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Assessment of Some Chemical Constituents in Selected Energy Drinks in Ilorin, Nigeria

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Abstract

The study compared and evaluated the physicochemical characteristics and some chemical components of some energy drinks. Samples of five (5) different energy drink brands were bought at random. The physicochemical characteristics of all the samples (pH, turbidity, conductivity, and total dissolved solids), as well as the levels of aspartame, sugar, and caffeine, were examined. The physicochemical properties (pH, turbidity, conductivity, and total dissolved solids) were 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. The values were found to be moderate and it was found to be within the FDA's suggested range. All energy drinks contained iron, calcium, zinc, and potassium, with concentrations ranging from 2.0 to 2500 mg/L, 2.763 ± 0.0009 - 19.310 ± 0.0015 mg/L, 0.045 ± 0.0001 - 13.887 ± 0.0037 mg/L, and 1.961 ± 0.0003 - 0.294 ± 0.0005 mg/L, respectively. Energy drinks had concentrations of copper, lead, and manganese ranging 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. While lead had a value over the maximum contaminant level (MCL) of 0.01 mg/L, copper and manganese concentrations were below the maximum contaminant level of 1.0 mg/L and 0.05 mg/L, respectively. With the exception of sample E, which had a value of 0.102 ± 0.0003 mg/L and was higher than the maximum contaminant level (MCL) of 0.005 mg/L, cadmium was not found in any energy drink. The concentrations of sugar, aspartame, and caffeine were 1.11 mg/L to 2487.13 mg/L, 6.51 mg/L to 1491.19 mg/L, and 16.98 mg/L to 1686.73 mg/L, respectively. The amounts of aspartame and caffeine in each energy drink sample were less than the FDA's recommended levels of 400 mg/L and 3000 mg/L, respectively. It is crucial to keep an eye on the consumption pattern of these drinks in order to reduce the risk of consuming excessive amounts of dangerous ingredients and avoid the side effects that have been documented.

Keywords: Energy drinks, chemical constituents, physicochemical parameter, Ilorin

INTRODUCTION

Energy drinks refer to drinks with high caffeine content and other legal stimulants that are thought to be an energy source, such as taurine, carbohydrates, glucuronolactone, inositol, niacin, panthenol, and β-complex vitamins (Attila and Çakir, 2009). The majority of consumers of easily available energy drinks are young adults, and their usage has increased dramatically. Energy drinks have a history that began in 1987 when Red Bull was released in Austria. After being introduced to the US, its popularity increased in the 1990s. The sales of this drink have skyrocketed since then. The market for energy drinks increased by 80% in 2006. (Foran *et al.*, 2011). This is due to the manufacturers' claims that the drinks can increase physical endurance, focus, and reaction time in addition to increasing energy levels (Van den

Eynde et al., 2008). Energy drinks of many kinds have been available in Nigerian markets in recent years, either as nutritional supplements or as an energy boost. The target market for these beverages is children and young adults. There are several uses for these goods. According to a survey done among college students, 67% of them said they used energy drinks to deal with getting too little sleep, 65% said they used them to boost their energy, and 54% said they used them for fun at parties; 50% said they used them for studying or finishing a big course project; 45% said they used them while driving for an extended amount of time; and 17% said they used them to cure hangovers (Malinauskas et al., 2007).

These goods have also been employed to raise one's social standing or lessen the depressant effects of drinking (Ferreira et al., 2004; Kaminer, 2010). Numerous energy drinks are marketed as nutraceutical meals that enhance health, provide energy, or have other desirable qualities. Health experts are a little concerned that these drinks and the drinking habits of the intended audience could be harmful to their health. According to Clauson et al. (2008), tachycardia, headache, sleeplessness, and anxiousness are the most often reported side effects. In a recent study, hospitalization of people with pre-existing mental illness and new onset seizures in four patients (Iyadurai and Chung, 2007) were linked to heavy energy drink usage (Chelben et al., 2008). In response to customer desire for a dietary supplement that would result in greater energy, energy drinks initially made their appearance in Europe and Asia in the 1960s (Reissig et al., 2008). One of the first energy beverages, Lipovitan D, was introduced in 1962 by Taisho Pharmaceuticals, a Japanese business, and it continues to dominate the Japanese market. The market for energy drinks has expanded into a multimillion dollar sector since the 1960s and is said to be the beverage industry's fastest-growing section. Energy drinks are widely used in the morning, afternoon, and night by consumers in general and by those between the ages of 18 and 34 in particular, indicating that they have established a viable position in the beverage market. (Lal, 2007).

