DETERMINATION OF CAFFEINE IN BEVERAGES AND SOFT DRINKS

BY

JAMILU ABDULRAUF

B.Sc Applied Chemistry

SPS/12/MCH/00008

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, BAYERO UNIVERSITY KANO, IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.), DEGREE IN ANALYTICAL CHEMISTRY

SEPTEMBER, 2016

DECLARATION

I hereby declare that this work is the product of my own research effort, undertaken under the supervision of Prof M. I. Muhammad and has not been presented and will not be presented elsewhere for the award of a Degree or Certificate. All sources have been duly acknowledged."

_____

Jamilu Abdulrauf

SPS/12/MCH/00008

_____ _____

Dr. H. Musa **Signature/Date**

Head of Department

CERTIFICATION

This is to certify that this Dissertation has been examined and approved for the award of the Degree of Masters of Science (M.Sc.) in Analytical Chemistry.

_____ _____

Prof. M. I. Muhammad **Date**

(Supervisor)

APPROVAL

This research work titled "DETERMINATION OF CAFFEINE IN BEVERAGES AND SOFT DRINKS" by Jamilu Abdulrauf; SPS/12/MCH/00008 has been duly read and approved as meeting the requirements for the award of Master of Science (M.Sc.) Degree in Analytical Chemistry.

Prof. U.A Birnin Yawuri External Examiner	**Signature/Date**
Dr. M.D Saeed Internal Examiner	**Signature/Date**
Prof. M.I Muhammed Supervisor	**Signature/Date**
Dr. Haruna Musa H.O.D/Chief Examiner	**Signature/Date**
Dr. Nafi'u Hussain PG Rep.	**Signature/Date**

DEDICATION

This research work is dedicated to my late father Alh. Abdulrauf may his gentle soul rest in Aljannatul fir´daus amiin, and my caring mother Malama Maryama Abdulrauf for her support and motherly care throughout my activities may Allah (SWT) reward her with Aljannatul firdaus amin.

AKNOWLEDGEMENT

In the name of Allah (S.W.T) the most Beneficent and the most Merciful, All praise be to Allah the Lord of the entire universe Who taught mankind how to use pen. His blessing and salutation be upon Muhammad (S.A.W), his progeny and companions and his followers up to the day of Judgement.

My sincere gratitude goes to my Supervisor Prof. Musa Ibrahim Muhammad, an intelligent, experienced and most accommodating teacher, whose suggestions and objective criticisms made this work into reality.

My regards also goes to my lecturers Prof. A.A Audu| Prof.M.A Kurawa|Dr.H. Musa|Dr. S.M Dagari|Prof. M.B Ibrahim,|Dr Umar Sani|Dr M.D Saeed|Dr. Umar Sani and the rest of the lecturers in the Department.

My sincere greetings go to my principal, Alhaji Lawal Musa, his two vice principals: Mal. Aminu Kado and Mal. Habibu Abdulkadir, for their strong support, encouragement and exhortations throughout my study. May Allah (SWT) reward them with Aljannatul- Firdaus, amiin.

I also acknowledge the effort made by my wife Malama Safarau for adjusting her needs to ensure the success of the study. May Allah (SWT) reward her with Aljannatul firdaus amiin. I thank my elder and younger brothers and sisters for their prayers and financial assistance, may Allah (SWT) bless them abundantly amiin.

TABLE OF CONTENT

ABSTRACT

This study was carried out to determine the pH and level of caffeine in various brands of soft drinks, energy drinks, tea and coffee-based beverages available in Kano state, Nigeria. Different brands of these products were purchased from different shops in Kano metropolis. Caffeine was carefully extracted from each product and analysed by ultraviolet/visible spectrophotometric methods. The results showed that the caffeine content of the soft and energy drinks ranged from 9.01 to 12.20mg/100mL, and 34.65 to 40.88mg/100mL respectively. While INS and RGL coffee ranged from 34.15 to 47.16mg/100mL and 9.19 to 40.64mg/100mL respectively. The mean caffeine content of tea-based beverages analysed from six different countries ranged from 10.876 to 24.459mg/100mL in Hong Kong and Nigeria samples respectively. From the analysis of variance, the results obtained did not shows any significant differences. Like-wise the mean pH of carbonated soft and energy drinks were highly acidic ranging from 3.178 to 3.406 respectively, while that of coffee and tea samples were generally low ranging from 5.86 to 6.40 respectively. The reason behind the low pH values in carbonated drinks could be as a result of the presence of carbon(iv) oxide gas or other acids such as phosphoric acid, malic acid, ascorbic acid, citric acid and tartaric acid used as preservatives by the manufacturers of these product.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Beverages

A beverage is a drink specially prepared for human consumption either at meal or leisure times (Ogah and Obebe, 2012). There are varieties of beverages that can be broadly classified in to alcoholic and non-alcoholic beverages. Alcoholic beverages contain alcohol in varying proportion while the non alcoholic beverage comprises of soft drinks, energy drinks, fruit juices and hot beverages. Soft drinks, energy drinks, fruit juices may contain caffeine arising from the raw materials used for its preparation or from its deliberate addition. Hot beverages often contain caffeine. They are termed hot because they are usually served hot by addition of hot water or milk. This group consists of cocoa, tea and coffee based product which are commercially available in the Nigerian markets (Ogah and Obebe, 2012).

1.2 CAFFEINE

Caffeine (1,3,5,-trimethyl xanthine),is a naturally occurring alkaloid found in tea leaves, kola nut, cocoa beans, coffee and so on. It is used as a flavouring agent in a variety of beverages, including some soft drinks and energy drinks (Dobrina et al., 2012)

Caffeine (Fig.1.1) causes various physiological effects, such as relaxation of bronchial muscle, stimulation of central nervous system, gastric acid secretion and dieresis. (Dobrinas et al., 2012)

Table 1.1: Properties OF Caffeine

Molecular formula	$C_8H_{10}N_4O_2$
Molar mass	194.19g/mol
Appearance	Odourless, white powder
Density	$1.23g/cm^3$
Melting point	$227\text{-}228^oC$

Sources: Wanyika et al., 2010

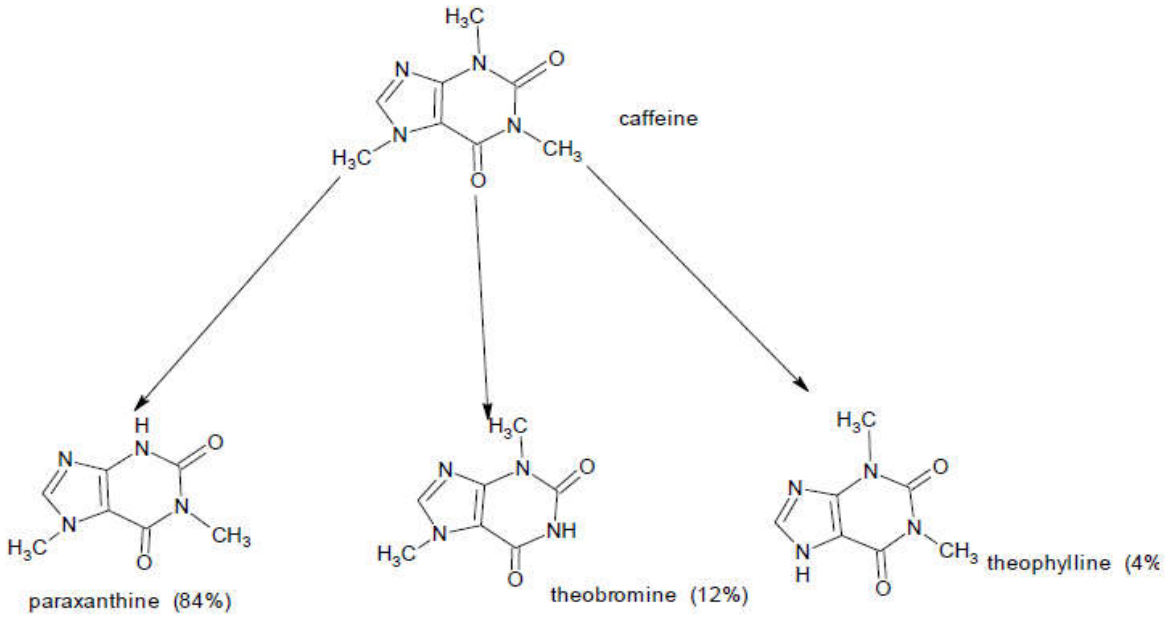


Figure 1.1: Caffeine and its main metabolic product (Wanyika et al., 2010)

1.3 Tea (*Camellia Sinensis L.*),Hausa name: (Ganyen shayi):

Tea is grown on different land elevation which is the oldest and best beverage in the world next to water (Plate.1.2). There are 3 types of tea: green, oolong, and black. Green and Oolong tea are the most widely consumed beverages in Asian countries and has been familiar in China and Japan from centuries. Oolong tea is semi-fermented during processing, whereas green tea is not fermented and black tea is fully fermented. Semi-fermented tea is especially good for digestion; hence it is advisable to have after a large

meal. It is also useful for diabetes patients by reducing high blood pressure, cancer, weight loss etc (Rahman et *al.,* 2013).

The chemical composition of semi-fermented oolong tea is counted between the ranges of non-fermented green tea to fully fermented black tea. The tea liquor contains quantitatively important polyphenols, minerals, alkaloids, carbohydrates, proteins, and amino acids, vitamins and traces of lipids. The major constituents of green and oolong tea include theaflavins, thearubigins, ascorbic acid, polyphenolase enzyme, and flavanols. (Rahman *et al.,* 2013).

There are two major kinds of tea, black tea and green tea.The Caffeine content is 1 to 5% of its dry weight depending on type, brand and brewing method. The polyphenol contents are one of the major constituents of tea which is reported to account for up to 40% of the dry weight. The lipids content in tea has a significant difference due to variety and climatic difference moreover the region also influence the results. Lipids present in the protoplasm up to 6 to 10% by weight may or may not have any significant role of quality in the manufacturing process. The most commonly known sources of caffeine are coffee, and tea leaves (Rahman *et al.,* 2013).

1.4 Coffee beans

Coffee is the most important traded commodity in the world after oil (Plate.1.3). It is also one of the most popular beverages in the world for its characteristic taste, aroma and for its potential beneficial effects on human health. The name coffee probably originated from the name of the province ‘Keffa’ where shepherds from Abyssinia/Ethiopia discovered the coffee beans in the 6th century. In the 13th century coffee’s restorative powers were known and spread throughout the Islamic world. Two hundred years latter coffee was sold in Europe, thus introducing the new beverage into Western life and custom (Belete, 2008).

The popularity of coffee as a beverage is increasing, despite the fact that there are reports that it is not necessarily good for human health. There have been numerous reports on diseases associated with coffee consumption. Recent knowledge has put coffee in more positive light, and more and more reports suggest that coffee consumption has beneficial effects on human health (Belete, 2008). Coffee is commercially available as one of two varieties known as Arabica and Robusta. The coffee beverages are made from Arabica and Robusta roasted beans or blends of these two. Arabica coffees are generally considered of better quality and consequently they command higher prices. By taking this into account and considering that in recent times there is an increasing practice of selling coffees based on their variety and/or geographic origin, it is important to have methods to characterize these two varieties. (Belete, 2008).

1.5 Cola Drinks, Hausa name :(Lemun kwalba):

Cola like drinks account for 80 to 90% of the caffeine added to food today; these products with caffeine artificially stimulate the body and increase heart rate (Plate.1.3). While this artificial stimulation temporarily arouses the intellect and fatigue seems to disappear, its short lived. The excess stimulation depletes the body of vital energy as it struggle to deal with this poison that has entered its system. In the amount presently consumed, it can cause insomnia, nervousness, irritability, anxiety, and disturbance in the heart rate and rhythm. There are other effects from the consumption of caffeine including increased incidence of bladder and stomach cancer, raised of blood pressure and it aggravate diabetes and damage the lining of the stomach. (Violeta et al.,2010).Therefore the determination of caffeine compound in soft and energy drinks, coffee and tea samples for assurance of food safety and quality control is mandatory.

1.6 AIM AND OBJECTIVES

1.6.1 AIM: The aim of this research is to determine the level of caffeine in different brands of coffee, tea, soft and energy drinks.

1.6.2 OBJECTIVES:

i**.** To ascertain whether they are safe and healthy for their consumers.

ii. To determine the average caffeine level of each beverage under study in order to ascertain the level of caffeine in each.

iii. To compare between the caffeine level determined in the beverages, energy and carbonated soft drinks under study and the recommended level set by NAFDAC, and International food information council.

CHAPTER TWO

2.1 LITERATURE REVIEW

Amos-Tautua *et al* (2014), reported the ultraviolet spectrophotometric determination of caffeine in soft and energy drinks available in Yenagoa, Nigeria. They determined the pH and levels of caffeine in eight brands of carbonated soft and energy drink available in local markets in Yenagua, Nigeria. The quantitative analysis of caffeine was performed by UV spectrophotometric method, using carbon tetrachloride as an extracting solvent. Results show that the pH of the beverages were slightly acidic ranging from 4.53- 7.20. The minimum caffeine level was observed in the carbonated soft drink Coca-Cola (43.7$\pm$ 0.55ppm), the energy drink Red Bull showed the highest caffeine content (58.31$\pm$0.35ppm). The carbonated soft drinks showed caffeine levels in the range of 43.71 to 45.83ppm with average concentration of 44.52ppm, whereas in energy drinks it ranged from 47.56 to 58.31ppm with a mean concentration of 52.24ppm.

Alpdogan, *et al* (2002), reported the derivative spectrophotometric determination of caffeine in some beverages, they revealed a UV spectrophotometric method cannot be used directly for the determination of caffeine in beverages owing to the matrix effect of UV-absorbing substance in the sample matrix. To overcome this difficulty,some tedious background correction techniques have been used. The result obtained using derivative spectrophotometric have shown that the Cola has the highest proportion of caffeine ranging from 149.32$\pm$0.68µgml^{-1} compared to coffee and tea which have1.36$\pm$0.03% and 1.53$\pm$0.03% respectively.

The work of Oliveira (2010), for the quantification of catechins and caffeine from green tea (*camellia sinensis)* infusions, extract, and ready to drink beverages, using High performance liquid chromatography (HPLC).revealed that the tea bag had the highest concentration of

total catechins with 5 to 9.5% followed by the extract with 3.64 to 4.88% and ready to drink green tea beverage showed low levels of catechins from 0.14 to 0.26%. As for caffeine content, green tea extract had higher concentration (1.96 to 3.54%) compared to the tea bags (1.39 to 1.57%). Tea bags were found the most suitable for consumption because it contains higher amount of catechins and smallest amounts of caffeine.

Bal- Ram *et al* (1998), reported the determination of caffeine content in coffee using FT-IR spectroscopy in combination with attenuated total reflectance technique. The observed band at 1665cm^{-1} was used to estimate caffeine in standards and in the samples. The sensitivity of the technique was 5ppm.

Violeta *et al* (2010), reported the chromatographic determination of caffeine contents in soft and energy drinks available in the Romania market. Different brands of soft and energy drinks, carbonated beverages available in the Romania market were analysed for caffeine by HPLC with a diode array UV-visible detector at 217nm. The caffeine contents in energy drink samples ranged from 16.82mg/100ml to 39.48mg/100ml while the carbonated soft drink group showed caffeine content in the range of 9.79 to 14.38mg/100ml. In addition, the concentrations of caffeine have been converted into the daily intake doses based on beverages consumption. The mean values of caffeine daily intake were 124mg and 49mg through the ingestion of energy drinks and soft drinks respectively. violeta *et al*(2010)

Belete (2008), reported the determination of caffeine level of coffee using camag thin layer chromatography scanner 3 and quantitative proton Nuclear Magnetic Resonance (qHNMR) spectroscopy. The caffeine values of coffee, tea and soft drink samples quantified by both methods were found to be in agreement with those reported in the literature. Caffeine content were found to be: in green and roasted coffees (0.8-1.1%), tea (1.9-2.1%), soft drinks (5-7mg per 100ml), machine brewed coffees (80-103mg per 150ml) and home-brewed coffees

(Ethiopian traditional style) 12-39mg per 150ml. Both methods can be recommended for routine analysis.

