50% for studying or completing a major course project, 45% used it while driving a car for a long period of time and 17% for treating hangover (Malinauskas *et al.,* 2007). These products have also been used to reduce the depressor effect of alcohol or even to gain social status (Ferreira *et al.,* 2004; Kaminer, 2010).

Many energy drinks are promoted as being nutraceutical foods, boosting health, energy, or otherwise having sought-after benefits. There is some concern among health professionals that these beverages, and the drinking behaviours of the targeted consumers, may in fact have adverse health consequences. The most commonly reported adverse effects include insomnia, nervousness, headache, and tachycardia (Clauson *et al.,* 2008). In a recent study, heavy consumption of energy drinks was attributed to new onset seizures in four patients (Iyadurai and Chung, 2007) and hospitalization of individuals with pre-existing mental illness (Chelben *et al.,* 2008).

1.1 Energy Drinks

Energy drinks first appeared in Europe and Asia in the 1960s in response to consumer demand for a dietary supplement that would result in increased energy (Reissig *et al.,* 2008). In 1962, a Japanese company, Taisho Pharmaceuticals, launched Lipovitan D, one of the very first energy drinks, which is still dominating the Japanese market. Since the 1960s, the energy drink market has grown into a multibillion dollar business which has been reported as being the fastest growing segment in the beverage industry. Energy drinks have established a viable position in the beverage market as evidenced by their commonplace consumption in the morning, afternoon, and night, not only by the general consumer, but those of age 18 to 34 in particular (Lal, 2007).

The popularity of energy drinks and the growth in their consumption among adolescents and young adults have brought worries regarding general health and well being of these consumers. Adolescents and young adults are often uninformed about the content of energy drink (Rath, 2012).

1.2 Contents of Energy Drinks

There are hundreds of energy drinks available in the market, many share very similar ingredient profiles. Most of these energy drinks consist mainly of caffeine, Taurine, Guarana, Ginseng, B vitamins, Ginko Biloba, L-carntine, sugars, Antioxidants, Glucuronolactone, Yerba Mate, Creatine, Acai Berry, Milk Thistle, L-theanine, Inositol and artificial sweetners (Babu *et al.*, 2008).

1.2.1 Caffeine

Caffeine is probably the most frequently ingested pharmacologically active substance in the world. It is one of the main ingredients of stimulant drinks and it is also present in tea, coffee and other beverages and foods. Caffeine is extracted from the raw fruit of over sixty species of coffee plants (coffea Arabica), all part of the methylxanthine family. The dimethylxanthine derivatives, theophylline and theobromine, are also found in a variety of plants. It is also extracted from tea, kola nuts, and cocoa. The average total intake of caffeine in the Republic of Ireland and the UK is estimated to be 214 and 278 mg per person per day, respectively (FSPB, 2010). Data from the consumption survey, based on weekly intake, indicates that among stimulant drink consumers, the average daily caffeine intake from stimulant drinks alone would be approximately 35 mg, rising to about 90 mg among the highest consumers (FSPB, 2010). This does not appear excessive. However, when the consumption of stimulant drinks in a single session was investigated, the average caffeine consumed was approximately 240 mg (3 cans), rising to about 640 mg (8 cans) among the highest consumers (FSPB, 2010). Such large intake levels among the highest consumers are a cause of concern, particularly in relation to the known potential acute health effects of caffeine such as tachycardia, increases in blood pressure and dehydration,

as well as behavioural and cognitive effects. The health effects of chronic or habitual caffeine consumption remain uncertain.

1.2.2 Taurine

Taurine (2-aminoethyl sulfonic acid) is a sulfur containing amino acid that is the most abundant amino acid found naturally in our bodies, primarily in the retina and skeletal and cardiac muscle tissue (Timbrell *et al.,* 1995; Imagawa *et al.,* 2009). Taurine is derived from the metabolism of methionine and cysteine (Huxtable, 1992; Stipanuk, 2004). It is also present in common food items such as meat and fish. The data on stimulant drink intake among stimulant drink consumers indicate that average daily taurine intake from stimulant drinks was approximately 0.4 g, increasing to about 1 g among the highest consumers (FSPB, 2010). The most taurine consumed from stimulant drinks in a single session was averaged at approximately 3 g, rising to about 8 g by the highest consumers (FSPB, 2010). Stimulant drink intake at the maximum level of intake provides taurine far in excess of that from other foods or beverages in the diet. Data available indicate no evidence of adverse effects of taurine at such intakes and in a recent report the EU Scientific Committee for Food (SCF) was unable to conclude that the '*safety-in-use*' of taurine in the concentration range reported for stimulant drinks has been adequately established (EU SCF, 1983).

1.2.3 Guarana

Guarana (*Paullinia cupana*) is a native South American plant containing guaranine, a substance chemically similar to caffeine with comparable stimulant effects. Guarana is often added to stimulant drinks, either in combination with caffeine or on its own. The stimulant effect of guarana is related to its caffeine content; one gram of guarana contains

as much caffeine (40 mg) as a medium strength cup of coffee. The precise source and nature of the stimulant activity of guarana is not well understood. However, it has been reported that guarana exerts a more prolonged effect than an equivalent amount of caffeine. The Food and Drinks Administration (FDA) in the USA currently prohibits the use of guarana in food and drinks while awaiting further clarification on its safety (USFDA, 2011).

1.2.4 Glucuronolactone

This is a naturally occurring substance produced in small amounts within the body. Supplementation with d-glucarates, including glucuronolactone, may favor the body's natural defense mechanism for eliminating carcinogens and tumor promoters and their effects (Zołtaszek *et al.,* 2008). The data from the consumption survey indicate that average daily glucuronolactone intake from stimulant drinks was approximately 0.25 g, rising to about 0.7 g among the highest consumers (FSPB, 2010). The most glucuronolactone consumed from stimulant drinks in a single session was averaged at approximately 1.8 g, rising to about 4.8 g among the highest consumers (FSPB, 2010). These maximum levels of intake provide more glucuronolactone than would otherwise be achieved through other foods or beverages in the diet. There is very little information available for risk assessment of glucuronolactone at such intakes. There is no indication from the available data that there is any risk to health from consumption of high amounts of glucuronolactone, although these data are limited.

1.2.5 Ginseng

Ginseng is a herb that has been used for over 2000 years by people in East Asian countries including China, Japan, and Korea as a remedy for various diseases and for promoting longevity (Lee *et al.*, 2005; Nam *et al.*, 2005). *Panax ginseng* is the primary commercial species and is often referred to as Korean or Asian ginseng. Siberian ginseng (*Eleutherococcus senticosus*) is not truly a ginseng since it contains eleutherosides as its active constituent and no ginsenosides. *P. ginseng* is a small, shade loving perennial shrub that reaches about 60cm in height and belongs to the plant family Araliacae. The entire ginseng plant has been used for medicinal purposes; however, the root is the most prominent and dominates the commercial sales. Ginseng has been incorporated into a variety of energy drinks although little medical literature supports these uses. Adverse effects associated with ginseng use tend to be mild. However more serious complications have been reported, including diarrhoea, vaginal bleeding, severe headache, and Stevens-Johnson syndrome (Enerst, 2002; Dega *et al.*, 1996). Many of these effects may be attributed to contaminants. Agranulocytosis in 4 patients taking ginseng had been linked to unreported phenylbutazone and aminopyrine contained in the preparation (Ries *et al.*, 1975). A ginseng abuse syndrome, characterized by morning diarrhoea, hypertension, rashes, insomnia, and irritability had been reported (Siegel, 1979). Little is known regarding the effects of ginseng in children and adolescents (Braganza and Larkin, 2007).

1.2.6 Vitamins

B vitamins are a group of 8 individual water-soluble vitamins, usually referred to as the B complex when grouped together, and all play essential roles in cellular processes. B vitamins are incorporated into many of the mainstream energy drinks. A typical can of 250

ml may contain 360% of the recommended daily allowance (RDA) of B6, 120% of B12, and 120% of B3 (niacin). The container size varies among brands and it may hold multiple servings. The addition of excess amounts of B vitamins is also observed in the more extreme energy drinks like 5-Hour Energy which contains 8333% of the RDA for vitamin B12 and 2000% of the RDA for B6. It is claimed that the consumption of these large amounts of B vitamins increases mental alertness and focus, as well as improves mood. The average person, however, consumes the RDA of B vitamins from a typical diet since B vitamins are found in a variety of foods including bananas, lentils, potatoes, tuna, and turkey. Vitamins B2 (riboflavin), B3 (niacin), B6 (pyridoxine, pyridoxal, pyridoxamine), and B12 are the most common of the B vitamins that are incorporated into energy drink formulations (Wardlaw and Smith, 2009).

Vitamin B2 is a coenzyme in the metabolism of carbohydrates. Vitamin B3 plays a major role as a coenzyme in energy metabolism, fat synthesis, and fat breakdown (Wardlaw and Smith, 2009). Vitamin B6 is a group of 3 structurally similar compounds that all can be converted into the vitamin B6 coenzyme which aids in the utilization of carbohydrates, fats, and proteins (Wardlaw and Smith, 2009). Vitamin B12 assists in folate metabolism and in nerve function (Wardlaw and Smith, 2009). Since all of the B vitamins are water soluble, once the RDA has been met, the excess vitamins are excreted from the body via urine. Although the consumption of a large amount of B vitamins does not possess any adverse health effects, the logic behind the extreme amounts of B vitamins in these beverages is not well rationalized (Wardlaw and Smith, 2009).

1.2.7 Sugar

Sugar is one of the most common as well as most dangerous additives on the market today. It's sweet taste and short-term positive effects lead health experts to consider the possibility of sugar addiction. Natural sugars, such as those found in fruits and dairy products, provide an easily digestible form of good-for-you energy. The synthetic copycats like refined sugar, sucrose, fructose and glucose do far more harm than good (Nash, 1992).

More than 32 g of extra sugar a day can cause a myriad of health problems (Bauer, 2011) such as raised cholesterol levels, suppressed immune system, hyperactivity, anxiety, difficulty concentrating, crankiness, decreased emotional stability, a raised level of neurotransmitters, hypoglycemia, increased blood pressure, interference with protein absorption, and impaired DNA structure (Appleton, 2011). Just one serving of Red Bull contains 27 g of sugar (Red Bull Energy drink USA), and the average cup of coffee contains 32 g (Starbecks coffee company) (Nash, 1992).

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1.3 Justification

Energy drinks have established an enviable position in the beverage market as evidenced by their commonplace consumption. There are a number of scientific reports on the adverse consequences of excessive consumption of these drinks. Many of these products do not provide the complete chemical composition, and the caffeine content and other ingredients present are unknown to the consumer. Hence there is need to quantify the major content of these energy drinks and compare with those of accepted standards. Also, energy drinks occur mostly in liquid and powdered forms. There has been little or no research on the powdered products as more attention has been given to those in liquids.

These powdered products are usually dissolved in water by consumers before intake. It is therefore imperative to determine the caffeine, aspartame and other energizers of the powdered products and compared them with those of the liquid products. It will also be necessary to determine some other physicochemical properties of the energy drinks.

1.4 Aim and Objectives

The aim of this work is to carry out comparative study on the physicochemical properties and some chemical constituents of selected energy drinks. This will be achieved through:

a. determination of the caffeine and aspartame concentrations of the energy drinks;
b. determination of the carbohydrate (sugar) contents;
c. determine the physicochemical properties of energy drinks;
d. determination of the level of heavy metals (Cu, Zn, Pb, Cd, Mn, Fe) in them;
e. determination of micronutrients (K, Ca,) present in them;
f. using Statistical Analysis to analyse and compare between the powdered and liquid forms of energy drinks;
g. comparing the obtained results with set standards by regulatory bodies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Review Work on Caffeine

The term “energy drinks” refers to beverages that contain caffeine in combination with other ingredients such as taurine, guarana, and B vitamins, and that claims to provide its consumers with extra energy. There is limited evidence that consumption of energy drinks can significantly improve physical and mental performance (Scholey and Kennedy, 2004), driving ability when tired (Reyner and Horne, 2002), and decrease mental fatigue during long periods of concentration (Kennedy and Scholey, 2004). Unfortunately, the body of literature is limited and it is not known whether these improvements are due to the caffeine, other herbal ingredients, or as a result of the combination of the ingredients found in a beverage (Scholey and Kennedy, 2004).

The caffeine content of a single serving of energy drink (8 to 12 fl oz) can range from 72 to 150 mg; however, many bottles contain 2-3 servings, raising the caffeine content to as high as 294 mg per bottle. In comparison, the caffeine content, per serving (8 fl oz.), of brewed coffee, tea, and cola beverages ranges between 134-240 mg, 48-175 mg, and 22-46 mg respectively (Nawrot *et al.,* 2003). A recent literature review determined that consumption of up to 400 mg caffeine daily by healthy adults is not associated with adverse effects (Nawrot *et al.,* 2003). However, groups that are at risk, such as women of reproductive age and children, should limit their daily consumption of caffeine to a maximum of 300 mg for the former and 2.5 mg/kg body weight for the latter; (Nawrot *et al.,* 2003) thus they may need to avoid consuming energy beverages with a higher caffeine content. A recent survey

of 78 youth (11-18 years) found that 42.3 percent of participants consumed energy drinks (O'Dea, 2003). However, the effects of ingredients found in energy drinks on children and an adolescent has raised concern. In adolescents, caffeine consumption has been associated with an increase in blood pressure (Savoca *et al.*, 2004). Based on the limited data regarding safety, it is not recommended that children or adolescents consume energy drinks.

2.2 Caffeine

Caffeine (1,3,7-trimethylxanthine), theophylline (3,7- dimethylxanthine), and theobromine (1,3-dimethylxanthine) are in the family of alkaloid. Caffeine is an odourless, white solid that has the form of needles or powder. It has a bitter taste and a molar mass of 194.19 g/mol. It is slightly soluble in water due to its moderate polarity. It is a natural central nervous system stimulant, having the effects of reducing drowsiness and recovering alertness. Since it is widely consumed by humans, caffeine is considered the most frequently used psychoactive substance in the world (Lovett, 2005).

The majority of energy drinks contain caffeine as an active ingredient due to its stimulatory effect on the central nervous system (Pennay *et al.*, 2011). The main mechanism of action of caffeine, in concentrations typically achieved after the consumption of a caffeinated beverage, is to act as an adenosine receptor blocker in the brain (Dunwiddie and Mansino, 2001; Pettenuzzo *et al.*, 2008). Caffeine has a similar chemical structure to that of adenosine allowing it to attach to the adenosine receptors. The blockage of adenosine to the neurons causes the sleep promoting effects of adenosine to stop, resulting in the neurons speeding up instead of slowing down (Ferre, 2008).

