
Original Research Article

Assessment of pH, titratable acidity, and caffeine content of some brands of energy drinks

Uyi M Ogbeide*, Daniel E Irene, Henry A Okeri

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, PMB 1154, Benin City, 300001, Nigeria

* For correspondence: Email: uyi.ogbeide@uniben.edu, Tel: +23470380586764

Abstract

Purpose: Caffeinated beverages that contain high levels of stimulant ingredients, usually caffeine, as well as sugar and often supplements, such as B vitamins or carnitine, and that are promoted as a product capable of enhancing mental alertness and physical performance are referred to as energy drinks. Energy drinks are generally consumed by adolescents and athletes. This study aims to evaluate the endogenous pH, titratable acidity, and caffeine content in selected brands of energy drinks commonly consumed by athletes and adolescents in Benin City.

Methods: Eight (8) commonly consumed energy drinks were evaluated. A digital pH meter was used to measure the pH of the selected energy drinks and the titratable acidity was evaluated by titrating 0.1N sodium hydroxide solution against 30 ml of each energy drink till the end-point was reached. The

caffeine content was determined by titrating the caffeine-iodine complex formed against standard sodium thiosulphate using iodometric back titration.

Results: All the energy drinks were acidic with a pH ranging from 2.61 to 3.48 which is less than the critical pH (5.5). The titratable acidity of the energy drink samples ranged from 5.1 to 15.6 g/100 ml and the caffeine content varied from 0.029 to 0.165 mg/ml.

Conclusion: The outcome of this study confirms that energy drinks had low pH values and high titratable acidity except for ED G and ED H with low titratable acidity and low caffeine content.

Keywords: Caffeine, endogenous, energy drinks, pH, titratable acidity.

Indexing: Index Copernicus, African Index Medicus

Introduction

Energy drinks are beverages that have in their content other than calories, caffeine in combination with other presumed energy-enhancing constituents such as herbal extracts, B vitamins and taurine. They first appeared in Europe and Asia in the 1960s in response to consumers' demand for a dietary supplement that would result in increased energy [1], but only became popular in 1987 when the most widely known brand, Red Bull, was released in Austria; hitting the US market in 1997 [2].

Initially, athletes were the primary consumers of energy drinks. However, as the energy drink market grew and expanded into various niche

markets, athletes were no longer the primary target. Most of the energy drinks are now targeted at teenagers and young adults between 18-34 years today due to this generation's on-the-go lifestyle and receptiveness to advertisements for these types of products [1].

The primary concern about the health risks associated with the consumption of caffeinated energy drinks is the excessive intake of caffeine. Caffeine occurs naturally in various plants such as coffee beans, kola nuts, tea leaves, cocoa beans, and many other plants. It is one of the favourite legal drugs, used as an ingredient in beverages

such as soft drinks, energy drinks and pharmaceutical formulations [3].

Caffeine, a xanthine derivative alkaloid with the chemical name 1,3,7-trimethylxanthine has a molecular formula $C_8H_{10}N_4O_2$ (Fig. 1), molecular weight 194.19 g/mol, melting point 237°C , density 1.05 g/cm and pK_a 10.4 at 40°C . Pure caffeine takes the form of a white hexagonal crystal, is odourless and has a bitter taste. The nature of caffeine has been reported to be highly soluble in chloroform and dichloromethane compared to other organic solvents investigated such as benzene, diethyl ether etc. Caffeine is partly soluble in water at room temperature (2 g/100 ml) but is very soluble in boiling water (66 g/100 ml) [4].

Caffeine has a similar chemical structure to that of adenosine allowing caffeine to attach to the adenosine receptors. The main mechanism of action of caffeine, in concentrations typically achieved after the consumption of a caffeinated beverage, is to act as an adenosine receptor blocker in the brain [5]. The blockage of adenosine to the neurons causes the sleep-promoting effects of adenosine to stop, resulting in the neurons speeding up instead of slowing down. Caffeine is also known to increase the secretion of adrenaline, which can lead to a variety of secondary metabolic changes that can positively affect physical or mental performance. Once ingested, caffeine is rapidly absorbed from the gastrointestinal tract and undergoes dealkylation which results in paraxanthine (84%), theobromine (12%), and theophylline (4%); with the xanthenes theobromine and theophylline having very similar chemical structures compared to caffeine. [6]

