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Analysis Of Chemical Components And Reducing Sugar Content In Popular Energy Drinks In Nigeria: A Comprehensive Study

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Abstract
The study undertook a thorough examination of the chemical components present in soft drinks, focusing particularly on popular energy drink brands in Nigeria and their potential health effects using established chemical analysis techniques. All the sampled soft drinks exhibited acidity attributed to acidic preservatives, with pH levels ranging from 2.78 to 3.28, impacting both taste and shelf life. The Lane and Eynon titration method was employed to determine sugar content, revealing the presence of both reducing sugars (glucose, fructose) and non-reducing sugars (sucrose), which ranged from 15.28 to 23.71 g/100 ml. Energy drinks, notable for their higher caffeine content to counteract bitterness, were found to contain caffeine levels ranging from 325.11 to 405.12 mg/L, measured using UV-visible spectrophotometry. Thin-layer chromatography identified synthetic food colorants across different drinks, which serve to enhance visual appeal and influence consumer perception. The study underscores the importance of consumer awareness regarding the chemical composition of soft drinks, particularly energy drinks, and their potential health implications.
Keywords: *soft drinks, energy drinks, chemical constituents, consumer awareness*

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I. Introduction

A soft drink, also referred to as soda, pop, tonic, mineral, or carbonated beverage, is a quenching beverage typically crafted from water, sweeteners, and flavoring agents. The sweetening elements may encompass sugar, high-fructose corn syrup, or sugar substitutes in the context of diet drinks. Additionally, soft drinks may incorporate caffeine, fruit juice, or a fusion of both [1]. It is essential to note that these beverages are non-alcoholic and distinctly differ from *hard drinks*, which contain alcohol.

In recent times, the Nigerian market has witnessed a notable upsurge in the consumption of energy drinks, a specific subtype of soft drinks tailored towards children and young adults. These energy drinks, enriched with caffeine, herbal extracts, B vitamins, and taurine, have garnered popularity, especially among the youth demographic. However, concerns have emerged regarding the potential impact of these soft drinks on consumer well-being.

The chemical composition of soft drinks plays a pivotal role in their overall acceptance among consumers. The acidity of soft drinks, predominantly attributed to carbon dioxide gas or various acids such as phosphoric, malic, ascorbic, citric, and tartaric acids used for preservation, has been identified as a contributing factor to enamel erosion and dental health issues associated with prolonged consumption [2].

Caffeine, (1,3,7-trimethylxanthine), a prevalent ingredient in energy drinks, is a natural stimulant found in plants like coffee beans, kola nuts, and tea leaves, widely utilized in soft drinks, energy beverages, and pharmaceuticals. Upon ingestion, caffeine stimulates the central nervous system and is swiftly absorbed from the gastrointestinal tract, undergoing demethylations that result in paraxanthine (84%), theobromine (12%), and theophylline (4%). The xanthines, theobromine, and theophylline, sharing chemical structures with caffeine, contribute to the metabolic changes associated with caffeine consumption [3]. It is crucial to acknowledge that while caffeine can enhance physical or mental performance by elevating the secretion of epinephrine, its prevalent presence in energy drinks requires careful consideration of potential health implications associated with prolonged and excessive consumption [19].

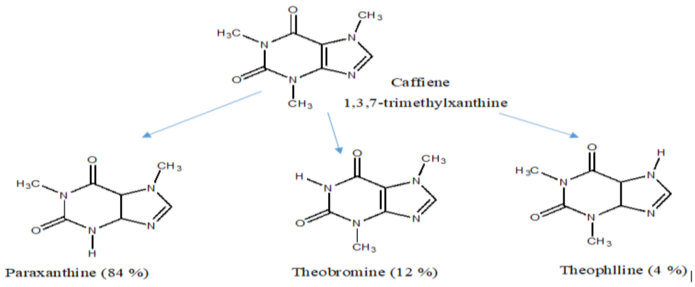

Figure 1: Chemical structure of caffeine and its metabolites.

