J. Bangladesh Acad. Sci. 49(2); 205-214: December 2025 DOI:https://doi.org/10.3329/jbas.v49i2.76778

Journal of Bangladesh Academy of Sciences

Journal homepage: http://www.bas.org.bd/publications/jbas.html

Research Article

Spectrophotometric analysis of caffeine and sugar content in energy drinks commonly consumed in Dhaka City, Bangladesh: A public health concern

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ARTICLE INFO

Article History

Received: 09 October 2024

Revised: 20 August 2025

Accepted: 20 August 2025

Keywords: Caffeine, Carbohydrate, Energy drinks, Solid mass, UV-Visible spectrometer.

ABSTRACT

Energy drink consumption has escalated, especially among the younger generation, credited to their stimulating properties, mainly because of their caffeine and carbohydrate contents. Given the potential health risks associated with excessive consumption of these substances, it is imperative to examine the composition of energy drink products on the market. This study used an analytical quantitative method to determine the mass concentrations of total solids, caffeine, and carbohydrates in eight brands of energy drink on sale in Dhaka, Bangladesh, using a composite, cost-effective experimental design. Total solid mass contents were 10.30 to 13.13 g per 100 mL of drinks, caffeine was found 233.30 to 546.48 mgL^{-1}, and carbohydrates were found 7.09 to 11.15 g per 100 mL energy drink using the double beam UV-visible spectrophotometer. The accuracy of the results was also indicated by the low standard deviation values of 1.04 to 4.39 for caffeine and 0.08 to 0.23 for carbohydrate, and the relative standard deviation of 0.26 to 1.75% for caffeine and 0.54 to 2.72% for carbohydrate. There was a need for proper health, legal, and standard considerations in energy drink manufacturing, given the results.

Introduction

Caffeine has long been used for thousands of years and is currently one of the most widely used active food compounds. Caffeine (1,3,7-trimethylxanthine; $C_8H_{10}N_4O_2$) is an organic xanthine alkaloid compound that occurs naturally (Committee on Military Nutrition Research, 2001; Heckman et al., 2010) and has mainly been discovered in coffee (*Coffea arabica*), tea (*Thea sinensis*), and cocoa (*Theobroma cacao*) plants. Due to its stimulant, antioxidant, anti-inflammatory, and analgesic effects (Cappelletti et al., 2015; Vieira et al., 2020), it has long been used as a vital compound for the formulation of over-the-counter medicines. Caffeine has remained a primary energetic compound, widely present in tea, soft drinks, chocolates, confectionery, and energy drinks, thereby making it one of the most commonly used substances worldwide (Błaszczyk-Bębenek et al., 2021; Saraiva et al., 2023). Recent health suggestions from administrations emphasize the health risks associated with the consumption of this stimulant for pregnant, lactating women; teenagers; children; youth; as well as those experiencing health conditions like cardiovascular disease (Wikoff et al., 2017). Such guidelines highlight significant differences in physiological responses to regular and excessive caffeine doses (Temple et al., 2017). The exact amount of caffeine needed to elicit adverse responses also varies from person to person, depending on body weight and caffeine sensitivity (Higdon & Frei, 2006). Sensitive persons are suggested to restrict their caffeine intake to less than 400mg daily to remain unaffected by the adverse responses of caffeine, including drowsiness, headaches, nausea, and anxiety (Smith, 2002). Caffeine works as an antagonist of adenosine receptors, resulting in the facilitation of the discharge of neurotransmitter chemicals like dopamine, noradrenaline, and acetylcholine, responsible for several psychoactive responses, including peripheral vasoconstriction, hypertension, thermogenesis, and augmentation of the functions of the kidneys and

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stomach, respectively (Benowitz, 1990; Riksen et al., 2009).