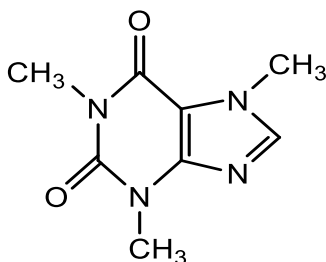


Fig. 1: Structure of caffeine

Other ingredients added to energy drinks include; Taurine, Guarana, B vitamins, Ginseng, Yerba mate, Ginkgo Biloba, Glucuronolactone, preservatives, colouring agents, flavouring agents etc. [7]

The concept of pH is unique among the commonly encountered physicochemical quantities [8]. pH is a measure of the hydrogen ion concentration in solution. The pH value is defined by the Sorenson Equation, as the negative logarithm of the H^+ concentration in a given solution. The range goes from 0 to 14, with 7 being neutral.

pH is a measurable parameter and the electronic device that is used for measuring the pH of a liquid (or in special cases of semisolid compounds) is called a pH meter. All pH meters are calibrated against buffer solutions of known hydrogen ion activity [9].

Titratable Acidity (TA) refers to the total concentration of free protons and un-dissociated acids in a solution that can react with a strong base and be neutralized. TA titration will generally use the strong base, NaOH, and either a chemical indicator or pH meter to signal when equivalent amounts of the base have been metered into the sample. The concentration of sodium hydroxide used is typically 0.1 N or less [10]. TA is a more accurate measure of the total acid content of drinks [11].

The frequent consumption of acidic drinks can erode dental enamel and promote caries. As many sports drinks on the market feature critically low pH values [12]. Previous research identified beverages with a low pH as the cause of enamel loss. The critical pH below which enamel begins to erode is 5.5. However, one study reported that TA was a more important indicator than pH value for determining the erosive potential of beverages [13].

The objectives of the study were to determine the pH, titratable acidity and caffeine content of energy drinks using a digital pH meter, acid-base titration and iodometric back titration respectively.

Methods

All reagents and chemicals used were of analytical grade and they include; sodium hydroxide, distilled water was obtained from a commercial source, deionized water was obtained from a chemical source, phenolphthalein indicator solution, methyl orange indicator, sulphamic acid, buffer solution (at pH 4.0, 7.0 and 10.0), sodium thiosulphate, iodine, potassium iodide, potassium iodate, starch indicator, Hydrochloric acid. Eight brands of energy drinks- Fearless (ED A), Predator (ED B), Blue bullet (ED C), Rox (ED D), Power horse (ED E), Monster (ED F), Coca-Cola (ED G) and Coca-Cola cherry (ED H) were assayed for their contents of caffeine. All solutions were prepared in distilled water and stored in an amber colour bottle at room temperature.

Preparation of solutions

A fresh starch indicator solution was prepared by dissolving 1.0 g of starch in 10 ml distilled water stirred and made up to 100 ml and boiled for 2 minutes. The solution was stirred and let cool at room temperature. Hydrochloric acid (4.0 M) was prepared by measuring 33.3 ml of 37% HCl and poured into a 100 ml volumetric flask containing distilled water to make up to volume. Potassium iodide (10%) was prepared by weighing 10 g of KI, dissolved in 20 ml of distilled water and made up to a 100 ml mark with distilled water.

Potassium iodate (0.1 N) was prepared by weighing 1.7833 g of KIO₃ powder and dissolved in 100 ml of distilled water and made up to a volume (500 ml) with more distilled water.

Sodium thiosulphate (0.1 N) was prepared by dissolving 24.8 g of sodium thiosulphate crystals diluted in 100 ml of freshly boiled and cooled distilled water. More distilled water was added to it up to 1000 ml.

Iodine solution (0.1 N) was prepared by weighing 20 g of KI into a 100 ml beaker and 40 ml of distilled water was added with a little heating. The mixture was cooled to room temperature and 12.7 g of solid iodine was dissolved in the same glass. The iodine solution was transferred into a 1000 ml volumetric flask, and then diluted with distilled water to the mark [4].

Sodium hydroxide solution (0.1 N) was prepared by dissolving 4.1240 g of sodium hydroxide pellets in 200 ml of distilled water in a 1000 ml

measuring cylinder and the mixture was stirred properly until it was dissolved and made up to the 1000 ml mark with distilled water [14].

Calibration of pH meter

The digital pH meter was calibrated using buffer solutions of pH 4.0, 7.0 and 10.0. 30 ml of buffer solution of pH 4 was measured into a 50 ml glass beaker. The electrode was rinsed in distilled water and dried with a piece of tissue, then submerged into the buffer solution. The knob of the electrode was adjusted and allowed to stay in the buffer solution until a stable reading was reached and the pH on the screen was equivalent to the pH of the buffer solution. The probe was rinsed again with distilled water and dried with a piece of tissue paper and the same procedure was carried out for buffer solutions 7 and 10 [13, 15, 16].

