Determination of pH, caffeine and reducing sugar in energy drinks available in Bangladesh

Md. Mozammel Hossain, Iffat Jahan, Mohammad Mahfuz Ali Khan Shawan, Afroza Parvin, Md. Mahmudul Hasan, Kazi Rasel Uddin, Salina Akter, Subrata Banik, Md. Ashraful Hasan, Mahbubul Morshed, Md. Nazibur Rahman, S. M. Badier Rahman

Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
mhossain@juniv.edu, mozammel1129@gmail.com

Abstract: Though the popularity of energy drinks is increasing day by day no considerable chemical studies on it has not yet been done in Bangladesh. This investigation was carried out to determine the pH, levels of caffeine and reducing sugar contents in five energy drinks available in local market in Rajshahi, Bangladesh. pH were measured by pH meter. Quantitative estimation of caffeine was performed by a simple and fast standard UV spectrophotometric method, using carbon tetrachloride as the extracting solvent. Reducing sugar content of the energy drink was also determined spectrophotometrically. Results showed that the pH of the beverages were perfectly acidic ranging from 2.85 to 3.11. The minimum caffeine level was observed in Brand-4 (40.34 mg/serving), while Brand-5 showed the highest caffeine content (244.57 mg/serving) showing a range from 40.34 to 244.57 mg/serving. The levels of caffeine in all energy drink samples are well below the maximum allowable limits set by the food regulatory bodies, except Brand-5. The reducing sugar content of the energy drinks ranged from 73.80 mg/serving to 136.08 mg/serving. Brand-1 has the lowest and Brand-4 has the highest reducing sugar providing an idea about the sugar contents of energy drinks. Further investigations are needed to determine the suitability of these energy drinks.

[Hossain MM, Jahan I, Shawan MMAK, Parvin A, Hasan MM, Uddin KR, Akter S, Banik S, Hasan MA, Morshed M, Rahman MN, Rahman SMB. **Determination of pH, caffeine and reducing sugar in energy drinks available in Bangladesh.** *N Y Sci J* 2015;8(2):92-96]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 17

Keywords: Energy drinks, pH, Caffeine, Reducing sugar, Bangladesh, Rajshahi

1. Introduction

Now, there has been an increase in the popularity of "energy drink" or "functional beverages" in Bangladesh. They are also known as nutraceutical foods, which are substances considered to be a food or part of a food that may provide some health benefit (Villasenor *et al.*, 2002). Socio-culturally, Bangladesh does not favor wine or whisky as a drink either. In the upper crust of society though, intake of liquid like this is not considered a vice. Then people newly acquiring wealth here are always in search of enjoyment. They view that their drinking habit will help to overcome their class difference. Such pull and push within society however exert an extra pressure on the young generation to emulate their elders. But limited affordability compels them to lay their hands on cheaper varieties of drinks. Quite a few companies and business houses have taken a round-about path to cash in on the young people's gullibility. They are marketing a number of what is called energy drink. The market growth rate of energy drinks is very high in Bangladesh. However, the growth drift has some eccentricity. The market is mostly dominated by low-income segment. Now a days energy drinks are getting more popularity among the teenagers.

Energy drinks are acidic in nature having lower pH values. The lower pH value is due to the presence of CO_2 gas or other acids such as phosphoric acid, malic acid, ascorbic acid, citric acid and tartaric acid used as preservative by the manufacturer of these energy drinks (Bassiouny and Yang, 2005; Ashurst, 2005). These acids inhibit the growth of microorganisms such as bacteria, mould and fungi which may contaminate beverages. Studies showed that drinking acidic beverages over a long period can cause erosion of tooth enamel and predisposition of the consumer to dental disease (Marshall *et al.*, 2003; Bassiouny and Yang, 2005).