Jenway (2014), reported the quantitative determination of Caffeine in beverages and soft drinks using UV wavelength spectroscopy, the results showed that the Nescafe has highest caffeine concentration of 74.8 ppm fallowed by red bull with 70.60 ppm the caffeine of P G tea, Coca-Cola, Pepsi cola were 49.20 ppm, 25.30 ppm and 27.90 ppm respectively. When ranking the drinks in terms of caffeine content per serving the order was Red Bull > Pepsi cola > instant coffee > coca cola > P G tea. Instant coffee and PG tea rise from third and fifth places to second and third respectively when the drinks are ranked in order of the caffeine content per ml.

Wanyika *et al* (2010), determined the level of caffeine content of tea and instant coffee brands found in Kenyan market using high performance liquid chromatography (HPLC) and UV-visible spectrophotometric methods. The levels of caffeine content in all the tea and coffee brands were found to be within the documented range. The order of caffeine concentration in tea samples was found as follows: Chaimaramoja > Finlays premium > Kericho gold > Sasini. In coffee. It was found that the caffeine content of africafe > Nescafe>dormans.

Ayala, *et al* (2009), reported the quantitative determination of caffeine and Alcohol in energy drinks and the potential to produce positive Transdermal Alcohol concentration in human subjects using gas chromatography/mass spectrometry (GC-MS), eleven energy drinks were quantitatively assayed for both ethanol and caffeine. Caffeine concentration of diluted samples ranged 2.74 to 5.31mg/L.

Tadelech, (2010), reported the characterization of caffeine and determination of caffeine in tea leaves using UV-visible spectrometer. In his research the optical transition properties of

caffeine were measured in different solvents (i. e dichloromethane, water, chloroform and ethyl acetate). The results showed that caffeine has the highest optical transition in dichloromethane than the other solvent.

Ogah and Obebe (2012),reported the uv/visible spectrophotometric determination of caffeine content of various brands of cocoa and coffee-based beverages available in Lagos,Nigeria.The result show that the caffeine content of the cocoa-based product ranged from 1.8 to 3.1 mg/g of the product while classic and decaffeinated coffee contained 8.4 and 2.3 mg/g of the product respectively.

Musallem and Alam (2013), reported the isolation and quantitative estimation of caffeine in two brand of coffee and three brands of tea available in the local markets of Muskat. The results showed that coffee contains higher amount of caffeine as compared to tea leaves. Caffeine content of coffee ranged from 39-41mg/g, while tea samples contained 20-26.5mg/g of the product respectively.

CHAPTER THREE

3.1 Materials and Methods:

All glassware were soaked overnight in the 0.2M nitric acid solution and washed thoroughly with water and detergent, then rinsed with deionised water dried in an oven at 105^{o}**C** and transferred to desiccators before used. The chemical and reagents used in this study were of analytical grade purity and no further purification was carried out and was obtained from sigma/Aldrich. Pure anhydrous caffeine was obtained from Sigma/Aldrich company. Different kinds of soft drinks, energy drinks, coffee and tea samples were obtained from different stores in Kano metropolis.

3.2 Sampling:

The samples of different types of soft drinks, energy drinks, tea, instant and regular coffees were obtained from different super Markets in Kano metropolis.

3.2.1 Sample preparation:

200cm^3 aliquots of boiling purified water was added to each of the 250cm^3 beakers containing 2g coffee or tea sample. The coffee or tea sample prepared were stirred for 2 minute and allowed to cool to room temperature. The soft and energy drinks were used as supplied by the manufacturers.

3.3 Preparation of reagents:

3.3.1 Na_2CO_3 (20%): 20g of powdered predried sodium trioxocarbonate (iv) was dissolved in a 250cm^3 beakers containing 40cm^3 of distilled water. On cooling the solution was transferred in to 100cm^3 volumetric flask and diluted to mark with distilled water.

3.4 Preparation of caffeine standard solution:

A 10mg/100ml stock standard of caffeine was prepared by dissolving 25mg of caffeine in 250cm^3 purified carbon tetrachloride (CCl_4) in a 250cm^3 volumetric flask. Working standards were prepared by pipetting 2, 4, 6,8,10 cm^3 aliquots of the stock standard solution in to separate 100cm^3 volumetric flasks and diluted to volume with carbon tetrachloride (CCl_4) to produce concentration of 2,4,6,8, and 10mg/100mL standard solution. The absorbance of each solution was measured at the wavelength of maximum absorption of 270nm using quartz curvette. The absorbance values were then plotted against concentrations to generate a standard calibration curve and the concentration of caffeine in the samples was obtained from it by interpolation.

3.5 pH determination:

pH of beverages was determined using Sartorius Jenway pH meter (3510 model), The pH of all the soft and energy drinks were determined by calibration the pH meter electrode and deep in to the portion of a liquid sample, while tea and coffee samples were boiled for two minute with distilled water(200cm^3) and allowed to cool to room temperature, portion of the liquid samples were used to determine the pH by dipping the pH meter electrode in to it.

3.6 Caffeine extraction procedure:

An aliquot (5cm^3) of the drink sample was drawn with 10cm^3 pipette and placed into a 125cm^3 separatory funnel followed by addition of distilled water (10cm^3), 1cm^3 of 20% aqueous sodium trioxocarbonate (iv) (Na_2CO_3) solution and 20cm^3 analytical grade CCl_4. The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion, The non aqueous CCl_4 layers was transferred to a clean 50cm^3 volumetric flask. Another 20cm^3 portion of CCl_4 was added to the aqueous solution in the separatory funnel and the extraction procedure was repeated twice more, and the solvent layer

combined. This volume was made up to 50cm^3 in the volumetric flask with the solvent. This procedure was repeated for all the drink, coffee and tea samples. The absorbance of each resulting solution was measured on UV/Visible spectrophotometer at 270nm using 10mm curvette. Sample blank was prepared as above without the sample.

3.7 Quantitative Caffeine Determination:

Quantitative analysis of caffeine was performed by 6405 Jenway UV/Visible spectrophotometer. The maximum wavelength was determined by scanning the standard solution from 200-600nm and the obtained results gave the highest absorption value at 270nm.Standard linear calibration curve was run to obtain the linear range of sample analysis, correlation factor was with accepted value =0.9989 and the standard calibration curve was linear over the range of (2-10) ppm caffeine with equation(y=0.0749x +0.0082).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

The caffeine contents of energy and carbonated soft drinks are presented in Figures 4.1 and 4.2 below. From the figures it can be seen that the caffeine content of energy drinks obtained in this research ranged from 34.20mg/100mL to 41.44mg/100mL. The minimum caffeine content was observed in RBE energy drink sample 34.65±0.45mg/100mL while RTE energy drink showed the highest caffeine content 40.88±0.56mg/100mL.The soft drink samples analysed showed the caffeine content in the range of 9.01±0.05mg/100mL to 12.20±0.03mg/100mL.The minimum caffeine content in soft drinks was observed in PCL 9.01±0.05mg/100mL while CCL showed the highest caffeine content of 12.20±0.03mg/100mL. The mean caffeine level in analysed energy drinks and carbonated soft drinks was found to be 34.78±0.31mg/100mL and 10.29±0.13mg/100mL (Fig.4.3).From the analysis of variance the results obtained did not shows any significant difference. These values could also be considered low and safe when compared with the recommended daily intake set by International food information council (300mg/day) IFIC (2009), but most of energy drinks analysed exceeded the limit set by NAFDAC (32mg/100mL) NAFDAC (2015). Various values have been previously reported for caffeine content in energy drinks and carbonated soft drink samples, which includes (Violeta *et al.*,2010),reported the caffeine contents of energy drink samples ranged from 16.82 mg/100mL to 39.48 mg/100mL while the carbonated soft drink samples showed caffeine content in the range of 9.79 – 14.38 mg/100mL,(Tautua *et al.*,2014) reported the caffeine content of energy drinks ranged from 47.56ppm to 58.31ppm while the carbonated soft drink samples showed the caffeine content in the ranged of 43.71ppm and 45.83ppm. All over the world, the caffeine contents of drink samples varies according to the type of brand analysed, Violeta *et al.*, (2010). Clearly, the caffeine mean level in the analyzed samples are less than the values reported by other researchers.

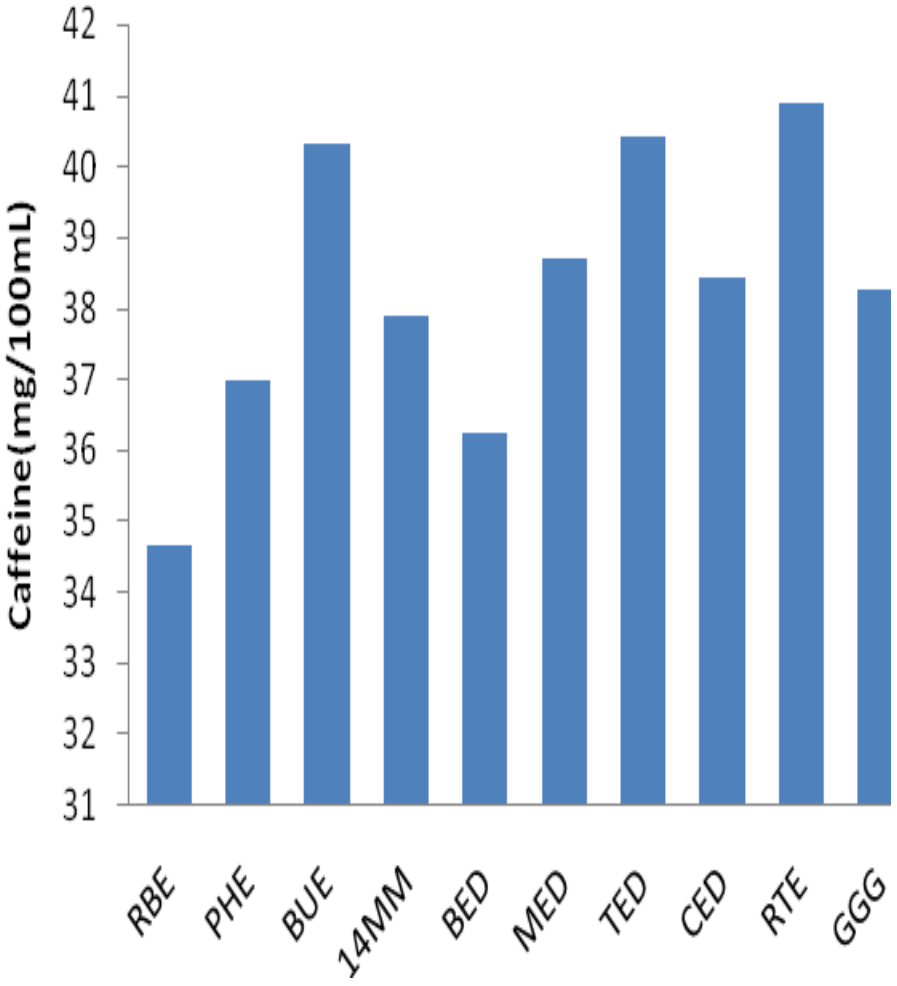


Figure:4.1 Caffeine Content of the Energy Drink Samples

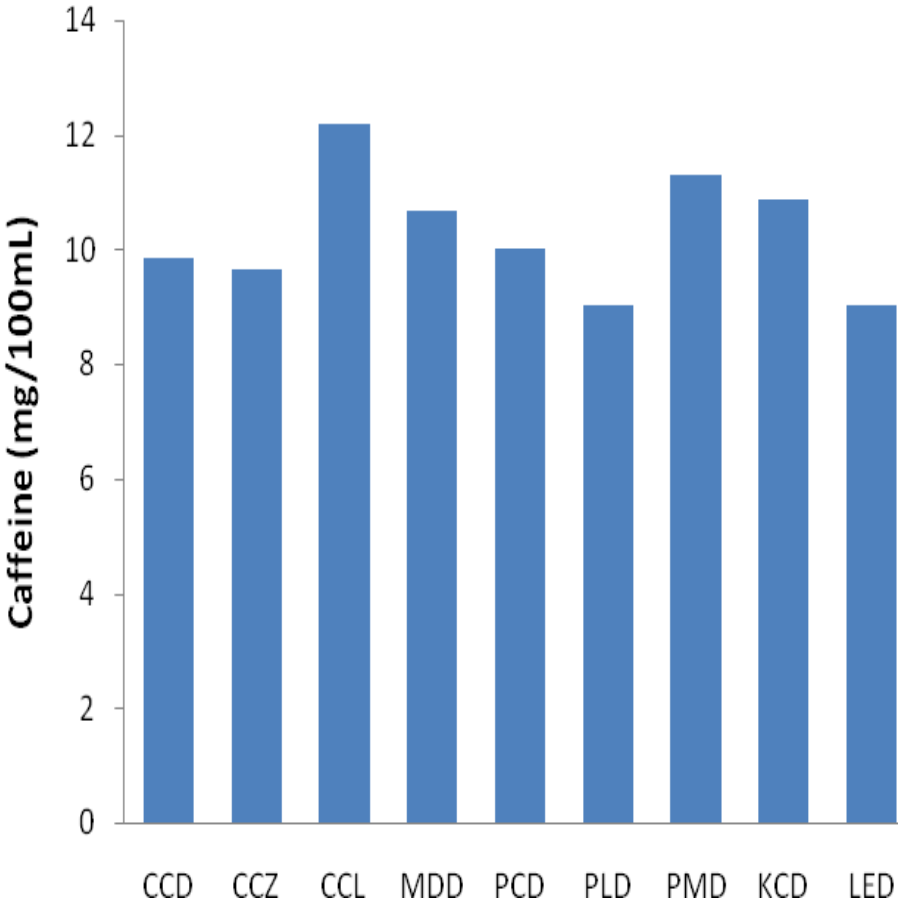


Figure: 4.2 Caffeine Content of the Soft Drink Samples.

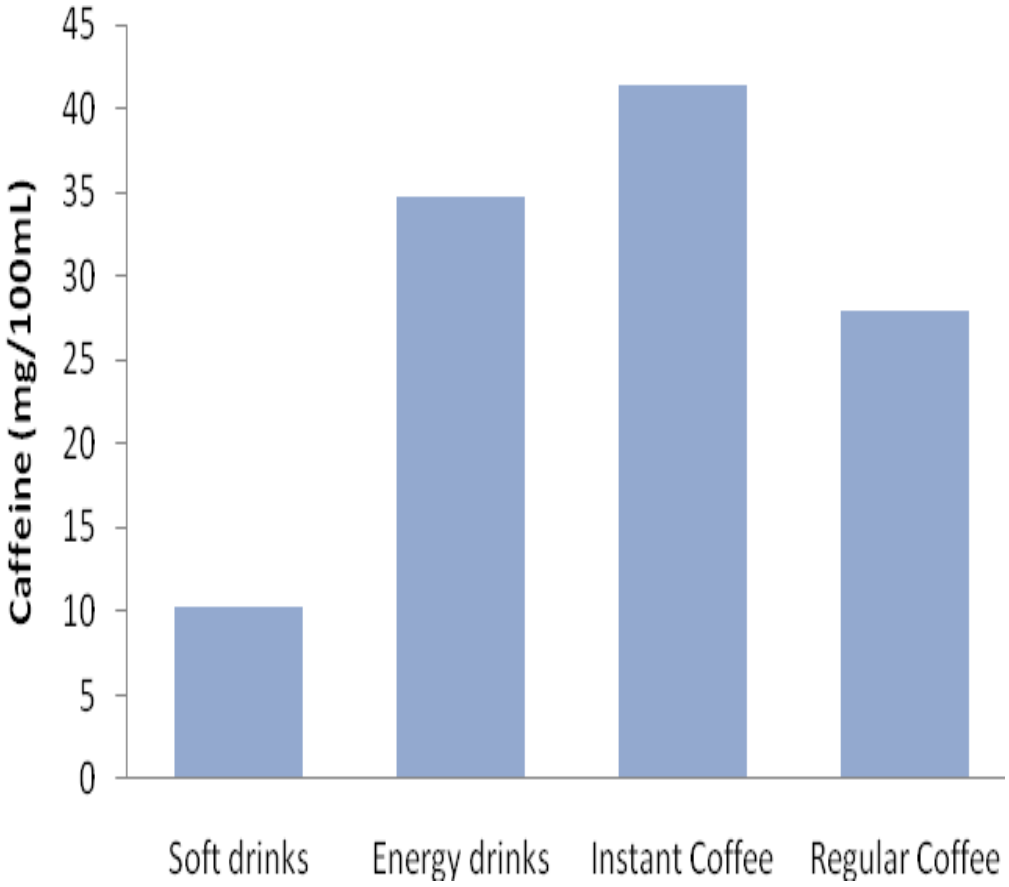


Figure: 4.3 Mean caffeine content of the samples.