Research on the cardiovascular effects of caffeine has delved into its impact on hemodynamic parameters. A comprehensive review indicates that, among healthy adults, a moderate daily caffeine intake of ≤ 400 mg (equivalent to 6 mg/kg/d for a 65 kg person) is generally not linked to adverse effects [4] [19]. However, exercising caution in daily caffeine consumption is recommended to mitigate potential negative consequences.

Food colorants constitute another crucial component of most soft drink brands, with exceptions being rare. These substances, including dyes, pigments, and other additives, contribute color to food and beverages. They exist in various forms such as liquid, gel, paste, or powder, categorized as natural, nature identical, synthetic, or inorganic [5]. Synthetic food colorants, manufactured in factories, are water-soluble and can be directly incorporated into foods without additional processing.

Food colorants have been implicated as a significant source of food intoxication, leading to surveys to identify non-permitted colors in different food products. The exclusion of certain food dyes is attributed to their toxicological, mutagenic, and carcinogenic properties. For instance, Sudan is identified as a harmful dye causing mutagenic changes and stomach damage [5] [6]. Complications associated with colorants include asthma, urticaria, abortion, hyperactivity in children, carcinogenicity, decreased intelligence quotient (IQ), anaphylactic reactions, idiosyncrasy, weakened immune system, decreased white blood cell (WBC) and lymphocyte count, and vitamin B6 deficiency [7].

Food colorants can be categorized into major groups as natural, nature identical, synthetic, and inorganic. Synthetic food colorants are chemical substances that do not occur in nature and are manufactured in factories. These colorants are typically water-soluble and can be used in foods without additional processing [5].

The maximum permissible limit of colorants to be added to any food shall be 0.1 g/kg of food as consumed. The Acceptable Daily Intake (ADI) is defined as the amount of a substance that can be consumed every day throughout an individual's lifetime without appreciable health effects. Even permissible artificial colorants, if consumed indiscriminately, pose potential risks. The ADI of erythrosine was reduced from 2.5 to 0.1 mg/kg body weight due to observed effects on thyroid function in short-term rat studies.

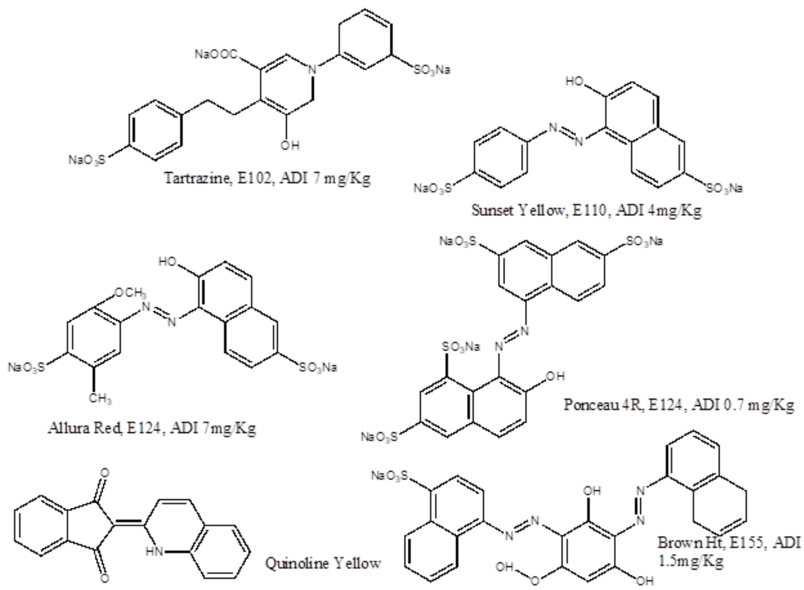

Figure 2: Chemical Structures Of Common Food Colourant, The European Union (E) Identification Numbers, And Their Acceptable Daily Intake (ADI)

Overweight and obesity, once primarily concerns in developed nations, have now become prevalent in developing countries. The Body Mass Index (BMI), calculated as the ratio of body weight (BW) in kilograms to the square of an individual's height in meters, serves as an indicator of whether someone is overweight (BMI > 25) or in an obese condition (BMI > 30). Lifestyle and dietary changes contribute to increased risk factors for metabolic diseases, including obesity, metabolic syndrome (MetS), type 2 diabetes (T2D), hypertension, dyslipidemias, stroke, and cardiovascular diseases (CVDs) mortality [7] [20].

