# Quantitative determination of artificial sweeteners and sucrose in energy drinks and mango juice available in Dhaka city

Kabirul Islam,[1] Yashna Tehjib Meghla,[1] Md. Akhtaruzzaman,[2] Abu Shara Shamsur Rouf,[3] Md. Shahadat Hossian,[3] Mahboob Hossain[4]

[1]Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka; [2]Institute of Food and Nutrition, University of Dhaka; [3]Department of Pharmaceutical Sciences, University of Dhaka; [4]Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh

## Abstract

Energy drinks and mango juice are popular beverages. Apart from the natural ingredients and some additives present in these drinks, sugar is an important component of both. It has been established that, other than providing sweetness, sugars are potent to bring about health consequences for their consumers. Sweeteners, both artificial (aspartame, sodium cyclamate, and saccharin) and natural (sucrose), were our centers of interest. This study aimed to determine the presence and levels of these sweeteners in energy drinks and mango juice. Spectrophotometric methods were used to determine the concentration of the mentioned sugars. For this purpose, a total of 42 samples of 7 different brands were collected from different locations in Dhaka city, Bangladesh. The methods were found to be linear over the concentration range of 10-26 µg/mL ($r^2$=0.9989), 137-320 µg/mL ($r^2$=0.9891), 2.5-24 µg/mL ($r^2$=0.9915) and 2354-2784 µg/mL ($r^2$=0.9985) for aspartame, sodium cyclamate, saccharin, and sucrose, respectively. Mango juice contained a relatively lower amount of saccharin compared to energy drinks. In the case of aspartame, one brand of energy drinks had the least amount. Moreover, both energy drinks and mango juice had a similar content of sodium cyclamate, but one brand of mango juice had a relatively low content of sodium cyclamate.

Correspondence: Mahboob Hossain, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, 66, Mohakhali, Dhaka-1212, Bangladesh.
Tel: +88.02222264051.
E-mail: mmhossain@bracu.ac.bd

Key words: artificial sweeteners, energy drink, mango juice.

Contributions: KI, YTM, SH, worked in the laboratory; MA, ASSR, MH, provided intellectual contributions and guidance.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval and consent to participate: not applicable, as neither animals nor human subjects are involved in this study.

Funding: none.

Availability of data and materials: data and materials are available from the corresponding author.

Received: 6 October 2022.
Accepted: 14 December 2023.
Early access: 25 January 2024.


*Italian Journal of Food Safety 2024; 13:10914*
*doi:10.4081/ijfs.2024.10914*



## Introduction

Energy drinks are a different class of beverage that is said to provide instant energy. Energy drinks are usually composed of caffeine, taurine, l-carnitine, glucuronolactone, vitamins, and other herbal supplements like ginseng and guarana, among others.

Artificial sweeteners come under the class of non-caloric sugar, which is also known as low-calorie sweeteners or intense sweeteners. Their demand is perpetual, and their usage has turned versatile by capturing the market within a short period due to their promotional tagging of being non-caloric. Saccharin is 300 times sweeter than sucrose and is mostly used in soft drinks, dessert mixes, yogurt, and as a tabletop sweetener. The Food and Drug Administration (FDA) recommended a ban on the use of saccharin in 1977, based on the findings of a group of researchers who concluded it to be a weak carcinogen of bladder cancer in rats (Arnold *et al*., 1980). At present, more than 200 million people worldwide consume aspartame as a sweetener and use it in nearly 6000 food products. Unlike other artificial sweeteners, it readily hydrolyzes to its constituent amino acids, phenylalanine (50%), aspartic acid (40%), and methanol (10%) in the gastrointestinal tract (Singh *et al*., 2013). People with phenylketonuria lack the enzyme that catalyzes phenylalanine to tyrosine, and as a consequence, excess phenylalanine is converted to phenyl ketones. If this condition remains untreated, it may lead to intellectual malfunction and other serious health problems (Medline Plus, 2017).