Caffeine is a common ingredient of energy drinks. It is deliberately added as a flavoring agent and to make the drinks addictive. It is a bitter white crystalline 'xanthine' alkaloid that acts as a mild psychoactive stimulant drug. It is found in varying quantities in the seeds, leaves or fruits of many plants species (Andrews *et al.*, 2007; Dionex, 2007; Wanyika *et al.*, 2010; Violeta *et al.*, 2010). The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. Caffeine stimulates CNS reducing physical fatigue and restoring mental alertness when unusual weakness or drowsiness occurs (Nehlig *et al.*, 1992; Maidon *et al.*, 2012). The US Food and Drug Administration (FDA, 2006) limits the maximum amount of caffeine in carbonated beverages to 6 mg/oz (200 ppm or 0.2 mg/ml). NSDA (1999) allows caffeine content in soft drinks in the range between 30 and 72 mg/355 ml (12 oz) or 8.45-

20.28 mg/100 ml. According to Natural Health Products (NHP) Regulations (2008) the caffeine content in energy drink is 80 mg per 250 ml serving. Clauson *et al.*, (2008) reported that energy drinks contained 80-300 mg of caffeine per 8 oz serving. Though a recent literature review determined that consumption of up to 400 mg caffeine daily by healthy adults is not associated with adverse effects, adverse effects associated with caffeine consumption in amounts of 400 mg or more include nervousness, irritability, sleeplessness, increased urination, abnormal heart rhythms (arrhythmia), decreased bone levels, and stomach upset. Badr *et al.*, (2013) found that oral administration of caffeine lead to significant reduction in serum Ca, Zn and Mn in growing rats. Caffeine binds to cell membranes in place of adenosine, an inhibitory neurotransmitter, causing changes in normal physiological processes (Committee on Nutrition and the Council on Sports Medicine and Fitness, 2011). However, groups that are at risk, such as women of reproductive age and children, should limit their daily consumption of caffeine to a maximum of 300 mg for the former and 2.5 mg/kg body weight for the latter; thus they may need to avoid consuming energy beverages with a higher caffeine content (Nawrot *et al.*, 2003). Adolescents should limit caffeine consumption, as intakes greater than 100 mg/day has been associated with elevated blood pressure (Savoca *et al.*, 2014). Combination of caffeine with alcohol, narcotics and some other drugs compounds produces a toxic effect, sometimes with lethal outcome (Mamina and Pershin, 2002; Ben Yuhas, 2002; Wanyika *et al.*, 2010). Based on these findings, consumption of energy drinks by pregnant or nursing women, adolescents and children is not recommended. Khayyat *et al.*, (2012) found that energy drinks induced an elevation of liver enzymes AST, ALT and ALP after two and four weeks of treatment in albino rats.

Another common ingredient of energy drink is refined sugar. A comprehensive study of energy beverages reported that the median sugar content of sugar-sweetened energy drinks was 27 g per 8 oz serving, comparable to sodas and fruit drinks, and higher than sportsdrinks and flavored water (Yale Rudd Center for Food Policy & Obesity, 2011). Clauson *et al.*, (2008) reported that energy drinks contained 35 g of processed sugar per 8 oz serving. Consumption of sugary beverages is associated with increased risk for dental caries, weight gain, overweight, obesity, diabetes, and heart disease (Pomeranz, 2011; Webb, 2013).

Though a number of energy drinks have been introduced to the Bangladeshi market no considerable chemical analysis on it has not yet been done to determine its suitability as a drink. As a little attempt in this matter the aims of the present investigation is to determine the levels of pH, caffeine and reducing sugar in energy drinks available in Bangladesh.

2. Material and Methods

Sample collection: Five available energy drink samples were collected from Saheb Bazar, Rajshahi and were marked as Brand-1, Brand-2, Brand-3, Brand-4 and Brand-5. The beverages were purchased from various confectionary stores of Saheb Bazar, Rajshahi. After collection samples were preserved in refrigerator.

Determination of pH: pH of the energy drinks were determined by using Hanna benchtop pH meter (HI 2314).