The rising consumption of soft drinks is linked to the onset of these health issues [18] [22]. Approximately 60% of sugar consumption in Nigeria is attributed to the food and soft drinks industries, while the remaining 40% is utilized by confectionaries, beverage, bakeries, pharmaceuticals, and dairy industries [8]. Nigeria's per capita sugar consumption in 2021 was around 8 kg, below the global average of 21.4 kg per person and the WHO-recommended 9.1 kg per annum.

Sweeteners can be categorized into two groups: artificial (synthetic) sweeteners, with little or no nutritional value, and natural sweeteners, also known as caloric sweeteners. Synthetic sweeteners have a sweetening power 30-500 times higher than sucrose, the commonly used sugar in drinks. To reduce the incidence of cardio-metabolic diseases, strategies such as controlling body weight by limiting calorie intake are recommended. A common approach is to decrease the consumption of drinks containing glucose or fructose/fructose syrup and replace sugars in the diet with low or non-caloric substitutes known as alternative sweeteners (AS) [9] [10] [11] [12]. Currently, ASs like acesulfame (used as acesulfame-potassium), saccharin, and aspartame play a crucial role in the food industry [13].

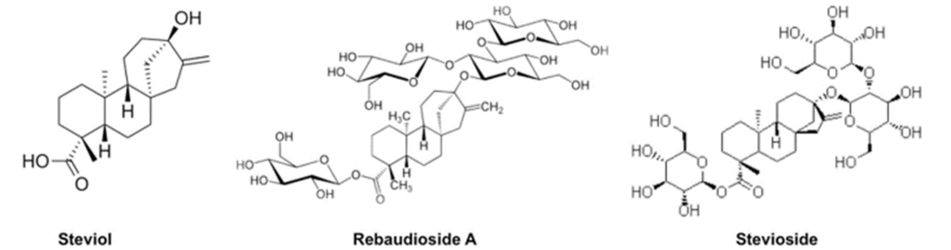

Figure 3: Chemical Structures Of Some Natural Sweeteners

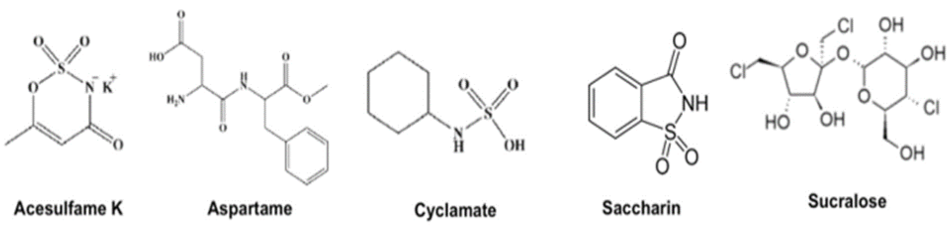

Figure 4: Chemical Structure Of Artificial Sweeteners

The sweeteners, whether it is high fructose corn syrup or unnecessarily-high amounts of sucrose, in carbonated sodas provide more calories than are generally needed by the average drinker.

This present study aimed to determine some chemical constituents of soft drinks (carbonated drinks, fruit and energy drinks) and their concentrations in other to provide health information for the consumers.

Table 1: Natural and Artificial Alternate Sweeteners (AS) Approved by FDA and EU

Sweetener Origin	Name	Chemical Formula	Sweetness*	ADI (FDA) mg/Kg	ADI (EU) mg/Kg
Artificial	Aspartame	$C_{14}H_{18}N_2O_5$	200	50	40
	Saccharin	$C_7H_5NO_3S$	300x	15	5
	Cyclamate	$C_6H_{12}NNaO_3S$	30-50x	Not approved for consumption	0 - 11
	Sucralose	$C_{12}H_{19}Cl_3O_8$	600x	5	15
Natural	Steviol	$C_{20}H_{30}O_3$	30 -300x	4	4
	Rebaudioside A	$C_{44}H_{70}O_{23}$	30 -300x	4	4
	Stevioside	$C_{38}H_{60}O_{18}$	30 -300x	4	4

II. Methodology

Sampling Procedure for Energy Drinks

Twelve soft drink brands, representing both carbonated and energy drinks were selected based on high consumer preference for laboratory analysis. Subsequently, 12 products were procured from the markets for further examination.