Figure: 4.4 shows the pH values of energy drink samples. From the figure it can be seen that PHE energy drink had the lowest pH value of 2.99±0.017, followed by RBE 3.20±0.010,both TED and MED drinks have pH value of 3.33, the highest pH value was observed in RTE energy drink 3.98±0.012.The pH of energy drink analysed ranged from 2.99±0.017 to 3.98±0.012,with a mean of 3.178±0.017(Fig.4.5). Various values have been previously reported for the pH of energy drinks which include Sarmad *et al.,*(2012),who reported the pH of PBE, PHE, WTE, BBE and KED as 3.38±0.03, 3.42±0.09, 3.18±0.12, 3.40±0.07and 3.66±0.01respectively.Tautua *et al.,*(2013),determined the pH of energy drinks i.e. PHE,LBE and RBE as 5.85±2.03, 5.79±1.04, and 6.44±0.76 respectively. Mozammel *et al.*,(2015), determined the pH of five different brands of energy drinks as fallows Brand 1,Brand 2,Brand 3,Brand 4, and Brand 5, as 2.98, 3.01, 2.85, 3.11,and 3.09 respectively. The reason behind the low pH value of carbonated energy drinks could be as a result of presence of Carbon (iv) oxide and other acid such as ascorbic acid, phosphoric acid used as preservative by the manufacturers of these product.(Tautua *et al.*,2014).

Figure 4.6 shows the pH of the soft drink samples. From the figure it can be seen that CCL has low pH value of 2.62±0.021 fallowed by KCD drink 2.91±0.015, The PCL showed the highest pH value of 3.59±0.025.The mean pH of soft drink samples analysed is 3.406±0.061 (Fig 4.5).From the result it can be seen that all carbonated soft drink samples analysed have low pH value. Various values have been previously reported for the pH content of soft and energy drinks which include (Sarmad *et al.*, 2012),who reported that all beverages were low in its pH values, PCD was the lowest pH (2.75), while KCD has the highest pH (3.66).Erickson *et al.*,(2001), determined the pH of soft drinks as followed CCC, SSD, 7UR, 7UD, PRD, PDD 2.50, 3.42, 3.19, 3.67, 2.49, 3.22 respectively. The reason behind the low pH values could be as a result of the presence of CO_2 gas or other acids such as phosphoric acid, malic acid, ascorbic acid, citric acid and tartaric acid used as preservatives by the manufactures of these beverages (Tautua *et al.,* 2013). These acids inhibit the growth of microorganisms such as bacteria, mould and fungi which may contaminate beverages. Studies revealed that drinking acidic beverages over a long period can erode tooth enamel and predisposes the consumer to dental disease. (Tautua *et al.*, 2013).The values obtained in this research are in agreement with those reported in the literature

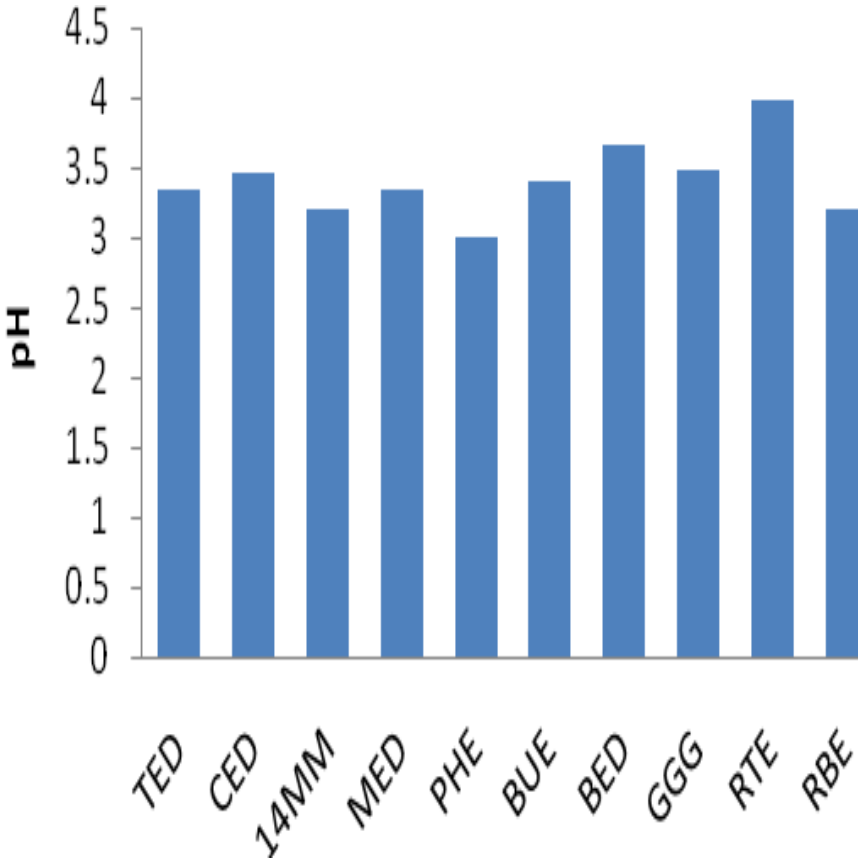


Figure:4.4 pH of the Energy Drink Samples

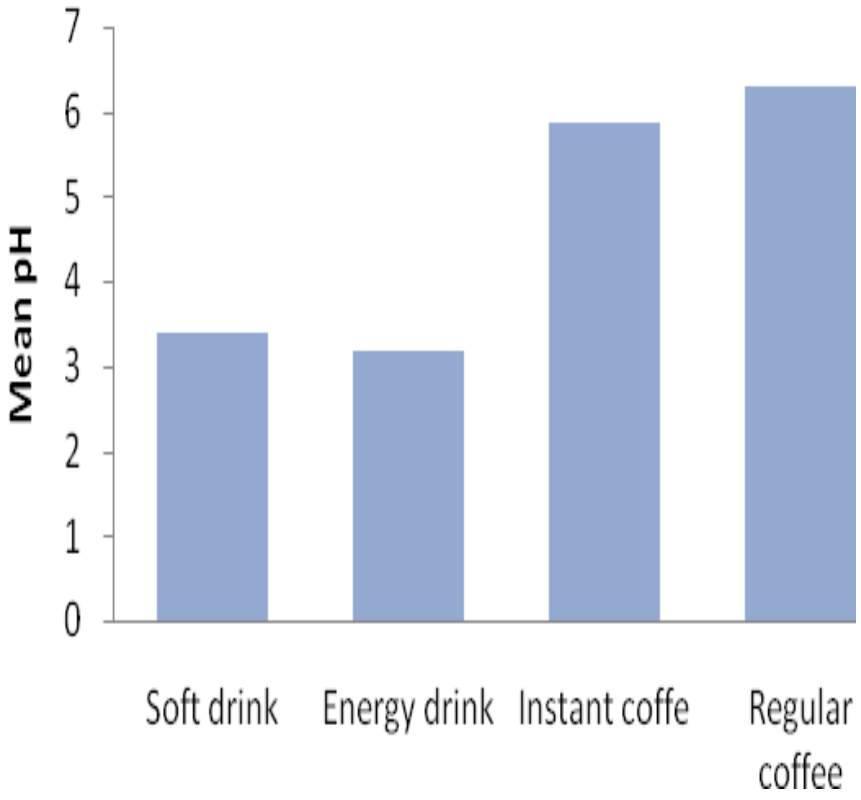


Figure: 4.5 Mean pH of the samples.

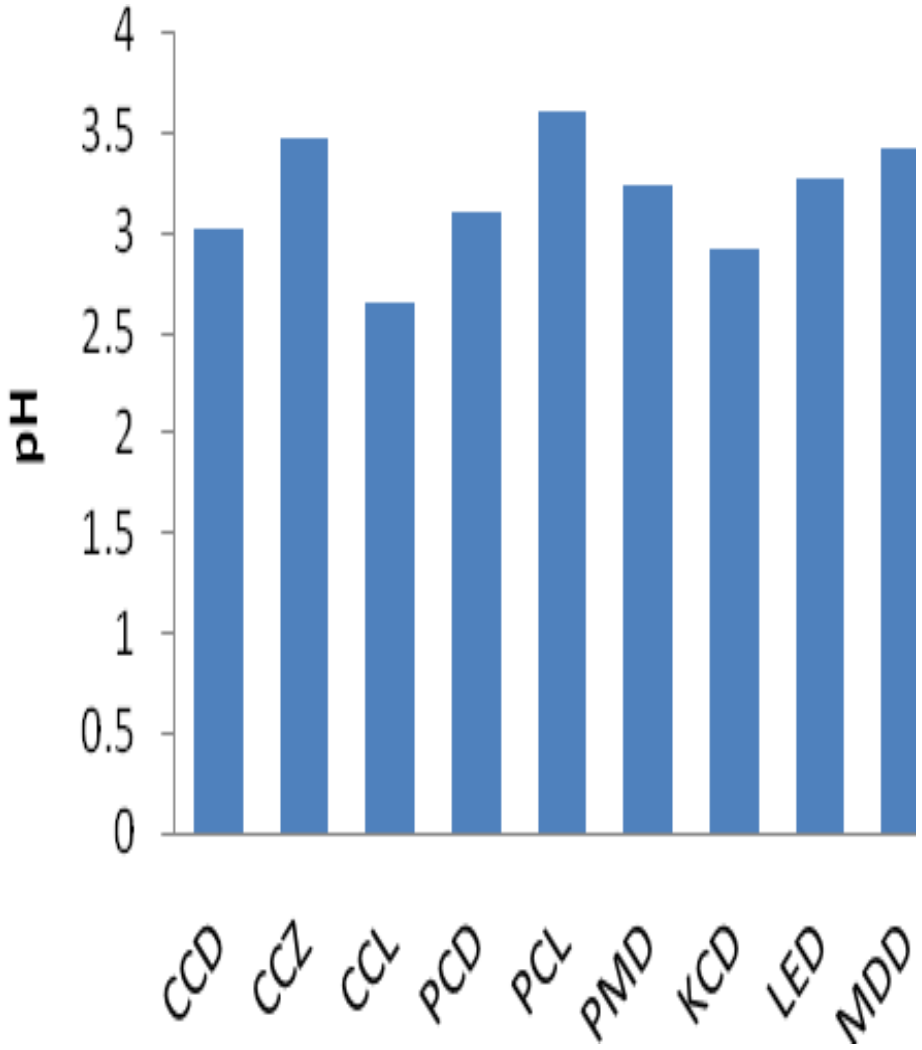


Figure: 4.6 pH of soft drink samples.

Figure 4.7 shows the caffeine content of instant coffee. From the figure it can be seen that the caffeine content of instant coffee ranged from 34.28mg/100mL to 47.293mg/100mL.The lowest caffeine content was observed in CTI (34.15±0.122mg/100mL) and the highest caffeine content was observed in UCI (47.16±0.133mg/100mL).The mean caffeine level of the analysed instant coffee was found to be 41.43±0.228mg/100mL (Fig.4.8).Various values have been previously reported for the caffeine content of instant coffee which include Jenway(2014) reported the caffeine content of NCI 74.8ppm.Wanyika *et al.*,(2010), reported the caffeine content of Dormant, Nescafe, and Africafe as 327.80±2.40, 624.53±2.64 and 684.56±24.35ppm respectively. Musallem M. A and Alam. S. K (2013) reported the caffeine content of Nescafe as 41mg/g.Figure:4.9 showed the caffeine content of regular coffee, from the figure it can be seen that the caffeine content ranged from 9.19±0.09mg/100mL to 40.64±0.501mg/100mL respectively. The lowest caffeine content was observed in CMR (9.19±0.09mg/100mL) and the highest caffeine content was observed in MCR (40.64±0.501mg/100mL). The mean caffeine content of regular coffee was found to be 27.98±0.222mg/100mL (Fig.4.8). Various value have been reported for the caffeine content of regular coffee which include Musallem M. A and Alam. S. K (2013) who reported the caffeine level of Coffee as 39mg/g. The values obtained in this research are in agreement with those reported in the literature. Figure: 4.10 showed the caffeine content of the CHN green tea samples analysed, from the figure it can be seen that the caffeine content ranged from 10.13mg/100mL to 23.277mg/100mL. The lowest caffeine content is observed in RKM 10.146±0.016mg/100mL and the highest caffeine content was observed in ZNB, both TDD and SIM showed similar caffeine content of 20.146±0.44mg/100mL and 20.198±0.637mg/100mL respectively. The mean caffeine content of CHN green tea was found to be 20.189±0.223mg/100mL (Fig.4.11). Figure: 4.12 showed the caffeine content of SLK tea analysed, from the figure it can be seen that the caffeine content ranged from 20.648mg/100mL to 30.94mg/100mL. The lowest caffeine content was observed in JLS tea (20.928±0.28mg/100mL) and the highest caffeine content was observed in SLT tea (30.824±0.28mg/100mL), the mean caffeine content of SLK tea was found to be 24.066±0.072mg/100mL (Fig.4.11). From the analysis of variance the results obtained did not shows any significant difference. The level of caffeine determined in this research with the exception of tea and soft drink samples were above the limit set by NAFDAC(2015) but could also be considered low and safe by considering the daily intake set by IFIC, (2009).

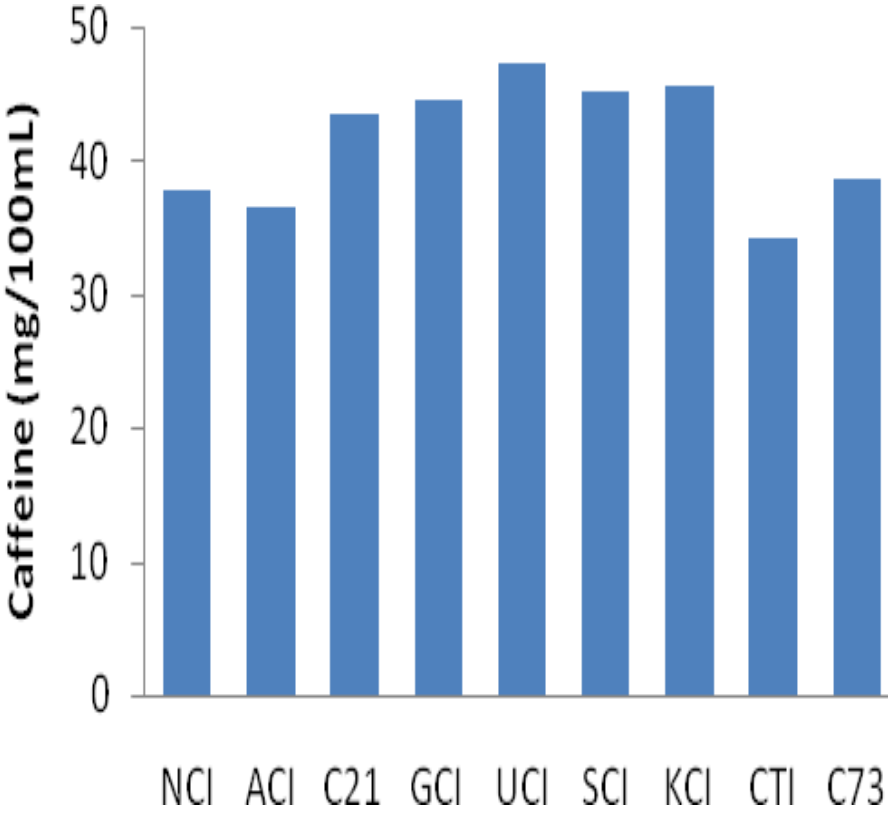


Figure: 4.7 Caffeine content of the instant coffee samples.

