



Spectrometric Determination of Sugar and Caffeine content in Soft Drinks sold in Ede Metropolis

M.A. Abibu¹, O.G. Dawodu², A. A.T. Taleat³, B.O. Adeniyi⁴ and I. M. Olanipekun⁵

^{1,3,4,5} Department of Chemical Science, Federal Polytechnic Ede, Osun State, Nigeria

²Department of Biochemical Science, Federal Polytechnic Ede, Osun State, Nigeria

Corresponding email: mabibu09@yahoo.com

Abstract: Nowadays, the growing interest in the consumption of soft drinks for refreshment by many Nigerian populaces has necessitated the quality control and quality assurance debate on their health supremacy. A study was carried out to determine and compare sugar and caffeine content in five selected industrial soft drinks namely coca-cola, fanta, sprite, fearless and pepsicola drinks sold in Ede metropolis Osun-state, Nigeria. Samples were purchased randomly from local shops (Timi and Total Oke-Gada markets) within Ede North local Government, Osun-state, Nigeria. The sugar and caffeine content in soft drink were determined by Uv/visible spectroscopic method while pH value determined by AOAC conventional method with pH meter was achieved. The results showed consistent pattern with respect to pH in acidic medium. Thus, no significant differences were observed in pH value of coca-cola, fanta, fearless and pepsicola samples of pH ranges between 2.1-2.6 while sprite drink showed highest pH value of 3.1. Among all soft drink samples fearless had highest caffeine content of 32.3 ± 0.00 mg/L. The concentration of sugar ranges between 1.6 ± 0.04 – 3.3 ± 0.01 mg/L. Sugar concentrations in selected soft drinks were in the order pepsicola > coca-cola > sprite > fearless > fanta. The soft drinks were all acidic at 25°C to prevent color degradation. The differences still fall within the acceptable limit. This provides a blue print for the selected industrial soft drinks, hence, it's recommended that despite the sugar and caffeine content in these drinks are within the recommended limits, and little to moderate consumption is thereby advised.

Keywords: Caffeine, Sugar, soft drink samples, Spectroscopic method

1. Introduction

Good nutrition is the foundation of good health. The growing interest in the consumption of industrial soft drinks and other non-alcoholic beverages for refreshment by many Nigerian populaces has necessitated the need for quality control and quality assurance information on their health supremacy. In a published paper, by Idumah *et al.*, (2020) reported that the high rate in consumption carbonated/soft drinks is associated to the lack of portable water and climate change (hot weather) among others. Apart from drinking water, soft drinks including sodas, energy drink, chocolate, ice-cream and fruit drinks are good source of hydration to quench thirst and cool the body during hot summer. Most soft drinks intended for human consumption are cooled by electric refrigeration for consumption on the premises. There is no single definition available for soft drink, but in general term it is a complex mixture that encompasses non-alcoholic liquid, fruit acids, sweetening agents and natural or artificial flavored, colorings water based drink that have carbon dioxide added to them to make them bubbly or fizzy (Chaudhary, 2018). Ingredients formulations in soft drinks of different brands are typically composed of sugar, caffeine, coloring, dilute phosphoric acid and flavoring agents. (Damle *et al.*, 2011). These chemical compositions of soft drink give a tangy taste which is liked by everyone. However, a large percentage of these ingredients, for example, on the basis of sugar and caffeine contents may be hazardous to human health if consumed in large quantities, and there is widespread concern generally with regard to negative health issues, preservatives and sweeteners (Kregiel, 2014). Chemically, sugar as glucose Figure 1a is a sweet water-soluble carbohydrate to enhance the flavor of a drink and gives a satisfying sensation in human diet. On the other hand caffeine, Figure 1b, is a tasteless white crystalline 1,3,7-trimethylxanthine, an active alkaloid compound used for mild psychoactive therapy which is legal and unregulated consumed in most parts of the

world to activate pulse, brain and for body system stimulation. It even increased incidence of cancer and effects on male fertility.