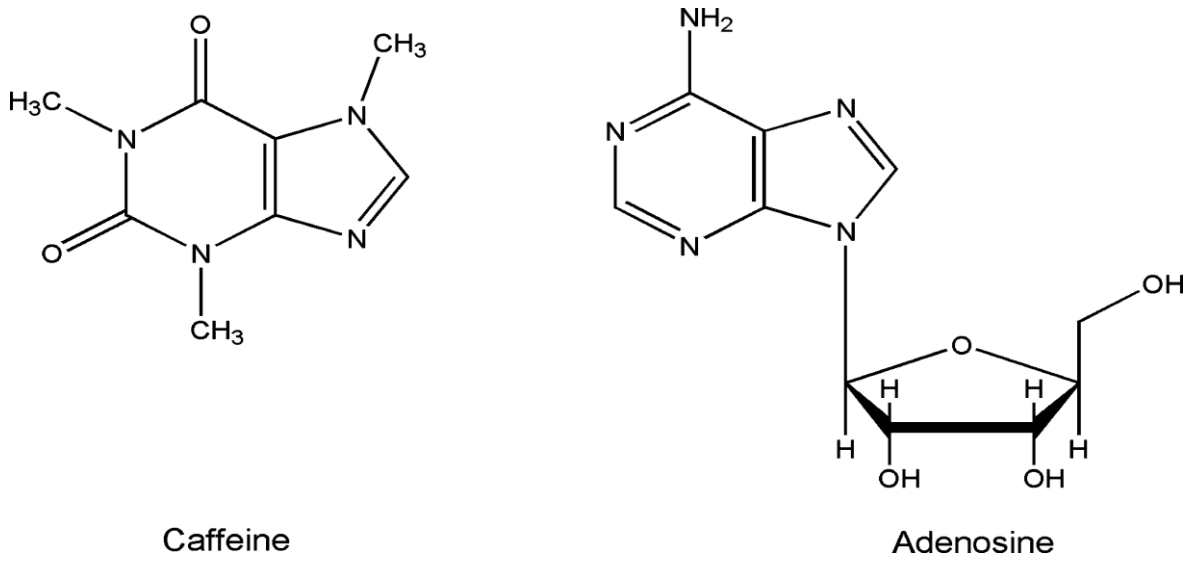


Figure 2.1: Chemical structure of caffeine and adenosine (Spiller, 1998)

2.2.1 Metabolism of caffeine

The main mechanism of caffeine at in take is to exert most of its biological effects through the antagonism of the A_1 and A_{2A} subtypes of the adenosine receptor (James, 2004). Adenosine (Figure 2.1) is an endogenous neuromodulator with mostly inhibitory effects, and adenosine antagonism by caffeine results in effects that are generally stimulatory. Some physiological effects associated with caffeine administration include central nervous system stimulation, acute elevation of blood pressure, increased metabolic rate, and dieresis (Carrillo and Benitez, 2000). Caffeine is rapidly and almost completely absorbed in the stomach and small intestine and distributed to all tissues, including the brain. Caffeine metabolism occurs primarily in the liver, where the activity of the cytochrome P450 isoform CYP1A2 accounts for almost 95% of the primary metabolism of caffeine. CYP1A2-catalyzed 3-demethylation of caffeine results in the formation of 1,7-dimethylxanthine (paraxanthine) (Figure 2.2). Paraxanthine may be demethylated by

CYP1A2 to form 1-methylxanthine, which may be oxidized to 1-methyluric acid by xanthine oxidase. Paraxanthine may also be hydroxylated by CYP2A6 to form 1,7-dimethyluric acid, or acetylated by *N*-acetyltransferase 2 (NAT2) to form 5-acetylamino-6-formylamino-3-methyluracil, an unstable compound that may be deformylated nonenzymatically to form 5-acetylamino-6-amino-3-methyluracil (figure 2.2) (Crews *et al.*, 2001; Krul and Hageman, 1998).

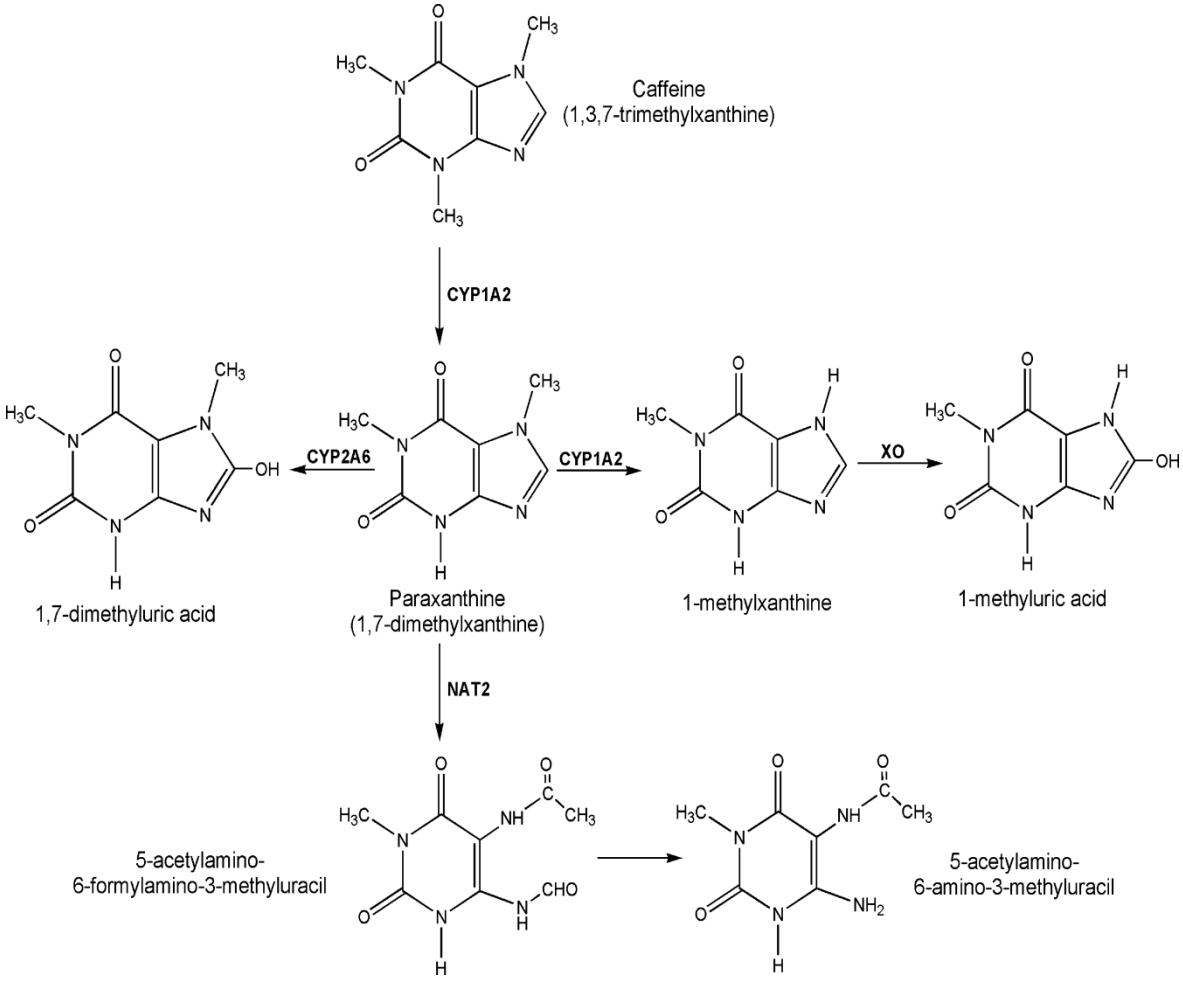


Figure 2.2: Major pathways in caffeine metabolism

2.2.2 Caffeine and health

Caffeine has been widely studied in a variety of areas regarding human health and performance and it is evident that caffeine consumption can increase energy utilization (Smith and Rogers, 2002). Many studies also confirm its ability to enhance mood and alertness (Kaplan *et al.,* 1997; Smith and Rogers, 2002; Lorist and Tops, 2003), exercise performance (Graham, 2001; Doherty and Smith, 2004), the speed at which information is processed, awareness, attention, and reaction time (Cysneiros *et al.,* 2007). Caffeine has also been shown to reduce some of the negative side effects associated with sleep loss. Some studies suggest that caffeine can contribute to improved alertness and performance at doses of 75 to 150 mg after acute sleep loss and doses of 200 to 600 mg after a night or more without sleep (Bonnet *et al.,* 2005).

Caffeine also has a stimulatory effect on thermo genesis (Acheson *et al.,* 1980; Dulloo *et al.,* 1989; Astrup *et al.,* 1990; Bracco *et al.,* 1995). In addition, caffeine consumption has also been linked to reduced food intake (Tremblay *et al.,* 1988; Lima *et al.,* 2005) and to promote lipolysis in both animals and humans (Hasegawa and Mori 2000; Zheng *et al.,* 2004; Lopez-Garcia *et al.,* 2006). Caffeine also has a diuretic effect regardless of its consumption as energy drink, tea, or coffee (Riesenhuber *et al.,* 2006). The cardiovascular effects of caffeine have also been studied and suggest that caffeine likely has an effect on hemodynamic parameters (Karatzis *et al.,* 2005; Hartley *et al.,* 2004; Hodgson *et al.,* 2005). A review regarding caffeine consumption concluded that among the healthy adult population, a moderate daily caffeine intake of ≤400 mg (equivalent to 6 mg/kg/d for a 65 kg person) was not associated with any adverse effects (Nawrot *et al.,* 2003). Nonetheless, caution should be exercised in regard to the amount of caffeine consumed per day.

2.2.3 Research works on caffeine

The determination of concentration of caffeine in foods drinks and pharmaceuticals using different methods of analysis have been reported widely in literature. A quantitative determination of caffeine and alcohol in energy drinks have been reported by Ayala *et al.*, (2009). Quantitative analysis of caffeine was performed by gas chromatography–mass spectrometry (GC–MS) using an Agilent 5975 MSD. Linear regression analysis of calibrators in the range 0–10 mg/L yielded an R^2 value of 1.000 and the control sample fortified with 1.0 mg/L caffeine produced a calculated concentration of 1.02 mg/L (102%). Quantitative caffeine determinations in diluted samples yielded concentrations ranging from 2.74 to 5.31 mg/L. These correspond with caffeine doses of 65–126 mg per 8-oz serving.

Rachel *et al.,* (2006) reported the determination of caffeine contents of energy drinks, carbonated sodas and other beverages by gas chromatography with nitrogen-phosphorus detection. Caffeine was isolated from the beverages by liquid-liquid extraction, and the final extracts were subjected to gas chromatographic analysis utilizing nitrogen-phosphorus detection. Quantization of caffeine was based on a calibration curve prepared in a concentration range 25 – 250 mg/L for the caffeinated samples and 10 – 100 mg/L for the decaffeinated samples, with the limit of quantization arbitrarily set at the concentration of the lowest standard. The caffeine concentration of the caffeinated energy drinks ranged from none detected (i.e., zero (0) to 141.1 mg/serving. The caffeine content of carbonated sodas ranged from none detected (i.e., zero (0) to 48.2 mg/serving while other beverages ranged from <2.7 to 105.7 mg/serving.

A Simple High Performance Liquid Chromatographic (HPLC) method validated for determination of the content levels caffeine in beverages was reported by Mei *et al.,* (2012). Shim-pack VP-ODS column was used with methanol: water (30:70) % (v/v) eluent. The detector wavelength was set at 270 nm. Linearity of the method was check from 10-100 ppm and the correlation coefficient was 0.9999. The method detection limit was 0.023 ppm and the precision was 1.25% at 40 ppm caffeine concentration. The spiked recoveries for caffeine were 99%, 105%, 99.2%, 102% and 102% in Pepsi Cola, Coca Cola, Red Bull, Gazaltain black tea and Coffee samples respectively. The caffeine contents in tea samples ranged from 440 ppm to 473 ppm with average concentration of 458.6 ppm. The caffeine concentrations in energy drinks samples ranged from 170.6 ppm to 324 ppm with average concentration of 255.6 ppm. The coffee sample contains 252.4 ppm. The carbonated soft drinks showed caffeine content in the range of 32.4 ppm to 133.3 ppm with average concentration of 96 ppm.

2.3 Aspartame

Aspartame is a white crystalline powder having no odour, but is intensely sweet. It is approximately 200 times sweeter than sucrose, the accepted standard for sweetness. It is a synthetic non nutritive sweetener (a methyl ester of a dipeptide) composed of aspartic acid and phenylalanine. It has a molecular formula of $C_{14}H_{18}N_2O_5$ and a molecular weight of 294.31 g/mol. The chemical name for aspartame is (*S*)-3-amino-*N*-[(*S*)-1-methoxycarbonyl-2-phenylethyl] succinamic acid. It has been reported that at pH 7 and room temperature, aspartame is soluble in water of approximately 10 g/L (Homler, 1984). Aspartame is soluble in ethanol of 0.37% and is insoluble in oil. Aspartame has two melting points, melting initially at 190°C, solidifying and re-melting at 246-247°C. A

possible explanation for this behaviour is that the intra-molecular reaction of the primary amine with the methylester takes place at the initial melting point to generate 5-benzyl-3, 6-dioxo-2-piperazine acetic acid (DKP) and methanol (Prankerd, 2002). There are two forms of aspartame, an alpha and a beta form. Only the alpha form is sweet. Although aspartame yields the same caloric intake as sugar on a weight to weight basis (i.e., 4 kcal/g), it can be added at almost 200 times lower levels and achieve the same sweetness, thereby providing a far lower net caloric intake. This attribute has resulted in the use of aspartame as a low calorie or non nutritive sweetener in foods and beverages worldwide.

2.3.1 Chemistry of aspartame

The intestinal absorption and metabolism of aspartame have been intensively studied, in rodents, pigs, primates and humans. Several excellent reviews have been published (Stegink, 1987; Butchko *et al.*, 2001). In all species examined, aspartame is metabolized in the gastrointestinal tract by esterases and peptidases into three components: the two constituent amino acids, aspartic acid and phenylalanine, and methanol. Aspartame may be completely hydrolyzed to these three components in the gastrointestinal lumen and absorbed into the general circulation, or may be hydrolyzed to methanol and aspartylphenylalanine dipeptide. In this case, the dipeptide is absorbed into the gastrointestinal mucosa cells and then cleaved into amino acids (Stegink, 1987).

Aspartame can also be absorbed into the mucosal cells prior to hydrolysis, and be cleaved within the cell, to its three components, which then enter circulation (Matthews, 1984). The available evidence indicates that aspartame does not enter the circulation prior to hydrolysis. Metabolism of the three cleaved components of aspartame has been shown to

be identical to the metabolism of the components given individually (Stegink, 1987). Consequently, studies in which aspartame is administered parenterally are not representative of oral administration, which is how aspartame is always consumed by humans. The metabolism of the sweetener provides approximately 4 kcal/g of energy (Gougeon *et al.*, 2004). The contribution of energy to the diet overall, however, is negligible as the high intensity sweetening power of aspartame (approximately 200 sweeter than sucrose by weight) means that little is needed to be added to foods to achieve sweetness.

2.3.2 Biochemical data

The use of aspartame by diabetic individuals is increasing. It is widely used in the weight loss regime, and approximately 200 million people consume aspartame worldwide (Shapiro, 1988). Aspartame has a biological effect even at the recommended daily dose (Gombos *et al.*, 2007). Approximately 50% of the aspartame molecule is phenylalanine, 40% is aspartic acid and 10% is methanol (Newsome, 1986). Aspartic acid is a metabolite of aspartame that is an excitatory amino acid and is normally found in high levels in the brain. These levels are controlled by the blood–brain barrier, which protects the brain from large fluctuations in plasma aspartate (Maher and Wurtman, 1987). Phenylalanine is an amino acid essential to the production of monoamines in the brain and is found in nearly all protein foods (Fernstrom *et al.*, 1983). On the other hand, due to the high levels of phenylalanine in blood, consumption of aspartame may cause brain damage (Haschemeyer and Haschemeyer, 1973). Certain brain amino acid levels have been shown to be increased after aspartame consumption (Dailey *et al.*, 1991; Diomede *et al.*, 1991; Yokogoshi *et al.*, 1984). Although methanol is released during aspartame digestion, it only accounts for 10%

of the aspartame molecule (Stegink *et al.*, 1983), and among the metabolites, methanol is a toxicant that causes systemic toxicity (Kruse, 1992). Various neurochemical effects due to aspartame consumption have been reported (Coulombe and Sharma, 1986; Pan-Hou *et al.*, 1990; Goerss *et al.*, 2000). Their adverse neurological effects include headache (Johns, 1988), insomnia and seizures after ingestion of aspartame, which are also accompanied by the alterations in regional concentrations of catecholamine (Coulombe and Sharma, 1986). Previous finding have shown that aspartate may lead to neurotoxicity through sustained contact with the receptors, such as glutamate producing an excitotoxic effect (Olney *et al.*, 1980). Large doses of both aspartame as well as these individual metabolites have been tested in humans and other animals, with controversial reports. It has been reported that not only the metabolites of methanol, but methanol per se as well, is toxic to the brain (Jeganathan and Namasivayam, 1998).