Confirming the solid mass of an energy drink is vital for evaluating its nutritional profile and quality. Quantifying the total mass will give manufacturers a means to determine the amounts of each carbohydrate, vitamin, mineral, and herbal supplement they have added for dietary improvement, and to enhance their ability to maintain product formulation and comply with regulations. Consumers also benefit from this determination, as they can make more informed decisions about what they consume and how the nutritional components of an energy drink will affect their dietary requirements. Carbohydrates are the primary organic compounds and the most significant energy source, and all carbohydrates, including non-digestible carbohydrates, are considered fundamental elements of a healthy diet (Grosch et al., 2008; Belitz et al., 2009). Most energy beverages contain a large percentage of carbohydrates, along with caffeine and various other stimulants in chocolates and candies (Campos et al., 2022), thus resulting in an increasing amount of research conducted to determine how these different ingredients may affect cognitive function (Seifert et al., 2011; Boyle et al., 2018). Studies have indicated that the combined effect of caffeine and carbohydrates is a positive influence on both sustained attention (over 30 minutes) and short-term attention (less than 30 minutes) (Kennedy and Scholey, 2004; Aniţei et al., 2011). Not only do caffeine and glucose improve mental energy by relaying an increase in mental alertness, achievement of mood elevation, and motivation to perform tasks (described as effective mental energy) (Childs and De Wit, 2008; Gorby et al., 2010), but the consumption of caffeine with glucose has been shown to enhance perceptions of alertness, stimulation, and arousal (Smit et al., 2006; Howard et al., 2010). This quantifiable energy correlate is essential for evaluating energy, creating nutrition labels, and identifying adulterations. Additionally, by understanding the composition of carbohydrates consumed, one is better positioned to correlate the carbohydrate intake with health outcomes (Menezes et al., 2004).

Energy drinks are distinguished from conventional soft drink products because they are generally formulated with significantly higher concentrations of both caffeine and sugar, and their contents have been analyzed. Researchers have specifically examined the total solid mass of energy drinks and the amounts of caffeine and carbohydrates in each drink to evaluate the health risks associated with their consumption. Energy drinks are marketed to give users an energy boost by containing a combination of caffeine, the natural stimulant, sugars, glucuronolactone, amino acids, herbs, and vitamins, and most contain very high levels of caffeine and sugar (O'Brien et al., 2008). The growth rate of caffeine-based energy drinks globally has been extremely rapid since the start of the millennium (Spaeth et al., 2014). The energy drink market in Bangladesh is projected to grow from USD 128.5 million in 2024 to USD 185.40 million in 2029. The increased sales of energy drinks in Bangladesh are primarily due to rising demand for ready-to-drink beverages, particularly among working-class individuals with busy schedules. Due to the widespread availability of energy drinks through convenience stores, supermarkets, and local retailers in Bangladesh, there is a greater demand and increased consumer acceptance of these products (Bangladesh Energy Drinks Market Insights, 2023). The primary purpose of this research project is to develop a low-cost, easy-to-use, and quick method for measuring caffeine and carbohydrate (sugar) levels in energy drinks sold in Bangladesh. The objective of this project is to determine the caffeine and carbohydrate content of energy drinks and to educate consumers about the limits of daily energy drink consumption without adverse health effects.

Materials and Methods

Chemicals, glassware, and instruments

Deionized water was used for all analyses carried out throughout the project. The glassware used included micropipettes (1-1000 µL), pipettes (5-10 mL), volumetric flasks (5, 10, 20, 50, and 100 mL), funnels, beakers, spatulas, and vials. This glassware was cleaned with detergent and water, rinsed 3 times with distilled water, then rinsed with acetone, and finally baked in an oven set to 60 ^{0}C. The baked glassware was wrapped in aluminum foil, and Mini-uniprep™ vials were used to store standard and sample preparations. For the experimental examination, the equipment used included an electrical balance (ATY124, SHIMADZU), an oven (GSM 11/8, Hope Valley, S336RB, England), a UV-visible spectrophotometer (UV-1800, SHIMADZU), a vortex mixer (Stuart), and a rotary vacuum evaporator (VP30, Lab Tech).

Sample collection and storage

Energy drink samples of different brands (n=8) were Braver, Fighter, Power, Oscar, Royal Tiger, Speed, Red Bull, and Bull Dozer, collected from other areas of Dhaka city for analysis (Table 1). The sample collection was conducted from April 2023 to August 2023. All collected samples were refrigerated to preserve their chemical composition without degradation or alteration (Ouhakki et al., 2024). Each sample was procured and analyzed within the recommended time for consumption to ensure the accuracy and relevance of the analytical results.