Obuzor and Ajaezi (2010) examined the pH, conductivity, turbidity, total dissolved solids, total soluble solids, level of bitterness, reducing sugar, vitamins A and C, and minerals of five well-known commercial brands of malt drinks made in Nigeria. The results showed that the pH range for malt drinks was 4.4–4.6, with the highest conductivity of 2.93 μS/cm and a TDS of 1480 mg for Maltina. The levels of bitterness varied between 11–13 Bu (Grand Malt) and 15–17 Bu (Amstel Malta). The reducing sugar content was high, ranging from 693.45 to 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. Al-Mayaly (2013) reported on the concentration of heavy metals in 20 samples of various artificial fruit juices found in Iraqi marketplaces. The findings showed that 100% of the examined samples had levels of cadmium and copper that were over regional and global allowable limits, whereas 60% of the samples had acceptable nickel levels. Additionally, it was discovered that 15% of the examined samples had high lead contents above the Iraqi norm and that almost 35% exceeded WHO limit values. For each sample, the zinc values remained within the permissible range.

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 metals concentration limit.

Energy drinks have established an enviable position in the beverage market as evidenced by their commonplace consumption. There are several 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 a need to quantify the major content of these energy drinks and compare with those of accepted standards. Also, energy drinks are mostly imported or locally produced. There has been little or no research on the locally produced products as more attention has been given to those imported.
Determining the amount of caffeine, aspartame, and other energizers in locally made goods and contrasting them with those imported is therefore essential. Determining the energy drinks' additional physicochemical characteristics will also be important.

METHODOLOGY

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-dinitrosalicylic acid , Sucrose stock solution solution.

Apparatus
Spectronic 20 spectrophotometer and cuvette, Digital pH meter digital (JENWAY 3505), Digital TDS/conductivity meter Sensation 5, Digital Turbidity Meter (HACH DR/890, Colorimeter pH Meter (Orion 320), Digital TDS/conductivity Meter (HACH), HPLC L-2200, Auto Sampler from Hitachi, Hitachi pump L-2130, Oven L-2300 with Hitachi UV-VIS detector L-2400, Dell display And Laser Jet O1006 inkjet printer, Chromatographic examination was performed isocratically using the following: Wavelength: 214 nm Filter Column: Waters Spherisorb C18, 5μm ODS2, 4.6 x 250 mm, Flow rate 1.0 ml/minute, mobile phase: 125 ml methanol and 225 ml acetonitrile in 650 ml of Buffer, Pyrex digestion flasks, Sonnicator/vortex mixer Pasteur pipettes and bulbs Mohr pipettes And bulbs.

Preparation of stock solution

Preparation of standard solution for AAS
A stock solution of each of the elements 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 of 0-10 μg/ml in 100 ml volumetric flasks were prepared for each of the elements. This was used to prepare a standard calibration curve for each element.

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 stock standard. 4 ml was pipetted into a 25 ml volumetric flask, and distilled water was added to the mark. Pipetted into a 10-milliliter volumetric flask, the intermediate standard (1 ml, 2 ml, 3 ml, 4 ml, and 5 ml) was then made up to mark with distilled water and labeled as the 1st, 2nd, 3rd, 4th, and 5th levels, respectively. The Whatman filter paper was used to filter each level into a beaker or centrifuge bottle. In triplicate, the filtrate was injected into HPLC after being moved into auto-sampler vials with a stopper. A standard calibration curve was plotted using the absorbance values for the standard solutions.