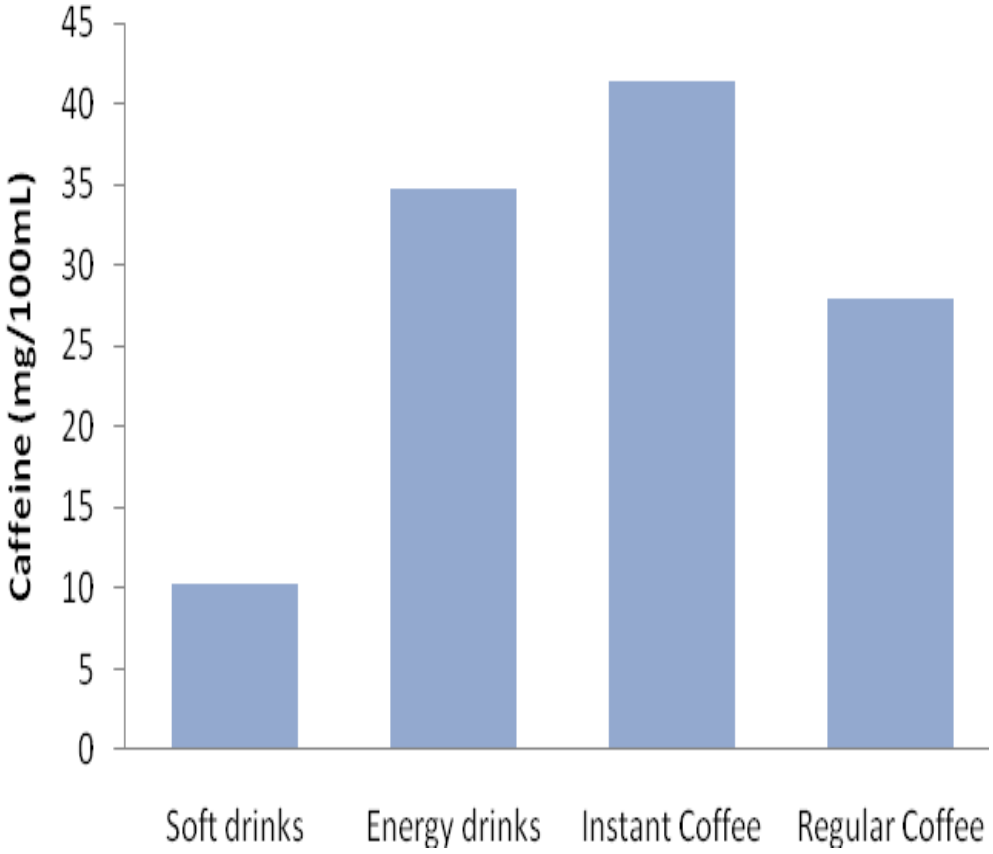


Figure: 4.8 Mean caffeine content of the soft and energy drinks, instant and regular coffee samples.

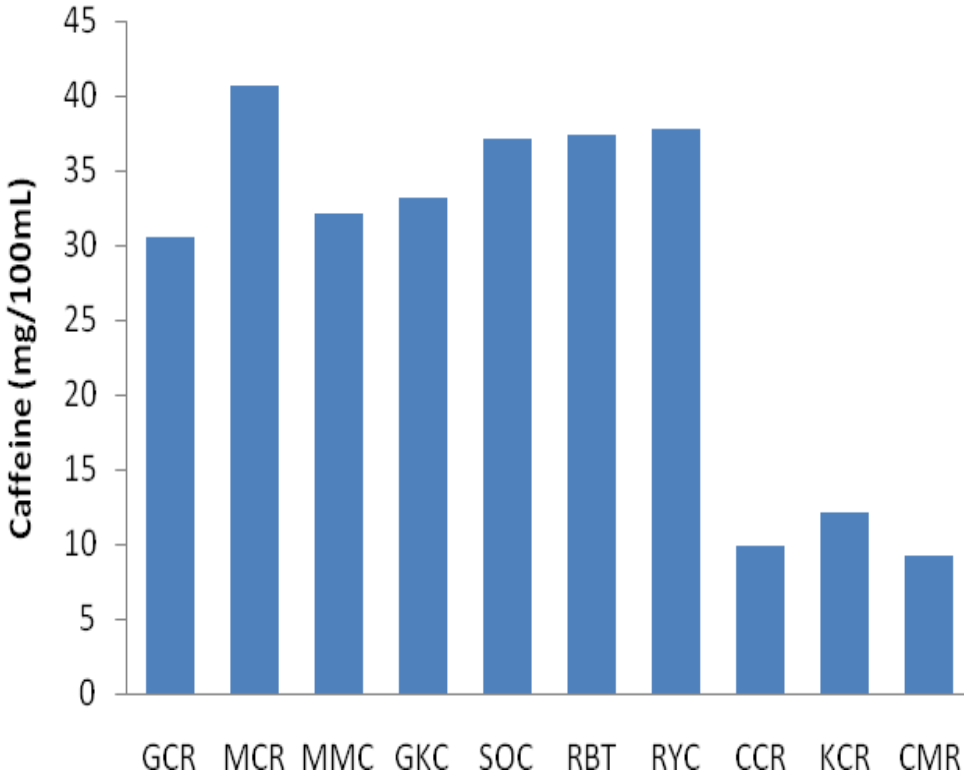


Figure: 4.9 Caffeine Content of the Regular Coffee Samples.

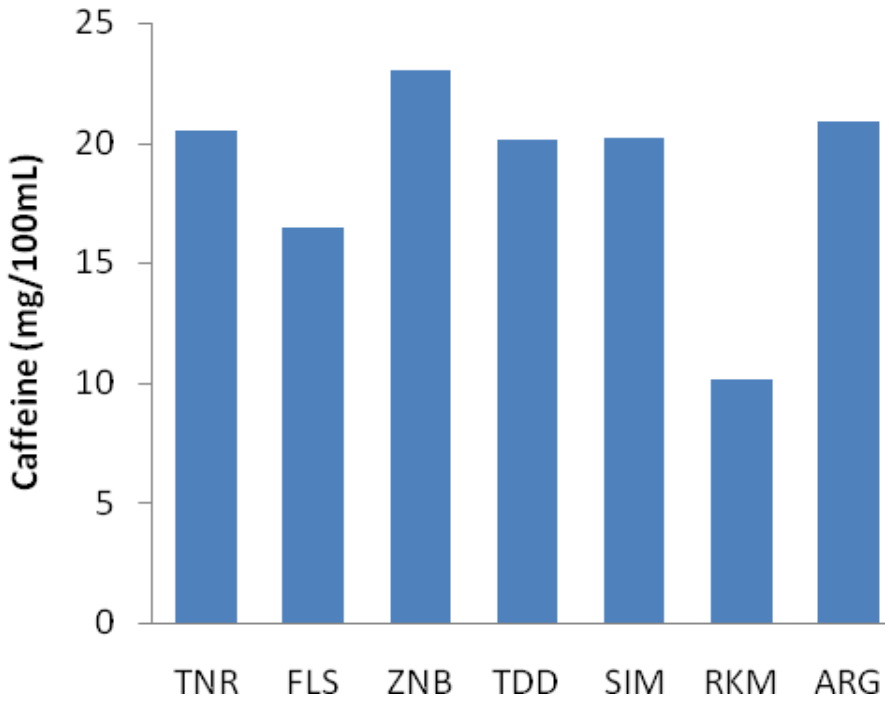


Figure: 4.10 Caffeine contents of the CHN green tea samples.

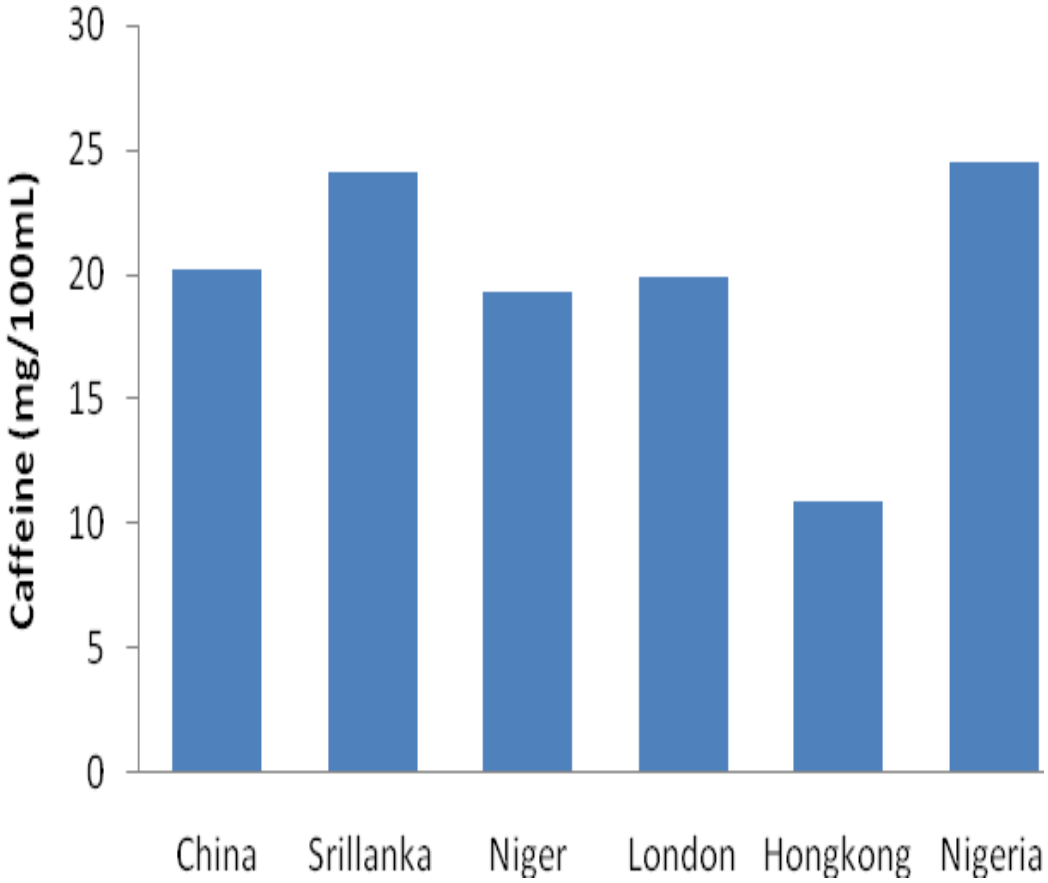


Figure: 4.11 Mean caffeine contents of the CHN,SLK,NGR,LND,HNK,NGA tea samples.

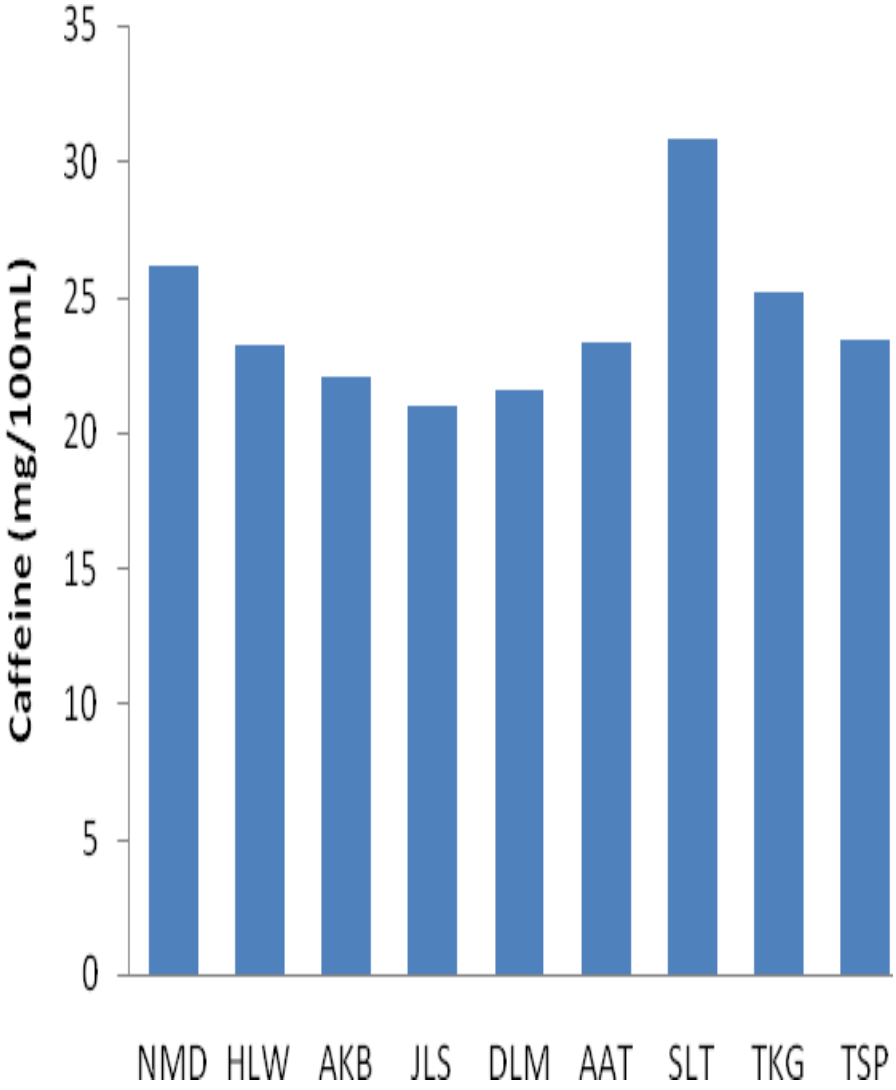


Figure: 4.12 Caffeine contents of the SLK tea samples.

Figure 4.13 shows the caffeine content of the NGR green tea, from the figure it can be seen that the caffeine content ranged from 17.272mg/100mL to 22.504mg/100mL, the lowest caffeine content was observed in BUT (17.280±0.008mg/100mL) and the highest caffeine content was observed in ADO (22.264±0.24mg/100mL), The mean caffeine content of NGR green tea was found to be 19.254±0.112mg/100mL (Fig.4.11). Figure 4.14 shows the caffeine content of the LND black tea samples analysed, from the figure it can be seen that the caffeine contents ranged from 18.189 mg/100mL to 22.732mg/100mL, the lowest caffeine content was observed in BTP (18.197±0.008mg/100mL) and the highest caffeine content was observed in ENG1 (22.699±0.033mg/100mL), the mean caffeine content of LND tea is 19.871±0.04mg/100mL(Fig.4.11). Figure 4.15 shows the caffeine contents of HNK tea samples. From the figure it can be seen that the caffeine contents of HNK tea ranged from 4.988mg/100mL to 25.815mg/100mL, the lowest caffeine content was observed in TSN5 (05.083±0.095mg/100mL) and the highest caffeine content was observed in TSN2 (25.789±0.026mg/100mL), the mean caffeine content of HNK tea was found to be 10.876±0.045mg/100mL(Fig.4.11). Figure 4.16 shows the caffeine content of the NGA tea samples. From the figure it can be seen that the caffeine content of NGA tea samples ranged from 05.426mg/100mL to 35.753mg/100mL, the lowest caffeine content was observed in YLT tea 05.460±0.034mg/100mL and the highest caffeine content was observed in TBR tea (35.720±0.033mg/100mL),the mean caffeine content of NGA tea samples analysed was found to be 24.459±0.167mg/100mL (Fig.4.11). From the analysis of variance the results obtained did not shows any significant difference. Earlier studies revealed that Caffeine content is associated to origin, genetic, environmental variability, harvesting time and processing manner of plant materials(Komes *et al.,* 2009).Various values have been reported for the caffeine content of tea samples which include 24mg/g , 26.5mg/g, and 20.00mg/g in black tea (Lipton), black tea (red label) and green tea respectively Musallem M. A and Alam. S. K (2013).Caffeine contents of tea samples were reported in the ranged of 20.00mg/g to 26.5mg/g (Ushir *et al.*,2011), Generally all the soft drinks and tea samples analysed in this research are within the ranged set by NAFDAC(2015) with the exception of two Nigerian tea i.e TBR and BCT tea which showed the caffeine content of 35.720±0.033mg/100mL and 34.67±0.473mg/100mL respectively, likewise with the exception of CCR, KCR, and CMR, all the energy drinks and coffee samples analysed have crossed the limit set by NAFDAC. But all of the samples analysed in this research were in agreement with those reported in the literature and were within the recommended daily intake set by the IFIC, (2009).