Determination of caffeine: 10 mg of caffeine was dissolved in 100 ml purified carbon tetrachloride (CCl_4) in 100 ml volumetric flask to get 100 ppm stock standard of caffeine solution. Working standards were prepared by pipetting 5, 10, 15, 20 and 25 ml, respectively aliquots of the stock standard solution into separate volumetric flasks (50 ml) and diluting to volume with purified carbon tetrachloride to produce concentrations of 10, 20, 30, 40 and 50 mg/l, respectively standard solution. The absorbance of each solution was measured at absorption maximum of 270 nm using 1cm quartz cuvette. The absorbance values were then plotted against concentrations to generate a standard calibration curve. The caffeine of the energy drink was extracted with carbon tetrachloride (CCl_4) (Tauta *et al.*, 2014). The absorbance of each sample solution was measured at absorption maximum of 270 nm using 1cm quartz cuvette. Hitachi double beam spectrophotometer (U-2900) was used for taking absorbance of standards and samples.

Determination of reducing sugar: The reducing sugar content of the energy drink was determined spectrophotometrically by measuring absorbance at 575 nm (Miller, 1959).

3. Results and Discussions

The pH values of the energy drinks are given in Table 1, which ranges from 2.85 to 3.11. All the energy drinks are acidic. Brand-3 has the lowest and Brand-4 has the highest pH value. Tauta *et al.*, (2014) found that beverages are acidic having a pH range from 5.92 to 6.44. The ideal (neutral) pH of the mouth ranges from 6.5-7.5. A pH of 5.5 is considered to be the threshold level for the development of dental decay. Both soft drinks and sports drinks have been shown to have a pH between 2.5 and 3.5. Demineralization of tooth enamel will occur more rapidly if the pH drops below the critical 5.5 level for long periods of time and if the pH is dropped below the critical level frequently. Citric acid is the most

aggressive acid linked to tooth demineralization (Oltjen). Lin *et al.*, (2003) showed that all kinds of soft drinks are acidic and that cola drinks especially make our bodies poor in oxygen.

Table 1. pH of energy drinks

Sl. No.	Name	Serving size (ml)	pH
1	Brand-1	250	2.98
2	Brand-2	250	3.01
3	Brand-3	250	2.85
4	Brand-4	270	3.11
5	Brand-5	250	3.09

The caffeine content in the in the energy drink samples ranged from 40.34 to 244.57 mg/serving (Table 2). The average caffeine content in the energy drinks was found to be 89.21 mg/serving. The lowest caffeine content was found in Brand-4 (40.34 mg/serving), while Brand-5 showed the highest caffeine content (244.57 mg/serving). Tauta *et al.* (2014) showed that caffeine content in energy drink samples of Nigeria ranged from 47.56 to 58.31 ppm and the average caffeine quantity in the energy drinks was found to be 52.24 ppm. Mei *et al.* (2012) found that caffeine concentrations in energy drinks samples of Sudan ranged from 170.6 ppm to 324 ppm with average concentration of 255.6 ppm. Consumption of 300 mg of caffeine per day is generally considered as safe (Rogers and Dernoncourt, 1998; Smith, 2005). This amount typically corresponds to 3 cans of energy drink, especially 1 can for Brand-5. Caffeine content in beverage drinks varies by brand from 10 to 60 mg of caffeine per serving (Violeta *et al.*, 2010). In our investigation, the levels of caffeine in all energy drink samples are well below the maximum allowable limits set by the above food regulatory bodies, except Brand-5 containing 244.57 mg of caffeine per serving (0.978 mg/ml or 978.28 ppm). This value is very high from maximum allowable limits set by the above food regulatory bodies such as US Food and Drug Administration (FDA, 2006), NSDA (1999) and Natural Health Products (NHP) Regulations. Consumption of 3 to 4 cans of these energy drinks (Brand-1,2,3,4) cross the maximum allowable limit causing adverse effects, where consumption of only one can of Brand-5 is enough to cause that.