Three different compounds, cyclamic acid, calcium cyclamate, and sodium cyclamate, are commonly known as cyclamate (Mortensen, 2006). Cyclamate is 30 times sweeter than sugar (Sardesai and Waldshan, 1991). Kojima and Ichibagase (1966) reported that cyclamate can be metabolized by gut bacteria to cyclohexylamine. As a consequence, the FDA has removed cyclamate from the generally recognized safe list. Price *et al.* (1970) stated that cyclamate at higher concentrations with saccharin caused bladder cancer in rats, and therefore, its usage as a food additive in the United States of America was banned by the FDA.

## Consequences of consuming artificial sweeteners or non-caloric sweeteners

### *Risk of cancers*

The relationship between the consumption of artificial sweeteners and the risk of lymphoma and leukemia was discussed by Schernhammer *et al.* (2012).

### *Glucose intolerance*

A recent paper published in Nature states that artificial sweeteners can cause glucose intolerance in consumers by altering the gut microflora (Suez *et al.,* 2014).

### *Weight gain*

The San Antonio Heart Study observed changes in weight between men and women over 7-8 years. According to Fowler *et al.* (2008), the possibility of putting on weight and obesity has a positive correlation with the consumers of artificially sweetened beverages (ASB) compared to non-consumers of ASB, regardless of being normal weight or overweight at baseline.

### *Metabolic syndrome*

There have been reports of metabolic syndrome resulting from the consumption of ASB in a variety of cohort studies (Swithers, 2013). A study of coronary artery risk development in young adults showed a positive guild of metabolic syndrome with ASB, and it also stated that the risk increases approximately by 17% (Duffey *et al.,* 2012).

### *Type 2 diabetes*

A study of European Enhanced Tactical Humanoid 3rd Revision showed that the risk of type 2 diabetes (T2D) was more than doubled for frequent consumers of ASB compared to non-consumers, and sugar-sweetened beverage consumption was also linked with an increased likelihood of T2D (Bhupathiraju *et al.*, 2013).

# Materials and Methods

## Sample collection

In total, 42 samples were collected (two samples for each batch and three batches of seven brands). Out of the seven brands, there were four brands of energy drinks (three of them were national and one was international) and three brands of mango juice (all of them were national). As the samples were from different companies, to avoid conflict, they were given codes Sp, Po, Tg, Rb, Fo, Fr, and Mg instead of their brand names.

## Sample preparation

All the energy drink samples were decarbonated using an ultrasonic bath at 40 kHz (Human Lab Instrument Co., Korea) frequency for 10 minutes in a test tube. Fruit juice samples were treated with several chemicals to obtain the desired result (artificial sweetener). Initially, 5 mL of the sample was dissolved in distilled water to make a total volume of 50 mL (10× dilutions). It was then filtered with Whiteman type 1 filter paper. A pale yellow or white filtrate was obtained and stored for further analysis.

### *Saccharin*

A simple extractive spectrophotometric method was used for the determination of saccharin from energy drinks and mango juice. This method was proposed by Mathew *et al.* (2006).

Preparations were as follows: i) saccharin standard solution: 50 mL of 1 mg/mL stock solution was prepared by dissolving 50 mg of saccharin powder in 50 mL of water. Serial dilution was performed to prepare a standard solution of concentration ranging from 2-34 $\mu$g; ii) equal volume of formic acid and water was added to prepare 50% v/v, *i.e.*, to prepare 50 mL of 50% v/v formic acid; iii) 1% w/v of potassium iodide was prepared by dissolving 1 g of potassium iodide in 100 mL of water; iv) a stock of Cetyl trimethyl ammonium bromide (CTAB) 0.1 M was prepared by dissolving 1.822 g of CTAB in 50 mL water.

The stock solution was diluted accordingly to prepare a solution of 1 mM; v) concentrated sulfuric acid (98%) was diluted to 10% using the formula $C_1V_1=C_2V_2$; vi) concentrated hydrochloric acid (37%) was converted to 5% using the same formula; vii) sodium hydrogen carbonate: 2 g of $NaHCO_3$ were dissolved in 100 mL of water to prepare a 2% solution of $NaHCO_3$.