Determination of solid mass

To quantify the solid mass of energy drink samples, an aliquot of 10 mL was accurately measured with a volumetric flask and transferred into a cleaned, dried, and pre-weighed 100 mL round-bottom flask (RBF). The RBF was then attached to a rotary evaporator at a temperature setting of 35-40 °C. The liquid fraction was considered to have evaporated when it left only a viscous solid residue adhering to the walls of the RBF; the RBF was then transferred to a freeze dryer. Once the solid mass in the RBF was arid, it was weighed along with the final weight of the RBF holding the solid residue. The mass of the solid was measured and expressed in grams per 100 grams of sample.

Preparation of a standard solution of caffeine

Caffeine standards were kept in a vial wrapped and stored in a refrigerator at 0 °C. To make the stock solution, 0.01 g of the caffeine standard was dissolved in deionized water in a 100 mL volumetric flask to yield a solution at 100 mgL^{-1}. This solution was assigned the name of the standard used and the concentration. It was stored in the refrigerator at 0 °C. Subsequent concentrations of 60.0, 40.0, 20.0, 10.0, 4.0, 2.0, and 1.0 mgL^{-1} were obtained after the serial dilution process. The absorbance of these solutions was measured using a double-beam UV spectrophotometer to construct a calibration curve. The UV-Visible spectrum of standard caffeine was recorded at λmax 273 nm (Bhawani et al., 2015; Habtamu and Belay, 2020). The calibration curves were obtained by serially diluting working standard solutions of caffeine and plotting absorbance against concentration in Microsoft Excel. The calibration curve is shown in Fig. 1.

Sample preparation for the determination of caffeine

At first, 20 mL of an energy drink was transferred to a cleaned volumetric flask, filtered, and degassed

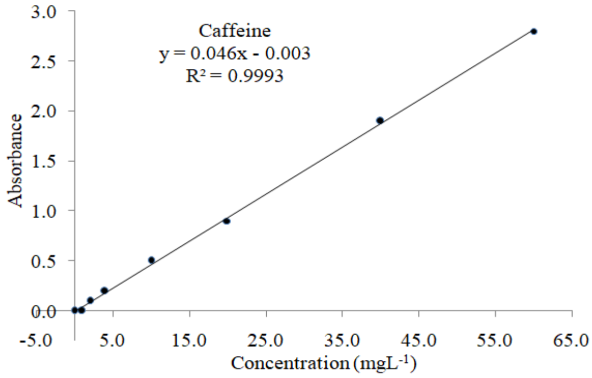

Fig. 1. Standard Calibration Curve of Caffeine

over about 10-15 min. A known volume (5 mL) of this degassed sample was transferred to a volumetric flask for dilution. This sample was diluted several times with deionized water. All sample solutions were prepared similarly, and absorbance was measured for each solution using a UV-Visible spectrophotometer (Amos-Tautua et al., 2014).

The UV spectrometer was initially run with a blank solution (deionized water) for caffeine determination. A wavelength range of 240-350 nm was used for caffeine determination. At these fixed wavelengths, the absorbance of each sample solution was measured at λmax = 273 nm. Absorbance spectra of two samples are shown in Fig. 2.

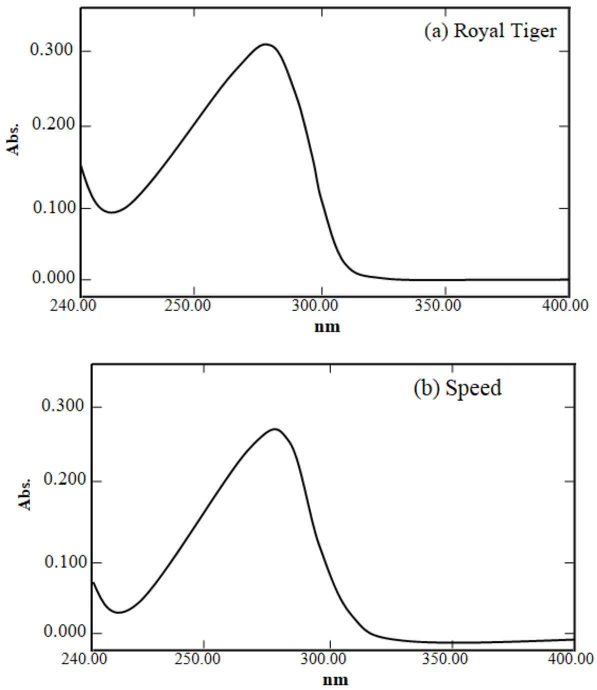

Fig. 2. UV-spectrum of sample solutions (a) Royal Tiger and (b) Speed energy drinks