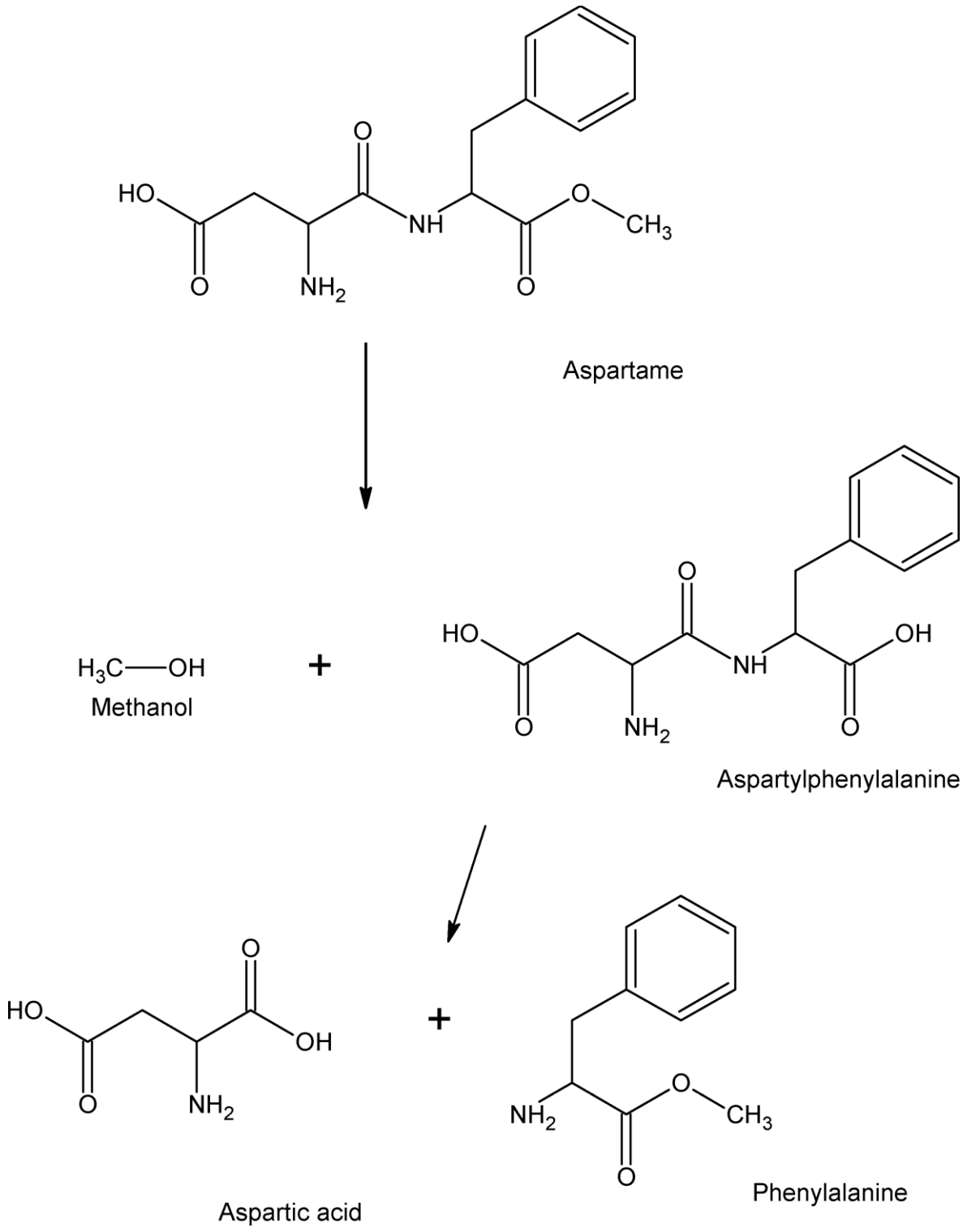


Figure 2.3: Metabolism of aspartame (Oppermann *et al.*, 1973; Ranney and Oppermann, 1979)

2.4 Heavy Metals

Heavy metals are potential environmental contaminants with the capability of causing human health problems if present to excess in the food we eat. Environmental pollution is the main cause of heavy metal contamination in the food chain. Emissions of heavy metals to the environment occur *via* a wide range of processes and pathways, including to the air (*e.g.* during combustion, extraction and processing), to surface waters (*via* runoff and releases from storage and transport) and to the soil (and hence into ground waters and crops). Atmospheric emissions tend to be of greatest concern in terms of human health, both because of the quantities involved and the widespread dispersion and potential for exposure that often ensues. People may be exposed to potentially harmful chemical, physical and biological agents in air, food, water or soil. However, exposure does not result only from the presence of a harmful agent in the environment but also by exposure to contact. There must be contact between the agent and the outer boundary of the human body, such as the airways, the skin or the mouth. As a result of the soil, atmosphere, underground and surface water pollution, our foods and beverages are getting contaminated with heavy metals (Krejpcio *et al*. 2005).

Heavy metals are given special attention throughout the world due to their effects even at very low concentrations (Das, 1990). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the World Health Organization (WHO).

Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Jarup *et al.*, 1998). Heavy metal composition of food is of interest because of their essential or toxic nature. For example, iron, zinc, copper, chromium, cobalt, and manganese are essential, while lead, cadmium, nickel, and mercury are toxic at certain levels (Onianwa *et al.*, 1999).

2.4.1 Lead

Lead is a potentially harmful metal that have aroused considerable concern (Cabrera *et al*. 1995). Lead affects humans and animals of all ages, however, the effects of lead are most serious in young children. The symptoms of acute lead poisoning are headache, irritability, abdominal pain and various symptoms related to the nervous system. Lead encephalopathy is characterized by sleeplessness and restlessness. Children may be affected by behavioural disturbances, learning and concentration difficulties. Acute exposure to lead is known to cause proximal renal tubular damage (WHO, 1995). Long-term lead exposure was positively correlated with duration of exposure to lead from automobile exhaust, blood lead and nail lead which resulted to kidney damage and urinary excreation of N-acetyl-beta-glucosamynidase (*NAG*) (Mortada *et al.*, 2001).

2.4.2 Cadmium

Cadmium is a toxic and carcinogenic element (Krejpcio *et al*., 2005; Rubio *et al*., 2006). Because of their high toxicity, arsenic, lead and cadmium need to be quantified in food and beverages (Barbaste *et al*., 2003). Cadmium intake in relatively high amounts can be detrimental to human health. Over a long period of intake, cadmium may accumulate in the kidney and liver and, because of its long biological half life, may lead to kidney damage

(Maduabuchi *et al*., 2006). Inhalation of cadmium fumes or particles can be life threatening, and although acute pulmonary effects and deaths are uncommon, sporadic cases still occur (Seidal *et al.*, 1993; Barbee and Prince, 1999). Long-term high cadmium exposure may cause skeletal damage, first reported from Japan, where the itai-itai (ouch-ouch) disease (a combination of osteomalacia and osteoporosis) was discovered in the 1950s. The exposure was caused by cadmium-contaminated water used for irrigation of local rice fields. A few studies outside Japan have reported similar findings (Jarup *et al.*, 1998).

2.4.3 Copper

Copper is one of the essential trace elements. The deficiency of this element is manifested by impaired haematopoesis, bone metabolism, disorders of the digestive, cardiovascular, and nervous systems. The acute exposure to copper containing dust is manifested by metal fume fever (Křižek *et al*. 1997). In humans copper is necessary for the development of connective tissue, nerve coverings, and bone. Copper also participates in both Fe and energy metabolism. Copper acts as a reductant in the enzymes superoxide dismutase, cytochrome oxidase, lysil oxidase, dopamine hydroxylase, and several other oxidases that reduce molecular oxygen. It is transported in the organism by the protein ceruloplasmin. There is about 80 mg of copper in the adult body (highest concentrations in liver and brain) and median intake of copper ranges between 1.0 and 1.6 mg/day in adults (US data). Good sources of dietary copper are liver and other organ meats, oysters, nuts, seeds, dark chocolate, and whole grains. Copper deficiency in humans is rare, but when it occurs leads to normocytic, hypochromic anemia, leucopenia and neuropenia, and inclusive osteoporosis in children (Kanumakala *et al.*, 2002). Excessive dietary zinc can cause

copper deficiency. Chronic copper toxicity is rare in humans, and mostly associated with liver damage. Acute copper intoxication leads to gastrointestinal effects characterized by abdominal pain, cramps, nausea, diarrhea, and vomiting.

2.4.4 Zinc

Zinc is involved in the activity of about 100 enzymes, e.g. RNA polymerase, carbonic anhydrase, copper–zinc superoxide dismutase, angiotensin I converting enzyme. Also it is present in Zn-fingers associated with DNA. Zinc is mainly transported by ceruloplasmin. There is 2–3 g of zinc present in the human body (second to iron in body content) and about 1 mg/L in plasma. Zinc deficiency is common in underdeveloped countries and is mainly associated with malnutrition, affecting the immune system, alcoholism, and malabsorption, causes dwarfism, hypogonadism, dermatitis, wound healing, the senses of taste and smell, and impairing DNA synthesis (Onianwa *et al*., 2001). Zinc seems to support normal growth and development in pregnancy, childhood, and adolescence. It is found in red meat and poultry, beans, nuts, seafood (oysters are extremely rich in zinc), whole grains, fortified breakfast cereals, and dairy products. Its toxicity due to excessive intake has been seen in both acute and chronic forms. Intakes of 150–450 mg of zinc per day have been associated with low copper status, altered Fe function, reduced immune function, reduced levels of HDL (Hamilton *et al.*, 2000).

2.4.5 Manganese

Manganese is associated with bone development, and with amino acid, lipid, and carbohydrate metabolism. Manganese is found in different enzymes, e.g. mitochondrial mangaese superoxide dismutase, glutamine synthetase, arginase, and activates several

hydrolases, transferases and carboxylases. It is transported in the body by transferrin and by macroglobulins and albumin (Davis and Greger, 1992; Rabin *et al.,* 1993). Sources of dietary manganese include grain, rice, tea, and nuts. Manganese is toxic in excess; in brain it can cause a Parkinson-type syndrome (Aschner, 2000).

2.4.6 Iron

Iron is in four classes of proteins: Iron-heme proteins (e.g. hemoglobin (2/3 body iron), myoglobin, catalase, cytochromes); iron–sulfur enzymes (e.g. aconitase, fumarate reductase); proteins for iron storage and transport (transferrin, lactoferrin, ferritin, hemosiderin), and other iron-containing or iron-activated enzymes (e.g. NADH dehydrogenase, succinate dehydrogenase, alcohol dehydrogenase, cyclooxygenases). Total iron intake ranges from 14.4 to 20.2 mg/day (Chanarin, 1999). Serum iron is about 1.3 mg/L, mostly bound to transferring. Iron content in an adult man is about 4 g, decreasing to about 3 g in menstruating women. Fe deficiency causes anemia. Sources of heme iron (15% of consumption) are hemoglobin and myoglobin from animals. Sources of non-heme iron are cereals, seeds of leguminous plants, fruits, vegetables, and dairy products. One of the most serious forms of iron overload is acute iron poisoning. Chronic iron intoxication occurs frequently associated to genetic and metabolic diseases, repeated blood transfusions, or excessive intake (Fraga and Oteiza, 2002).

2.4.7 Calcium

Calcium is an essential nutrient that is sparse in many diets. Calcium is vital to the structure of bones and teeth, contraction of muscles, enzymes activity, regulation of heart beat, release of neurotransmitters and clothing of blood. Calcium supplementation has been

shown to help reduce bone loss in post-menopausal women and give significant protection against hip fractures (Reid, 1995; Devine, 1999). As with all nutrients, bioavailability is the key to calcium effectiveness. Research has pointed to calcium supplementation as a means of relieving high blood pressure (Meese, 1987; Sowers, 1989; Takagi, 1991). Recent studies have also indicated that calcium supplementation can help improve mood, concentration and behavior in premenstrual women, and it has also been found to be beneficial in the reduction of water retention (Penland and Johnson, 1993; Thys-Jacos, 1989).

2.4.8 Potassium

Potassium is an essential element that functions in the maintenance of electrolytes and osmosis balance. It works to offset the excessive negative charges of organic constituents within the cells. As a component in the production of ATP, potassium also plays a role in the excitability of the nerves and muscles. Potassium is beneficial for the prevention and treatment of hypertension, and cardiovascular diseases as well as supporting the muscles, kidney, adrenals and nerve functions. Many studies have shown that supplementing with potassium can help lower blood pressure. This appears to be particularly relevant to the over-sixty age group (Fotherby and Potter, 1992). There is ample research to suggest that potassium supplementation compare favourably to blood pressure reducing drugs and without the negative side effect (Khaw and Barret-Connor, 1984). A potassium-rich diet can be highly beneficial to those suffering from diabetes. Supplementing of food state potassium leads to improve insulin sensitivity, responsiveness and secretion (Norbiato *et al.*, 1984). It also reduces the risk of heart disease, atherosclerosis and cancer.

2.5 Carbohydrates

Carbohydrates can be subdivided into several categories based on the number of sugar units present. A monosaccharide consists of one sugar unit such as glucose or fructose. A disaccharide (e.g., sucrose, lactose, and maltose) consists of two sugar units. Oligosaccharides, containing 3 to 10 sugar units, are often breakdown products of polysaccharides, which contain more than 10 sugar units. Oligosaccharides such as raffinose and stachyose are found in small amounts in legumes. Examples of polysaccharides include starch and glycogen, which are the storage forms of carbohydrates in plants and animals, respectively. Finally, sugar alcohols, such as sorbitol and mannitol, are alcohol forms of glucose and fructose, respectively. The classification of carbohydrates according to their chemical structure is given in appendix A.

2.5.1 Sugar

The term "sugars" is traditionally used to describe mono- and disaccharides (FAO/WHO, 1998). Sugars are used as sweeteners to improve the palatability of foods and beverages and for food preservation (FAO/WHO, 1998). In addition, sugars are used to confer certain functional attributes to foods such as viscosity, texture, body, and browning capacity. The monosaccharides include glucose, galactose, and fructose, while the disaccharides include sucrose, lactose, maltose, and trehalose. Some commonly used sweeteners contain trisaccharides and higher saccharides. Corn syrups contain large amounts of these saccharides; for example, only 33 percent or less of the carbohydrates in some corn syrups are mono- and disaccharides; the remaining 67 percent or more are trisaccharides and higher saccharides (Glinsmann *et al.*, 1986). This may lead to an underestimation of the intake of sugars if the trisaccharides and higher saccharides are not included in an analysis.