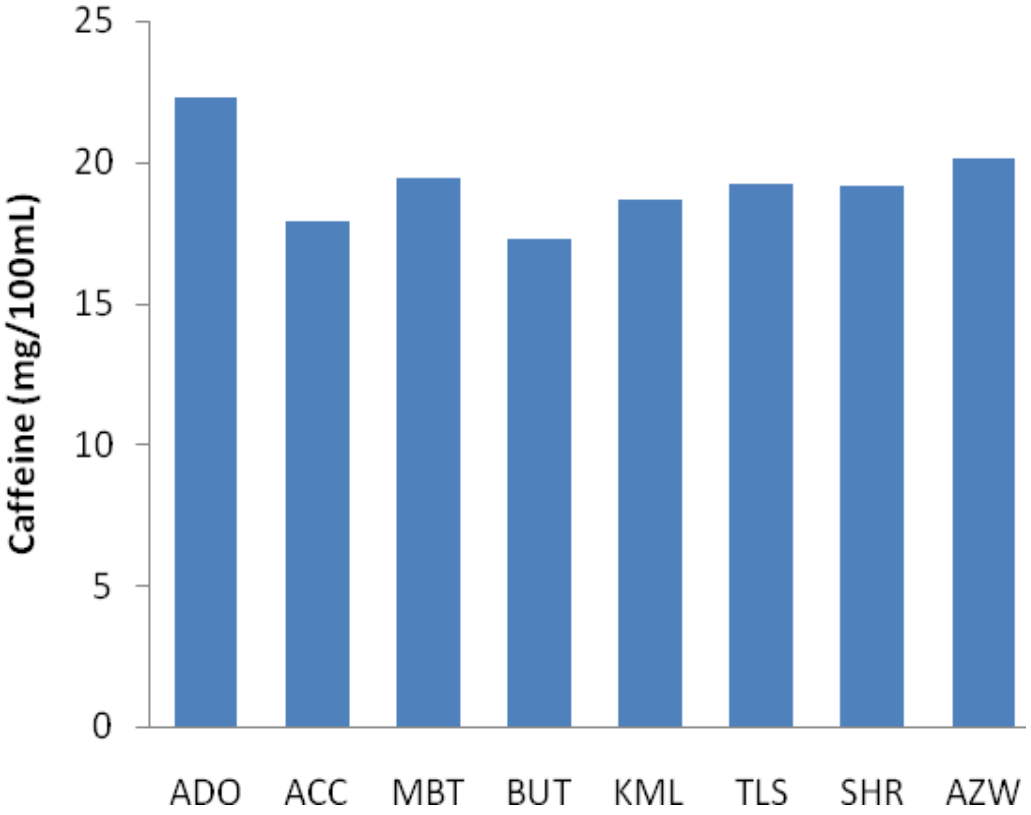


Figure: 4.13 Caffeine contents of the NGR green tea samples.

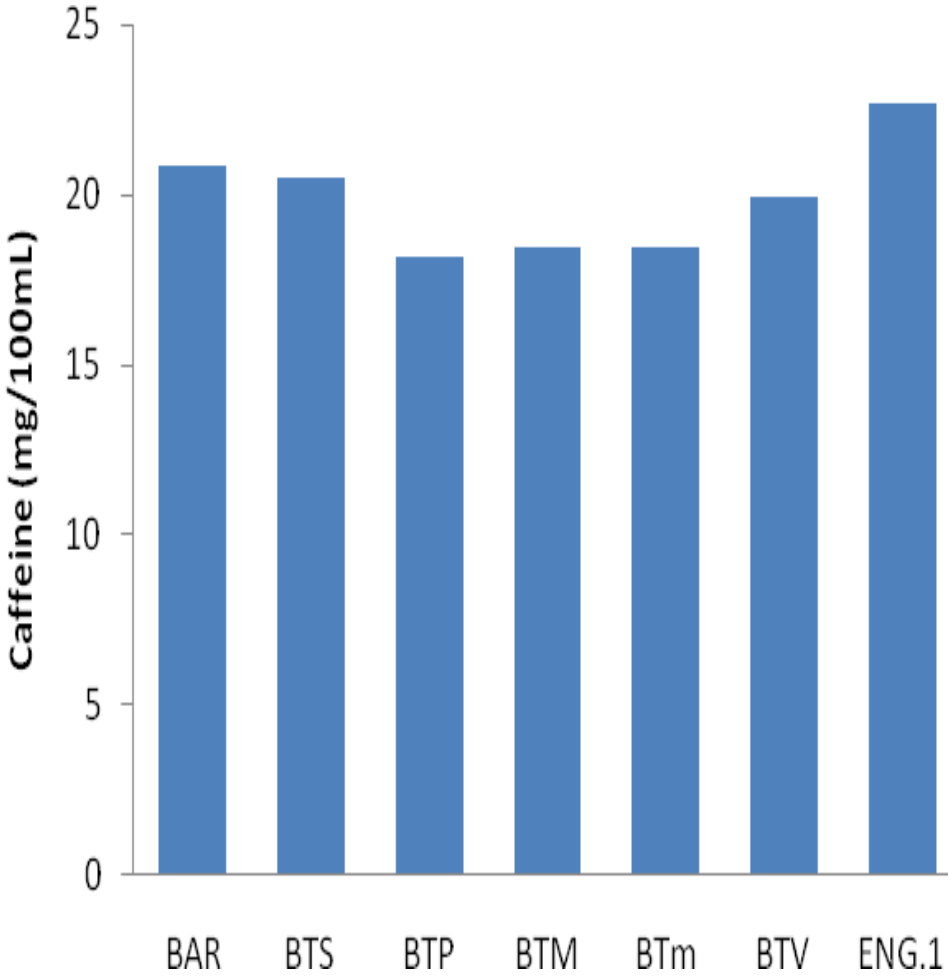


Figure: 4.14 Caffeine content of the LND black tea samples.

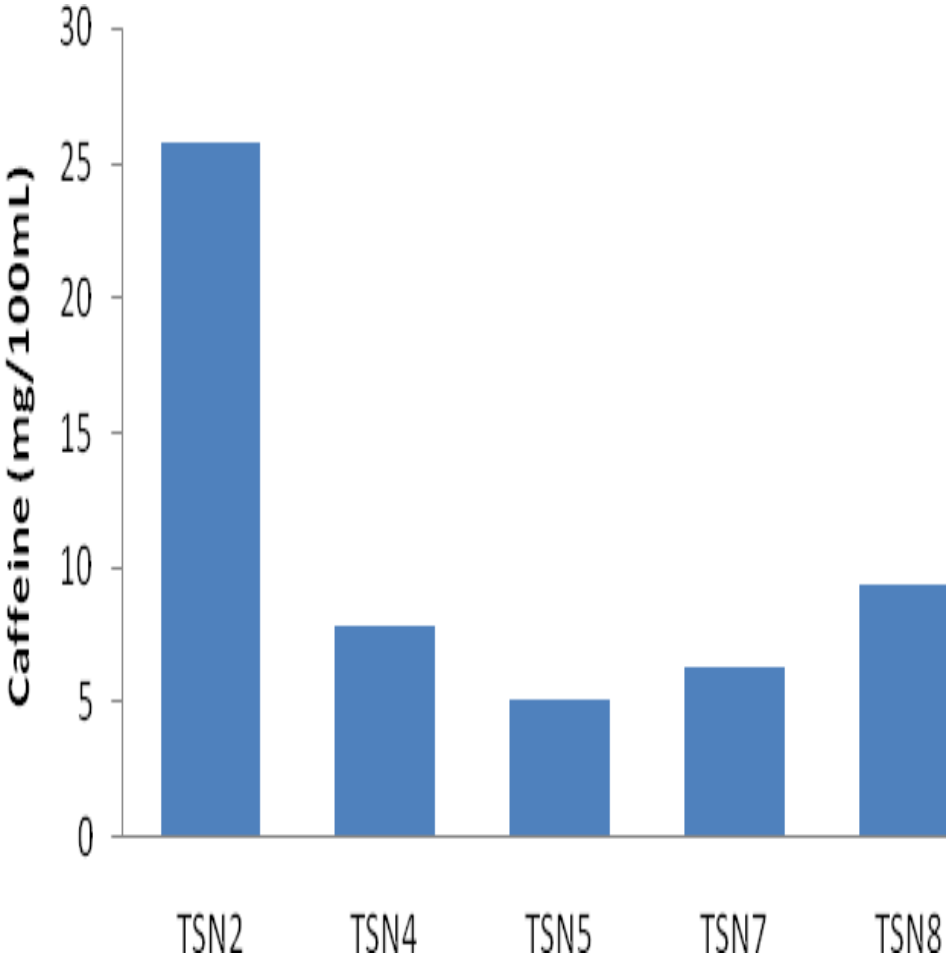


Figure:4.15 Caffeine content of the HNK tea samples.

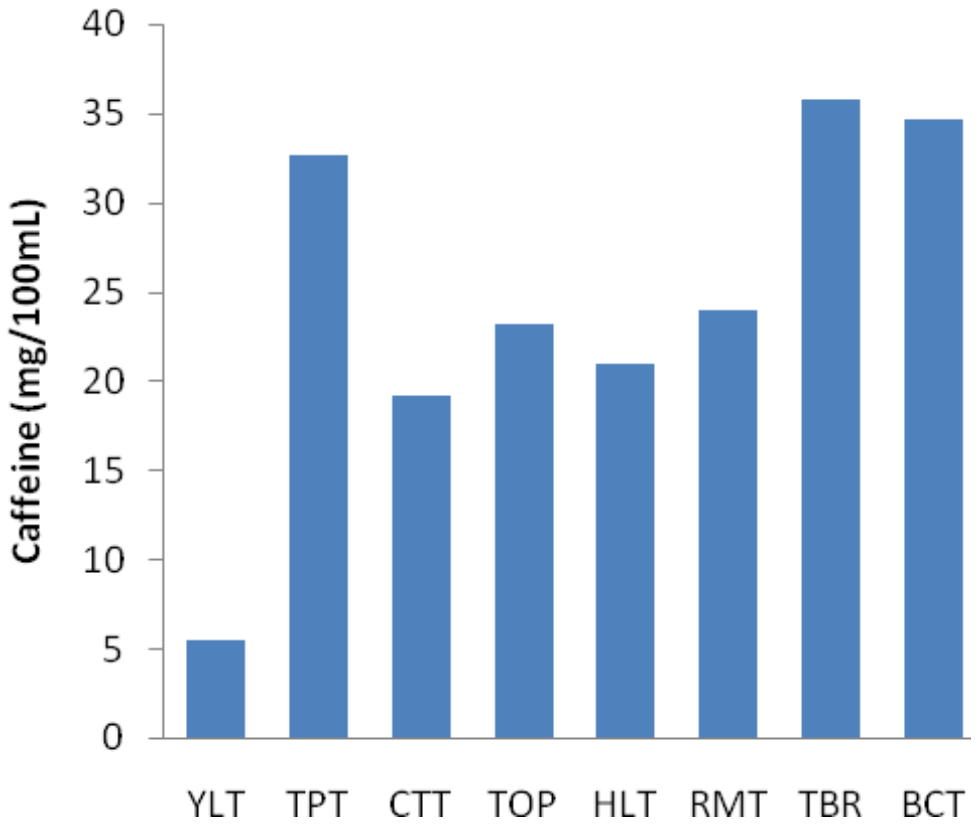


Figure: 4.16 Caffeine contents of the NGA tea samples.

Figures: 4.17 and 4.18 shows the pH of instant and regular coffee samples. From the figures it can be seen that the pH of instant coffee ranged from 5.203 to 6.796.Likewise that of regular coffee ranged from 5.680 to 6.956, the mean pH of instant and regular coffee were found to be 5.86±0.074 and 6.30±0.020,(Fig 4.19).From Figure: 4.20 to figure:4.26 showed the pH level of tea samples. From the figures it can be seen that the pH of CHN green tea ranged from 5.651 to 6.44, while that of SLK tea ranged from 5.445 to 5.936, both NGR, LND, HNK and NGA tea samples have pH in the ranged of 6.065 to 6.785, 5.53 to 6.685, 5.614 to 6.42 and 4.823 to 6.546 respectively. The mean pH of tea samples from different countries ranged from 5.663 to 6.418. The results obtained is slightly higher than the critical pH value below which enamel erosion takes place (pH 5.5) Serap and Aysen (2010). All the tea samples analysed were slightly acidic this might be due to the presence of organic acids such as citric, malic, and oxalic acid Serap and Aysen (2010). Similar results were obtained by other researchers which include Brunton and Hussain,(2001) stated that both conventional black tea and herbal tea eroded dental enamel. They also stated that the pH of conventional black tea and herbal tea have been measured to be lower than the critical pH(5.5) that is necessary for demineralisation of enamel. Our results are higher than the finding of Brutun and Hussain(pH 3.5) and are in agreement with the result of Phelan and Rees,(2003) (pH 3.1 to 7.1), Rahman *et al.*,(2013)(pH 5.58 to 5.71) and Serap and Aysen (2010)(pH 6.47 to 7.24).The results obtained in the research were within the range reported by other researchers.

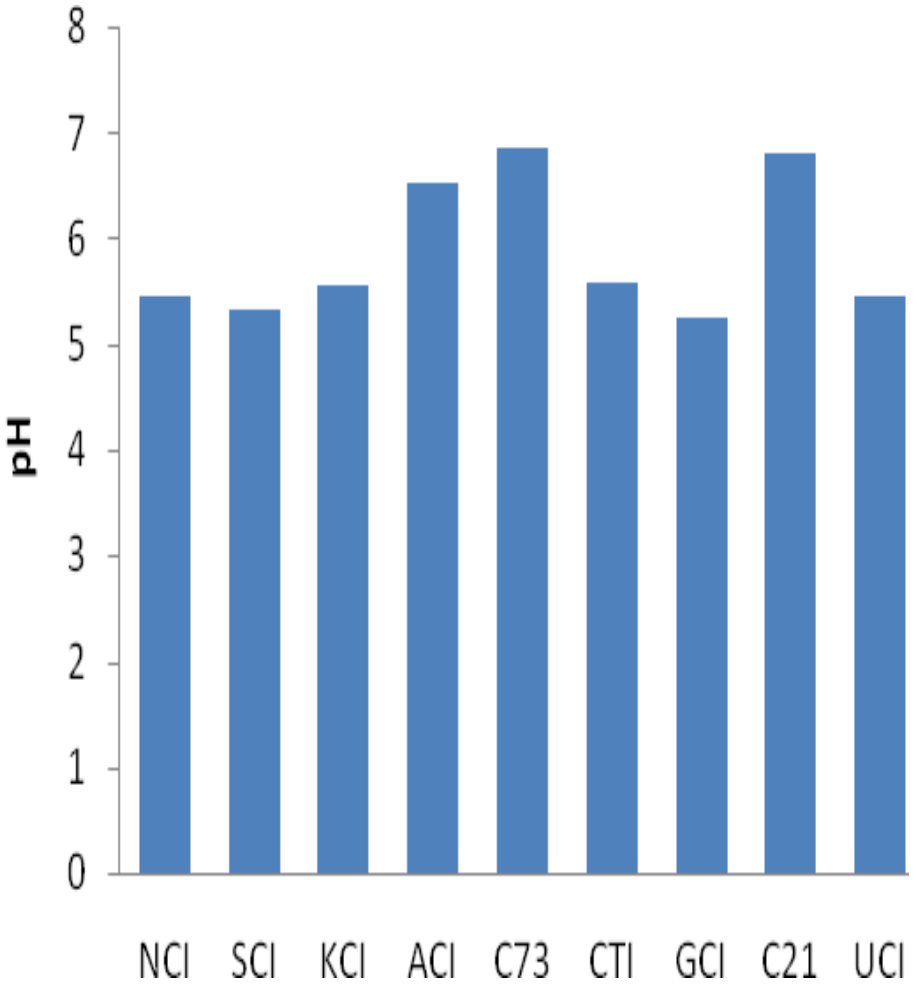


Figure: 4.17 pH of instant coffee samples.