Table 2. Caffeine content of energy drinks

Sl. No.	Name	Serving size (ml)	Caffeine (mg/serving)
1	Brand-1	250	51.68
2	Brand-2	250	50.43
3	Brand-3	250	59.03
4	Brand-4	270	40.34
5	Brand-5	250	244.57

The reducing sugar content of the energy drinks are given in Table 3, which ranges from 73.80 mg/serving to 136.08 mg/serving. Brand-1 has the lowest and Brand-4 has the highest reducing sugar. The high concentrations of refined carbohydrates in energy drinks make it harder for saliva to clear this product from the teeth and as such the pH remains more acidic for a longer period of time. Refined sugars play a pivotal role in the caries (tooth decay) process. The evidence linking sugar and tooth decay is overwhelming. An increase of 1 oz in daily soda pop consumption increases a child's risk of having extensive caries by 26%. In addition to potential tooth destruction, high soft drink consumption can lead to excessive caloric intake, which may contribute to childhood obesity, a growing problem in the U.S. Type 2 diabetes, cardiovascular diseases, and asthma are all conditions linked to obesity and nutritional imbalances (Oltjen).

Table 3. Reducing sugar content of energy drinks

Sl. No.	Name	Serving size (ml)	Reducing sugar (mg/serving)
1	Brand-1	250	73.80
2	Brand-2	250	115.20
3	Brand-3	250	85.05
4	Brand-4	270	136.08
5	Brand-5	250	103.95

4. Conclusion

In Bangladesh, the popularity of energy drink is increasing day by day and our young generation is adopting in it. From the study it is found that the pH values of available energy drinks are lower than that of other countries, which is a matter of concern. It is shown from the results of this study that the concentration of caffeine in the energy drinks was significantly lower than the maximum authorized level, except Brand-5 which may cause health hazards. In this investigation reducing sugar content provides an idea about the sugar contents of energy drinks. However since caffeine is an additive substance and because of health concerns arising from its consumption, it seems appropriate that warning labels, indication of the presence and amounts of caffeine should accompany all caffeinated beverages. As health hazards are also associated with low pH and sugar content of these drinks, the amount of these parameter should presented on the labels. None of the drinks evaluated in this study were so labeled. Energy drink manufacturer can follow US Food and Drug Administration (FDA), Nutrition Labeling and Education Act of 1990 (NLEA) and Dietary Supplement Health and Education Act of 1994 (DSHEA) for proper labeling. Further investigations

are needed to determine the appropriateness of these energy drinks.

Acknowledgements:

Authors are grateful to the BCSIR Laboratories, Rajshahi of Bangladesh for Lab support to carry out this work.

Corresponding Author:
Md. Mozammel Hossain
Department of Biochemistry and Molecular Biology
Faculty of Biological Sciences
Jahangirnagar University
Savar, Dhaka-1342, Bangladesh
Cell No.: +88-01717635098, +88-01689048616
E-mail: mhossain@juniv.edu
mozammel1129@gmail.com