2.5.2 Added sugars

Added sugars are defined as sugars and syrups that are added to foods during processing or preparation. Major sources of added sugars include soft and energy drinks, cakes, cookies, pies, fruitages, fruit punch, dairy desserts, and candy (USDA/HHS, 2000). Specifically, added sugars include white sugar, brown sugar, raw sugar, corn syrup, corn-syrup solids, high-fructose corn syrup, malt syrup, maple syrup, pancake syrup, fructose sweetener, liquid fructose, honey, molasses, anhydrous dextrose, and crystal dextrose. Added sugars do not include naturally occurring sugars such as lactose in milk or fructose in fruits.

2.5.3 Sugar-sweetened beverage

Sugar-sweetened beverages (SSBs) include all sodas, fruit drinks, sport drinks, energy drinks, low-calorie drinks and other beverages that contain added caloric sweeteners, such as sweetened tea, rice drinks, bean beverages, sugar cane beverages, and non-alcoholic wines/malt beverages.

Over nearly the past 30 years, U.S. children and adolescents have dramatically increased their consumption of sugar-sweetened beverages (SSBs), which have become the main source of added sugar for many adults and youth,(Brownwell *et al.,* 2009; Brownwell and Frieden, 2009; Craig, 2010; Johnson *et al.,* 2009) with energy drinks, soft drinks, and fruit juices receiving the most attention.

The Australian National Nutrition Survey (NNS) revealed a substantial increase in the consumption of SSB over the past four decades, rising from 47 liters per person in 1969 to 119.9 liters per person in 1999 (Gill *et al.,* 2006; Levy and Tapsell, 2007). This change represents the largest increase of added sugar in diet and subsequently contributes to

excessive energy intake. In Australia, sucrose is the main sweetener added to beverages including SSB, and is also the largest contributor to their energy contents (Crowe *et al.,* 2004), whereas high fructose corn syrup is the main sources of sweetener I such beverages in the U.S (Hawkes, 2010). One standard serve (375mL) of a typical SSB contains 42g of sugar (630KJ) (Ludwig *et al.,* 2004). Subsequently, regular SSB consumers drinking one serve of SSB daily will have energy intake up to 10% greater than non SSB consumers (Krachler *et al.,* 2006; Ludwig *et al.,* 2001; Striegel-Moore *et al.,* 2006).

A similar uptrend in SSB consumption has been observed in the U.S, where more than 75% of SSB sold are caffeinated (Jacobson, 2005). By 2000, production of SSB per annum in the U.S had increased to nearly 225 Litre per person (representing 33% of added sugar in the American diet) (Murray *et al.,* 2005), compared with only 34 litre per person per annum in the mid 20th century (Jocobson, 2005). Mean annunal consumption of SSB is estimated to be 1.2 drinks per adult per day and as many as two drinks per child per day, adding 800kJ or 1,470kJ to the diet respectively (Nestle, 2005).

2.5.4 Sugar-sweetened beverages and health risks

SSB are classified as a high glycemic index food, a group of food characterized by high sugar, white flour or artificial sweetener contents (Brand-Miller *et al.,* 2002). High Glycemic index foods rapidly increase blood sugar, heart rate and blood pressure, and induce fatigue and lethargy after consumption (Brand-Miller *et al.,* 2002). Those who consume SSB are more likely to gain weight than non consumers (Krachler *et al.,* 2006; Ludwig *et al.,* 2001; Malik *et al.,* 2006; Striegel-Moore *et al.,* 2006). SSB has been shown to be a factor in the development of over weight (Berkey *et al.,* 2004: Vartanian *et al.,*

2007), dental caries (Rangan *et al.,* 2008) and obesity (Ebbeling *et al.,* 2006; Vartanian *et al.,* 2007) in children and adolescents. By affecting energy balance and thus body weight, SSB consumption increases risk of obesity related diseases such as type 2 diabetes (Schulze *et al.,* 2004), bone fractures, tooth decay (Shenkin *et al.,* 2003), pancreatic cancer (Schernhammer *et al.,* 2005), gastro esophageal reflux (Jensdoltir *et al.,* 2004), cardiovascular diseases (Astrup *et al.,* 2008), Metabolic syndrome (Dhingra *et al.,* 2007) and hypocalcaemia (Vartanian *et al.,* 2007). The consumption of added sugar, soft drinks, energy drinks and sweetened fruit soups or stewed fruit was positively associated with the risk of pancreatic cancer (Larsson *et al.,* 2006; Genkinger *et al.,* 2012) and also associated with increased risk of symptomatic prostate cancer (Drake *et al.,* 2012). Choi *et al.,* (2010) revealed that the consumption of fructose-rich beverages, such as SSBs, is associated with an increased risk of gout in women. The consumption of SSBs and fructose was strongly associated with an increased risk of gout in men (Choi and Curhan, 2008). SSB consumption is associated with increased serum uric acid levels, and frequency of hyperuricemia, the precursor of gout (Choi *et al.,* 2008).

2.6 Review Works on Beverages

Heavy metal concentration of 20 samples of different artificial fruits juices in Iraqi markets were reported by Al-Mayaly, (2013). The results indicated that all the studied samples (100%) exceeded the local and international permissible values of Cadmium and copper, while 60% of them exceeded the acceptable values of Nickel. Also, it was found that 15% of the studied samples were with high concentrations of lead which exceeded the Iraqi standard and about 35% of them exceeded the limits values of WHO. Zinc concentrations were still with the acceptable range for all the samples.

Obuzor and Ajaezi (2010) analyzed five popular commercial brands of malt drinks (Maltina, Malta Guinness, Amstel Malt, Hi-Malt and Grand Malt) produced in Nigeria for pH, conductivity, turbidity, total dissolved solids, total soluble solids, level of bitterness, reducing sugar, vitamins A and C and minerals. Malt drinks were found to be acidic with pH range of 4.4 - 4.6. Maltina had the highest conductivity of 2.93 µS/cm and a TDS of 1480 mg. Bitterness level ranged from 11 - 13 Bu (Grand Malt) and 15 - 17 Bu (Amstel Malta). The reducing sugar content was found to be high and it ranged from 693.45 - 923.37 mg/dl. Vitamin A content of the drinks were in the range of 40.99 (Grand Malt) – 49.51 mg (Malta Guinness) and Vitamin C ranged from 5.69 (Grand Malt) - 9.97 (Maltina); these values were adequate, meeting Dietary Reference Intakes (DRIs). The content of iron, zinc, cadmium, calcium, copper, chromium, manganese, nickel and lead was negligible, while the content of calcium and sodium was low.

Maduabuichi *et al.*, (2008) analyzed iron, manganese and nickel using atomic Absorption Spectroscopy (AAS) in 50 beverages sold in Nigeria. The results showed that iron levels ranged from 0.020-2.460 mg/L for canned, and 0.020-2.090 mg/L for non-canned beverages. In 95.24% of the canned beverages, iron level exceeded the maximum contaminated limit (MCL) of 0.30 mg/L, while 75.86% of non-canned beverages had iron levels exceeding the MCL. Manganese levels ranged from 0.001-0.730 mg/L for canned beverages, and 0.001-0.209 mg/L for non-canned beverages. 42.86% of the canned beverages exceeded MCL of 0.05 mg/L while 51.72% of non-canned beverages had manganese levels exceeding the MCL. Nickel levels ranged from 0.013-0.993 mg/L for canned and 0.009-0.938 mg/L for non-canned beverages. 80.95% of the canned beverages

exceeded the MCL of 0.005 mg/L while 72.41% of non-canned beverages exceeded the MCL.

Bengol *et al., (*2010) determined qualitatively the heavy metal concentration in 104 canned soft drinks collected from several regions in Turkey using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) method. The mean levels (± SE) of arsenic, copper, zinc, cadmium, and lead were found to be 0.037 ± 0.002 mg/kg, 0.070 ± 0.009 mg/kg, 0.143 ± 0.012 mg/kg, 0.005 ± 0.0003 mg/kg, and 0.029 ± 0.002 mg/kg, respectively, in soft drinks. The data revealed that arsenic, copper, zinc, cadmium, and lead mean levels found in all soft drinks, collected from several regions in Turkey, were within the Turkish Food Codex (TFC) values.

A rapid and simple UV spectrophotometric procedure applied for the determination of the content levels of some food additives in 29 different beverage samples commercially available in Riyadh local markets was reported by Alghamdi *et al.*, (2005). The analytical measurements were undertaken primarily to assess the compliance of content levels of the investigated food additives and their daily intake doses with the permissible levels. The results obtained from the study indicated that the average quantity levels of aspartame, caffeine and sodium benzoate in the analyzed beverages were 246.7 ppm, 18.99 ppm and 16.14 ppm, respectively. In addition, the concentrations of these food additives were converted into the daily intake doses based on beverages consumption. It was estimated that the mean daily intakes of aspartame, caffeine and sodium benzoate by the adult population of Riyadh city through the consumption of the analyzed beverages were 92.5

mg, 6.3 mg and 6.46 mg, respectively. None of the analyzed beverage samples was found to violate the current legal limits practiced in the Saudi food regulations.

Mohammed *et al.*, (2012) analyzed ten brands of beverages (soft and energy drinks) consumed in Basrah governorate/Iraq, for its pH, trace minerals and caffeine contents. Results showed that the pH of the beverages ranged from 2.75-3.66. Iron and Calcium were found in all beverages, Cadmium was found in (Boom Boom, Pit bull, Kalaschnikow and Barbican). Lead was found only in O_2, Power horse, Kalaschnikow and Riviere. Manganese was found in Boom Boom, Wild Tiger, Pit bull and Riviere. Caffeine was found in all beverages except Riviere and Barbican, the highest level of caffeine was in Kalaschnikow (103.13 mg/100 ml) when compared with the other beverages.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Buffer tablets (pH 4.00) – (Reagent grade) (pH Range 3.98 – 4.02)

Buffer tablets (pH 7.00) – (Reagent grade) (pH Range 6.95 – 7.04)

Caffeine (Reagent grade) – HPLC/STD/012

Aspartame (Reagent grade) – HPLC/STD/009

6 *M* HCl (50 ml in 100 ml of water)

2.5 *M* NaOH (100 g in 1000 ml of water)

Aqua regia

0.050 *M* 3, 5-dinitrosalicylic acid

Sucrose stock solution solution

3.1.2 Preparation of stock solution

3.1.2.1 Preparation of standard solution for AAS

A stock solution of each of the element was prepared by dissolving an appropriate amount (1.000 g/ 1000 ml) for Cu, Zn, Pb, Mn, P, and Fe, and (2.542 g/ 1000 ml), (2.497 g/ 1000 ml), (1.907 g/ 1000 ml) for NaCl, KCl, CaCl respectively to get a concentration of 1000 ppm. Five standard solutions covering the range 0-10 µg/ml in 100 ml volumetric flasks were prepared for each of the element. This was used to prepare standard calibration curve for each element.

3.1.2.2 Preparation of standard solution for HPLC

Caffeine and aspartame stock solutions were prepared by dissolving 10.0 mg of caffeine and 18.0 mg of aspartame standard into a 25 ml volumetric flask each of distilled water to give a concentration of 0.4 g/dm^3 and 0.72 g/dm^3 of caffeine and aspartame respectively. From each individual stock standard, 4 ml was pipetted into 25 ml volumetric flask and make up to mark with distilled water. Intermediate standard (1 ml, 2 ml, 3 ml, 4 ml, 5 ml) respectively was pipetted into 10 ml volumetric flask and made up to mark with distilled water and named (1st , 2nd , 3rd , 4th , 5th levels respectively). All levels were filtered with whatman filter paper into a beaker or centrifuge bottle. The filtrate were transferred into the auto sampler vials and cork and injected into HPLC in triplicate. The absorbance for the standard solutions were recorded and used to plot a standard calibration curve.

3.1.2.3 Preparation of standard solution for UV

Sucrose stock solution was prepared by dissolving 1 g of sucrose in 100 ml volumetric flask of distilled water to give a concentration of 10 g/dm^3. Sucrose standard solutions were prepared by suitable dilution of the stock solution. Stock solution of 20 ml was pipetted into a clean 100 ml volumetric flask. Distilled water was added to a point about 1 cm below the calibration mark. A Pasteur pipette was carefully used to add water until the bottom of the meniscus is exactly on the line. The flask was covered with Parafilm and shake well to mix. In a similar fashion, 40:100, 60:100, and 80:100 dilutions were prepared. Each sucrose standard (2 ml) was pipetted into a large test tube; also 2 ml of distilled water was pipetted into a test tube for the blank solution. Two millilitres of 6 *M HCl* was pipetted into each test tube and place in a boiling water bath for 10 minutes. The test tubes were removed and 8 ml of 2.5 *M NaOH* was carefully pipetted into each, then

2.00 ml of 0.050 *M* 3, 5-dinitrosalicylic acid (DNSA) into each. As soon as the DNSA was added, it was shaken to mix the solution thoroughly, and the tubes were placed in a boiling water bath for 5 minutes. Each tube was made to remain in the boiling water for the same amount of time. After removal from the boiling-water bath at the proper time, the test tubes were quickly placed in an ice-water bath for 10 minutes. The blank and prepared standard solutions were poured into a clean, dry cuvette and placed in a spectrometer and the absorbance readings were taken. The absorbance for the standard solutions were recorded and used to plot a standard calibration curve.

3.1.3 Apparatus and equipment

Spectronic 20 spectrophotometer and cuvettes

Digital pH meter (JENWAY 3505)

Digital TDS/conductivity meter (HACH) Sension 5

Digital turbidity meter (HACH DR/890 Colorimeter

pH meter (Orion 320)

Hitachi Auto Sampler HPLC L-2200

Hitachi pump L-2130

Hitachi UV-VIS detector L-2400

Column Oven L-2300

Dell monitor and laser Jet O1006 Printer

Chromatographic analysis was carried out isocratically using the following:

Wavelength: 214nm

Column: Waters Spherisorb C18, 5µm ODS2, 4.6 x 250 mm

Flow rate: 1.0 ml/minute

Mobile phase: 125 ml methanol, 225 ml Acetonitile in 650 ml buffer

Pyrex digestion flasks

Sonnicator/vortex mixer

Pasteur pipettes and bulbs

Mohr pipettes and bulbs

3.1.4 Sample collection

Fourteen (14) brands of energy drinks samples, three (3) powdered and eleven (11) liquids were randomly purchased from the market and analyzed. The samples were refrigerated prior to analysis. The samples were labeled SO, HC, LB, EV, PH, XL, JW, WD, KM, RB, PA, EJ, AL, BU respectively.

3.1.5 Sample preparation

3.1.5.1 Sample preparation for AAS

The energy drink samples were shaken before opening. Liquid samples (30 ml) and powdered samples (1.0 g) were measured into a clean 250 ml dry Pyrex digestion flask. Concentrated aqua regia (25 ml) was added. The digestion flask was heated gently until frothing subsided. The samples were then heated to dryness, dissolved in 30 ml distilled water and filtered with filter paper. The solution was made up to volume in a 100 ml flask.

3.1.5.2 Sample preparation for HPLC

The energy drink samples were shaken before opening. Liquid samples (2 ml) and powdered samples (2.0 g) were measured into 10 ml volumetric flask. Deionised water of

5 ml was added and shaken for 5 minutes using vortex mixer/sonnicator. They were made up to mark with deionised water and filtered with whatman filter paper.