Preparation of standard solution for UV

4 stock solution was made by dissolving 1 g of sucrose at a concentration of 10 g/dm^3 in a 100 ml volumetric flask of distilled water. The stock solution was appropriately diluted to create sucrose-standard solutions. A 20-milliliter stock solution was pipetted into a spotless 100-milliliter volumetric flask. The calibration mark was depleted by approximately 1 centimeter after adding distilled water. Water was gradually added with a Pasteur pipette until the meniscus's bottom was precisely on the line. After wrapping the flask in Par film, give it a good shake to combine. 40:100, 60:100, and 80:100 dilutions were made similarly. A big test tube was filled with two milliliters of each sucrose standard, and another test tube was filled with two milliliters of distilled water to serve as the blank solution. Each test tube was pipetted with two milliliters of 6 M HCl, and it was then submerged in a bath of boiling water for ten minutes. After removing the test tubes, 2.00 ml of 0.050 M 3, 5-dinitrosalicylic acid (DNSA) and 8 ml of 2.5 M NaOH were gently pipetted into each test tube. After adding the DNSA, the tubes were put in a boiling water bath for five minutes, and the mixture was completely mixed by shaking the tubes. Every tube was designed to spend the same length of time in the boiling water. The test tubes were promptly placed in an ice-water bath for ten minutes after being removed from the boiling-water bath at the appropriate time. The prepared and blank standard solutions were added to a dry, clean cuvette, which was then put in a spectrometer to measure absorbance. A standard calibration curve was created by recording the absorbance for the standard solutions.

Sample collection

Five (5) samples of various energy drink brands that were chosen at random from the market and examined. The samples were chilled before being examined. The samples were given the following labels: A, B, C, D, and E.

Sample preparation for AAS

The energy drink samples were shaken before opening. The samples (30 ml) were weighed out and transferred into a dry, 250 ml Pyrex digestion flask. 25 ml of concentrated aqua regia was added. The digesting flask was gradually heated until the foaming stopped. Once the samples were completely dry, they were diluted in 30 milliliters of distilled water and passed through filter paper. In a 100 ml flask, the solution was added up to volume.

Sample preparation for HPLC

Before being opened, the energy drink samples were shaken. Using a vortex mixer/sonicator, 2 ml of the samples and 5 ml of deionized water were added, and the mixture was agitated for 5minutes. Deionized water was used to make them up to par, and whatman filter paper was used for filtering.

Sample preparation for UV

A clean 100 ml volumetric flask was pipetted with 2 ml of each energy drink. To a point approximately1 centimeter below the calibration mark, distilled water was added. Water was gradually added with a Pasteur pipette until the meniscus's bottom was precisely on the line. Each of the diluted energy drink samples was processed in the same way as the standards using aliquots (2 ml).

For every dilution, the determination was made in triplicate

Physicochemical Parameters Analysis

pH Determination

Using a digital pH meter (JENWAY 3505), the pH was measured. Before using the probe on the sample, it was properly cleaned with distilled water. 50 milliliters of each energy drink sample were put in a beaker, the pH meter probe was inserted, and the pH values were noted.

Conductivity Determination

A digital TDS/conductivity meter (HACH) Session 5 was used to measure the electrical conductivity. Before using the probe on the sample, mixer, or sonicator, it was completely cleaned with distilled water and put up to specification. The TDS/conductivity meter probe was introduced into each 50 ml energy drink sample, which was then placed in a beaker, and the conductivity readings were noted.

Turbidity Determination

A digital turbidity meter (HACH DR/890 Colorimeter) was used to measure the turbidity. Before using the probe on the sample, it was properly cleaned with distilled water. 50 cc of each energy drink sample were put in a beaker, the digital turbidity meter's probe was inserted, and the turbidity readings were noted.

Total dissolved solid Determination

The measurement of total dissolved solids was made with a digital TDS/conductivity meter (HACH) Sension 5. Before using the probe on the sample, it was properly cleaned with distilled water. 50 milliliters of each energy drink sample were put in a beaker, the TDS/conductivity meter probe was inserted, and the TDS results were noted.

Elemental Analysis

Cu, Zn, Pb, Mn, Ca, P, Cd, and Fe were determined using an atomic absorption spectrophotometer, while K was determined using flame emission spectroscopy. Standard solutions were used to standardize and calibrate the instruments. The concentration of metals in each sample was measured separately after confirming that the instrument was calibrated correctly and that the standard values were within the confidence limit.

Determination of Carbohydrate (Sugar)

Miller (1959) described the spectrophotometric (colorimetric) method used to measure the amount of sugar (carbohydrates) in energy drinks. The color that results from sugars reducing 3,5-dinitrosalicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid is the basis for the procedure. The concentration of sugar was ascertained by applying the Beer-Lambert rule to the measured concentration of the colored product that resulted from the reaction of glucose with DNSA.