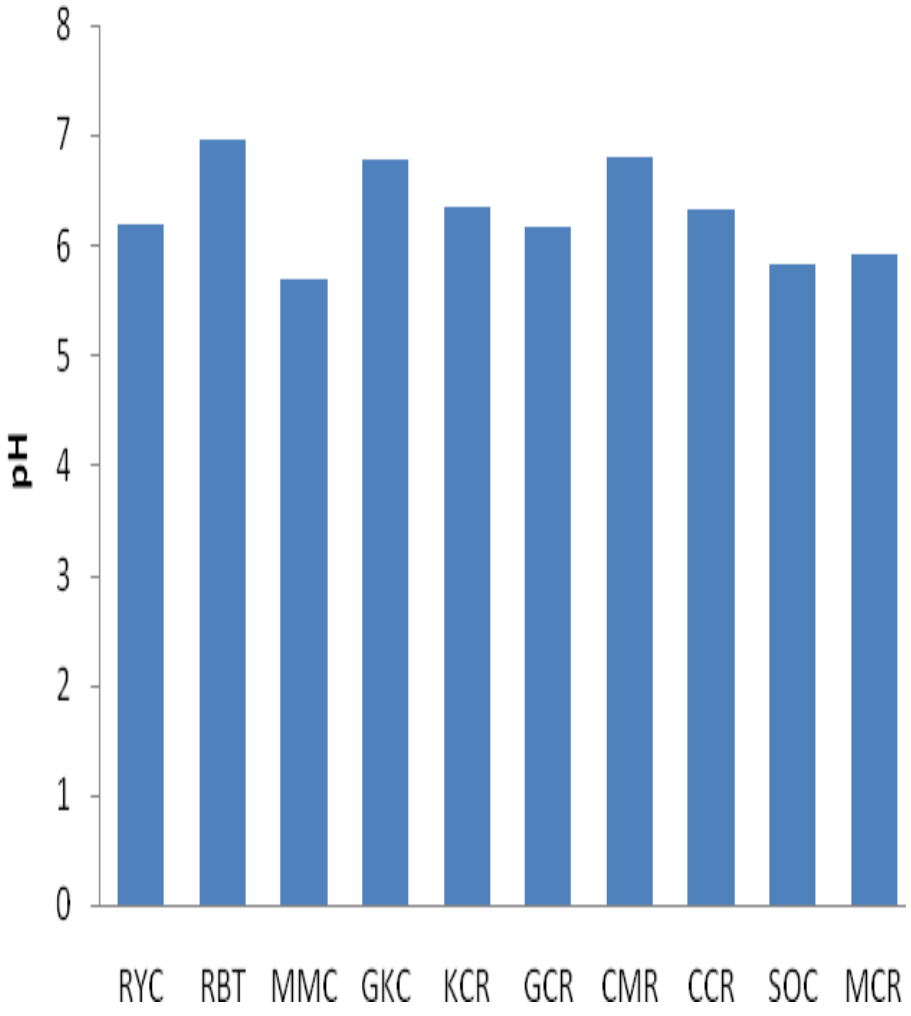


Figure: 4.18 pH of regular coffee samples.

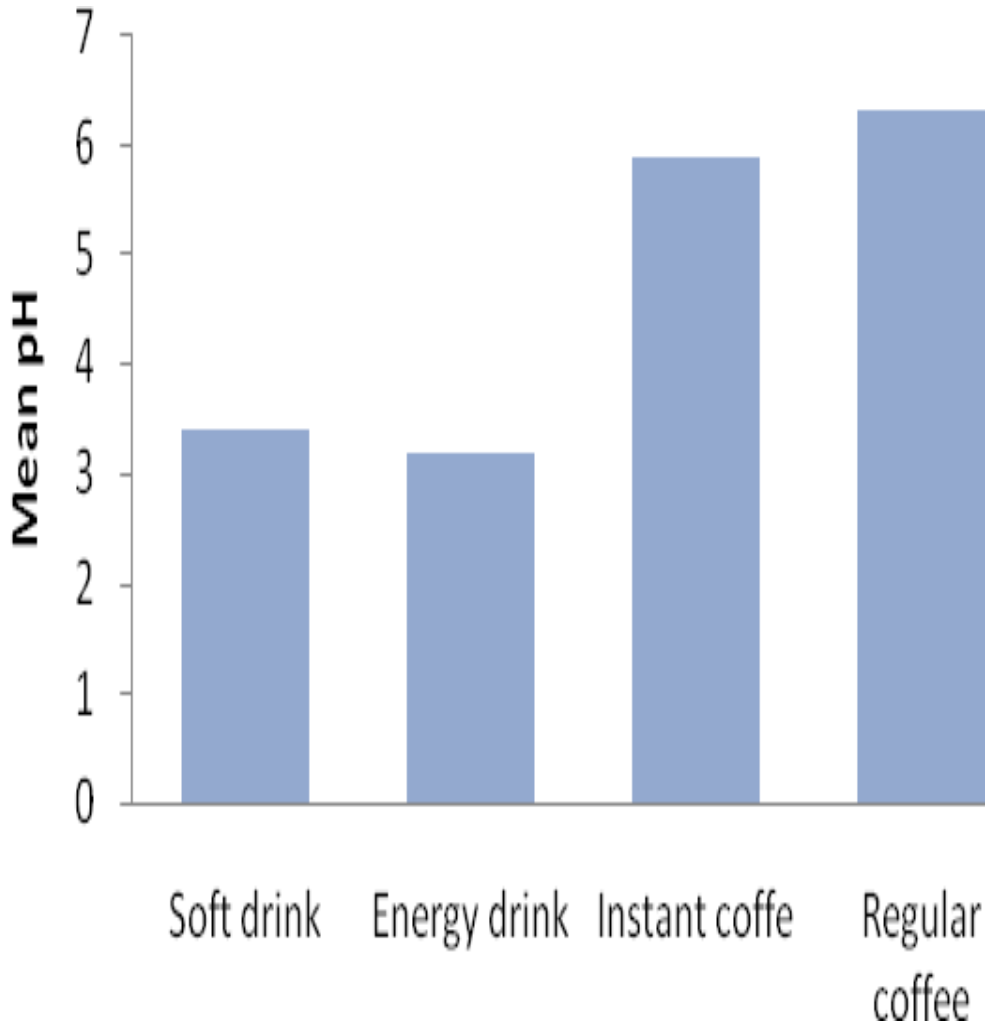


Figure: 4.19 Mean pH of soft drinks, energy drinks, instant and regular coffee samples.

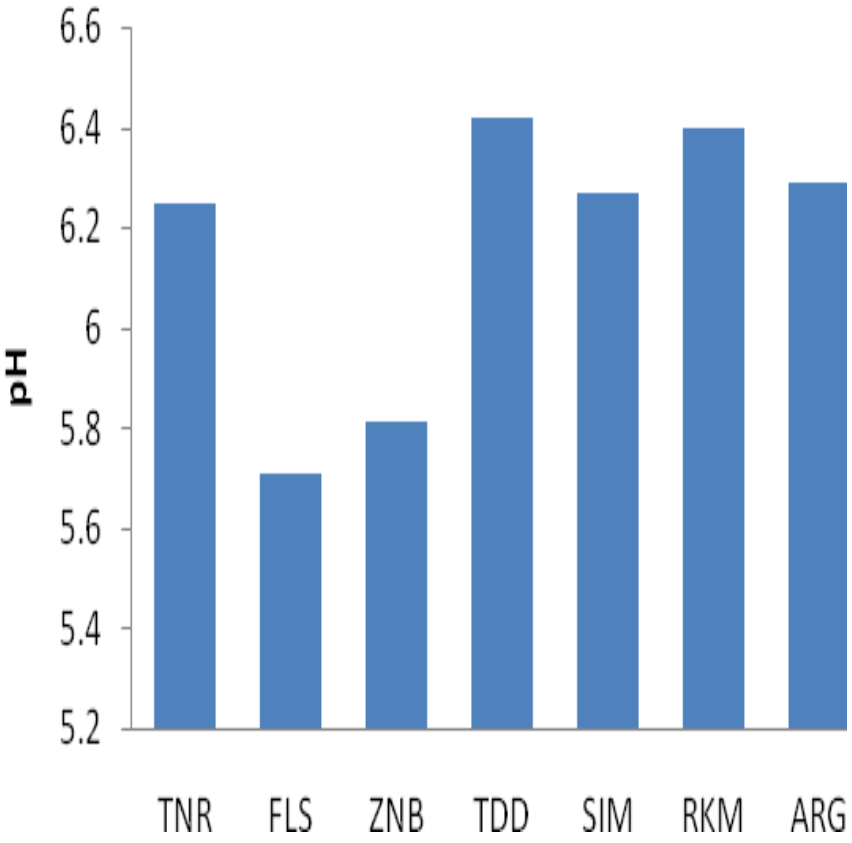


Figure: 4.20 pH of CHN green tea samples.

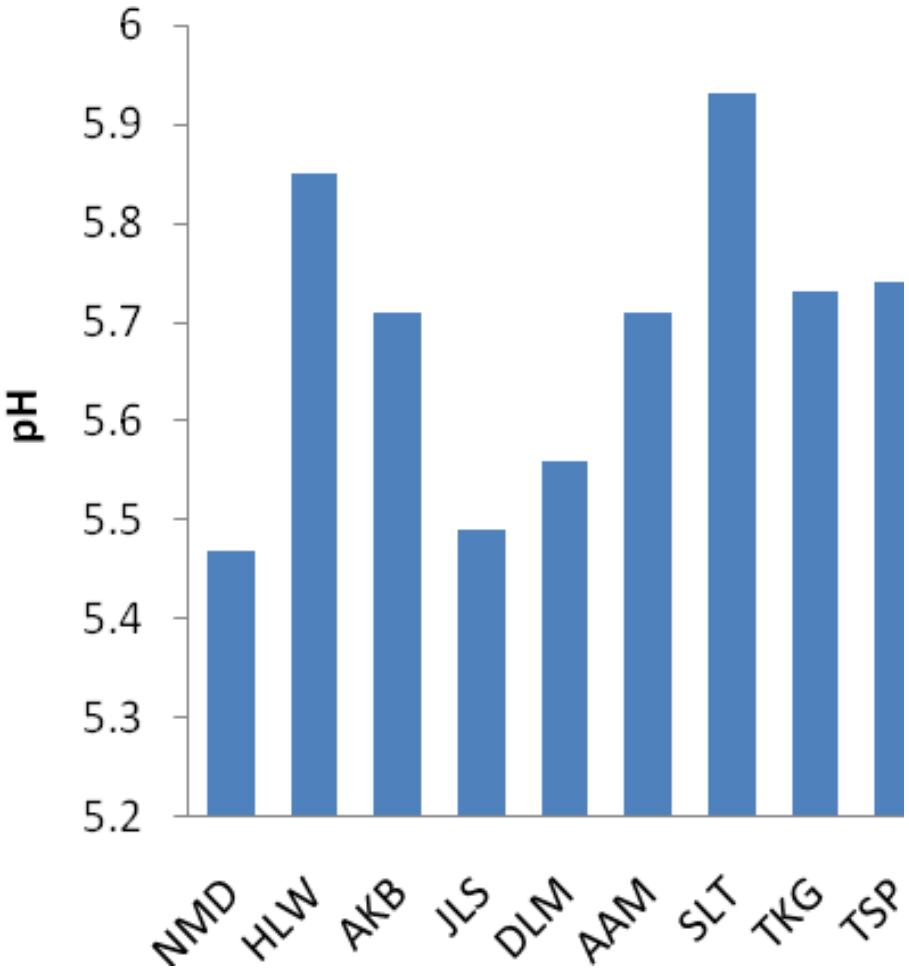


Figure: 4.21 pH of SLK tea samples

Figure: 4.22 pH of NGR green tea samples.

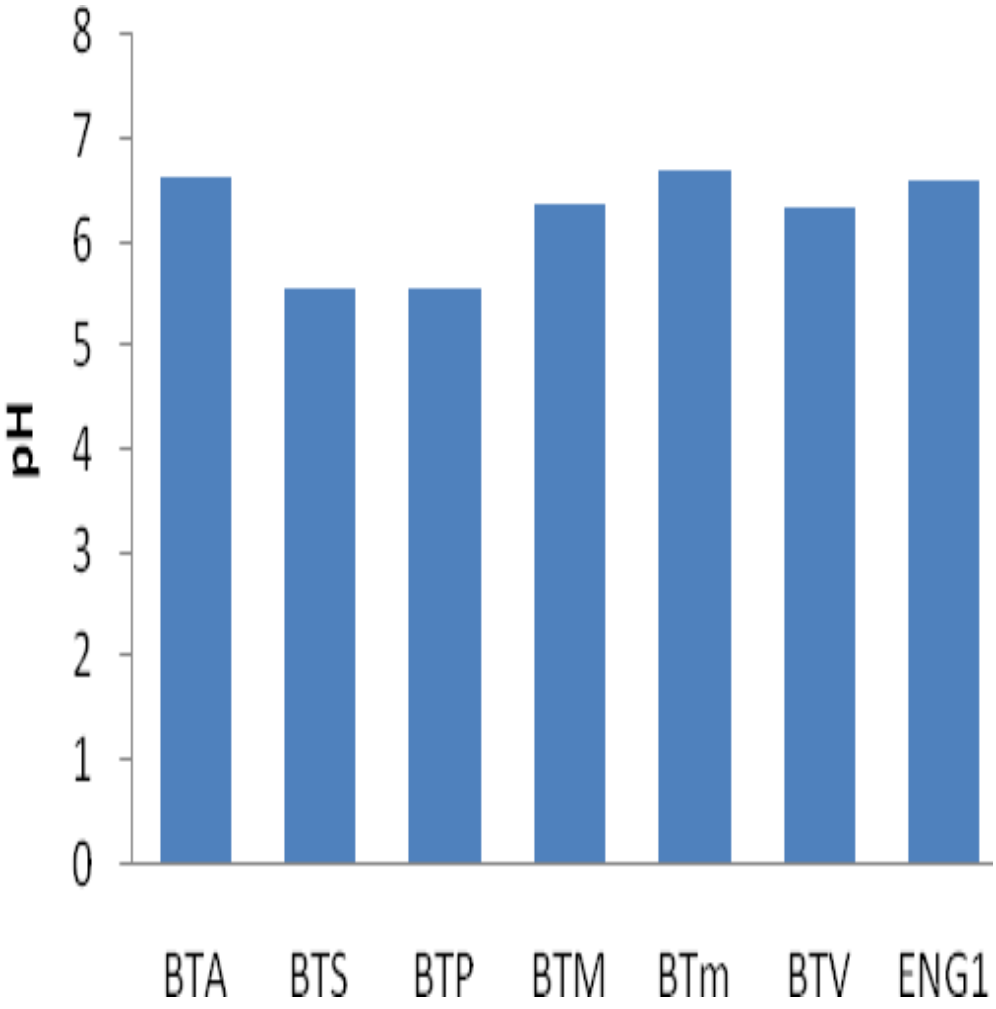


Figure: 4.23 pH of LND black tea samples.

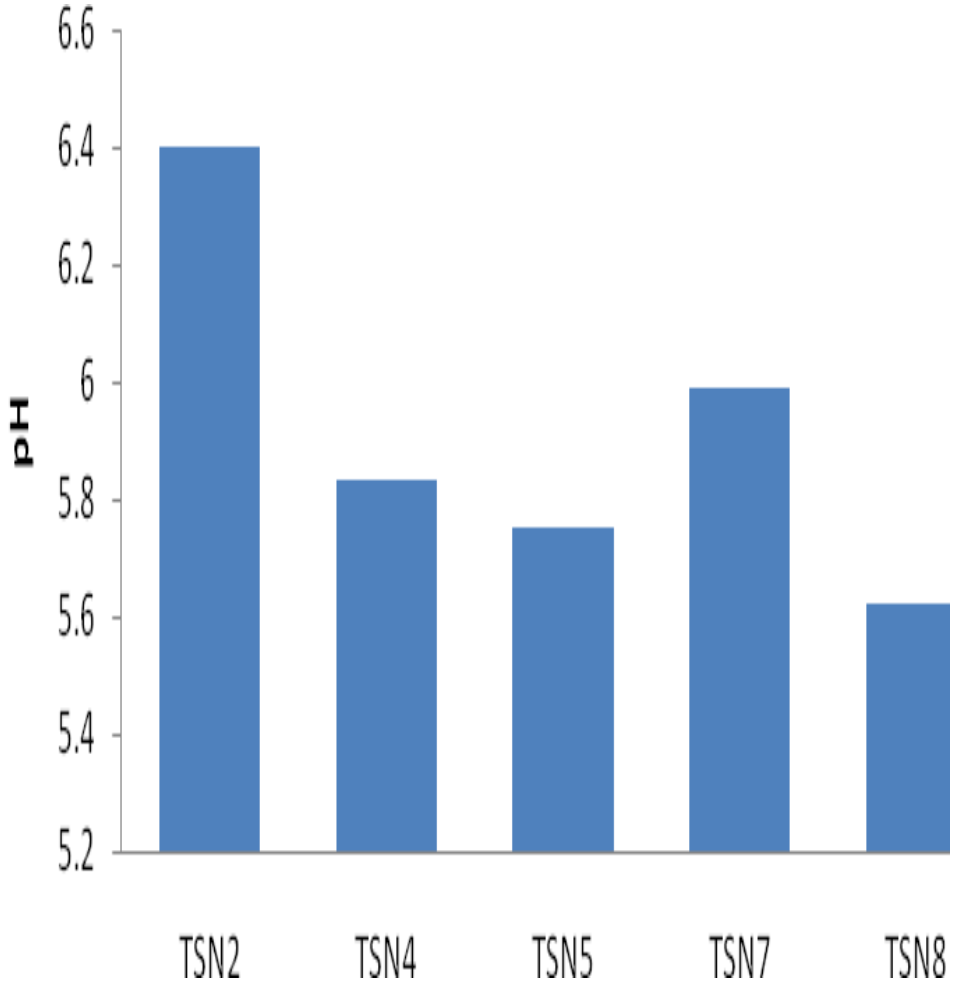


Figure: 4.24 pH of HNK tea samples.