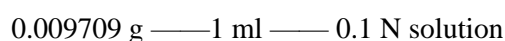
Determination of pH of energy drinks

The electrode was rinsed in distilled water and dried with cotton wool to absorb any remaining water. A volume, of 30 ml of the drink sample was measured into a 50 ml glass beaker and the electrode was immersed into the sample in a beaker and the electrode was left in the sample for 2 minutes until the meter reading was allowed to stabilize and the final pH value was recorded. The same procedure was repeated for all the drink samples and determination was done in triplicates and the mean pH was calculated and recorded [10, 15, 16].

Evaluation of titratable acidity

Standardization of 0.1 N sodium hydroxide solutions

Weights of 0.2436 g and 0.2608 g of sulphamic acid were accurately weighed into a beaker and dissolved in 25 ml of freshly boiled and cooled water, after complete dissolution, 2 drops of methyl orange indicator were added and the solution was then titrated against 0.1 N NaOH solution from the burette until an endpoint was reached (colour change) [17]. The determination depends on the following equation of the reaction



Determination of titratable acidity

A volume (30 ml) of drink sample was measured into a 50 ml conical flask. The burette was set up and filled with the 0.1N sodium hydroxide (NaOH) solution needed for the titration. 5 drops

of phenolphthalein indicator were added to the content in the conical flask. A concentration (0.1 N) NaOH solution was titrated against 30 ml of the drink until the endpoint was reached. The volume of NaOH was then recorded. The same procedure was done for all drink samples and titrations were done in triplicates for all the drink samples and the mean titre value was calculated and recorded [10, 18].

The titratable acidity for each sample was then calculated using this formula:

$$\% \text{ acid} = \frac{(\text{volume of NaOH used}) \times (0.1\text{N NaOH}) \times (100)}{\text{volume of sample used}(30 \text{ ml})}$$

Determination of caffeine by iodometric back titration

Standardization of thiosulphate solution

A volume (10 ml) of KIO_3 0.1 N was transferred into a 100 ml Erlenmeyer flask, 10 ml of 10% KI

solution and 2.5 ml of 4.0 N HCl were added. This solution was immediately titrated with a standard solution of 0.1 N sodium thiosulphate till the yellow solution was almost gone (pale yellow). Starch indicator (1 ml) was added and the titration was continued until the blue colour of the solution disappears. The titration was done in duplicates [4].

Determination of caffeine levels

A volume (25 ml) solution of 0.1 N iodine solutions was acidified and added to 25 ml of the energy drink in a conical flask. The resultant solution was titrated against a solution of 0.1N thiosulphate using starch as an indicator. A blank titration involving iodine and thiosulphate solutions was carried out. The difference between both determinations was to determine the caffeine content of the energy drinks. The assay was done in duplicates [19].

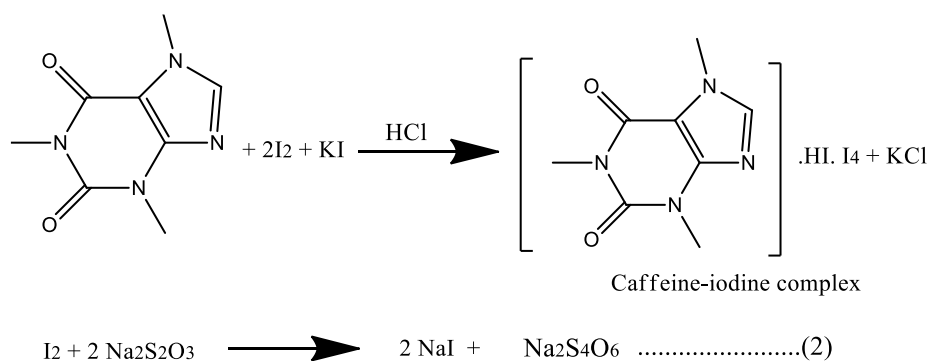


Fig. 2: Equation of reaction to determine caffeine levels

Results

The result of pH, titratable acidity and caffeine content of some brands of energy drinks are shown in **Table 1**. From the results, it can be seen that ED G had the lowest pH (2.61 ± 0.01) while

ED C had the highest pH (3.48 ± 0.02). The titratable acidity was highest for ED E (15.60) and lowest for ED H (5.10). The determination of the caffeine content of the various brands of energy drinks showed that ED A had the highest caffeine content (0.165 mg/ml) and ED H as well as ED F had the lowest caffeine content (0.029).