#### *Procedure: standard curve establishment*

The extraction and determination procedure for the standard and sample were based on the method described by Mathew *et al.* (2006).

#### *Working with sample extraction of saccharin from the sample*

10 mL of decarbonated energy drinks were mixed with 1 mL of 10% sulfuric acid. The mixture was extracted twice with 6 mL of diethyl ether, the upper ether layer was extracted twice with 2% sodium hydrogen carbonate, then the ether layer was discarded. The aqueous layer was acidified with 2 mL of 5% hydrochloric acid and extracted twice with 5 mL of diethyl ether into a test tube. Then the test tube was heated in a water bath at 80ºC so that the ethereal extract evaporated. The residue on the test tube was dissolved in water, and the volume was made up to 5 mL. Using this dissolved extract, the procedure mentioned earlier was repeated, and the absorbance reading was recorded. From the plotted standard curve, the concentration or amount of saccharin present in the sample was calculated.

### *Aspartame*

The spectrophotometric method was developed by Lau *et al.* (1988) for the determination of aspartame in soft drinks based on the reaction of aspartame with ninhydrin.

Preparations were as follows: a) acetate buffer was prepared by mixing 50 mL of 0.2 M acetic acid solution with 50 mL of 0.2 M sodium acetate solution; ii) 4% ninhydrin solution was prepared by dissolving 4 g of ninhydrin in 100 mL absolute ethanol; iii) aspartame standard solution was prepared by dissolving 10 mg of aspartame powder in 10 mL propylene carbonate to prepare a stock of 1 mg/mL. Accurate serial dilutions were performed to prepare a standard solution of concentration ranging from 5 µg/ml to 50 µg/mL.

#### *Procedure*

The extraction and determination procedure for the analysis of samples was based on the method described by Lau *et al.* (1988) and Celik *et al.* (2014).

#### *Working with aspartame standard solution*

To an aliquot of 0.75 mL of standard aspartame solution, 0.5 mL of acetate buffer of pH 3.5 was added in a centrifuge tube. 3 mL of propylene carbonate and 2 mL of absolute ethanol were added to the centrifuge tube. The mixture was placed in an ultrasonic bath for 5 minutes at a frequency of 40 kHz. Then it was centrifuged at 5000 rpm for 5 minutes for the extraction of aspartame with the assistance of propylene carbonate. 3.5 mL of the lower phase was collected and dried with anhydrous sodium sulfate. After 20 minutes of settling, 2 mL of the dried solution was carefully taken into another screw-capped test tube, followed by the addition of 2 mL of 4% ninhydrin solution. The reaction mixture was boiled in a hot water bath at 80ºC for 20 minutes. The solution was then cooled, followed by dilution with ethanol to a volume of 5 mL. Absorbance reading was taken at 585 nm and recorded. Using the absorbance value and the corresponding concentration, a standard curve was plotted.

#### *Working with the sample*

The same procedure was followed with decarbonated energy drinks and treated fruit drinks to obtain the concentration of aspartame present in them.

### *Cyclamate*

A new method has been developed using ultrasound-assisted emulsification micro-extraction for the determination of cyclamate. The cyclamic acid formed is extracted as fine droplets in the presence of chloroform as an extraction solvent that contains a rhodamine B (RhB) reagent. A highly colored ion-pair complex of cyclamate ($RhBH^+$) was formed when the extracted cyclamic acid further reacted with RhB. In turn, the $RhBH^+$ formed can be used to spectrophotometrically detect the amount of cyclamate present.

Preparations were as follows: i) cyclamate standard solution was prepared by dissolving 10 mg of cyclamate in 10 mL of water. Using appropriate serial dilution, a standard solution of 25 $\mu$g/mL to 200 $\mu$g/mL was prepared; ii) to obtain 100 mL of 0.1 M sulfuric acid, 8 mL of concentrated (18.4 M) acid was dissolved in 92 mL of water. 10 mg of RhB powder was dissolved in 104.2 mL of chloroform to prepare a RhB solution of concentration $2\times10^{-4}$ M.