Preparation of a standard solution of carbohydrate

Exactly 2.0 mg of standard glucose was weighed into a 100 mL volumetric flask to prepare a standard solution. Different concentrations of solution, such as 12.5, 60, 100, 150, and 200 mgL^{-1}, were prepared from the standard solution. For each experiment, 3 mL of each solution was collected, and 50 µL of phenol was added. Then, 3.0 mL of H_2SO_4 was carefully added. A reddish-brown colored solution was obtained, and the absorbance was measured at 489 nm (Sultana et al., 2012; Gerwig, 2021). The calibration curve (Fig. 3) was obtained by measuring the absorbance of serially diluted glucose standards and plotting absorbance against concentration in Microsoft Excel.

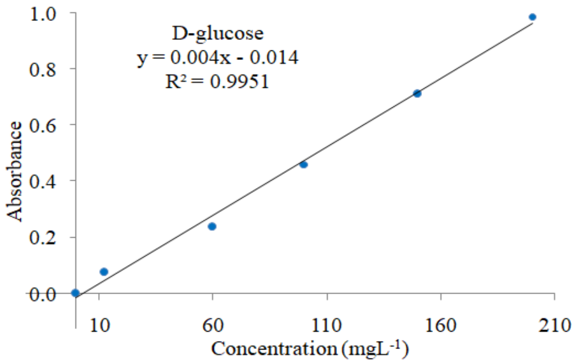

Fig. 3. Calibration curve for standard D-glucose

Sample preparation for the determination of total carbohydrate

Exactly 20 mg of the solid mash from each sample was dissolved in 100 mL of water, and the mixture was homogenized using a vortex mixer. From this solution, 3 mL was taken in a test tube. Then 50 µL phenol (80 %) and 3 mL H_2SO_4 (con.) solution were added to the test tube. All of the sample solutions were prepared similarly, and the absorbance of each solution was measured in a UV-Visible spectrophotometer using a 1 cm quartz cell (Albalasmeh et al., 2013).

The UV spectrometer was initially run with a blank solution of sulfuric acid for carbohydrate determination. The wavelength range of 400-600 nm was used for carbohydrate determination. At these fixed wavelengths, the absorbance of each sample solution was taken at λmax 489 nm. Absorbance spectra of two samples are shown in Fig. 4.