Table 1: Result of mean pH, titratable acidity and caffeine content of selected energy drinks

Sample	Mean pH \pm S.D	Titratable acidity (g/100 ml)	Caffeine content (mg/ml)
ED A	2.74 ± 0.02	8.60	0.165 ± 0.01
ED B	3.29 ± 0.02	11.50	0.107 ± 0.01
ED C	3.48 ± 0.02	13.50	0.097 ± 0.05
ED D	3.47 ± 0.01	13.10	0.087 ± 0.04
ED E	3.17 ± 0.01	15.60	0.116 ± 0.05
ED F	3.46 ± 0.01	14.80	0.029 ± 0.01
ED G	2.61 ± 0.01	5.20	0.107 ± 0.04
ED H	2.90 ± 0.01	5.10	0.029 ± 0.01

Discussion

The study primarily focuses on the pH, titratable acidity, and caffeine content of eight brands of energy drinks. The evaluated energy drinks were acidic (pH <7) within a range of 2.61 – 3.48. The pH was lowest for ED G with a pH of 2.61 and highest for ED C with a pH of 3.48. ED E had the highest TA of 15.6 g/100 ml while ED H had the lowest TA (5.1 g/100 ml). Caffeine content was highest for ED A (0.165 mg/ml) while ED F and ED H had the lowest caffeine content of 0.029 mg/ml.

The growing intake of ED has been connected to a rise in dental erosion nevertheless; there is normally a widespread innocence about the mutilating effects of acid erosion due to packaged ED. There are numerous symptoms of dental erosion like notable loss of enamel and sensitivity. The drop in the pH of the oral cavity below pH 5.5 leads to demineralization of dental hard tissue leading to dental erosion [19]. Several acids have been used in the manufacture of soft drinks (energy drinks); lactic and tartaric acids. However, commonly used acids are citric, malic and phosphoric acids [2]. Energy drink-induced dental erosion is primarily caused by either citric and/or phosphoric acids. Chelation or ligand-promoted dissolution by anionic citrate contributes to enamel demineralization by the removal of Calcium ions (Ca^{2+}) at a higher pH range approaching 6 [20]. Also, these acids exhibit buffering capacity that can keep up pH below baseline value i.e. at acidic pH values.

As the TA of a drink gets higher, the hydrogen ion availability for interaction with tooth surfaces also increases and therefore, the higher its erosive potential. While the pH value identifies the erosive potential in the first few minutes of an erosion test, the TA gives a measure of the total acid content of a drink, thus better characterizing the erosive potential with longer exposure times [21].

From the result of the study in Table 1, ED E needed the highest volume of 0.1 N NaOH. While ED H needed the lowest amount of 0.1 N NaOH. This shows ED H which is presented with the lowest titratable acidity of 5.1 g/100 ml is not as erosive as ED E with a TA of 15.6 g/100 ml. Modification by adding calcium and phosphate to these drinks may be helpful to reduce the erosive potential of the drinks [17]. Raising the pH may also resolve the trouble, but altogether, it

increases spoilage risks or the presence of pathogenic bacteria. This has left health professionals no option but to emphasize correct drinking habits in counselling sessions and health programmes on oral health to reduce the adverse effect and impact of these drinks on oral health and health in general.

Iodometric titration is an easy and precise method to ascertain the quantity of caffeine in an aqueous solution. From the result of the study in Table 1, the highest caffeine content was found in ED A while ED F and ED H had the lowest caffeine content. In caffeine, there are double bonds that can be added by iodine and caffeine reacts with iodine in an acidic environment. The quantity of caffeine can be ascertained by adding excess iodine solution. The equation for the chemical reaction that occurs is shown in Fig. 2. The caffeine content in ED A was nearly six times that in ED F and ED H and nearly twice that in ED D. Generally, 300-400 mg is the recommended daily intake of caffeine. The maximum caffeine intake per day of 400 mg would only be exceeded for ED A and ED F if more than 5 and 32 bottles were respectively consumed [18].