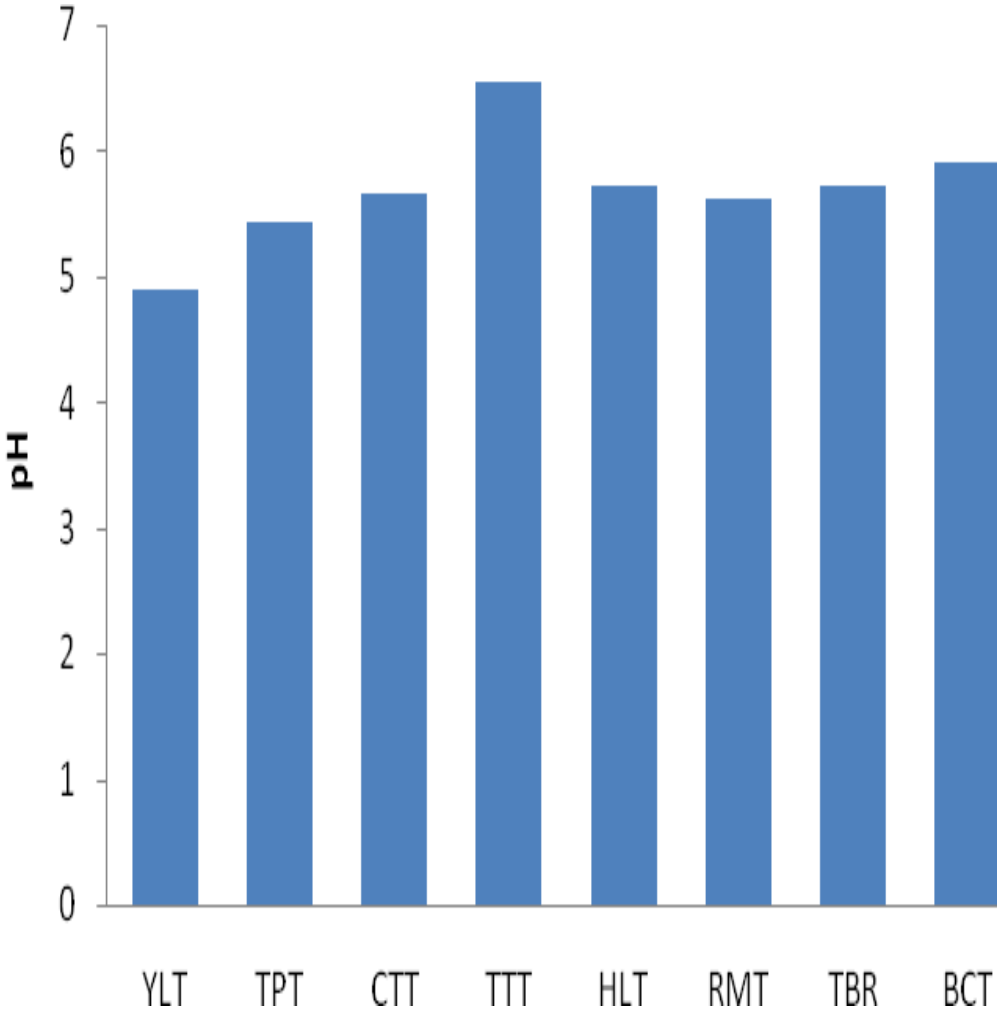


Figure: 4.25 pH of the NGA tea samples.

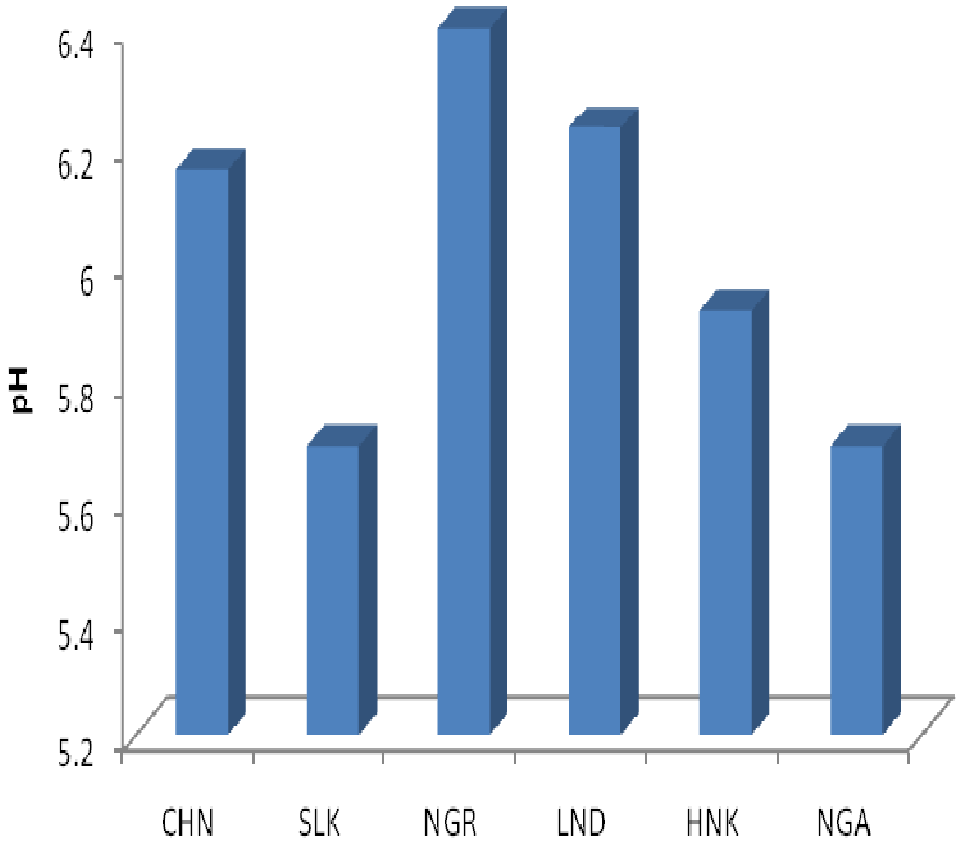


Figure: 4.26 Mean pH of the tea samples from CHN,SLK,NGR,LND,HNK and NGA.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

It is shown from the results of this study that coffee and energy drinks based beverages widely consumed by the public, especially the adults, contain higher amount of caffeine relative to soft drink and tea based beverages. However, the amount of caffeine found in most of the products analysed were well below the regulatory level of NAFDAC 32mg/100mL,NAFDAC(2015), but are generally considered safe for healthy consumers by considering the recommended daily intakes set by the IFIC (300mg/day), IFIC(2009).

5.2 RECOMMENDATION

The UV/Visible spectrophotometric method employed in this study for the quantification of caffeine in the samples analysed was found to be relatively easy, fast, sensitive and cheaper. The major instrument required is a modern computerized UV/Visible spectrophotometer which can be acquired at an affordable price. This analytical method may therefore be recommended for the rapid, accurate and sensitive quantification of caffeine in beverages by any educational institution in developing countries.

It is recommended that people who need caffeine restriction due to health conditions should choose products with lower caffeine contents. Also, since caffeine is an additive substance, Manufacturers of caffeine-containing products should be required by regulatory bodies to indicate its presence and amount on package labels for the information of the consuming public.

REFERENCES

Alpdogan, A., Kadir, K., & Sidika, S. (2002). Derivative Spectrophotometric Determination of Caffeine in Some Beverages, *Turk j Chem.*, 26, 295-302.

Amos-Tautua., Bamidele, W. M., and Diepreye, E. R. E. (2014). Ultra-violet Spectrophotometric Determination of Caffeine in soft and Energy Drinks Available in Yenagua, Nigeria. Advanced Journal of Food Science and Technology, 6(2), 155-158.

Ayala J.Simon K., and Kerrigan S. (2009). Quantitative Determination of Caffeine and Alcohol in Energy Drinks and the Potential to Produce Positive Transdermal Alcohol Concentration in Human Subjects. Journal of Analytical Toxicology,33(1)27-33.

Bal- Ram S, Margret, A. W., Yuhong, H. and Catherine, L. (1998). Determination of Caffeine Content in coffee using Fourier transform Infra-red Spectroscopy in combination with attenuated total reflectance technique. Biochemical education, vol. 26, 243-247.

Belete, A. (2008). Determination of Caffeine Level in Coffee. Research work conducted in Department of Chemistry, Addis Ababa University. Msc research work, pp 1-13

Brunton PA, Hussaine A.*(2001)* Erosive effect of herbal tea on dental enamel. Journal of Dentistry,vol.29:517-520.

Dobrinas,S.,Soceanu,A.,Popescu,V.,Stanciu,G.,andSmallberger,S.(2012):Optimization of a uv/visible spectrometric method for caffeine analysis in tea, coffee and other beverages, Journal of Chemistry and Chemical Engineering, Biotechnology, Food Industry.vol.14(2),pp071-078.

Erickson P.R., Alevizos D.L., Rindelaub D.J.,(2001) Soft drinks hard on teeth, North-West Dentistry.pp17-18.

IFIC (2009), International Food Information Council Foundation: Questions and Answers About Energy Drinks and Health, 2009, http://www.foodinsight.org/Resources/

Jenway (2014), The Quantitative Determination of Caffeine in beverages and soft drinks Using UV Wavelength Spectroscopy, Journal of Bibby Scientific.pp 2-4

Komes D., Horlic, D., Belscak, A. Kovacevic, K G. & Baljak. (2009). Determination of Caffeine content in Tea and mate'tea by using different method, *Czech J. food Sci.,vol.* 27.

Musallem M. A and Alam. S. K (2013):Isolation and quantitative estimation of caffeine content in different brands of coffee and tea leaves. Academic Journal of Bioscience,vol.1(3)pp67-68.

Mozammel M.H., Iffat j., Mohammad M. A,. Khan S., Afroza p., Mahmudul M.H., Kazi R. U., Salina A., Subrata B., Md. Ashraful H.M., Mahbubul M., Nazibur R. M., Badier S.M.R.,(2015).Determination of pH,Caffeine,and reducing suger in energy drinks avoilable in Bangladesh,New York science journal 8(2).

NAFDAC (2015) regulatory level of caffeine in beverages available at:

http://m.facebook.com/Nafdac Nigeria/posts/764179520269444.Retrieved on 21/08/2015

Ogah C.O and Obebe O.T.(2012) Caffeine content of cocoa and coffee beverages in Lagos, Nigeria. Journal of Innovative Research in Engineering and Sciences 3(1)404-411

Oliveira R., M. M. (2010). Quantification of Catechins and Caffeine from green tea (Camellia Sinensis) Infusions, extract and ready-to-drink beverages Cienc. *Technology Aliment., Campinas*, 32(1), 163-166.

Phelan J., Rees J. (2003) Erosive potential of some herbal tea. Journal of Dentistry vol.31:241-246.

Rah man M.M., Kalam M.A., and Islam M.M.(2013):Change of chemical compositions in semi-fermented tea on land elevation. African Journal of Agricultural Research, Vol. 8(25) pp. 3224-3228.

Sarmad G. M., Alaa G. A. And Al-Hussainy K. S.,(2012). Determination of Caffeine and Trace Minerals Contents in Soft and Energy Drinks Available in Basrah Markets. Pakistant Journal of Nutrition 11(9):747-750.

Serap A., and Aysen Y.,(2010) pH and Neutralisable acidity of the most consumed Turkish Fruit and Herbal Teas.Academic Journal of Bioscience,vol.(2) pp 75-77

Tadelech, A. & Gholap, A. V. (2010). Characterization of Caffeine and the determination of Caffeine in tea leave Using UV-visible Spectrometer. African Journal of Pure and Applied Chemistry, 5(1), 1-8.

Ushir Y,Luha A, Abhang S. and Vadalia K.,(2011) Estimation of secondary metabolites in different tea and coffee brands from Indian market, International Journal of Pharmacy and Life science,vol.2(3):599-600.

Violeta N., Ion, T., & Mira, E. I. (2010). Chromatographic Determination of Caffeine Contents in Soft and Energy Drinks Available in the Romanian Market. Chemistry and Chemical Engineering. Biotechnology Food Industry, 11 (3), 351-358.

Wanyika, H. N., Gatebe, E. G., Gitu, L. M. Ngumba, E. K & Maritim, C. W. (2010). Determination of Caffeine Content of tea and Instant Coffee Brands Found in Kenyan Market. African Journal of Food Science, 4(6), 353-358.

APPENDIXES

Appendix i: Caffeine contents of the soft and energy drink samples analysed(mg/100mL)

SOFT DRINKS SAMPLES CODES	Caffeine content*(mg/100mL)*	*ENERGY DRINKS SAMPLES CODE*	*Caffeine content(mg/100mL)*
CCD	*9.85±0.124*	*RED*	*34.65±0.455*
CCZ	*9.66±0.333*	*PHE*	*37.00±0.455*
CCL	*12.20±0.031*	*BUE*	*40.32±0.653*
MDD	*10.68±0.223*	*14MM*	*37.90±0.267*
PCD	*10.00±0.193*	*BED*	*36.25±0.213*
PLD	*9.01±0.054*	*MED*	*38.71±0.432*
PMD	*11.30±0.031*	*TED*	*40.41±0.141*
KCD	*10.87±0.145*	*CED*	*38.44±0.161*
LED	*9.02±0.145*	*RBE*	*40.88±0.561*
		GGG	*38.28±0.192*

Appendix ii: Caffeine contents of the instant and regular coffee samples analysed(mg/100mL).

INSTANT COFFEE SAMPLES CODES	*Caffeine content(mg/100mL)*	*REGULAR COFFEE SAMPLES CODES*	*Caffeine content(mg/100mL)*
NCI	*37.76±0.122*	*GCR*	*30.52±0.223*
ACI	*36.46±0.667*	*MCR*	*40.64±0.501*
C21	*43.53±0.453*	*MMC*	*32.16±0.109*
GCI	*44.55±0.036*	*GKC*	*33.12±0.135*
UCI	*47.16±0.347*	*SOC*	*37.12±0.424*
SCI	*45.16±0.028*	*RBT*	*37.43±0.184*
KCI	*45.51±0.059*	*RYC*	*37.73±0.432*
CTI	*34.15±0.122*	*CCR*	*09.83±0.100*
C73	*38.58±0.201*	*KCR*	*12.05±0.021*
		CMR	*9.19±0.090*

Appendix iii: Mean Caffeine content of the drinks and coffee samples analysed(mg/100mL).

SAMPLES NAME	*Caffeine content(mg/100mL)*
Soft drinks	*10.288±0.133*
Energy drinks	*34.784±0.313*
Instant coffee	*41.429±0.226*
Regular coffee	*27.979±0.222*

Appendix iv: Caffeine contents of the CHN and SLK green tea samples analysed (mg/100mL).

China green tea samples codes	*Caffeine content(mg/100mL)*	*Srillanka tea samples codes*	*Caffeine content(mg/100mL)*
TNR	*20.518±0.151*	*NMD*	*26.108±0.083*
FLS	*16.440±0.014*	*HLW*	*23.224±0.087*
ZNB	*23.021±0.257*	*AKB*	*22.042±0.008*
TDD	*20.146±0.440*	*JLS*	*20.928±0.280*
SIM	*20.198±0.637*	*DLM*	*21.552±0.020*
RKM	*10.146±0.016*	*AAT*	*23.336±0.034*
ARG	*20.850±0.048*	*SLT*	*30.824±0.116*
		TKG	*25.182±0.008*
		TSP	*23.394±0.016*

.