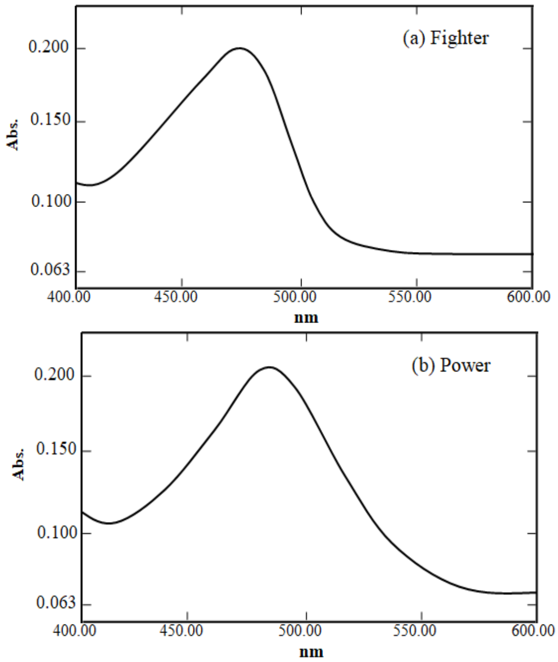

Fig. 4. UV-spectrum of sample solutions (a) Fighter and (b) Power energy drinks

By comparing the absorbance at specific λmax for both the caffeine (273 nm) and carbohydrate (489 nm) in the sample with those of the standard caffeine and carbohydrate, they were identified. From the calibration curves, the amount of caffeine and carbohydrate present in the samples was calculated using the formula y = mx+c, where y =absorbance, x =amount of caffeine or carbohydrate, m = slope of calibration curve, and c = intercept on the Y-axis. In this study, the standard deviation (S) and relative standard deviation (RSD) were computed to evaluate the determination of caffeine and total carbohydrate content in commercially available energy drink samples in Dhaka city, Bangladesh. Each energy drink sample was analyzed in triplicate. Microsoft Excel was employed for these calculations, utilizing standard mathematical formulas to determine the SD and RSD. The RSD values were instrumental in assessing the analytical method's consistency, thereby ensuring the reliability of the UV-Visible spectrophotometric technique used in this research. The following statistical equations are used to calculate S (Eq. 1) and RSD (Eq. 2).

$$S = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}} \text{ ------------ (Eq.1)}$$

$$RSD\,(\%) = \frac{S}{Mean} \times 100 \text{ ------------(Eq. 2)}$$

Where x = values of individual data, $\bar{x}$ = Mean value of the data set, and n = number of test values. LOD and LOQ were calculated from the calibration line at low concentrations using the standard deviation of the responses and the slope (Shrivastava and Gupta, 2011). The following equations were used for calculating LOD (Eq. 3) and LOQ (Eq. 4):

$$LOD = \frac{3.3 \times \text{Standard deviation of the response}}{\text{Slope of the calibration curve}} \text{------------(Eq. 3)}$$

$$LOQ = \frac{10 \times \text{Standard deviation of the response}}{\text{Slope of the calibration curve}} \text{------------(Eq. 4)}$$

The SD of the response was also calculated using the SD of the y-intercept of the regression line (y = mx + c), assuming a linear relationship between the response (y) and x over a specified range of concentrations (Suydam, 2000). The equation can be written as y = mx + c.

Results and Discussion

The solid mass of various energy drink samples was determined and is presented in Fig. 5. The solid mass ranged from 10.3 g/100 mL for Power energy drink to 13.13 g/100 mL for Oscar energy drink. This slight variation clearly indicates substantial differences in the values for non-volatile compounds across brands. Braver, Fighter, Speed, and Royal Tiger energy drinks registered values of 12.10 g/100 mL, 11.90 g/100 mL, 12.30 g/100 mL, and 11.30 g/100 mL, respectively. This highlights considerable uniformity within this designated group. It is pertinent to note that the solid mass for Red Bull and Bull Dozer was 11.30 g/100 mL and 11.10 g/100 mL, respectively. This clearly highlights compositional variability in these commercial energy drinks.

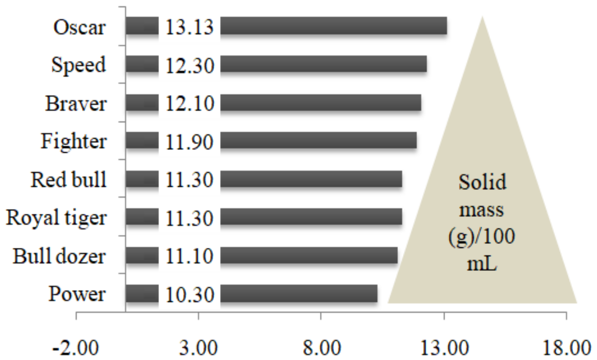

Fig. 5. Amount of total solid mass in the targeted energy drink samples.