Chemicals and Instruments Used

Various chemicals and instruments, including Fehling A solution, Fehling B solution, methylene blue aqueous solution, sucrose, glucose (AR grade), NaOH, HCl, sulphuric acid, and phenol acetic acid (BHD Chemicals, Sussex, England), phenolphthalein indicator, n-butanol, ammonia solution, acetic acid solution (*Pharmacos*, Sussex England), standard color samples, glacial acetic acid, distilled water were utilized (VWR *Prolabo* Chemicals, Sussex, England) and Instruments such as a UV-visible spectrophotometer, silica-coated aluminum TLC plates (Sigma-Aldrich) and a *Brix* meter were employed.

Analysis for Sugar Content

Sugar content was determined through both the *Lane and Eynon* titration method. This method involved titration to measure the concentration of reducing sugars. Non-reducing sugar (sucrose) was converted into monosaccharide using strong acid (inversion). The titration was carried out according to the [14].

Standardization of Sucrose Solution

Dilute invert sugar standard solution (10 %) was prepared and the pH was adjusted to 8.0. It was titrated with 10.0 mL of boiling Fehling's A and Fehling's B solution. The end point was determined by mixing 1% methylene blue until the blue color of the indicator disappeared to a brick red end point. The titration was completed within 3 minutes and blank titration was performed without hydrolyzing sucrose sample. The triplicate titration was performed for each sample and the average values were recorded.

Determination of Initial Reducing Sugar Content

Each sample was accurately measured (5 ml), diluted solution (50 times) and the pH was adjusted to 8.0. This solution was transferred into a burette. Titration was performed as the above procedure.

Hydrolysis of the Sample and Analysis of Total Sugar Content

About 5 mL of sample was mixed with 3 mL of conc. HCl and kept at 68°C for 30 minutes. The pH of the mixture was adjusted to 8.0, and 50 times diluted solution was prepared. This hydrolyzed sample solution was transferred into a burette. Titration was performed as the above procedure.

Method Validation and Quality Control

Samples were spiked with known volumes of invert sugar standard solution, and titrations were conducted. Validation included spiking samples with known amounts of sucrose.

Calculation:

$$\text{Sugar content } \left(\mathrm{g}\frac{100}{\mathrm{mL}}\right) = [E + (\mathrm{AB} - \mathrm{C}) * \frac{1}{\mathrm{D}}$$

∗ Dilution factor

where weight of the standard sucrose = A mg/mL. Required volume of hydrolyzed standard sucrose solution to titrate with Fehling A and B = B mL. Calculated dextrose factor = (A∗B) mg. Dextrose factor from table = C mg. Correction factor = (A∗B) – C mg. Required volume of sample to titrate with Fehling A and B = D mL. Dextrose factor of sample from table = E mg.

Determination of Sucrose Content in Energy Drink Samples

The sucrose content was calculated based on the difference between total reducing sugar content in the original and hydrolyzed samples. The total reducing sugar content of the hydrolyzed drink sample and reducing sugar content of original sample were subtracted and the value was taken as %w/w. Then, this value was multiplied by 0.95. Sucrose content of the sample was obtained through this calculation as %w/w. These non-reducing sugars are usually expressed in terms of sucrose and 0.95 g sucrose on hydrolysis yields 1 g invert sugar (glucose + fructose). Factor 0.95 was used for the calculation. Then sucrose percentage was multiplied by weight (g) of the 100 mL fruit drink sample.

$$\text{Sucrose content can be observed as} \frac{\mathrm{g}}{100}\mathrm{mL} =$$

(%) w/w – Initial reducing sugar ([$Totla\ reducing\ sugar$(%) w/w $-$ $initial\ reducing\ sugar$ %) w/w] x 0.9

All the calculations were done according to the reference table (invert sugar table for 10 mL of Fehling's solution). To avoid the effect of errors happening during the whole procedure, standard sucrose solution (1.9 mg/mL) was used and a correction factor was developed with comparing both calculated value and the table value of standard sugar content. This correction factor was used for the further calculations of samples. The whole titration procedure was done under the boiling condition and titration should be completed within 3 minutes because it takes more than 3 minutes for the back reaction to take place. End point of the titration should be determined very carefully. For high precision, titration was done in triplicate.