Determination of Caffeine and Aspartame
The National Agency for Food and Drug Administration Control (NAFDAC) revealed the use of high performance liquid chromatography to assess the presence of caffeine and aspartame in energy drinks.

RESULTS AND DISCUSSION

Physicochemical Parameters of Samples
The mean concentration ± SD of the physicochemical parameters. Table 1.1 displays the variables (pH, turbidity, TDS, and conductivity) of the energy drinks that were sampled.

Table 1.1: Energy drink pH, turbidity, total dissolved solids (TDS), and conductivity

Samples	Sample type	Ph	Turbidity (NTU)	TDS (mg/L)	Conductivity (µS/cm)
A	Liquid	4.53±0.012^{c}	126±1.732^{h}	473±0.577^{d}	1025±1.732^{f}
B	Liquid	5.18±0.023^{h}	68±0.577^{d}	914±2.309^{j}	1881±0.577^{l}
C	Liquid	4.47±0.012^{a}	82±2.209^{g}	550±0.577^{e}	1151±0.577^{g}
D	Liquid	4.54±0.012^{b}	592±1.155^{j}	243±0.577^{a}	487±1.155^{b}
E	Liquid	4.60±0.006^{c}	450±0.577^{i}	477±1.732^{c}	999±1.732^{e}

The result shows the mean ± SD of the pH of the sampled energy drinks. The pH ranged from 4.47 ± 0.012 - 5.18± 0.023. Sample C had the lowest pH while sample B 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 range 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). 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).

The turbidity of energy drinks ranged from 68 ± 0.577 – 592 ± 1.155 NTU with sample B having the least and sample D having the highest turbidity. These are lower than the results reported by Obuzor and Ajaezi (2010) for malt drinks which had turbidity values above detection limits (>1000NTU). Sample B was the least turbid while sample D had the highest turbidity. Turbidity is the measure of the degree to which water loses its transparency due to the 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.

The total dissolved solids (TDS) ranged from 243 ± 0.577 – 914± 2.309 mg/L as shown in Table 1.1. These values were within the range of 327.37 – 1480 mg/L reported by Obuzor and Ajaezi (2010) for malt drinks. Sample B had the highest TDS while sample D 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.

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 487±1.155 - 1881 ± 0.577 µs/cm. These values were similar to 2.93 – 1999 µs/cm reported by Obuzor and Ajaezi (2010) for malt drinks. Sample D is least conductive while sample B has the highest conductivity.

Heavy metal concentration
Table displays the mean concentration ± standard deviation of the heavy metals (cadmium, copper, zinc, lead, and manganese) in the energy drinks that were sampled.

Table 1.2: Energy drink concentrations of cadmium, copper, zinc, lead, and manganese

Samples	Sample type	Cd (mg/L)	Cu (mg/L)	Zn(mg/L)	Pb (mg/L)	Mn (mg/L)
A	Liquid	ND	ND	0.061±.0005	ND	0.002±.0001
B	Liquid	ND	ND	0.051±.0004	0.045±.0005	ND
C	Liquid	ND	0.041±.0003	0.174±.0004	0.073±.0003	0.007±.0005
D	Liquid	ND	0.002±.0002	0.049±.0009	0.054±.0010	ND
E	Liquid	ND	0.007±.0002	0.085±.0002	0.028±.0005	ND

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). Table 1.2 shows the mean ± SD for the concentration of heavy metals in the sampled energy drinks. Cadmium was not detected in all energy drinks and it was a great indication that the energy drinks are safe to consume. A 0.01mg cadmium level in an energy drink was 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.

The copper concentration of energy drinks ranged from 0.041 ± 0.0003 - 0.002±.0002 mg/L. Sample D had the lowest concentration while sample C had the highest concentration of copper. Copper was not detected in samples A and B as shown in Table 1.2. 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 the non-alcoholic beverage. The samples had concentrations below the MCL of copper of 1.0 mg/L (WHO 1993).

Zinc is such a critical element in human health that, 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 of zinc was within in range of 0.049± 0009- 0.174±.0004 mg/L. Sample C had the highest concentration while sample D had the least concentration. These values were similar to those reported by Bengol *et al.,* (2010) and Krejpcio *et al*. (2005). The zinc concentration of all sampled energy drinks was below the MCL 5.0 mg/L of zinc

(WHO, 1993).