#### *Procedure*

The extraction and determination procedure for the standard and sample was based on the method described by Hashemi *et al.* (2015).

#### *Working with standard cyclamate solution*

To an aliquot of 5 mL of cyclamate standard solution, 5 mL of 0.1 M sulfuric acid was added in a centrifuge tube. The tubes were immersed in an ultrasonic bath for 1 minute. 200 $\mu$L of RhB solution was added to the solution. Emulsification and extraction were performed at 40 kHz of ultrasonic frequency for 20 seconds at 25±1°C. As a result, oil-in-water emulsions of chloroform in water were formed. After that, the emulsion was centrifuged at 3500 rpm for 5 minutes. In the case of the blank, 5 mL of water was used instead of the standard cyclamate solution. Then, 100 $\mu$L of the settled organic layer was dissolved in 2.9 mL of chloroform (30× dilution) to take the spectrophotometric reading. Absorbance readings were recorded, and the standard curve was plotted against concentration and absorbance readings to determine the concentration of cyclamate present in various samples.

#### *Working with the sample*

Decarbonated samples of energy drinks should be 5× diluted and the diluted solution should be treated in the same way as described earlier.

### *Sucrose*

The method for the determination of sucrose in beverages has been developed by Gimba *et al.* (2014).

Preparations were as follows: i) standard sugar solution: 100 mg of sucrose was dissolved in 100 mL of water to prepare a standard solution of 1 mg/mL. Serial dilution was performed to produce a standard solution with a concentration ranging from 100 $\mu$g/mL to 1000 $\mu$g/mL; ii) hydrochloric acid: 180 mL of concentrated hydrochloric acid (10.14 M) was added with 82 mL of water to prepare an acid solution of 6 M of 200 mL; iii) sodium hydroxide: 10 grams of sodium hydroxide pellet was dissolved in 100 mL of water to produce a solution 2.5 M strength; iv) 3, 5-dinitrosalicylic acid (DNSA) solution preparation: 1.1406 g of DNSA powder was dissolved in 100 mL of water.

#### *Working with sucrose standard solution*

2 mL of sucrose standard solution was pipetted into a test tube, and 2 mL of water was added to it. 2 mL of 6 M hydrochloric acid was added to the test tube. Then, it was placed in a boiling water bath for 10 minutes at 80ºC. The test tubes were removed, and 8 mL of 2.5 M sodium hydroxide was added, followed by 2 mL of 0.05 M 3,5-DNSA. After the addition of DNSA, it was immediately vortexed, followed by inversion for proper mixing of the reacting components, and again incubated in a water bath. The mixture was quickly transferred to an ice water bath for 10 minutes after removing it from the boiling water bath. For the blank, 2 mL of water was added instead of the standard sucrose solution. The solutions were diluted 10 times before taking the spectrophotometric reading. Absorbance reading was taken at 580 nm.

#### *Working with samples and data analysis*

The samples were treated in the previously described way. All the data were analyzed using Microsoft Office Excel version 2016 (Microsoft, Redmond, WA, USA).

## Results

The research work here focuses on the determination of the amount of sugar and three artificial sweeteners, aspartame, saccharin, and cyclamate, present in three local and one international energy drinks, along with three local mango juices. The concentration was obtained from the standard curve plotted for each sugar. The results found were completely experimental and might vary from batch to batch for each of the brands.

### Determination of sucrose concentration

Standard curves (Figure 1) were plotted by using the coefficient of multiple determinations for multiple regression or $r^2$ statistics in Microsoft Excel (Microsoft, Redmond, WA, USA) to obtain the best-fit line. The averages of the three absorbances were used for plotting the standard curve for each known concentration of standard. The standard curve of sucrose had an equation of $y=0.00009x+0.0094$. The value of absorbance was inserted into the equation to obtain the unknown concentration. The $r^2$ value of the graph was 0.9985, and the mean recovery percentage was 98.51±2.31%. The mean sucrose content in Rb was the highest (2784.44 $\mu$g/mL). Variations in mean content for batches were more in Fr. Lastly, the range of mean sucrose content was 2353.89 $\mu$g/mL to 2784.4 $\mu$g/mL (Figure 2).