Colorant Extraction from Energy Drinks

The volume of the sample (5 ml) was acidified using 10 mL of 2 M glacial acetic acid. The sheep-wool was put into the conical flask and placed in water bath at 100 °C for 1 hour and 30 minutes. Then, the sheep-wool was taken out and washed under running tap water for 10 minutes. The washed wool was placed in conical flask and ammonia solution (2 M) was added to color stained wool and placed in water bath. When all colors were released from wool to ammonia solution, the wool was removed. Temperature of the water bath was maintained at 100°C to evaporate all ammonia from mixture. Then, the concentrated color was used for further analysis.

Identification of Extracted Colorants

The extracted colorants were identified using thin-layer chromatography (TLC) method as described by [23]. n-Butanol: water: glacial acetic acid (20 :10 :10 v/v) system was used. Standard colours and extracted colours were dissolved in 60% ethanol. Samples were spotted with standards (Carmosine (*E122*), Ponceau 4R (*E124*), Allura Red (*E129*), Sunset Yellow FCF (*E110*), Tartrazine (*E102*), in the same TLC plate [16]. Retention factor (R_f) values of each sample were calculated and compared with color standards. R_f values were calculated for all standards and samples.

Analysis of Caffeine

The UV/Vis spectrophotometer (*Secomam, UVi Light-XTD5, Ales*, France) [17] was used for the analysis of caffeine in different samples of soft drinks. The wavelength at which caffeine absorbs maximum was determined by scanning the range of 190-400 nm. The wavelength at which caffeine absorbs maximum was found to be 274 nm which was selected for further analyses.

Calibration solutions preparation

Caffeine stock solution (100 mg/L) was prepared by dissolving 0.01 g of recrystallized caffeine in 100 mL of chloroform in the volumetric flask. Different standard concentrations (1 to 25 mg/L) were prepared from the stock solution and their absorbances were measured at the wavelength of 274 nm in cuvettes in triplicate for each standard solution. The average values of absorbances measured are given in Table 1. Calibration curve for caffeine analysis was plotted from the measured absorbance values as shown in figure 6.

Extraction of caffeine samples and preparation of the sample solutions.

Samples were heated and then boiled for 10 minutes to remove carbon(iv)oxide used as the preservative in the drink samples. The samples were left to cool to room temperature after which 10 mL of each sample was measured into placed in the separatory funnel. 1 mL of sodium carbonate solution (20 %), 5 mL of chloroform was added to each sample and the caffeine was extracted by shaking the funnel for a few minutes and then the lower layer to separate. An aliquot (0.1 mL) of each extract was mixed with 5 mL of chloroform and placed in a cuvette for absorbance measurement at 274 nm. The experiment was performed in triplicate for each sample and average values were recorded.