Lead concentration of energy drinks ranged from 0.028 ± 0.0005 - 0.073±.0003 mg/L with sample E having the least and sample C having the highest concentration. Lead was not detected in sample A as shown in Table 1.2. The lead concentration of energy drinks was lower than the 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 ± 0.08 ppm in fruit juice in Nigeria. Maduabuchi *et al.,* (2006) also reported lead levels of 0.002 – 0.0076 mg/L in canned drinks and 0.092 mg/L in non-canned drinks. These were similar to the values determined in energy drinks. Lead detected in samples was above the MCL of 0.01 mg/L (WHO 1993).

Manganese was not detected in all the samples except for samples A and C as shown in Table 1.2. Their concentration ranged from 0.007 ± 0.0005 - 0.002 ± 0.0001 mg/L. 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). The concentrations of manganese in all the samples detected were below the MCL of 0.05 mg/L (WHO, 1993).

Concentrations of essential metals
The mean concentration ± SD of essential metals (iron, calcium and potassium) Table 1.3 displays the energy drinks that were sampled.

Table 1.3: Concentrations of iron, calcium, and potassium in energy drinks

Samples	Sample type	Fe (mg/L)	Ca (mg/L)	K (mg/L)
A	Liquid	0.534±.0008	13.667±.00197	35.00
B	Liquid	0.372±.0006	19.310±.0015	1500.00
C	Liquid	0.480±.0008	13.143±.0021	3.50
D	Liquid	0.316±.0005	19.310±.0029	5.00
E	Liquid	0.789±.0005	6.206±.0010	80.00

Mineral elements are important building blocks needed for regenerating tissues such as blood and bone. Minerals are inorganic substances essential for organ systems and the entire body (Ryan-Harshman and Aldoori, 2005). Some of these minerals, such as calcium, exist in large amounts in our body, 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 1.3 shows the mean ± SD for the concentration of essential metals in the sampled energy drinks.

The iron level of energy drinks ranged from 0.316±.0005 mg/L -0.789±.0005, with sample E having the highest value and sample D having the least as shown in Table 1.3. 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 concentrations higher than the MCL of 0.03 mg/L (WHO, 1993).

The calcium concentration of energy drinks ranged between 6.206±.0010- 19.310 ± 0.0015 mg/L, with sample E having the least and samples B and D share the same value as highest as shown in Table 1.3. All other samples varied in their concentration of calcium. The calcium concentration of energy drinks was low compared to 0.28 – 262 mg/L reported by Obuzor and Ajaezi (2010).

Potassium concentrations of the energy drinks were relatively low compared to the recommended daily intake (RDI) of potassium (WHO, 1993). It ranged from 3.0 to 1500 mg/L as shown in Table 1.3. The RDI of potassium ranged between 1600-5000mg/day. Only sample B had a concentration of 1500 mg/L and was close to the recommended RDI of potassium. All other samples were low, ranging between 3.00 to 735 mg/L. Potassium is the major intracellular ion, intimately related to sodium movement out of the cell via Na/K ATPase.

Concentrations of Caffeine, Aspartame and Sugar

Table 1.4 displays the caffeine, aspartame, and sugar concentrations of the sampled energy drinks.

Table 1.4: Sugar, caffeine, and aspartame content

Samples	Sample Type	Sugar (mg/L)	Caffeine (mg/L)	Aspartame (mg/L)
A	Liquid	942.90^{i}	67.08^{c}	624.84^{g}
B	Liquid	936.73^{i}	30.94^{b}	532.23^{f}
C	Liquid	938.27^{i}	200.10^{h}	788.13^{h}
D	Liquid	1686.73^{k}	190.22^{g}	876.06^{i}
E	Liquid	845.68^{f}	1.11^{a}	956.82^{j}

The caffeine and aspartame concentrations of the energy drinks were calculated using calibration curves obtained from the caffeine and aspartame analysis using HPLC. The sugar concentration of the energy drinks was calculated using calibration curves obtained from the sugar analysis using a UV spectrophotometer in Appendix C.