3.1.5.3 Sample preparation for UV

Each of the energy drinks (2 ml) was pipetted into a clean 100 ml volumetric flask. Distilled water was added to a point about 1 cm below the calibration mark. A Pasteur pipette was carefully used to add water until the bottom of the meniscus is exactly on the line. Aliquots (2 ml) of each of the diluted energy drink samples were treated in the same manner as the standards. The determination was carried out in triplicate for each dilution.

3.2 Analysis of Physicochemical Parameters

3.2.1 Determination of pH

The pH was determined using a digital pH meter (JENWAY 3505). The probe was rinsed thoroughly with distilled water before use on sample. Each of the powdered energy drink sample (1 g) was measured into 100 ml volumetric flask. Distilled water (50 ml) was added and shaken for 5 minutes using vortex mixer/sonnicator and made up to mark. For each energy drink samples (liquid and dissolved powder), 50 ml was placed in a beaker, the probe of the pH meter was inserted and the pH values were recorded.

3.2.2 Determination of conductivity

The electrical conductivity measurement was determined using a digital TDS/conductivity meter (HACH) Sension 5. The probe was rinsed thoroughly with distilled water before use on sample. Each of the powdered energy drink sample (1 g) was measured into 100 ml volumetric flask. Distilled water of 50 ml was added and shaken for 5 minutes using vortex

mixer/sonnicator and made up to mark. Each energy drink samples (liquid and dissolved powder) (50 ml) was placed in a beaker, the probe of the TDS/conductivity meter was inserted and the conductivity values were recorded. .

3.2.3 Determination of turbidity

The turbidity measurement was determined using a digital turbidity meter (HACH DR/890 Colorimeter. The probe was rinsed thoroughly with distilled water before use on sample. Each of the powdered energy drink sample (1 g) was measured into 100 ml volumetric flask. Distilled water of 50 ml was added and shaken for 5 minutes using vortex mixer/sonnicator and made up to mark. For each energy drink samples (liquid and dissolved powder), 50 ml was placed in a beaker, the probe of the digital turbidity meter was inserted and the turbidity values were recorded.

3.2.4 Total dissolved solid

The total dissolved solids measurement was determined using a digital TDS/conductivity meter (HACH) Sension 5. The probe was rinsed thoroughly with distilled water before use on sample. Each of the powdered energy drink sample (1 g) was measured into 100 ml volumetric flask. Distilled water of 50 ml was added and shaken for 5 minutes using vortex mixer/sonnicator and made up to mark. For each energy drink samples (liquid and dissolved powder), 50 ml was placed in a beaker, the probe of the TDS/conductivity meter was inserted and the TDS values were recorded.

3.3 Elemental Analysis

An atomic absorption spectrophotometer was used for the determination of the Cu, Zn, Pb, Mn, Ca, P, Cd, and Fe while a flame emission spectroscopy was used to determine K. The instruments were calibrated and standardized using standard solutions. After making sure the instrument is properly calibrated and results of standard are in confidence limit, concentration of metals in each sample were measured individually.

3.4 Determination of Carbohydrate (Sugar)

The amount of sugar (carbohydrates) in energy drinks was determined by spectrophotometric (colorimetric) method as described by Miller, (1959). The method is based upon the colour which forms when sugars reduce 3,5-dinitrosalicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid. The concentration of the coloured product formed by the reaction of glucose and DNSA was measured and the Beer-Lambert law was used to determine the concentration of sugar.

3.5 Determination of Caffeine and Aspartame

Caffeine and aspartame in energy drinks were determined using high performance liquid chromatography as described by National Agency for Food and Drug Administration Control (NAFDAC).

3.5.1 Preparation of pH 4.0 and pH 7.0 buffer solution

One buffer tablet each respectively was put into a 100 ml volumetric flask respectively**.** Distilled water of 20 ml was added to each of the flask and shaken until it dissolves**.** Each

of the flasks was made up to mark with distilled water. Conc. sulphuric acid was used for adjusting the pH of mobile phase.

3.5.4 Buffer preparation

Approximately 2.382 g potassium dehydrate orthophosphate (monobasic) (HPLC grade) was weighed into a 1000 ml volumetric flask. 750 ml of distilled water was added and shaken until it dissolves. The flask was made up with distilled water.

3.5.5 Mobile phase preparation

In a class A 250 ml measuring cylinder, 125 ml methanol (HPLC grade) was measured and poured into a 1000 ml class A volumetric flask. Into another class A 250 ml measuring cylinder, 225 ml acetonitrile was measured and poured into the same 1000 ml volumetric flask. The 1000 ml volumetric flask was made up to mark with potassium dehydrate orthophosphate buffer solution. The mobile phase was then transferred into instrument solvent reservoir bottle and sonnicated for 30 minutes. The pH of the mobile phase was adjusted to 4.0 with conc. Sulphuric acid after mixture.

3.6 Statistical Analysis

All results were analyzed statistically using one-way analysis of variance (ANOVA) with Duncan post hoc test and a paired sample T-test was used to compare the liquid and powdered forms of energy drinks. Differences were considered significant for $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Physicochemical Parameters of Samples

The mean concentration ± SD of the physicochemical parameters (pH, turbidity, total dissolved solids (TDS), and conductivity) of the sampled energy drinks (liquid and powdered) are shown in Table 4.1.

4.2 Metal Concentrations

4.2.1 Concentration of heavy metals

The mean concentration ± SD of heavy metals (cadmium, copper, zinc, lead and manganese) of the sampled energy drinks (liquid and powdered) are shown in Table 4.2.

4.2.2 Concentrations of essential metals

The mean concentration ± SD of essential metals (iron, calcium and potassium) of the sampled energy drinks (liquid and powdered) are shown in Table 4.3.

4.3 Concentrations of Caffeine, Aspartame and Sugar

The concentration of caffeine, aspartame and sugar of the sampled energy drinks are shown in Table 4.3. A comparison of the concentration of caffeine and aspartame in energy drinks with the maximum permitted standard of the US FDA is shown in Figure 4.1 and 4.2 respectively.

Table 4.1: pH, turbidity, total dissolved solids (TDS) and conductivity of energy drinks

Samples	Sample type	pH	Turbidity (NTU)	TDS (mg/L)	Conductivity (µS/cm)
SO	Liquid	4.63 ± 0.012^{c}	126 ± 1.732^{h}	483 ± 0.577^{d}	1025 ± 1.732^{f}
BU	Liquid	5.18 ± 0.023^{h}	68 ± 0.577^{d}	914 ± 2.309^{j}	1881 ± 0.577^{l}
HC	Liquid	4.47 ± 0.012^{a}	84 ± 2.209^{g}	550 ± 0.577^{e}	1151 ± 0.577^{g}
LB	Liquid	4.54 ± 0.012^{b}	592 ± 1.155^{j}	243 ± 0.577^{a}	497 ± 1.155^{b}
EV	Liquid	4.60 ± 0.006^{c}	450 ± 0.577^{i}	477 ± 1.732^{c}	999 ± 1.732^{e}
PH	Liquid	4.83 ± 0.017^{e}	$75\pm1.732^{e,f}$	762 ± 0.577^{g}	1577 ± 1.732^{i}
XL	Liquid	5.04 ± 0.023^{g}	77 ± 1.155^{f}	676 ± 1.732^{f}	1410 ± 2.887^{h}
JW	Liquid	4.99 ± 0.006^{f}	84 ± 2.309^{g}	849 ± 0.577^{i}	1761 ± 1.555^{k}
WD	Liquid	5.27 ± 0.017^{i}	$71\pm0.577^{d,e}$	940 ± 0.000^{k}	1935 ± 1.555^{m}
KM	Liquid	4.69 ± 0.006^{d}	53 ± 1.732^{c}	241 ± 0.577^{a}	516 ± 1.555^{c}
RB	Liquid	4.96 ± 0.017^{f}	$73\pm1.732^{e,f}$	963 ± 1.155^{l}	1975 ± 1.732^{n}
PA	Powder	5.96 ± 0.012^{k}	49 ± 0.577^{c}	1064 ± 0.577^{m}	2210 ± 0.006^{a}
EJ	Powder	5.44 ± 0.023^{j}	13 ± 1.732^{b}	832 ± 1.155^{h}	1748 ± 1.732^{j}
AL	Powder	4.54 ± 0.012^{b}	8 ± 0.577^{a}	410 ± 1.155^{b}	889 ± 0.577^{d}

Results are expressed as Mean±SD. a-m: Increasing order of TDS. a-n increasing order of conductivity. A-k: increasing order of pH. A-j: Increasing order of Turbidity.

Values with the same letters superscript in the column, by treatment, are not significantly different while those with different letters are significantly different at $p<0.05$ from each other.

Table 4.2: Cadmium, copper, zinc, lead and manganese concentrations of energy drinks

Samples	Sample type	Cd (mg/L)	Cu (mg/L)	Zn(mg/L)	Pb (mg/L)	Mn (mg/L)
EJ	Powder	0.012±.0005	0.102±.0003	0.352±.0009	0.209±.0009	0.024±.0002
PA	Powder	ND	0.018±.0002	0.643±.0021	0.122±.0001	0.015±.0002
PH	Liquid	ND	ND	0.062±.0005	ND	0.003±.0001
RB	Liquid	ND	ND	0.050±.0004	0.045±.0005	ND
AL	Powder	ND	0.041±.0003	0.172±.0004	0.083±.0003	0.007±.0005
XL	Liquid	ND	0.002±.0002	0.049±.0009	0.054±.0010	ND
EV	Liquid	ND	0.007±.0002	0.085±.0002	0.028±.0006	ND
BU	Liquid	ND	0.012±.0001	0.045±.0001	0.068±.0002	ND
JW	Liquid	ND	0.019±.0002	0.053±.0005	ND	ND
HC	Liquid	ND	0.018±.0001	0.052±.0006	0.050±.0000	ND
WD	Liquid	ND	0.021±.0001	0.050±.0002	0.078±.0005	ND
LB	Liquid	ND	0.024±.0003	0.049±.0003	ND	ND
KM	Liquid	ND	0.022±.0002	0.057±.0001	0.043±.0003	ND
SO	Liquid	ND	0.070±.0006	13.887±.0037	0.139±.0004	ND

Results are expressed as MeanSD. ND: Not Detected

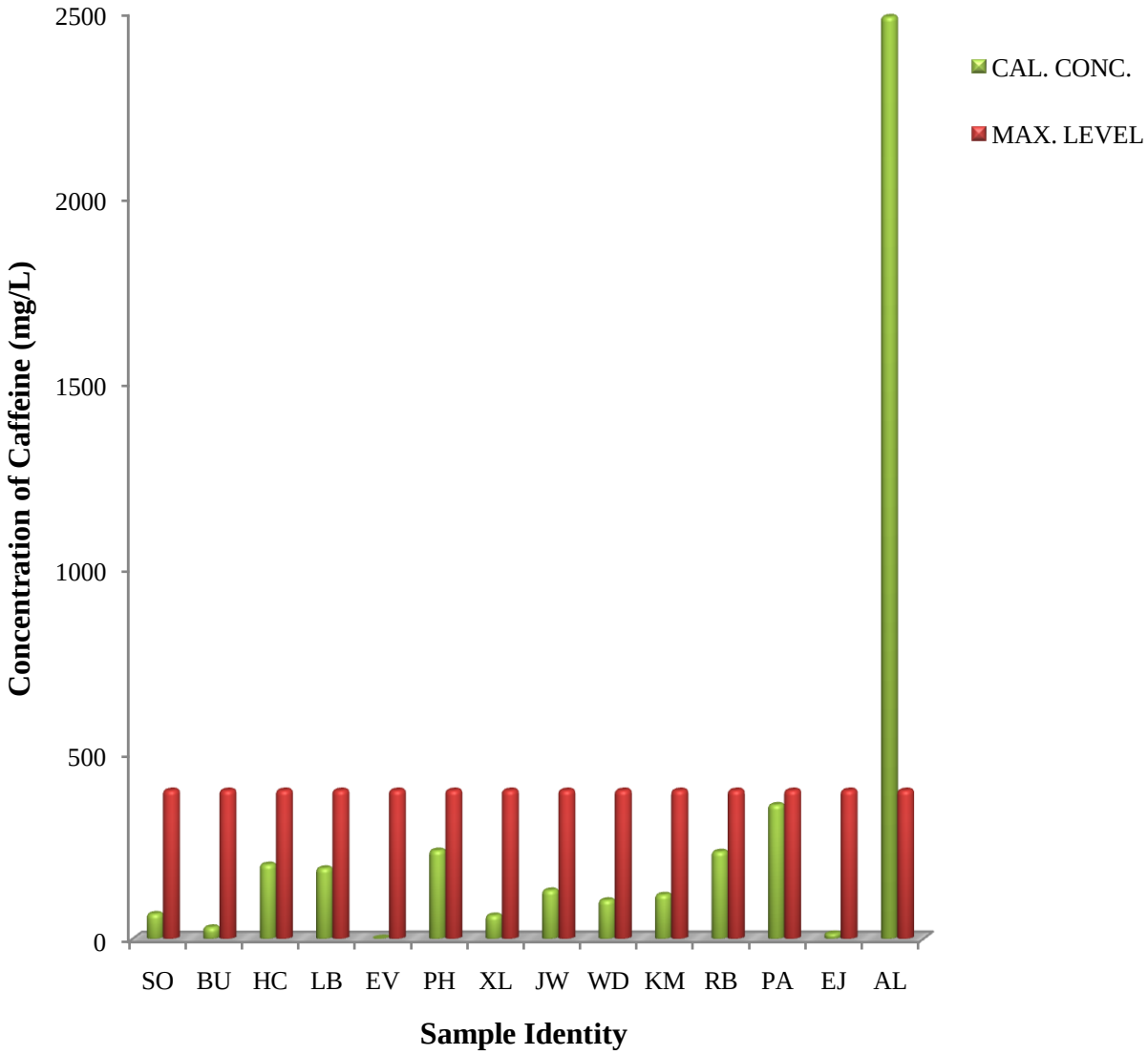
Table 4.3: Iron, calcium and potassium concentrations of energy drinks

Samples	Sample type	Fe (mg/L)	Ca (mg/L)	K (mg/L)
EJ	Powder	0.544±.0008	13.667±.0019	740.00
PA	Powder	0.392±.0006	19.310±.0015	2500.00
PH	Liquid	0.480±.0008	13.143±.0021	3.50
RB	Liquid	0.326±.0005	19.310±.0029	5.00
AL	Powder	0.799±.0005	6.206±.0010	80.00
XL	Liquid	0.418±.0002	5.386±.0005	3.50
EV	Liquid	0.539±.0005	7.402±.0020	6.50
BU	Liquid	0.294±.0005	2.831±.0011	2.00
JW	Liquid	0.412±.0003	2.763±.0009	16.00
HC	Liquid	0.326±.0006	8.531±.0013	6.50
WD	Liquid	0.386±.0002	8.290±.0012	5.50
LB	Liquid	0.586±.0010	4.707±.0008	110.00
KM	Liquid	0.353±.0007	3.546±.0004	2.00
SO	Liquid	1.961±.0003	5.117±.0001	25.50

Table 4.4: Concentration of sugar, caffeine and aspartame

Samples	Sample type	Sugar (mg/L)	Caffeine (mg/L)	Aspartame (mg/L)
SO	Liquid	942.90^{i}	67.08^{c}	624.84^{g}
BU	Liquid	936.73^{i}	30.94^{b}	532.23^{f}
HC	Liquid	938.27^{i}	200.10^{h}	788.13^{h}
LB	Liquid	1686.73^{k}	190.22^{g}	876.06^{i}
EV	Liquid	845.68^{f}	1.11^{a}	956.82^{j}
PH	Liquid	868.83^{g}	237.95^{i}	876.21^{i}
XL	Liquid	922.84^{h}	62.84^{c}	332.64^{d}
JW	Liquid	91.05^{d}	130.58^{f}	423.52^{e}
WD	Liquid	1098.76^{j}	103.97^{d}	283.84^{c}
KM	Liquid	660.50^{e}	118.63^{e}	599.93^{h}
RB	Liquid	867.28^{g}	234.84^{i}	6.51^{a}
PA	Powder	16.98^{a}	361.1^{c}	209.89^{b}
EJ	Powder	80.25^{c}	14.25^{a}	1491.19^{k}
AL	Powder	29.32^{b}	2487.13^{j}	198.59^{b}

a-k: Increasing order of sugar and aspartame. a-j: Increasing order of caffeine.
Values numbers with the same letters in the column, by treatment, are not significantly different while those with different letters are significantly different at $p<0.05$ from each other.