The analysis of caffeine and carbohydrate content in energy drink samples from various brands available in Dhaka city, Bangladesh, reveals significant variations in both parameters (Table 1). Caffeine concentrations ranged from 233.30 mgL^{-1} in the Power sample to 546.48 mgL^{-1} in the Oscar sample. The SD and RSD for caffeine content were low, indicating high precision in the measurements. For instance, Oscar exhibited the highest caffeine concentration (546.48 mgL^{-1}) with an SD of 1.42 and an RSD of 0.26%, demonstrating the consistency of the analytical method. Similarly, Braver and Fighter also showed high caffeine contents (418.77 mgL^{-1} and 500.72 mgL^{-1}, respectively) with low RSD values (0.45% and 0.42%, respectively), underscoring the reliability of the data. A recent study from the University of Dhaka showed that the caffeine content in various energy drinks ranged from 147.84 to 846.78 mgL^{-1} (Refat et al., 2022). Hossain et al. analyzed four different energy drinks available in Bangladesh and found caffeine concentrations ranging from 149.41 to 978.28 mgL^{-1} (Hossain et al., 2015). Vuletić et al. (2021 analyzed five energy drink samples from the local markets in Croatia and found caffeine concentration 394.670-173.574 mgL^{-1} (Vuletić et al., 2021). Amos-Tautua et al. (2014 analyzed four energy drink samples from the local markets in Yenagoa, Nigeria, and found caffeine concentration 47.56-58.31 mgL^{-1} (Amos-Tautua et al., 2014). The caffeine levels in the energy drinks determined in this study are consistent with reported research. Compared to previous studies, the caffeine content in the current study's samples is generally higher than that found in Yenagoa, Nigeria, and overlaps with the ranges reported in Dhaka and Croatia.

In terms of carbohydrate content, the values ranged from 7.09 g/100g in Fighter to 11.15 g/100g in Oscar (Table 1). The variability in carbohydrate content is further illustrated by the RSD values, which, although slightly higher than those for caffeine, still indicate acceptable precision. For instance, Power and Bull Dozer exhibited relatively high RSDs for carbohydrate content (2.35% and 2.72%, respectively), suggesting greater variability in these samples. Nonetheless, the overall RSD values remained within acceptable limits, confirming the robustness of the analytical procedures. A recent study in Bangladesh showed that the sugar content in various energy drinks ranged from 16.16 to 338.33 mgL^{-1} (Refat et al., 2022). Hossain et al. analyzed four different energy drinks available in Bangladesh and found carbohydrate concentrations ranging from

Table 1. Caffeine and carbohydrate content in the targeted energy drink samples.

Brand Name	Packing size	Caffeine (mgL^{-1})			Carbohydrate (g/100g)		
		Average	SD	RSD (%)	Average	SD	RSD (%)
Braver	250 mL	418.77	1.88	0.45	11.09	0.08	0.68
Fighter	200 mL	500.72	2.08	0.42	7.09	0.11	0.54
Power	250 mL	233.30	1.04	0.45	8.36	0.20	2.35
Oscar	250 mL	546.48	1.42	0.26	11.15	0.14	1.25
Royal tiger	250 mL	251.27	4.39	1.75	9.12	0.14	1.59
Speed	250 mL	259.12	2.51	0.97	10.64	0.13	1.20
Red bull	250 mL	424.21	3.43	0.81	9.17	0.15	1.37
Bull dozer	250 mL	316.49	2.08	0.66	10.24	0.23	2.72

Note: Three replicates were done for each type of brand

295.20 to 504.00 mgL^{-1} (Hossain et al., 2015). The results of this study are consistent with these published literature values.

The European Food Safety Authority (EFSA) has established that a daily caffeine intake of up to 400 mg for adults, 200 mg for pregnant or lactating women, and 100 mg for children is considered within safe limits (Agostoni et al., 2015; AACAP, 2020). Exceeding these thresholds or abruptly discontinuing caffeine consumption can trigger a spectrum of adverse physiological reactions, including anxiety, insomnia, hallucinations, hypertension, headaches, gastrointestinal disturbances, diuresis, dehydration, tremors, palpitations, and cardiac arrhythmias, attributed to caffeine's stimulant effects (Addicott et al., 2009; Jahrami et al., 2020; Weibel et al., 2021). The EFSA has indicated that data on the safety profile of caffeine for children and adolescents are insufficient (Saraiva et al., 2023). All these sample packs were below the allowable maximum limit for caffeine, which is 400 mg/kg (Agostoni et al., 2015). These rules require that energy drinks contain only safe levels of caffeine, as people may consume them alongside other products or foods containing caffeine, which may result in adverse health consequences if caffeine is consumed at levels above the maximum allowed. Caffeine also finds application in energy drinks for flavoring and for their dependence-forming properties.