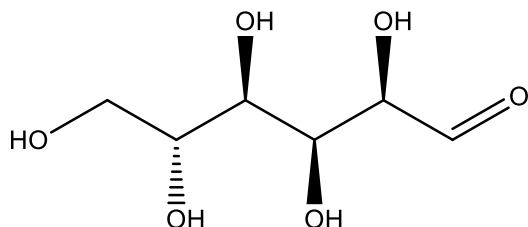


Figure 1.a Chemical structure of glucose (2014).

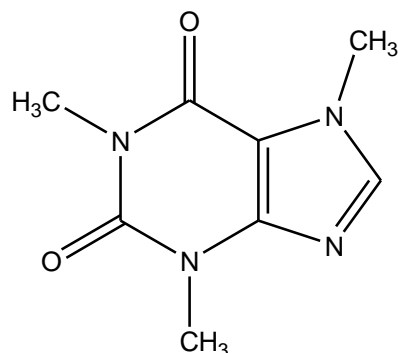


Figure 1.b Chemical structure of caffeine (Gerald et al., 2014).

Till today, there are different types of soft drinks and other non-alcoholic beverages within Nigeria from various manufacturers that many consumers believed some of them have high percentage of sugar and caffeine content and as such, these may result to negative health issues such as tooth erosion, changes in adult behavior and nervousness (Ohilebo and Blessing, 2020). In addition, it may also be associated with increases in blood sugar level and psychoactive performance of the people of all ages. The investigations regarding the safety, efficacy of the industrial soft drink products in Nigerian markets has become an essential part on human health in order to reduce the risk of non-communicable diseases among the populaces. As such reliable, sensitive method for the determination of sugar and caffeine in selected soft drinks, with shortest time is no longer a choice, but must in order to ensure food security. Therefore, this study aim to find out pH value and carry out the determination of sugar and caffeine content and from five (5) selected industrial soft drinks samples namely coca-cola (coke), fanta, sprite, fearless and pepsicola sold in Ede, Osun-state, by liquid-liquid extraction and analysed using UV/Visible spectrometric technique. The choice of UV/Visible spectrometric method is due to its simple operation, accurate data from the time-dependent reaction and it's available in our laboratory.

2. Materials and Methods

2.1. Chemicals and Materials

All chemicals and reagents were of analytical grades sucrose and anhydrous caffeine standards, chloroform (CHCl_3), sodium carbonate, HCl (95%), NaOH pellet, 3,5-dinitrosalicylic acid (DNSA) powder were purchased from BDH laboratory, Poole, BH5 1TD, England. All reagents solutions used in this study were prepared by using distilled water produced by Pure-Distiller SZ-96, Lab Science England. The values of pH of the samples were performed by using Sartorius pH meter.

2.2. Instrumentation

UV-Visible Spectrophotometer 6405 JENWAY (Hewlett Packard, United States) equipped with photo electric detector, using 1 cm quartz cell for the analysis. The absorption bands of the aqueous standard solutions were recorded over the wavelength 200-400 nm.

2.3. Wavelength selection

The wavelength at which caffeine absorbs maximum was determined by scanning the range of 200-800 nm. The wavelength at which caffeine absorbs maximum was found to be 270 nm caffeine and that of sugar is 580 nm.

2.4. Soft Drink Sample Collection

Five samples (Coca-cola, Fanta, Sprite, Pepsi, Fearless,) drinks were purchased randomly from local shops (Timi and Total Oke-Gada markets) within Ede North local Government, Osun-state, Nigeria. These entire products are registered and licensed in Nigeria by their respective companies.

2.5. Preparation of standard solution

First, 250 mL of 100 ppm caffeine standard stock solution was prepared in purified carbon tetrachloride (CCl₄). From which further serial dilutions takes place by pipetting 10, 20, 30, 40 and 50 mL in 100 mL carbon tetrachloride to create 10, 20, 30, 40 and 50 mg/L, standard solution respectively. The absorbance of each serial dilution was measured at a wavelength of 270nm using 10 mm quartz cuvette. Then, the graph of signal absorbance readings was plotted against working standard serial standard concentrations to generate a standard calibration curve.