CCC: Calculated concentration of caffeine.

Figure 4.1: A comparison of the calculated concentration of caffeine in energy drinks with the maximum US FDA standard

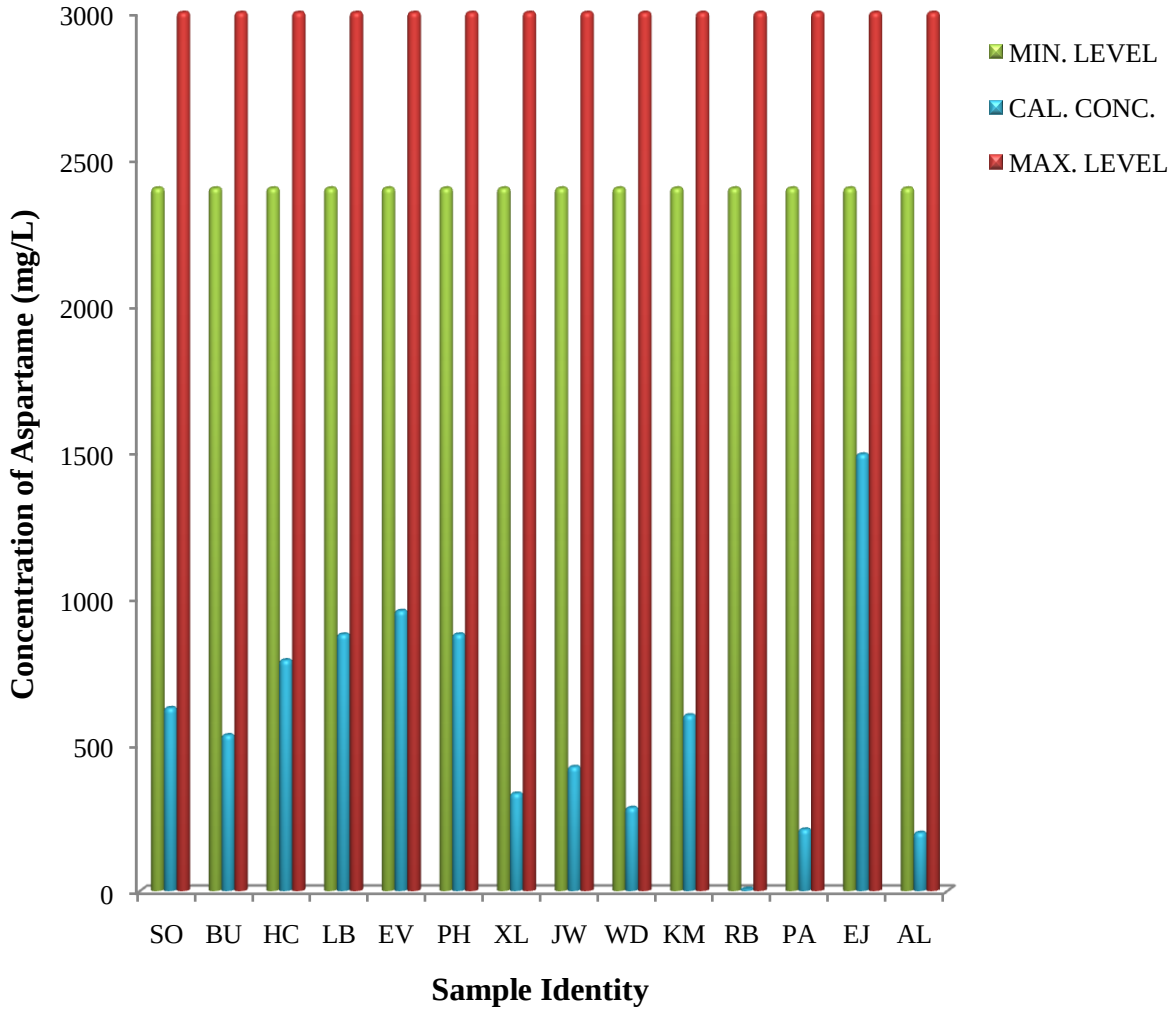


Figure 4.2: A comparison of the calculated concentration of aspartame in energy drinks with the minimum and maximum US FDA standard

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physicochemical Parameters of the Samples

5.1.1 pH

Table 4.1 shows the mean ± SD of the pH of the sampled energy drinks. The pH ranged from 4.47 ± 0.012 - 5.96 ± 0.012. Sample HC (liquid) had the least pH while sample PA (powdered) had the highest. The results were higher than pH values of 2.75 – 3.66 reported by Mohammed *et al.*, (2012) for soft and energy drinks in Basrah, Iraq. They were within the pH ranged of 4.2 – 6.3 reported by Adeleke and Abiodun (2010) for local beverages in Nigeria, and had similarities with pH values of 4.2 – 6.3 reported by Obuzor and Ajaezi (2010) for malt beverages. All samples pH are acidic (i.e. their pH values are less than 7). The reason behind the low pH values of these beverages may be attributed to the CO_2 gas used in the preservation of these beverages or the presence of other acids such as citric acid, phosphoric acid, ascorbic acid, malic acid, tartaric acid used as preservatives (Bassiouny and Yang, 2005; Ashurst, 2005). These acids inhibit the growth of microorganisms such as bacteria, mould and fungi which may contaminate the beverages. Drinking acidic beverages over a long period can erode tooth enamel and predispose the consumer to dental disease (Marshall *et al*., 2003; Bassiouny and Yang, 2005). A correct balance pH in the body is crucial to good health. Monitoring pH levels in beverages is important because unbalanced drinks can lead to tooth decay, an exposure to metallic chemicals and a weakening of the immune system. The pH of the analyzed energy drinks is in the range recommended by FDA for caffeinated drinks and coffee of 4.7 and 6.0 (FDA, 2003).

As shown in Table 4.1, mean pH are labeled a-k, which gives the order of increasing acidity, and represents the ANOVA analysis for the mean pH values of the sampled energy drinks. There was no significant difference in the mean pH values of samples AL and LB, and samples SO and EV. However, the mean pH values of other samples were all significantly different from each other at $p< 0.05$ confidence limit. There was also no significant difference in the pH values of liquid and powdered forms of energy drinks at $p<0.05$ confidence limit.

5.1.2 Turbidity

The mean ± SD of the turbidity readings of the sampled energy drinks is shown in Table 4.1. The turbidity of energy drinks ranged from 8 ± 0.577 – 592 ± 1.155 NTU with sample AL (powdered) having the least and sample LB (liquid) having the highest turbidity. These are lower than results reported by Obuzor and Ajaezi (2010) for malt drinks which had turbidity values above detection limits (>1000NTU). Sample AL is least turbid while sample LB had the highest turbidity. Turbidity is the measure of the degree to which water loses its transparency due to presence of suspended particles. The more total suspended solids in the water, the murkier it seems and the higher the turbidity (Maurice, 2010). Turbidity is considered as a good measure of the quality of water. The suspended particles help the attachment of heavy metals and other toxic organic compounds which may pose negative health effects to the consumers.

As shown in Table 4.1, the mean turbidity of energy drinks are labeled a-j, which gives the order of increasing turbidity of the drinks and represents the ANOVA analysis for the

mean turbidity values of the sampled energy drinks. There was no significant difference in the mean turbidity values of samples PA and KM, samples WD and BU, samples WD and PH, and samples PH and XL. The mean turbidity values of all other samples were significantly different from each other at $p<0.05$ confidence limit. There was significant difference between the mean turbidity of liquid and powdered forms of energy drinks.

5.1.3 Total dissolved solids

The total dissolved solids (TDS) ranged from 243 ± 0.577 – 1064 ± 0.577 mg/L as shown in Table 4.1. These values were within the range of 327.37 – 1480 mg/L reported by Obuzor and Ajaezi (2010) for malt drinks. Sample PA (powdered) had the highest TDS while sample LB (liquid) had the least TDS. Beverages with high values of TDS are likely to contain metals (essential and toxic) at high concentrations which may cause adverse health effects when consumed.

As shown in Table 4.1, the mean TDS values are labeled a-m, which gives the order of increasing TDS of the analyzed samples and represents the ANOVA analysis for the TDS values of energy drink samples. There was no significant difference between the mean turbidity values of samples LB and KM. the mean turbidity values of all other samples were significantly different from each other at $p<0.05$ confidence limit. There was also no significant difference between the mean turbidity of the liquid and powdered forms of energy drink samples.

5.1.4 Conductivity

Conductivity is the ability of electricity to pass through water using the impurities contained in the water as conductors. When water has a lot of impurities, it is more conductive, however, if water is pure, it is less conductive unless it is polarized (Maurice, 2010). Hence, energy drinks conduct electricity because it contains ions and it follows that energy drink with the highest concentration of ions will conduct the most. The conductivity of energy drinks ranged from 497±1.155 - 2210 ± 0.006 µs/cm. These values were similar to 2.93 – 1999 µs/cm reported by Obuzor and Ajaezi (2010) for malt drinks. Sample PA (powdered) is least conductive while sample RB (liquid) has the highest conductivity.

As shown in Table 4.1, the mean conductivity is labeled a-n, which gives the order of increasing conductivity of the samples and represents the ANOVA analysis for conductivity values of energy drink samples. The mean conductivity of all sampled energy drinks was significantly different from each other at $p<0.05$ confidence limit. There was no significant difference between the mean turbidity of the liquid and powdered forms of energy drink samples.

5.2 Metal Concentrations

5.2.1 Heavy Metals

Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the defining factor. Heavy metals are thus commonly defined as those having a specific density of more than 5 g/cm^3 (Duffus, 2002). The main threats to human health

from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid, but is usually classified as a heavy metal). Although adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing in some areas. Table 4.2 shows the mean ± SD for the concentration of heavy metals in the sampled energy drinks.

5.2.1.1 Cadmium

Cadmium was not detected in all energy drinks except for sample EJ which is a powdered form of energy drink and had a concentration of 0.012 ± 0.0003 mg/L as shown in Table 4.2. This was close to 0.01mg reported by Obuzor and Ajaezi (2010). Krejpcio (2005) and Al Mayaly (2013) reported cadmium levels of 0.004 – 0.060 mg/L and 0.005 – 0.05 ppm respectively while Maduabuchi *et al.*, (2006) reported cadmium levels of 0.003 – 0.081 mg/L for canned and 0.006 – 0.071 for non-canned beverages. These were higher values compared to those determined in the energy drinks. The cadmium concentration of energy drinks analyzed exceeded the maximum contaminant levels (MCL) of 0.005 mg/L set by US EPA (1985). Cadmium intake in relatively high amount can be detrimental to human health. Over a long period of intake, cadmium may accumulate in the kidney and liver, and because of its long biological half-life, may lead to kidney damage (Maduabuchi *et al.*, 2006). Cadmium is a non-essential metal and produces only significant adverse health effects.

5.2.1.2 Lead

Lead concentration of energy drinks ranged from 0.028 ± 0.0006 - 0.209 ± 0.0009 mg/L with sample EV (liquid sample) having the least and sample EJ (powdered sample) having the highest concentration. Lead was not detected in samples PH, JW and LB as shown in Table 4.2. The lead concentration in the powdered forms of energy was higher compared to those in liquid forms. The lead concentration of energy drinks was lower than lead concentrations of 0.020–0.46 mg/L reported by Krejpcio (2005). Onianwa *et al*., (1999) reported lead levels of 0.04 ± 0.01 ppm in carbonated soft drinks 0.06 ± 0.08ppm in fruit juice Nigeria. Maduabuchi *et al.*, (2006) also reported lead levels of 0.002 – 0.0073 mg/L in canned drinks and 0.092 mg/L in non-canned drinks. These were lower compared to the values determined in energy drinks. Lead detected in samples was above the MCL of 0.01 mg/L (WHO 1993). Lead toxicity causes many sign and symptoms such as abdominal pains, anaemia, anoxia, bone pair, brain damage, convulsion, dizziness, inability to concentrate (Satarug and Moore, 2004). Excessive exposure to lead may cause microcytic anemia, glycosuria, cognitive dysfunction, anorexia, metallic taste, insomnia and reticulocytosis. Target organs include the brain, bone, blood, kidneys, and thyroid gland (Satarug and Moore, 2004).

5.2.1.3 Copper

The copper concentration of energy drinks ranged from 0.002 ± 0.0002 - 0.102 ± 0.0003 mg/L. Sample XL (liquid sample) had the least concentration while sample EJ (powdered sample) had the highest concentration of copper. Copper was not detected in sample PH and RB as shown in Table 4.2. The powdered forms of energy drinks had higher

concentrations compared to those of the liquid forms. The concentration in energy drinks was low compared to the values 0.047–1.840 mg/L reported by Krejpcio *et al*. (2005) for fruit juice samples in Poland. The concentration was however higher than values 0.01 - 0.02 mg reported by Obuzor and Ajaezi (2010) in malt beverages, and values 0.0004 - 0.001 mg/kg, reported by (MAFF, 1998) determined in non-alcoholic beverage. Energy drinks had concentrations below the MCL of copper of 1.0 mg/L (WHO 1993). Copper is needed for proteins involved in growth, nerve function and energy release (Institute of Medicine, 2001). It is vital for the formation of some important proteins. It is a critical functional component of a number of essential enzymes, known as cuproenzymes. Two copper-containing enzymes, ceruloplasmin (ferroxidase I) and ferroxidase II are involved in iron metabolism (Attieh, 1999). Copper is stored in appreciable amounts in the liver. It also has anti-oxidant properties and involved in the regulation of gene expression. The deficiency of copper is manifested by impaired hematopoiesis, bone metabolism, disorders of digestive, cardiovascular and nervous systems (Křižek *et al.*, 1997).

5.2.1.4 Manganese

Manganese is both an important element and a potent neurotoxin. It accumulates in mitochondria, a major source of oxide, which can oxidize Mn^{2+} to the powerful oxidizing agent Mn^{3+}. Oxidation of important cells by Mn^{3+} has been suggested as a source of the toxic effects of manganese (Gunter *et al.*, 2004). Manganese is an actual component of manganese super oxide dismutase enzyme. It is a powerful antioxidant that searches the free radicals in human body and manages to neutralize these damaging particles and prevent any potential danger they may cause. Manganese was detected in all the powdered

samples. Only one of the liquid had manganese as shown in Table 4.2. Their concentration ranged from 0.003 ± 0.0001 - 0.024 ± 0.0002 mg/L. The liquid sample has a lower concentration of manganese compared to the powdered samples. These values were low compared to 0.001 – 0.730mg/L for canned and 0.001 – 0.209 mg/L for non-canned beverages reported by Maduabuchi *et al.,* (2006) but close to 0.01 mg reported by Obuzor and Ajaezi (2010) and 0.01 – 0.021 mg/L reported by Mohammed *et al.,* (2012). The concentrations of manganese in all the samples detected were below the MCL of 0.05 mg/L (WHO, 1993). Deficiency in manganese leads to various health problems, which may include bone malformation, eye and hearing problems, high cholesterol levels, hypertension, infertility, weakness, heart disorders, memory loss, muscle contraction, tremors, seizures (Institute of Medicine, 2001). It could also result in decreased learning ability in school-aged children and increase the propensity for violence in adult (Finley, 2004).