Conclusion

The energy drinks evaluated were acidic with pH within the range of 2.61 to 3.48 and titratable acidity ranged from 5.1 g/100 ml to 15.6 g/100 ml and the caffeine content ranged from 0.029 mg/ml to 0.165 mg/ml. A lot of people take energy drinks mostly for enhanced performance and to stay alert and a few others take or consume them because of the taste and for other personal reasons.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities on claims relating to the content of this article will be borne by the authors. Uyi Ogbeide designed the study, managed the data and wrote the manuscript which was reviewed by Henry Okeri. Daniel Irene did the laboratory work. All the authors read and approved the final manuscript.

References

1. Yunusa I, Ahmad IM. Energy-Drinks: Composition and health benefits. *Bayero J. Pure Appl. Sci.* 2011; 4(2):186-191.
2. Aslam HM, Mughal A, Edhi MM, Saleem S, Rao MH, Aftab A. Assessment of pattern for consumption and awareness regarding energy drinks among medical students. *Arch. Public Health.* 2013;71(1):1-11.
3. Hossain M, Jahan L, Shawan M, Parvin A, Hassan M, Udin K, Akter S, Banik S. Determination of pH, caffeine and reducing sugar in energy drinks available in Bangladesh. *N. Y. State J. Med.* 2015; 8(2):92-96.
4. Yazid EA, Pratama NMR. Analysis of caffeine in tablet dosage forms with spectrophotometric and iodometric back titration, *J. Trop. Pharm. Chem.* 2019; 4(6):271-280.
5. Pettenuzzo LF, Noschang C, Toigo VP, Fachin EA, Vendite D, Dalmaz C. Effects of chronic administration of caffeine and stress on feeding behaviour of rats. *Physiol. Behav.* 2008; 95:295-301.
6. Zandvliet AS, Huitema ADR, De Jonge ME, Hoed RD, Sparidans RW, Hendriks VM, van den Brink W, Van Ree JM, Beijnen JH. Population pharmacokinetics of caffeine and its metabolites theobromine, paraxanthine and theophylline after inhalation in combination with diacetylmorphine. *Basic Clin. Pharmacol. Toxicol.* 2005;96(1):71-79.
7. Visram S, Hashem K. Energy drinks. What's the evidence? Working Paper. The Food Research Collaboration, London. 2016:1-14.
8. Baucke FGK, Buck RP, Rondinimi S, Covington AK, Brett CMA. Measurement of pH, definition, standards and procedures (IUPAC Recommendations) 2002;74(11):2169-2200.
9. Karastogianni S, Girousi S, Sotiropoulos S. pH: Principles and measurement. *Encyclopedia of Food and Health.* 2016;4:333-338.
10. Gump, B. H. 'pH and titratable acidity. Available from: <https://www.umpqua.edu>. (Cited on 15th March, 2022).
11. Cairns AM, Watson M, Creanor SL, Foye RH. The pH and titratable acidity of diluting drinks and their potential effect on dental erosion. *J. Dent.* 2002;30:313-317.
12. Mettler S, Weibel E. Osmolality, pH, and titratable acidity of sports drink on the Swiss market. *Swiss Sports Exerc. Med.* 2018; 66(4):56-63.
13. Singh R. *Handbook of Practical Pharmaceutical Chemistry: A systematic approach to titrimetric analysis.* 2016; p. 49.
14. Thermo Fisher Scientific. pH calibration procedure for optimal measurement precision, (910001).
15. Ruff B. How to calibrate and use the pH meter.1-2. Available at <https://www.wikihow.com/Calibrate-and-Use-a-pH-Meter>.
16. Olaniyi AA, Olugungbamila FO. *Experimental Pharmaceutical Chemistry.* 6th ed Ibadan: Shaneson; 1991; pp. 4-5.
17. Batra M, Tangade P. Evaluation of titratable acidity of beverages as a risk factor for dental erosion. *J. Dent. Esthet. Function.* 2013;2(1):38-40.
18. Klu Michael W, David NM, Bright SA, John AA, Obeng G. Quantitative estimation of caffeine in some energy drinks on the Ghanaian market *Int. J. Nutr. Metab.* 10(3):16-22.
19. Bamise CT, Oderinu OH. Erosive potential: Laboratory evaluation of sports drinks available in Nigerian market. *Afr. J. Basic Appl. Sci.* 2013;5(3):139-144.
20. Reddy A, Norris DF, Momeni SS, Waldo B, Ruby J. The pH of beverages in the United States. *J. Am. Dent. Assoc.* 2016;147(4):255-263.
21. Benjakul P, Chuenarrom C. Association of dental enamel loss with the pH and titratable acidity of beverages. *J. Dent. Sci.* 6(3):129-133.