III. Results And Discussion

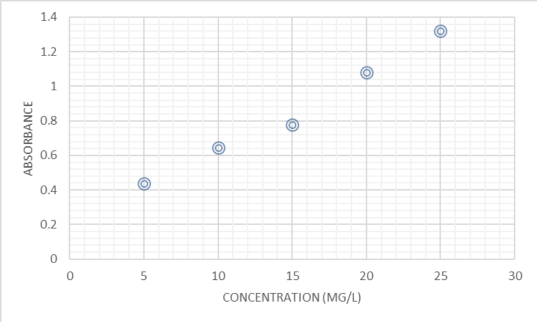

Figure 6: Standard Calibration Of Caffeine

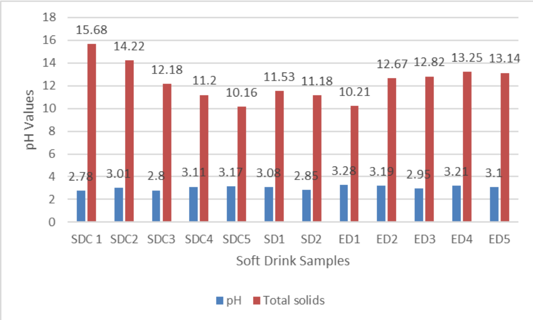

Figure 7: Ph And Total Solid Content Of Soft Drink Brands

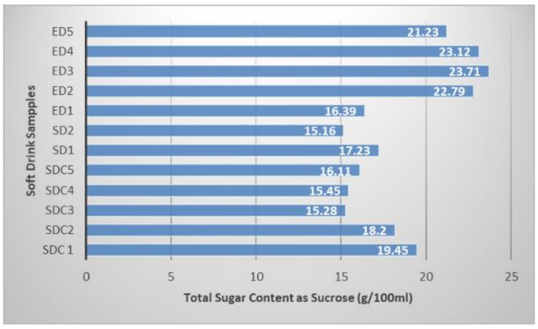

Figure 8: Total Sugar Content Soft Drink Brands

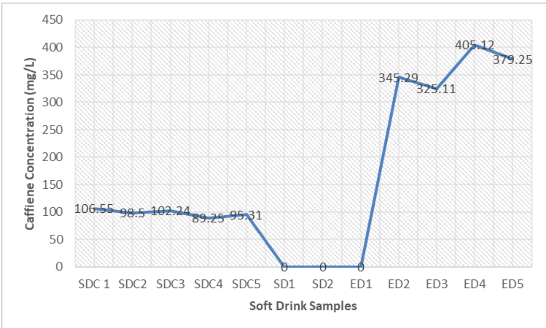

Figure 9: Caffeine Content Of Soft Drink Brands

The standard linear calibration curve obtained from the standard solutions of caffeine is presented in Figure 6. It showed a good linear relation between the absorbance and concentrations of standard solutions. Caffeine pH, total solids, sugar and caffeine content of carbonated soft drinks carbonated (SDC), Soft drinks (SD) and energy drinks (ED) samples were presented and illustrated in figure 7, 8 and 9 respectively.

The pH values across all samples range from 2.78 to 3.28. These acidic pH levels are typical for soft drinks and contribute to their flavor and preservation. SDC1 has the lowest pH, suggesting higher acidity. The acidity of drinks or any other food substances should be of concern to the consumers. Consumption of highly acidic drinks could lead to erosion of teeth enamel (6). Parents should therefore, prevent excessive consumption of soft drinks by children.

Total solids content varies from 10.16 to 15.68 g/100ml. SDC1 has the highest total solids, indicating a higher concentration of dissolved substances. This could affect mouth-feel and perceived sweetness of the drinks. Total sugar content ranges from 15.28 to 23.71 g/100ml. Samples ED2, ED3, ED4, and ED5 contain higher sugar levels, contributing to sweetness. SDC1 has the lowest sugar content, affecting sweetness perception. Samples ED series are energy drink brands having higher range of sugar content. The higher sugar content in Energy drinks may be due to the presence of caffeine in the energy drink formulation. Caffeine is bitter in taste and therefore required higher amount of sugar to attain the desired level of sweetness in the drinks brands. Caffeine is present in ED2, ED3, ED4, and ED5, ranging from 325.11 to 405.12 mg/L. Caffeine content contributes to the stimulant effect in energy drinks [15] [20]. SDC1 lacks caffeine, aligning with typical characteristics of non-energy soft drinks.

Various colorants are used in these soft drinks. SDC1 contains Sunset yellow and Tartarzine, while SD1 and SD2 use Caramel. The choice of colorants affects the visual appeal, and consumer perception of the product [5] [21].

SDC1 stands out with the highest acidity, lowest sugar content, and the presence of Sunset yellow and Tartarzine. This aligns with typical characteristics of citrus-flavored sodas. Energy drinks (ED2, ED3, ED4, ED5) exhibit higher sugar and caffeine levels, consistent with their energizing function. The presence of caramel color in SD1, SD2, and SDC2 contributes to their visual appeal but doesn't significantly affect pH or sweetness.

IV. Conclusion

This comprehensive analysis provides insights into the composition of the soft drink samples, considering pH, total solids, sugar content, caffeine levels, and colorants. The variation in these parameters reflects the diverse formulations and purposes of the different soft drink brands.

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