The caffeine levels in energy drinks vary among manufacturers, ranging from 10 to 50 mg per serving (Nour et al., 2010). The US Food and Drug Administration (FDA, 2006), however, limits caffeine in beverages to 6 mg per ounce. Consequently, the permissible caffeine content in soft drinks ranges from 30 to 72 mg per 355 mL (NSDA, 1999; Beauty and Oluwasanmi, 2022). According to BSTI (Bangladesh Standards and Testing Institution) regulations, the caffeine concentration in Bangladeshi energy drinks must be between 14.5 mg per 100 mL and 30 mg per 100 mL.

Table 2 provides a comprehensive analysis of the maximum allowable daily intake of eight energy drink brands available in Dhaka, Bangladesh, based on their caffeine content, with permissible consumption levels categorized for adults, pregnant women, and children according to established safe intake limits. For adults, with a maximum caffeine intake capped at 400 mg per day, Braver permits up to 955 mL daily, Fighter allows 799 mL, Power, having the lowest caffeine concentration, enables 1715 mL, Oscar restricts intake to 732 mL, Royal Tiger allows 1592 mL, Speed permits 1544 mL, Red Bull allows 943 mL, and Bull Dozer permits 1264 mL. For pregnant women, the limit on caffeine consumption is 200 mg daily. For Braver, the permitted level is 478 mL; for Fighter, 399 mL; for

Table 2. Maximum consumption levels of the targeted energy drink samples.

Brand Name (n=8)	Caffeine (mg/bottle)	Adult		Pregnant women		Children	
		Daily intake	Maximum Consumption ($mLday^{-1}$)	Daily intake	Maximum Consumption ($mLday^{-1}$)	Daily intake	Maximum Consumption ($mLday^{-1}$)
Braver	104.69	400 mg maximum	955	200 mg maximum	478	100 mg maximum	239
Fighter	100.14		799		399		200
Power	58.32		1715		857		429
Oscar	136.62		732		366		183
Royal tiger	62.82		1592		796		398
Speed	64.78		1544		772		386
Red bull	106.05		943		471		236
Bull dozer	79.12		1264		632		316

Power, 857 mL; and for Oscar, 366 mL. Additionally, Royal Tiger allows 796 mL, Speed 772 mL, Red Bull 471 mL, and, finally, Bull Dozer 632 mL.

Regarding children, with a recommended caffeine intake limit of 100 mg daily, Braver: 239 mL; Fighter: 200 mL; Power: 429 mL; and Oscar: 183 mL. Moreover, Royal Tiger will permit 398 mL, Speed 386 mL, Red Bull 236 mL, and finally Bull Dozer 316 mL. Of course, such varying levels emphasize how different in caffeine content these products are (Van Dam et al., 2020). Power, with the lowest caffeine content, has the highest intake among all consumer categories. On the contrary, Braver and Oscar, with higher caffeine content, have restricted intake levels so that the concern for cautious consumption, particularly for the vulnerable population of pregnant women and children, is highlighted. This data set underscores the need for consumer awareness of caffeine levels to avoid potential adverse health impacts.

Conclusion

In light of the above discussion, it is essential to conclude that the results obtained have highlighted variation in caffeine and carbohydrate content across different energy drink samples (n = 8) marketed in Dhaka, Bangladesh. Variations in caffeine content were observed in Power to Oscar, while variations in carbohydrates were observed in Fighters to Oscar. The accurate estimation of both contents in samples by UV-visible spectrophotometry underscores that both instruments exhibit less variability in their results, as indicated by low standard deviation and relative standard deviation values. These outcomes have assumed prime importance for consumer safety, particularly for pregnant women and children, as their estimates would help calculate their recommended safe daily doses. Additionally, such outcomes underscore the need for stringent control measures in the energy drink market to prevent potential health risks from excessive intake of both caffeine and carbohydrates.

Acknowledgment

The authors are grateful to the International Science Programme (ISP), Uppsala University, Sweden, for financial support.

Authors contribution

Othai Saha: Formal analysis, visualization, review and editing; Md. Mazharul Islam: Conceptualization, data curation, investigation, methodology, software, supervision, visualization and writing original draft; and Mohammad Shoeb: Data curation, funding acquisition, project administration, resources, review and editing.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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