References

1. Andrews K, Schweitzer A, Zhao C, Holden JM, Roseland JM, Brandt M, Dwyer J, Picciano M, Saldanha L, Fisher K, Yetley E, Betz J, Douglass L. The caffeine content of dietary supplements commonly purchased in the U.S.: Analysis of 53 products having caffeine containing ingredients. Anal Bioanal Chem 2007;389(1):231-239.
2. Ashurst PR. Chemistry and Technology of Soft drinks and Fruit Juices. 2nd Edn, Blackwell Scientific Publication, London, 2005;3(4):433.
3. Bassiouny MA, Yang J. Influence of drinking patterns of carbonated beverages on dental erosion. Gen Dentistry 2005;53:207-210.
4. Yuhas B. Determination of Caffeine Content in Beverages with HPLC. Chem 2002;384.
5. Clauson KA, Shields KM, McQueen CE, Persad N. Safety issues associated with commercially available energy drinks. J Am Pharm Assoc 2008;48(3):e55-63.
6. Committee on Nutrition and the Council on Sports Medicine and Fitness. Sports drinks and energy drinks for children and adolescents: Are they appropriate? Pediatrics.2011;127(6):1182–1189.
7. Dionex. Determination of Additives in Carbonated Beverages. Application Note 193, Dionex Corporation. 2007.
8. FDA. Food Additives Status List, Food and Drug Administration. 2006. Retrieved form: http://www. cfsan.fda.gov/dms/opa-appa.html.
9. Khayyat L, Sorour J, Rawi MA, Essawy A. Histological, Ultrastructural and Physiological Studies on the Effect of Different Kinds of Energy Drinks on the Liver of wistar Albino Rat. J Am Sci 2012;8(8):688-697.
10. Lin CI, Joseph AK, Chang CK, Wang YC, Lee YD. Estimation of caffeine in tea samples by spectrophotometrically. Anal Chimica Acta 2003;481:175-180.
11. Maidon ABMA, Mansoer AO, Sulistyarti H. Study of various solvents for caffeine determination using UV spectrophotometeric. J Appl Sci Res 2012; 8(5):2439-2442.
12. Mamina EA, Pershin VF. Structure of chemical compounds, methods of analysis and process control HPLC determination of caffeine in biological fluids in the presence of other purine derivatives. Pharma Chem J 2002;36:48-50.
13. Marshall TA, Levy SM, Broffit B, Warren JJ, Eichenberger-Gilmore JM, Burns TL, Stambo PJ. Dental carries and beverage consumption in young children. Paediatrics 2003;112:e184-189.
14. Mei MA, Mawahib E, Mohammed IT, Badawi AZ, Abdalla AE. Determination of caffeine in some Sudanese beverages by high performance liquid chromatography. Pak J Nutr 2012;11(4): 336-342.
15. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426.
16. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. Effects of caffeine on human health. Food Addit Contam 2003;20:1-30.
17. NSDA. National Soft Drink Association. What's in soft drinks: Caffeine in soft drinks? 1999. Retrieved form: http://www.nsda.org/ whatsincaffeinecontent.html.
18. Natural Health Products (NHP) Regulations, 2008, SOR/2003-196, Minister of Justice, Canada.
19. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev 1992;17(2):139-70.
20. Oltjen, J. Soft Drinks, Sports Drinks, Energy Drinks, and Flavored Waters: What is in Them? How Has Their Increased Use Impacted Oral Health? How Can You Limit the Damage? What All Parents Should Know, Oltjen Orthodontics, P.A.,www.oltjenbraces.com/docs/soda-rticle.pdf.
21. Badr OM, El-Masry SA, Mansor MA, Abdalla WM. Biochemical effects of caffeine on bone of growing rats. Nat Sci 2013;11(12):182-187.
22. Pomeranz JL. Advanced policy options to regulate sugar-sweetened beverages to support public health. Journal of Public Health Policy 2011;33(1):75–88.
23. Rogers PJ, Dernoncourt C. Regular caffeine consumption: A balance of adverse and beneficial effects for mood and psychomotor

performance. Pharmacol Biochem Behav 1998;59(4):1039-45.
24. Savoca MR, Evans CD, Wilson ME, Harshfield GA, Ludwig DA. The association of caffeinated beverages with blood pressure in adolescents. Arch Pediatr Adolesc Med 2004;158:473-7.
25. Smith AP. Caffeine at work. Hum Psychopharmacol 2005;20(6):441.
26. Tautua A, Martin WB, Diepreye ERE. Ultra-violet Spectrophotometric Determination of Caffeine in Soft and Energy Drinks Available in Yenagoa, Nigeria Adv J Food Sci Technol 2014;6(2):155-158.
27. Villasenor IM, Simon MK, Villanueva AM. Comparative potencies of nutraceuticals in chemically induced tumor prevention. Nutr 2002;44:66-70.
28. Violeta N, Trandafir I, Ionica ME. Chromatographic determination of caffeine contents in soft and energy drinks available on the Romanian market. St Cerc St CICBIA 2010;11:351-358.
29. Wanyika HN, Gatebe EG, Gitu LM, Ngumba EK, Maritim CW. Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. Afr J Food Sci 2010;4:353-358.
30. Webb D. The Truth About Energy Drinks. Today's Dietitian 2013;15(10):62.
31. Yale Rudd Center for Food Policy & Obesity. Sugary drink FACTS: Evaluating sugary drink nutrition and marketing to youth 2011. http://www.sugarydrinkfacts.org/resources/Sugary- DrinkFACTS_Report.pdf, accessed 26 September 2012.

2/20/2015