Caffeine

The mean ± SD concentrations of caffeine as determined in each of the sampled energy drinks are shown in Table 1.4. The results obtained showed that caffeine concentrations ranged from 1.11 mg/l – 200.10 mg/L. These were within the range of 170 ppm – 324 ppm for caffeine concentrations in energy drinks reported by Mei *et al.,* (2012), and lower than the range of 440 ppm – 473 ppm for caffeine concentration in tea samples reported by Mei *et al.,* (2012). One of the values was similar to 1.41 mg/serving reported by Rachel *et al.,* (2006) and those reported by Alghamdi *et al.,* (2005). Sample E had the lowest caffeine concentration while sample C had the highest caffeine concentration. 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, the 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, flavored nonalcoholic 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 Nigeria, there have been no set limits by the food regulatory bodies for caffeine in energy drinks as the majority of the energy drink products are usually imported into the country. As such, they usually used the set standards by international bodies such as the FDA. The acceptable daily intake of caffeine is 400 mg/day (US FDA, 2010). Caffeine concentrations in all the energy drinks sampled were below the FDA set standards except. A possible reason for this may be its usage as an analgesic. This implies that a daily consumption of one can of any of the sampled energy drinks, may not have any adverse effect on the consumer. However, daily consumption of two or more cans of the energy drink may have adverse effects as reported in the literature, especially on children and pregnant women.

Aspartame

The concentration of aspartame as determined in each of the sampled energy drinks is given in Table 1.4. The results obtained show that aspartame concentration ranged from 532.23 mg/L – 956.82mg/L. These values were different with 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 higher compared to 80.29 – 435.05ppm reported for fruit juices and 156.98 – 554.35 ppm reported for powdered drinks. The values were also higher 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 B had the lowest aspartame concentration while sample E had the highest aspartame concentration. 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). 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 unless multiple of drinks are consumed.

Sugar

The concentration of sugar as determined in each of the sampled energy drinks is given in Table 1.4. The results obtained show that the sugar concentration in the sampled energy drinks ranged from 845– 1686.73 mg/L. Frequent consumption of sugar-containing foods can increase the 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 based on 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).

CONCLUSION

The general discussion about the advantages and disadvantages of energy drinks has picked up steam recently. Researchers studying health issues concur that consuming large amounts of caffeine can have negative effects on one's health. Increased blood pressure, increased stomach acid, increased anxiety, panic episodes, irritation in the bowels, and insomnia are some of the most frequent side effects.

This study examined the amounts of sugar, aspartame, and caffeine in energy drinks sold in Nigeria. The energy drinks' caffeine contents were found to range from 1.11 mg/L to 2847.13 mg/L. All of the samples had caffeine concentrations less than the 400 mg daily threshold established by the FDA. Additionally, allegations have been made concerning the safety of aspartame, the main artificial sweetener included in most beverages. Energy drinks ranged in aspartame content from 6.5 mg/L to 1491.19 mg/L. This is less than the combined WHO/FDA approved daily intake (ADI) of aspartame, which is 40–50 mg/kg per body weight. This translates to 2400–3000 mg per day for an adult weighing 60 kg. All of the energy drink samples contained sugar, with concentrations ranging from 16.98 to 1686 mg/L. There is ample evidence that sugar has negative health impacts. However, the recommended daily intake (ADI) of sugar has no predetermined upper limit. Every energy drink contained potassium, zinc, iron, and calcium. These are necessary components for the body's overall health. Despite having lower levels than the permissible total intakes, they nonetheless contribute to the body's daily requirements for potassium, calcium, zinc, and iron. It was not possible to find cadmium in every sample. Cadmium is a non-essential element that only has major negative consequences. Energy drinks have lead concentrations between 0.028 ± 0.0006 and 0.209 ± 0.0009. Sample A did not contain any lead. Samples had lead levels above the MCL of 0.01 mg/L. Lead is a non-essential element that only has major negative consequences. Two (2) out of the five (5) energy drink samples had manganese in them. The concentration was below the manganese MCL, ranging from 0.003 ± 0.0001 to 0.024 ± 0.0002. Energy drinks had copper concentrations between 0.002 ± 0.0002 and 0.102 ± 0.0003. These were less than the 1.0 mg/L MCL for copper. Additionally, the physicochemical characteristics including conductivity, turbidity, pH, and total dissolved solids were examined. These met the requirements set forth by authorities including the FDA and WHO.

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