2.6. Sample Treatment

Samples were degassed by placing it in a sonication bath for some time at room temperature until no more bubbles appear in each of the sample before liquid-liquid extraction. The entire extraction-cleanup procedure and spectrometric analysis was completed within one (1) day.

2.7. Determination of pH

The method described by AOAC, 2010 was used to measured pH of selected soft drinks. In brief, about 5ml of degassed soft drink was dispensed in a beaker and by using calibrated pH meter pH was measured. The conventional method of calibrating pH method was employed with buffer 4 and 7.

2.8. Estimation of caffeine level in soft drink

Estimation of caffeine is as per the method described by Desai (2020) with slight modification. First of all, about 5 mL of coke sample was drawn with a 10mL pipette and placed into a 125mL separating funnel followed by the addition of 10 mL distilled water, then 1 mL of 20% aqueous Na₂CO₃ solution and 20 mL of carbon tetrachloride CCl₄ as extracting solvent in separating funnel. The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. Then separating funnel was taken and adjusted it in the stands with beakers. The non-aqueous CCl₄ layer was removed to a clean 50mL volumetric flask. Another 20mL portion of CCl₄ was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice more and the CCl₄ solvent layers combined. This volume was made up to 50mL with the solvent. This procedure was repeated for all the drink samples. The absorbance of the resulting solutions was measured on UV/Visible spectrophotometer at 270nm using 10 mm quartz cuvette. Similar procedure was performed with different samples of soft drink and quality of caffeine was observed in them.

2.9. Sugar extraction procedure

The method was based on the color developed which forms when sugar is reduced from 3,5-dinitrosalicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid. Procedure, five serial dilutions of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL from sucrose stock solution as standards were prepared for standard calibration curve. Two milliliters (2 mL) of each sample of soft drinks at each instance and 2ml of each sucrose standard was pipetted into a 10 mL test tube. Into a separate test tube, 2 mL of distilled water was added for the blank solution. This is followed by the addition of 2 mL of 6M HCl then placed in a boiling water bath for 10 minutes and left to develop color. Additions of 8 mL of 2.5 M NaOH solution and 2 mL of 0.05 M DNSA solution then cover the test tube with stopper and shake well to mix. It was then placed in boiling water bath for 5 minutes followed by ice cold water for 10 minutes. Then the color developed stop. The absorbance of the five standard solutions and sample solutions was measured at 580 nm. All the aforementioned steps were conducted within less than 30 minutes. The time between DNSA addition and measurements is the same for all test sample solutions. A blank solution was included in each analytical run to check for interferences (e.g., signal intensity difference) and to control the accuracy. The concentration was then calculated.

3. RESULTS AND DISCUSSION

This experiment was carried out for the determination of sugar and caffeine content in selected soft drinks. In this study amount of sugar and caffeine content present in five (5) selected soft drink (Coca-Cola, Fanta, Sprite, Fearless and Pepsi-Cola) samples was estimated. Along with sugar and caffeine concentrations pH of the soft drink samples was also estimated. The following results were obtained by the laboratory analysis

3.1. pH

Table 1 depicts the results of the pH of selected samples of soft drinks. The drop in pH was found in the range of 2.14-3.06 revealed acidic medium which may be responsible to prevent deteriorating during soft drinks storage by actions of various microorganisms. Amongst all the soft drink samples, Sprite has highest pH of 3.06 while Pepsi-Cola has least pH of 3.14. The difference in the pH value may be due to the type of acid used by the manufacturers. Khalid *et al.*, (2016) reported that the pH values of beverages or industrial soft drinks could be as a result of presence of carbon dioxide, phosphoric acids, malic acid, tartaric acid used as preservatives by manufacturers.

Table 1 pH of selected soft drinks

Sample	pH
A	2.60
B	2.56
C	3.06
D	2.30
E	2.14

Key: A=Coca-Cola, B=Fanta, C=Sprite, D=Fearless, E=Pepsi-Cola. Values are expressed as mean \pm SD (Standard Deviation) N=3.

3.2. UV-visible Spectrophotometric Detection.

The spectrometric analysis with a photo diode detector applied to the samples solution provides good signals intensity for caffeine standard at different concentrated solutions at 270 nm Table 2 similarly, intensity responses of sugar standard at different concentrated solutions at 580 nm Table 3.