5.2.1.5 Zinc

Zinc is such a critical element in human health of which, a small deficiency is a disaster. Zinc deficiency is characterized by growth retardation, loss of appetite and impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions (Ryan-Harshman and Aldoori, 2005). The concentration zinc was with in range of 0.045 ± 0.0001 - 13.887 ± 0.0037 mg/L. Sample SO (liquid sample) had the highest concentration of 13.887 ± 0.0037. These values were similar to those reported by Bengol *et al.,* (2010) and Krejpcio *et al*. (2005) except for sample SO. The zinc concentration of all sampled energy

drinks were below the MCL 5.0 mg/L of zinc (WHO, 1993). Only sample SO had a concentration higher than the MCL of Zinc. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division (Institute of Medicine, 2001; Prasad, 1995). A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Ryan-Harshman and Aldoori, 2005; Institute of Medicine, 2001).

5.2.2 Essential Metals

Mineral elements are important building blocks needed for renewal of tissues such as blood and bone. Minerals are inorganic substances that are essential for the functioning of organ systems and the entire body (Ryan-Harshman and Aldoori, 2005). Some of these minerals exist in large amounts in our body such as calcium, while others such as manganese exist in trace amounts but are, nonetheless, critical to our health and well-being (Ryan-Harshman and Aldoori, 2005). If mineral levels are excess in the body, such as sodium, they may facilitate negative effects in the body. High sodium levels may elevate blood pressure. If mineral levels are inadequate in the body, they may facilitate negative effects in the body. Several metal ions are crucial to the metabolism of cells at low concentrations but are toxic at high concentrations, resulting in bell-shaped dose-response relationships (Marschner, 1995). These metals are sometimes called micronutrients. Table 4.3 shows the mean ± SD for the concentration of essential metals in the sampled energy drinks.

5.2.2.1 Iron

Iron fortification in foods has been increased to tackle the increased incidence of iron deficiency anaemia, especially in the western countries (Kusumi *et al.,* 2006). Iron deficiency anaemia is the most common micronutrient deficiency affecting mostly the low socioeconomic populations of developing countries (WHO). Excessive iron intake has been associated with overall increased risk of colorectal cancer (Senesse *et al.,* 2004). The iron level of energy drinks ranged from 0.294 ± 0.0005 mg/L -1.961 ± 0.0003, with sample SO (liquid sample) having the highest value and sample BU (liquid sample) having the least as shown in Table 4.3. The liquid samples had higher concentration of iron compared to the powdered samples. The values were lower compared to 0.020 – 2.090 mg/L for non-canned and 0.020 – 2.460 mg/L for canned beverages reported by Maduabuchi *et al.,* (2006) but higher than values 0.11 – 0.28 mg/L reported by Obuzor and Ajaezi (2010). All the sampled energy drinks had iron concentration higher than the MCL of 0.03 mg/L (WHO, 1993). Iron is important in many biological processes because it is an ideal oxygen carrier and because it can function as a protein-bound redox element. Iron deficiency is common worldwide and in infants can cause severe neurological deficit (Prasad, 1995).

5.2.2.2 Calcium

Calcium concentration of energy drinks ranged between 2.763 ± 0.0009 - 19.310 ± 0.0015 mg/L, with sample JW (liquid sample) having the least and sample PA (powdered sample) and RB (liquid sample) sharing the same value as highest. There are close similarities between the calcium concentrations of samples EJ (powdered) and PH (liquid), and those of samples RB (liquid) and PA (powdered), BU (liquid) and JW (liquid) as shown in Table

4.3. All other samples varied in their concentration of calcium. The calcium concentration of energy drinks were low compared to 0.28 – 262 mg/L reported by Obuzor and Ajaezi (2010). Calcium is needed for the formation and maintenance of bones, the development of teeth and healthy gums. It is necessary for blood clotting, stabilizes many body functions and is thought to assist in preventing bowel cancer (Attieh, 1999). It has a natural calming and tranquilizing effect and is necessary for maintaining a regular heartbeat and the transmission of nerve impulses. The required amount include: 1,000 mg/day for people aged 19 - 50 years and 1,200 mg per day for people over the age of 51 years. The maximum level of calcium is 2.5 g/day (Ryan-Harshman and Aldoori, 2005). Rickets, tetany, and osteoporosis can result its deficiency. Hypertension and colon cancer may relate to chronic low intake.

5.2.2.3 Potassium

Potassium concentrations of the energy drinks were relatively low compared to the recommended daily intake (RDI) of potassium (WHO, 1993). It ranged from 2.0 to 2500 mg/L as shown in Table 4.3. The RDI of potassium ranged between 1600-5000mg/day. Only sample PA having a concentration 2500 mg/L was within the recommended RDI of potassium. All other samples were low, ranging between 2.00 to 740 mg/L. Potassium is the major intracellular ion, intimately related to sodium movement out of the cell via Na/K ATPase. As such, it participates in maintaining normal membrane potential in cells. Potassium is also a major factor in osmotic fluid dynamics in the body. Renal control mechanisms keep blood levels within a narrow range. Normal or increased potassium antagonizes uptake/retention. Potassium deficiency results to irregular/rapid heart beat,

High Blood Pressure (HBP), kidney disease, infrequent menstrual cycles, ovarian cysts, muscle spasms/cramps, joint pains, weakened immune system and hyperglycemia. Excess potassium results to hypotension, reactive hypoglycemia, and increased risk for cancer. Toxicity is possible via supplements at doses near 18 g or in kidney dysfunction.

5.3 Caffeine, Aspartame and Sugar Concentrations in Energy Drinks

The caffeine and aspartame concentration of the energy drinks were calculated using calibration curves obtained from the caffeine and aspartame analysis using HPLC as shown in appendices B and C respectively. The sugar concentration of the energy drinks were calculated using calibration curves obtained the sugar analysis using UV-Spectrophotometer in as shown in Appendix D.

5.3.1 Caffeine

The mean ± SD concentrations of caffeine as determined in each of the sampled energy drinks are shown in Table 4.4. The results obtained showed that caffeine concentrations ranged from 1.11 mg/l – 2487.13 mg/L. These were within the range of 170 ppm – 324 ppm for caffeine concentrations in energy drink reported by Mei *et al.,* (2012), and lower than the ranged of 440 ppm – 473 ppm for caffeine concentration in tea samples reported by Mei *et al.,* (2012). The values were also lower than 1.41 mg/serving reported by Rachel *et al.,* (2006) and those reported by Alghamdi *et al.,* (2005). Sample EV (Liquid) had the least caffeine concentration while sample AL (powdered) had the highest caffeine concentration. The caffeine concentrations in the powdered samples were higher compared to the liquid samples. However, sample AL (powdered) had a very high caffeine

concentration compared to values reported by Mei *et al.*, (2012) and Alghamdi *et al.*, (2005).

The safety of caffeine intake has been assessed by several national regulatory scientific committees for use at the levels of consumption estimated by their respective populations. According to the Food Standards Agency UK, drinks containing more than 150 mg/L of caffeine must be labeled with the term 'high caffeine content' in the same field of vision as the name of the food. This must be accompanied by an indication of the amount of caffeine per 100ml in the product. No other labeling is currently required by law and this labelling does not apply to drinks such as tea and coffee.

The recommended upper daily intake levels of caffeine have been set by the Korean Food and Drug Administration. For adults less than 400 mg of caffeine per day, for pregnant women less than 300mg and for children less than 2.5 mg/kg of body weight (Heckman *et al.*, 2010). In Taiwan, Upper limit of caffeine is 320 mg/L for beverages other than tea and coffee (Heckman, *et al*., 2010). Mexican regulations do not include any upper limit for the addition of caffeine to beverages. However, flavoured non alcoholic beverages containing more than 20 mg/100 ml are considered ‘beverages with added caffeine, which must be printed on the label (Heckman, *et al*., 2010).

In Brazil, beverages containing 80mg of caffeine are considered ‘liquid compounds ready for consumption’ and the regulations foresee an upper limit of 350 mg/L (Heckman, *et al*., 2010). Chile regulation classified beverages containing 80mg of caffeine (320 mg/L) as

sports drinks and the regulation does not include an upper limit. Rather, it states a daily consumption higher than 500 mg of caffeine should not be consumed (Heckman, *et al*., 2010).

According to Health Canada, (2010), the maximum recommended daily intake of caffeine is 45-85 mg per day for children (2-4 years), 125 mg per day for teenager (13 years) and 400 mg per day for adult.

US FDA has cited 400 mg/day as an amount not generally associated with dangerous, negative effects. It has however not set a level for children, but the American Academy of Pediatrics discourages the consumption of caffeine and other stimulants by children and adolescents.

In Nigeria, there have been no set limits by the food regulatory bodies for caffeine in energy drinks as majority of the energy drink products are usually imported into the country. As such, they usually used the set standards by the international bodies such as FDA.

The acceptable daily intake of caffeine is 400 mg/day (US FDA, 2010). Figure 4.1 shows a comparison between the concentrations of caffeine in the sampled energy drinks and the set standards of US FDA. Caffeine concentrations in all the energy drink samples were below the FDA set standards except for sample AL which is above the standard. A possible reason for this may be due to its usage as analgesic. This implies that a daily

consumption of one can of any of the sampled energy drink with the exception of sample AL, may not have any adverse effect on the consumer. However, a daily consumption of two or more cans of the energy drink may have adverse effects as reported in literature especially on children and pregnant women.

As shown in Table 4.4, mean concentrations of caffeine are labeled a-j which shows the order of increasing caffeine concentrations, and represents the ANOVA analysis of the sampled energy drinks. That there was no significant difference between concentrations of caffeine in samples EJ and EV, samples SO and XL, and samples PH and RB. The concentration of caffeine in all other samples differs significantly from each other at $p<0.05$ confidence limit. There was also no significant difference between the caffeine concentrations of the liquid and powdered forms of energy drinks at $p<0.05$ confidence limit.

5.3.2 Aspartame

The concentration of aspartame as determined in each of the sampled energy drink is given in Table 4.4. The results obtained shows that aspartame concentration ranged from 6.51 mg/L – 1491.19 mg/L. These values were with in the range of 153.69 – 876.42 ppm and 198.22 – 709.36 ppm reported by Serdar and Knežević (2011) for soft drinks and artificial flavored drinks respectively but lower compare to 80.29 – 435.05ppm reported for fruit juices and 156.98 – 554.35 ppm reported for powdered drinks. The values were also lower compared to 40.25 – 507.75 ppm reported by Mackenzie and Erik (2001) and 127.2 – 344.5 ppm reported by Alghamdi *et al.,* (2005). Sample RB (Liquid) had the least

aspartame concentration while sample EJ (powdered) had the highest aspartame concentration. The concentration of aspartame in the liquid forms of energy drinks were generally higher compared to the concentration in the powdered forms of energy drinks.

The safety of aspartame has been considered by a range of regulatory organizations, their expert advisory groups and interested scientists (FAO/WHO, 1980). An acceptable daily intake (ADI) of 40 mg/kg body weight was established for aspartame, while an ADI of 7.5 mg/kg body weight was established for diketopiperazine (FAO/WHO, 1980).

The European Commission's Scientific Committee on Food (SCF) set the acceptable daily intake (ADI) of aspartame at the same level as those set by Joint FAO/WHO Expert Committee on Food Additives (JECFA). The US Food and Drug Administration approved aspartame for use in 1984 and have subsequently reaffirmed its safety in 1996 (FDA, 1996). The FDA established a slightly higher ADI for aspartame of 50 mg/kg body weight. The acceptable daily intake of aspartame range between 40 - 50 mg/kg/body weight which is equivalent to 2400 – 3000 mg/day for a 60 kg adult (FDA, 1996; FAO/WHO, 1980).

Figure 4.2 shows a comparison between the concentration of aspartame in the sampled energy drinks and the US FDA set standard. Aspartame concentrations in all the energy drink samples analyzed were all below the FDA set standard. This is an indication that they will not have adverse effects on the consumers except multiples of the drinks are consumed.

As shown in Table 4.4, the mean concentrations of aspartame are labeled a-k which shows the order of increasing concentration of aspartame in the sampled energy drinks and represents the ANOVA analysis of the sampled energy drinks. There was no significant difference in the aspartame concentrations of samples PA and AL, and samples PH and RB. All other sampled energy drinks differ significantly in their aspartame concentration at $p<0.05$ confidence limit. There was also no significant difference between the aspartame concentrations of the liquid and powdered forms of energy drinks at $p<0.05$ confidence limit.

5.3.3 Sugar

The concentration of sugar as determined in each of the sampled energy drink is given in Table 4.4. The results obtained shows that the sugar concentration in the sampled energy drinks ranged from 16.98 – 1686.73 mg/L. Sample PA had the least concentration while sample LB had the highest concentration. The concentration of sugar in the powdered forms of energy drinks ranged 16.98 – 80.25 mg/L while the concentration of sugar in the liquid forms of energy drinks ranged 91.05 – 1686.73 mg/L. The sugar concentrations in the liquid forms of energy drinks were generally higher than those in the powdered forms of energy drinks.

Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when prophylactic measures, e.g. oral hygiene and fluoride prophylaxis, are insufficient. However, available data do not allow the setting of an upper limit (UL) for (added) sugars on the basis of a risk reduction for dental caries, as caries development

related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption, and various other factors (EFSA, 2010).

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight (EFSA, 2010).

Most short-term intervention studies on the effects of high intakes of sugars on glucose and insulin response do not find adverse effects at intakes of predominantly added sugars up to 20 to 25 E%, provided that body weight is maintained. Although there is some evidence that high intakes (>20 E%) of sugars may increase serum TG and cholesterol concentrations, and that intakes >20 - 25 E% might adversely affect glucose and insulin response, the available data are not sufficient to set an upper limit for (added) sugar intake (EFSA, 2010). Available data do not allow the setting of a Tolerable Upper Intake Level for total or added sugars, neither an Adequate Intake nor a Reference Intake range.

As shown in Table 4.4, the mean concentrations of sugar are labeled a-k which shows the order of increasing concentration of sugar in the sampled energy drinks and represents the ANOVA analysis of the sampled energy drinks. There was no significant difference in the sugar concentration of samples SO, BU and HC, and samples PH and RB. All other

sampled energy drinks differ significantly from each other in their concentration of sugar at $p<0.05$ confidence limit. There was significant difference between the sugar concentrations of the liquid and powdered forms of energy drinks at $p<0.05$ confidence limit.

CHAPTER SIX

6.0 Conclusion

Debate regarding the overall risks and benefits of energy drinks has gained momentum in recent times. Health researchers agree that caffeine consumption can have adverse health consequences, particularly at high doses. Among the most common negative effects are increased anxiety, panic attacks, increased blood pressure, increased gastric acid, bowel irritability, and insomnia.