Appendix v: Caffeine contents of the NGR green tea and LND black tea samples analysed(mg/100mL).

Niger green tea samples codes	*Caffeine content(mg/100mL)*	*London tea samples codes*	*Caffeine content(mg/100mL)*
ADO	*22.264±0.240*	*BAR*	*20.875±0.013*
ACC	*17.866±0.028*	*BTS*	*20.474±0.040*
MBT	*19.396±0.008*	*BTP*	*18.197±0.008*
BUT	*17.280±0.008*	*BTM*	*18.472±0.053*
KML	*18.668±0.090*	*BTm*	*18.481±0.008*
TLS	*19.238±0.020*	*BTV*	*19.896±0.127*
SHR	*19.184±0.027*	*Eng. 1*	*22.699±0.033*
AZW	*20.137±0.426*		

Appendix vi: Caffeine contents of the HNK and NGA tea samples analysed(mg/100mL).

Hongkong tea sample codes	*Caffeine content(mg/100mL)*	*Nigeria tea samples codes*	*Caffeine content(mg/100mL)*
TSN2	*25.789±0.026*	*YLT*	*05.460±0.034*
TSN4	*07.809±0.028*	*TPT*	*32.614±0.061*
TSN5	*05.083±0.095*	*CTT*	*19.162±0.077*
TSN7	*06.323±0.061*	*TTT*	*23.154±0.174*
TSN8	*09.376±0.016*	*HLT*	*20.938±0.237*
		RMT	*23.956±0.248*
		TBR	*35.720±0.033*
		BCT	*34.670±0.473*

Appendix vii: Caffeine contents of the tea samples analysed (mg/100mL).

Mean caffeine of tea from different countries	*Caffeine content(mg/100mL)*
China	*20.189±0.223*
Srillanka	*24.066±0.072*
Niger	*19.254±0.112*
London	*19.871±0.040*
Hong Kong	*10.876±0.045*
Nigeria	*24.459±0.167*

Appendix viii: pH of soft drink samples analysed.

Soft drinks sample codes	*pH*	*Energy drinks samples codes*	*pH*
CCD	*3.01±0.010*	*TED*	*3.33±0.006*
CCZ	*3.47±0.017*	*CED*	*3.45±0.183*
CCL	*2.62±0.021*	*14MM*	*3.21±0.162*
PCD	*3.09±0.015*	*MED*	*3.33±0.183*
PCL	*3.59±0.025*	*PHE*	*2.99±0.017*
PMD	*3.23±0.015*	*BUE*	*3.41±0.006*
KCD	*2.91±0.015*	*BED*	*3.67±0.012*
LED	*3.27±0.016*	*GGG*	*3.49±0.017*
MDD	*3.41±0.020*	*RTE*	*3.98±0.012*
		RBE	*3.20±0.010*

Appendix ix: pH of instant coffee samples analysed.

Instant coffee samples codes	*pH*	*Regular coffee samples codes*	*pH*
NCI	*5.45±0.010*	*RYC*	*6.18±0.006*
SCI	*5.32±0.400*	*RBT*	*6.95±0.006*
KCI	*5.55±0.050*	*MMC*	*5.69±0.010*
ACI	*6.51±0.017*	*GKC*	*6.77±0.032*
C73	*6.85±0.006*	*KCR*	*6.34±0.006*
CTI	*5.57±0.053*	*GCR*	*6.17±0.015*
GCI	*5.25±0.047*	*CMR*	*6.81±0.012*
C21	*6.79±0.006*	*CCR*	*6.33±0.023*
UCI	*5.45±0.060*	*SOC*	*5.82±0.020*
		MCR	*5.92±0.012*

Appendix x: Mean pH of drinks and coffee samples analysed.

Samples names	*pH*
Soft drink	*3.406±0.061*
Energy drink	*3.178±0.017*
Instant coffee	*5.860±0.072*
Regular coffee	*6.298±0.020*

Appendix xi: pH of China green tea samples analysed.

China tea samples codes	*pH*	*Srillanka tea samples code*	*pH*
TNR	*6.25±0.012*	*NMD*	*5.47±0.025*
FLS	*5.71±0.059*	*HLW*	*5.85±0.006*
ZNB	*5.81±0.012*	*AKB*	*5.71±0.010*
TDD	*6.42±0.020*	*JLS*	*5.49±0.026*
SIM	*6.27±0.021*	*DLM*	*5.56±0.006*
RKM	*6.40±0.066*	*AAM*	*5.71±0.240*
ARG	*6.29±0.012*	*SLT*	*5.93±0.006*
		TKG	*5.73±0.010*
		TSP	*5.74±0.021*

Appendix xii: pH of Niger green tea samples analysed.

London tea samples code	*pH*	*Niger green tea samples codes*	*pH*
BTA	*6.60±0.015*	*ADO*	*6.75±0.006*
BTS	*5.55±0.021*	*ACC*	*6.34±0.010*
BTP	*5.54±0.010*	*MBT*	*6.21±0.012*
BTM	*6.35±0.015*	*BUT*	*6.10±0.035*
BTm	*6.67±0.015*	*KML*	*6.27±0.015*
BTV	*6.30±0.006*	*TLS*	*6.56±0.021*
ENG.1	*6.57±0.012*	*SHR*	*6.75±0.035*
		AZW	*6.20±0.006*

Appendix xiii: pH of Hong Kong tea samples analysed.

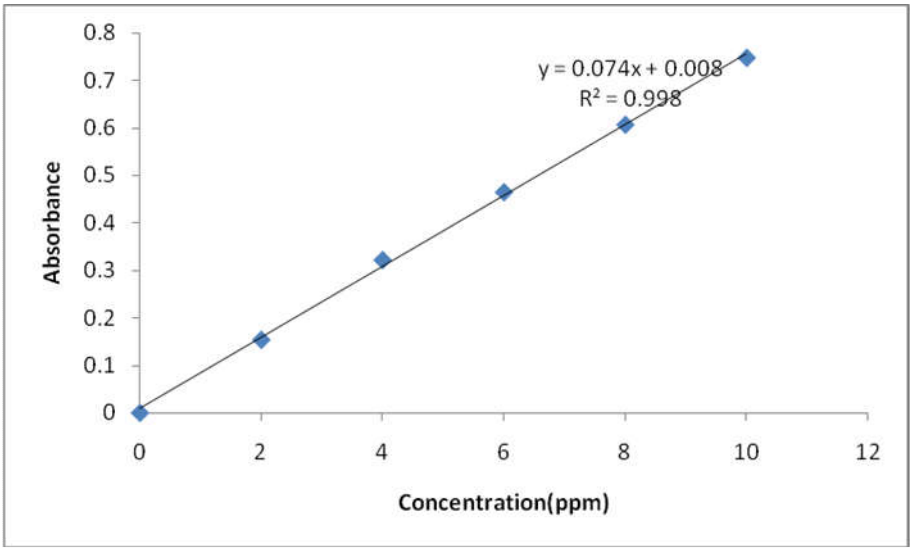
Hong Kong tea samples codes	*pH*	*Nigerian tea samples codes*	*pH*
TSN2	*6.40±0.020*	*YLT*	*4.89±0.067*
TSN4	*5.83±0.020*	*TPT*	*5.43±0.021*
TSN5	*5.75±0.021*	*CTT*	*5.66±0.015*
TSN7	*5.99±0.031*	*TTT*	*6.54±0.006*
TSN8	*5.62±0.006*	*HLT*	*5.73±0.006*
		RMT	*5.62±0.040*
		TBR	*5.72±0.042*
		BCT	*5.90±0.015*

Appendix xiv: Average pH of tea samples analysed.

Mean pH of tea samples from different countries	*pH*
China	*6.16±0.029*
Srillanka	*5.69±0.039*
Niger	*6.40±0.018*
London	*6.23±0.013*
Hong Kong	*5.92±0.020*
Nigeria	*5.69±0.027*

Concentration(ppm)	Absorbance
0	0
2	0.154
4	o.322
6	o.465
8	0.607
10	0.748

Appendix xv:Callibration curve for Caffeine standard.



GLOSSARY (samples Analysed Abbreviations)

Soft drinks	*Abbreviation*	*Energy drinks*	*Abbreviation*	*Instant coffee*	*Abbreviation*	*Regular coffee*	*Abbreviation*
Coca cola	CCD	Red Bull	*RBE*	Nescafe Classic	*NCI*	Ginseng Coffee	*GCR*
Coca cola Zero	CCZ	Power Horse	*PHE*	Alicafe	*ACI*	Mac coffee	*MCR*
Coca cola Life	CCL	Body up	*BUE*	Cafe21	*C21*	Max. Man Coffee	*MMC*
Mountain Dew	MTD	14mm	*14MM*	Good cafe	*GCI*	Goldkili Coffee	*GKC*
Pepsi Cola	PCD	Buffet	*BED*	Uni cafe	*UCI*	Shake off Coffee	*SOC*
Pepsi Light	PCL	More	*MED*	Star Café	*SCI*	Red Bubble Te	*RBT*
Pepsi Max	PCM	Tiger	*TED*	King Café	*KCI*	Red Yeast Coffee	*RYC*
Kally	KCD	Climax	*CED*	Café Tinno	*CTI*	Cowbell Coffee	*CCR*
Lucozade Energy	LED	Rock Top	*RED*	Café 73	*C73*	Kent Coffee	*KCR*
		Triple G	*GGG*			Coffee Mix	*CMR*

Countries name	*Abbreviation*
China	*CHN*
Srillanka	*SLK*
Niger	*NGR*
Hongkong	*HNK*
London	*LND*
Nigeria	*NGA*

China	*Abbreviation*	*Srillanka*	*Abbreviation*	*Niger*	*Abbreviation*
T e n e r e	*TNR*	N a m a r d e	*NMD*	A d o u a	*ADO*
F l e c h e	*FLS*	H i l l w a y	*HLW*	Achachi	*ACC*
Z a i n a b a h	*ZNB*	A k b a r	*AKB*	Maibuta	*MBT*
The daddy	*TDD*	J o l l y s u n	*JLS*	B u t a	*BUT*
S i m	*SIM*	D i l m a h	*DLM*	Kamala	*KML*
R a k i m	*RKM*	Alameer tea	*AAT*	Telemsi	*TLS*
Aragayga	*ARG*	S u l t a n t e a	*SLT*	S a h a r a	*SHR*
		T e a k i n g	*TKG*	Azawad	*AZW*
		T e a s h o p	*TSP*		

London	*Abbreviation*	*Hong Kong*	*Abbreviation*	*Nigeria*	*Abbreviation*
Black tea apple refresh	*BTA*	The santhe No. 2	*TSN2*	Yellow label	*YLT*
Black tea strawberry	*BTS*	The santhe No.4	*TSN4*	T e a p o t	*TPT*
Black tea peach & passion	*BTP*	The santhe No.5	*TSN5*	C i t y t e a	*CTT*
Black tea mint	*BTM*	The santhe No.7	*TSN7*	T o p t e a	*TTT*
Black tea mango magic	*BTm*	The santhe No.8	*TSN8*	Highland tea	*HLT*
Black tea vanilla tranquillity	*BTV*			Richmond tea	*RMT*
English No. 1	*Eng.1*			T a b a r a t e a	*TBR*
				Beyond comment tea	*BCT*

Soft drink sample

Anova: Single Factor	
CCD	9.85
CCZ	9.66
CCL	12.20
MDD	10.68
PCD	10.00
PLD	9.01
PCM	11.30
KCD	10.87
LED	9.02

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	9	45	5	7.5		
Column 2	9	92.59	10.28778	1.135319		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	125.8227	1	125.8227	29.14141	5.92E-05	4.493998
Within Groups	69.08256	16	4.31766			
Total	194.9052	17				

ENERGY DRINK	
RED	34.65
PED	37.00
BED	40.32
14MM	37.90
BUE	36.25
MED	38.71
TED	40.41
CED	38.44
RED	40.88
GGG	38.28

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	10	55	5.5	9.166667
Column 2	10	382.84	38.284	3.861049

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5373.953	1	5373.953	825.0032	1.73E-16	4.413873
Within Groups	117.2494	18	6.513858			
Total	5491.203	19				

	INSTANT COFFEE
NCI	37.76
ACI	36.46
C21	43.53
GCI	44.55
UCI	47.16
SCI	45.16
KCI	45.51
CTI	34.15
C73	38.58

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5971.788	1	5971.7 88	403.45 27	8.96 E-13	4.49399 8
Within Groups	236.8273	16	14.801 71			
Total	6208.615	17				

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	45	5	7.5
Column 2	9	372.86	41.4288 9	22.1034 1

REGULAR COFFEE

GCR	30.52
MCR	40.64
MMC	32.16
GKC	33.12
SOC	37.12
RBT	37.43
RYC	37.73
CCR	9.83
KCR	12.05
CMR	9.19

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	10	55	5.5	9.166667
Column 2	10	279.79	27.979	157.1973

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2526.527	1	2526.527	30.3735	3.11E-05	4.413873
Within Groups	1497.275	18	83.18196			
Total	4023.802	19				

SOFT DRINKS	10.288
ENER. DRINK	34.784
INSTANT COFFEE	41.429
REGULAR COFFEE	27.979

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	10	2.5	1.666667
Column 2	4	114.48	28.62	179.5128

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1364.509	1	1364.509	15.06251	0.008161	5.987378
Within Groups	543.5385	6	90.58975			
Total	1908.047	7				

CHN GREEN TEA

TNR	20.518
FLS	16.44
ZNB	23.021
TDD	20.146
SIM	20.198
RKM	10.146
ARG	20.85

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	7	28	4	4.666667
Column 2	7	131.319	18.75986	18.19784

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	762.4868	1	762.4868	66.69612	3.04E-06	4.747225
Within Groups	137.187	12	11.43225			
Total	899.6739	13				

SLK GREEN TEA

NMD	26.108
HLW	23.224
AKB	22.042
JLS	20.928
DLM	21.552
AAT	23.336
SLT	30.824
TKG	25.182
TSP	23.394

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	9	45	5	7.5
Column 2	9	216.59	24.06556	9.130381

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1635.729	1	1635.729	196.7158	2.08E-10	4.493998
Within Groups	133.043	16	8.31519			
Total	1768.772	17				

NGR GREEN TEA
SAMPLES

ADO	22.264
ACC	17.866
MBT	19.396
BUT	17.28
KML	18.668
TLS	19.238
SHR	19.184
AZW	20.137

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	36	4.5	6
Column 2	8	154.033	19.25413	2.290246

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	870.7368	1	870.7368	210.063	8.01E-10	4.60011
Within Groups	58.03172	14	4.145123			
Total	928.7685	15				

LND TEA SAMPLES

BAR	20.875
BTS	20.474
BTP	18.197
BTM	18.472
BTm	18.481
BTV	19.896
Eng.1	22.699

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	7	28	4	4.666667
Column 2	7	139.094	19.87057	2.676902

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	881.5626	1	881.5626	240.0911	2.68E-09	4.747225
Within Groups	44.06141	12	3.671784			
Total	925.624	13				

HNK

TSN2	25.789
TSN4	7.809
TSN5	5.083
TSN7	6.323
TSN8	9.376

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	15	3	2.5
Column 2	5	54.38	10.876	72.08568

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	155.0784	1	155.0784	4.158397	0.075766	5.317655
Within Groups	298.3427	8	37.29284			
Total	453.4212	9				

NGA TEA SAMPLES

YLT	5.46
TPT	32.614
CTT	19.162
TTT	23.154
HLW	20.938
RMT	23.956
TBR	35.72
BCT	34.67

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	8	36	4.5	6
Column 2	8	195.674	24.45925	100.1361

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1593.487	1	1593.487	30.02725	8.11E-05	4.60011
Within Groups	742.9524	14	53.06803			
Total	2336.439	15				

MEAN CAFFEINE CONTENT OF TEA SAMPLES

CHN	20.189
SLK	24.066
NGR	19.254
LND	19.871
HNK	10.876
NGA	24.459

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	6	21	3.5	3.5
Column 2	6	118.715	19.78583	23.99922

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	795.6851	1	795.6851	57.86965	1.82E-05	4.964603
Within Groups	137.4961	10	13.74961			
Total	933.1812	11				