Table 2: Caffeine and Sugar standards

Caffeine Conc. (mg/L)	Abs@270nm	Sugar Conc. (mg/ml)	Abs @ 580nm
10	0.33	0.2	0.043
20	0.71	0.4	0.067
30	1.07	0.6	0.116
40	1.46	0.8	0.136
50	1.78	1.0	0.189

3.3. Standard calibration curve

Plotting the absorbance against the concentration of standards generated the calibration curve. Within the concentration range studied, 10-50 mg/L and 0.1-1.0 mg/L linear plots were obtained for caffeine and sugar respectively. The mean correlation coefficients and equations obtained were $R^2 = 0.9991$ ($Y = 0.0635x - 0.025$) as shown in Figure 3a and 0.9805 ($Y = 0.1805x + 0.0019$) as shown in Figure 3b for caffeine and sugar respectively.

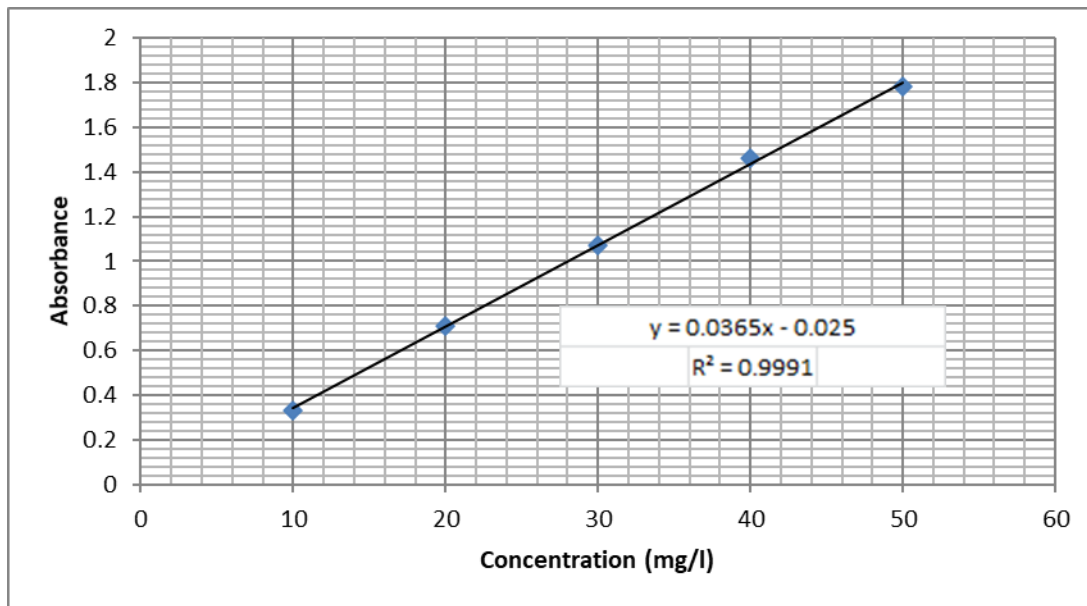


Figure 2a: Calibration curve for caffeine

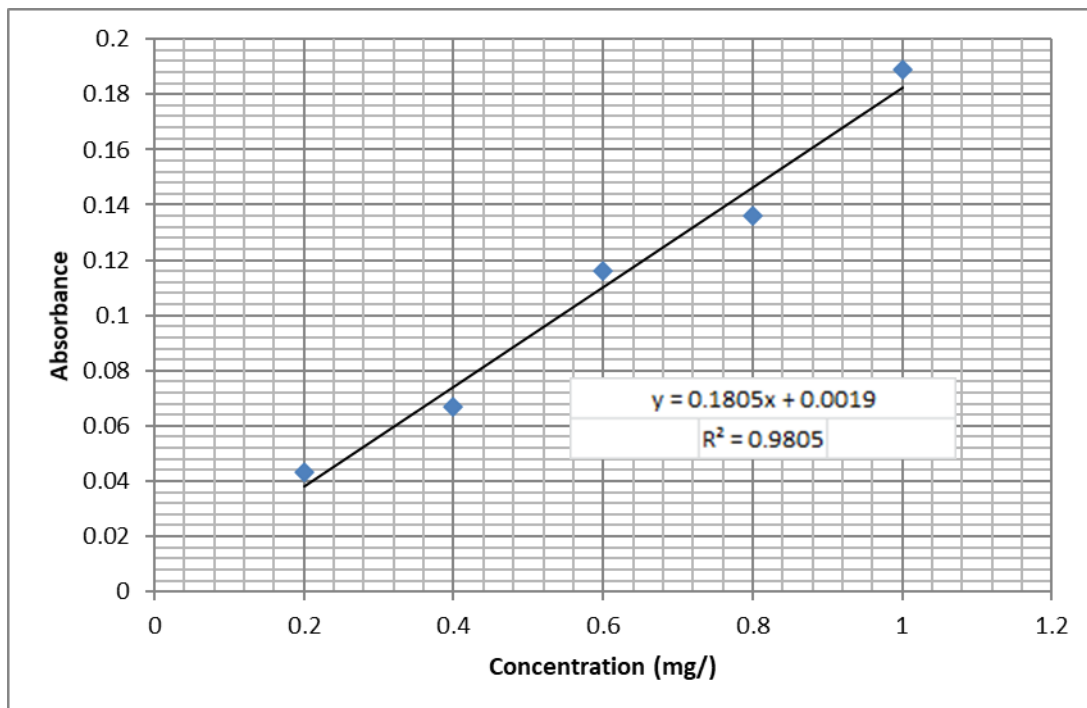


Figure 2: Calibration curve for Sugar

3.4. Concentration of caffeine and sugar content (mg/L)

The concentrations of caffeine and sugar in the samples were calculated using standard calibration curve shown in Figure 3. Caffeine concentration ranges in soft drink was found from 20.19mg/L to 32.27mg/L. Caffeine concentration in Coca-Cola was found to be 24.45mg/L, in Fanta was found to be 20.55mg/L, in Sprite was 20.19mg/L, in Fearless was 32.27mg/L, in Pepsi was 23.48mg/L. So among the entire sample the highest amount of caffeine was found in Fearless (32.27mg/L) and least amount of caffeine was found in Sprite (20.19mg/L). Also, sugar concentration ranges in soft drink was found from 1.602mg/mL to 3.337mg/mL. Sugar concentration in Coca-Cola was found to be 2.079mg/mL, in Fanta was found to be 1.602mg/mL, in