This study analyzed the caffeine, aspartame and sugar concentrations of energy drinks in the Nigerian market. Results for caffeine concentration in the energy drinks ranged between 1.11 mg/L – 2847.13 mg/L. Caffeine concentration in all the samples were below the FDA set standard of 400 mg per day except for sample AL which has the highest caffeine concentration of 2847.13 mg/L. There was no significant difference between the caffeine concentrations of the liquid and powdered forms of energy drinks.

There has also been reports regarding the safety of aspartame which is the predominant artificial sweetener used in most beverages. The aspartame concentration of energy drinks ranged from 6.5 mg/L to 1491.19 mg/L. This is below the acceptable daily intake (ADI) of aspartame set by joint WHO/FDA (40 – 50 mg/kg per body weight). This is equivalent to 2400 – 3000 mg/day for a 60 kg adult. There was no significant difference between the aspartame concentrations of the liquid and powdered forms of energy drinks.

Sugar was detected in all the energy drink samples and their concentration ranged from 16.98 – 1686 mg/L. Evidences abound on the adverse health effects of sugar. However, there are no set limits for the acceptable daily intake (ADI) of sugar. There was significant difference between the sugar concentrations of the liquid and powdered forms of energy drinks.

Iron, calcium, zinc and potassium were found in all the energy drinks. These are essential elements needed for the general well being of the body. Although their values were low compared to the acceptable total intakes, their presence contributes to the daily iron, calcium, zinc and potassium sources needed in the body.

Cadmium was detected only in sample EJ, having a concentration of 0.102 ± 0.0003 mg/L which exceeded the maximum contaminant level (MCL) of 0.005 mg/L set by the FDA. Cadmium is a non essential element and produces only significant adverse effects.

Lead concentration of energy drinks ranged from 0.028 ± 0.0006 - 0.209 ± 0.0009. Lead was not detected in samples PH, JW and LB. Lead detected in samples was above the MCL of 0.01 mg/L. Lead is also non essential element and produces only significant adverse effects.

Manganese was detected in four (4) of the fourteen (14) samples of the energy drinks. The concentration ranged from 0.003 ± 0.0001 - 0.024 ± 0.0002 which is below the MCL of

manganese. The copper concentration of energy drinks ranged from 0.002 ± 0.0002 - 0.102 ± 0.0003. These were below the MCL of copper (1.0mg/L).

The physicochemical properties such as pH, total dissolved solids, turbidity and conductivity were also investigated. These were in conformity with the standards set by regulatory bodies such as FDA and WHO.

The caffeine aspartame and sugar determined in the sampled energy drinks tested positive for majority of the energy drinks. Though they were mostly below the set standards, It is however important that the pattern of consumption of these drinks (i.e. not consuming more than two can on a daily basis) must be monitored to prevent taking excess doses of these substances to prevent the reported adverse effects.

6.1 Recommendations

Energy drinks have been associated with adverse health effects and the claims made by manufacturers about the benefits of energy drinks do not highlight risks associated with excessive consumption of a combination of the ingredients contained in energy drinks. Long term effects of energy drinks consumption of children and young people have not been adequately studied. Therefore, it is recommended that further research be carried out on the adverse effects of energy drinks on children. Research is also needed to be done on the effects of the combination of ingredients on health and excessive consumption of those ingredients to children and adolescents. People need to be educated and given proper awareness on the health risks associated with energy drinks.

It is recommended that it should be made compulsory for manufacturers to fully disclose the exact concentrations of ingredients including stimulants such as caffeine and guarana used on the labels of their products so that people are aware of what they consume.

Manufacturers should also provide prominent health and safety warning labels on each container, alerting consumers to the risks associated with consuming caffeine and other stimulants contained in energy drinks.

Agencies such as NAFDAC should conduct further research on the health and safety of energy drinks, focusing particularly on the impact on underage drinking. They should also develop a national media and public awareness campaign about the risks associated with energy drinks.

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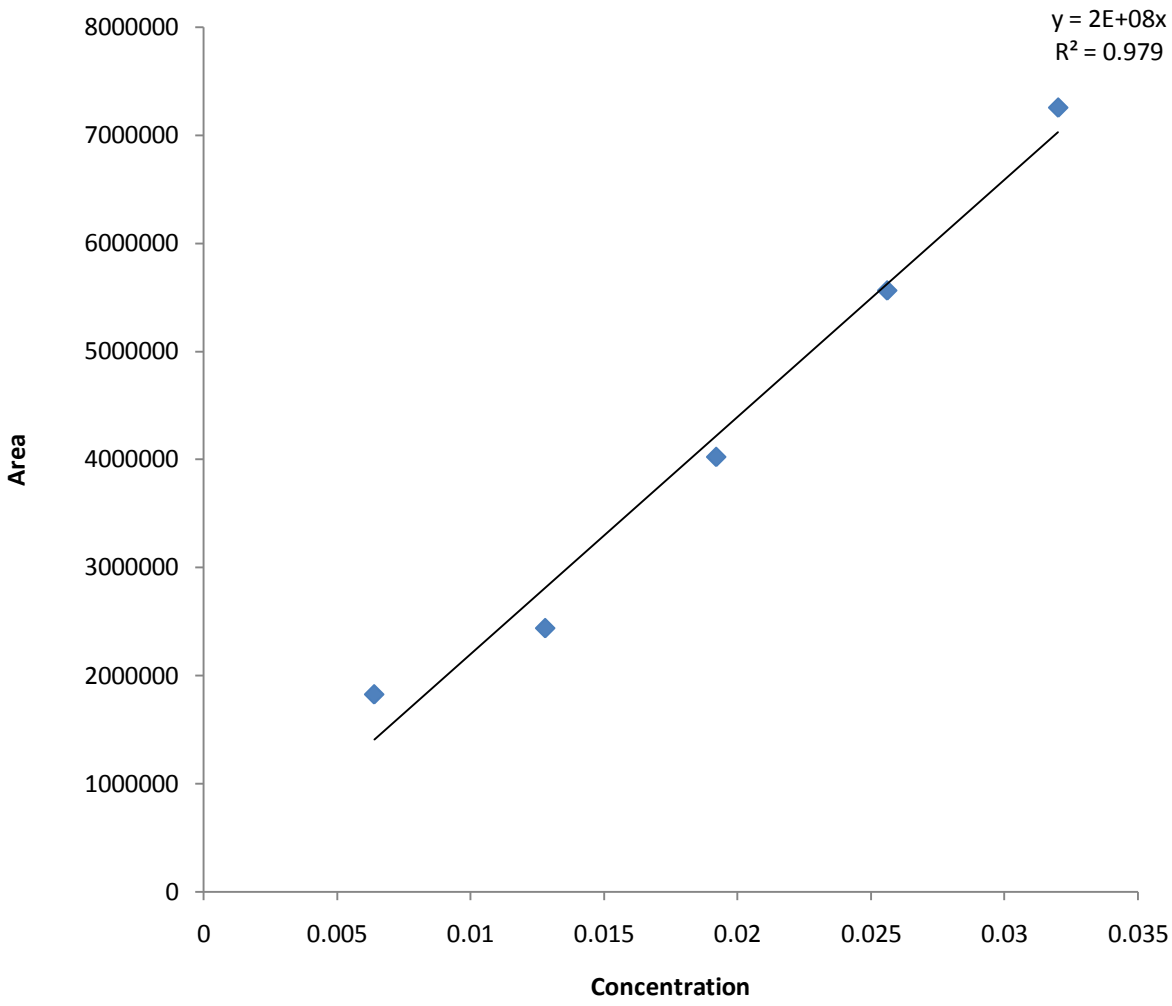
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APPENDICES

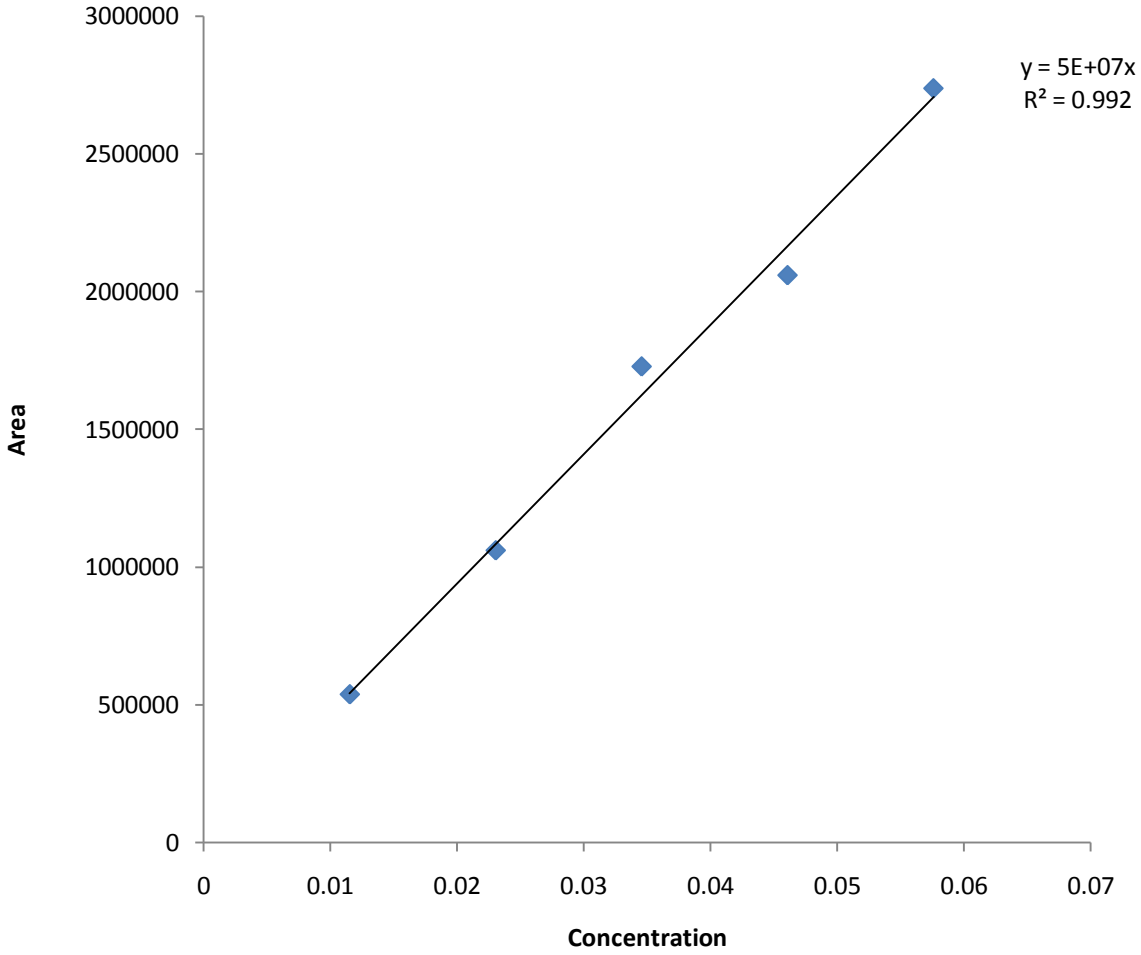
Appendix A: Classification of Carbohydrates According to their Chemical Structure

Class (DP*)	Sub-Group	Components
Sugars (1-2)	Monosaccharides	Glucose, galactose, fructose, mannose, tagatose
	Disaccharides	Sucrose, lactose, trehalose, maltose, isomaltulose
	Polyols	Sorbitol, mannitol
Oligosaccharides (3-9)	Malto-oligosaccharides	Maltodextrins
	Other oligosaccharides	Raffinose, stachyose, fructo-oligosaccharides, galacto-oligosaccharides
Polysaccharides (>9)	Starch	Amylose, amylopectin, modified starches, glycogen
	Non-starch polysaccharides	Cellulose, hemicellulose, pectins, hydrocolloids

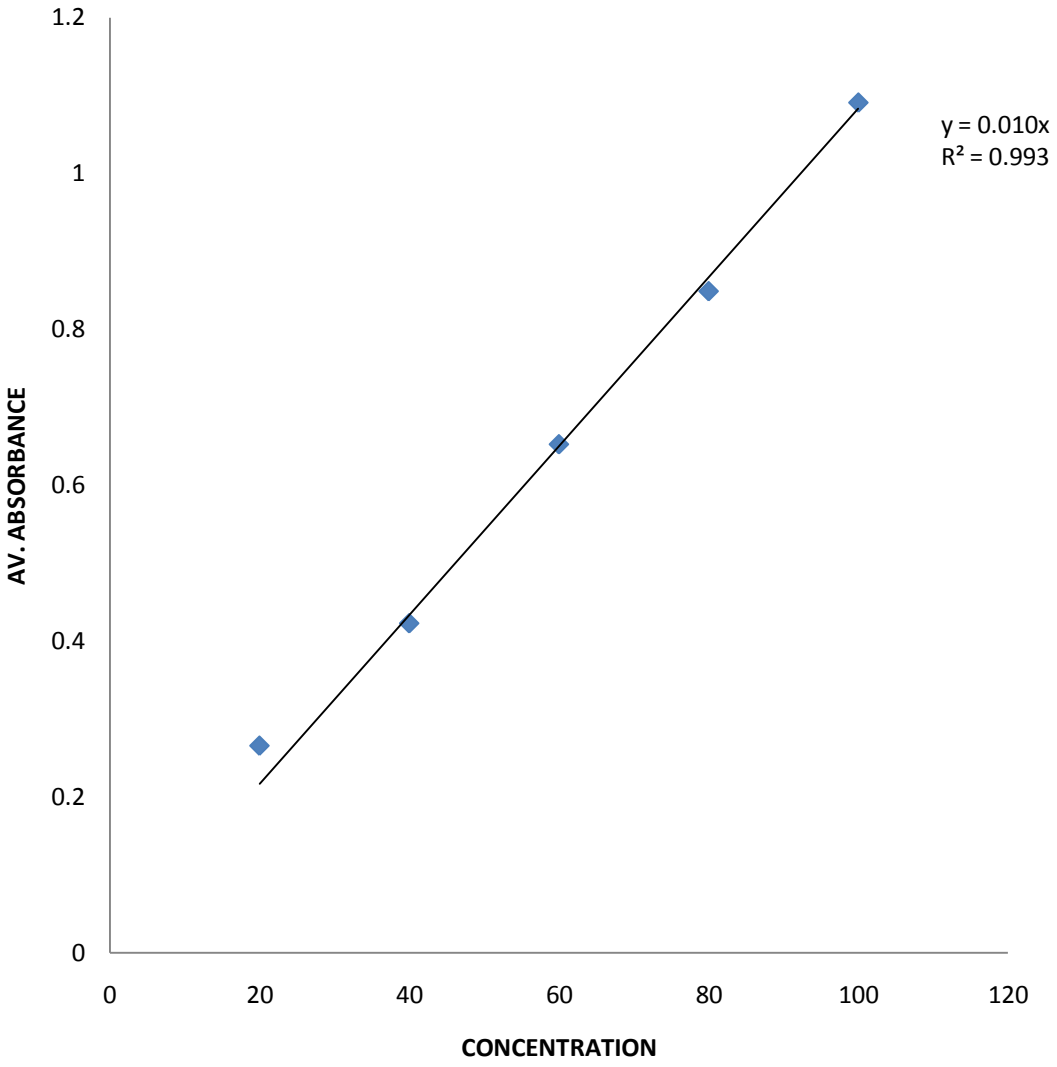
DP * = Degree of polymerization. Adapted from FAO Corporate Document Repository: Carbohydrates in Nutrition



Appendix B: Calibration curve obtained for caffeine



Appendix C: Calibration curve obtained for Aspartame



Appendix D: Calibration curve obtained for Sugar