Sprite was 1.956mg/mL, in Fearless was 1.899mg/mL, in Pepsi was 3.337mg/mL. Among the entire samples, the highest amount of sugar was found in Pepsi (3.337mg/mL) and least amount of sugar was found in Fanta (1.602mg/mL).

Table 3: Concentration of caffeine and sugar content (mg/L) with Recommended Daily In-take (RDI)

Samples	Concentration of caffeine (mg/L)	RDI(mg/day) by WHO (2015)	Sugar concentration (mg/mL)	RDI(mg/day) by WHO (2015)
A	24.45±0.01	400	2.079±0.09	2500
B	20.55±0.03	400	1.602±0.04	2500
C	20.19±0.06	400	1.956±0.02	2500
D	32.27±0.00	400	1.899±0.02	2500
E	23.48±0.15	400	3.337±0.01	2500

Key: A=Coca-Cola, B=Fanta, C=Spite, D=Fearless, E=Pepsi

4. Conclusion

The Spectrophotometric methods using in this study was useful and accurate to determine the concentration of caffeine and sugar in selected industrial soft drinks samples (Coca-Cola, Fanta, Spite, Fearless, Pepsi cola). High consumption of caffeine and sugar leads to adverse effect. So, determination of caffeine and sugar from soft drinks are necessary. The caffeine and sugar contents found from the soft drinks are lower than the specified limit (WHO, 2015). The quantity of caffeine and sugar in relation to the standard is generally reduced. These selected soft drinks are general highly acidic for preservation.

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