### Determination of saccharin concentration

An approximate best-fit line was obtained for the standard solution of saccharin (Figure 3); the curve had an equation of $y=0.0058x+0.1142$ with a $r^2$ value of 0.9915. The extraction method of saccharin had the lowest mean recovery of 96.25±12%

for standard. However, Fo and Fr were found to have the least amount of saccharin, viz., 2.46 and 2.62 $\mu$g/mL respectively (Figure 4). The highest concentration of saccharin was obtained in Po (23.81 $\mu$g/mL) (Figure 4). The mean saccharin content had a range of 2.46 $\mu$g/mL to 23.81 $\mu$g/mL (Figure 4).

## Determination of aspartame concentration

Unknown concentration was calculated using the equation y=0.002x+0.0231. The $r^2$ value of the curve was 0.9989 (Figure 5). The method applied had a mean recovery percentage for standard solutions of 99.0±2.4%. Moreover, all the unknown concentrations were calculated using Excel (Microsoft, Redmond, WA, USA) to avoid human error. Variation in aspartame content on different batches had to be observed; however, Rb had more consistency in mean aspartame level among batches of different brands.

Furthermore, Fr had the highest content of aspartame (25.95 µg/mL) (Figure 6), as well as the highest amount of aspartame among the brands that were tested. On the other hand, Po energy drinks had the least content of aspartame. The range of mean aspartame content in the sample was 10.2 µg/mL to 25.95 µg/mL (Figure 6).

## Sodium cyclamate

The equation for the standard curve of cyclamate showed a gradient of 0.0013 and an intercept of 0.269 (Figure 7). The mean recovery percentage was 97.4±5.8% for the standard, along with the $r^2$ value of 0.9891. The mean sodium cyclamate content for the batches of Mg was much closer to each other. Sp and Fr lie on two different poles based on mean cyclamate content; the latter had the least sodium cyclamate content (136.73 µg/mL) (Figure 8). The extent of mean cyclamate content among the three batches of different brands ranged from 136.73 µg/mL to 320.10 µg/mL (Figure 8).

## Approximate content of each sugar in a 250-mL bottle of the samples available in the market

Summing up the above results, the mean value of each of the sugars present in the different samples was calculated for better observation. Using the obtained mean values, the total content of each sugar in 250 mL was calculated. The histogram (Figure 9) showed sucrose, saccharin, aspartame, and sodium cyclamate content varied in a range of 585.86 mg to 637.31 mg, 1.29 mg to 5.53 mg, 2.73 mg to 6.18 mg, and 35.86 mg to 79.13 mg, respectively, in a container of 250 mL. In other words, it can be said that according to the present investigation, the amount of sugar a person consumed was illustrated in a 250-mL container (Figure 9).



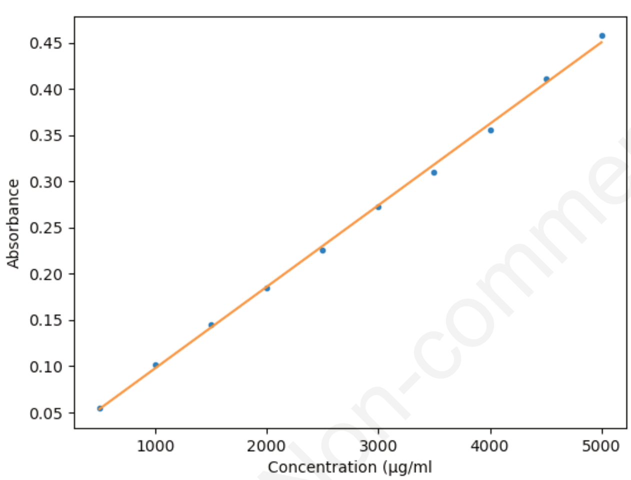


**Figure 1.** Standard curve of sucrose.


**Figure 2.** Average concentrations of sucrose in three batches of different brands.

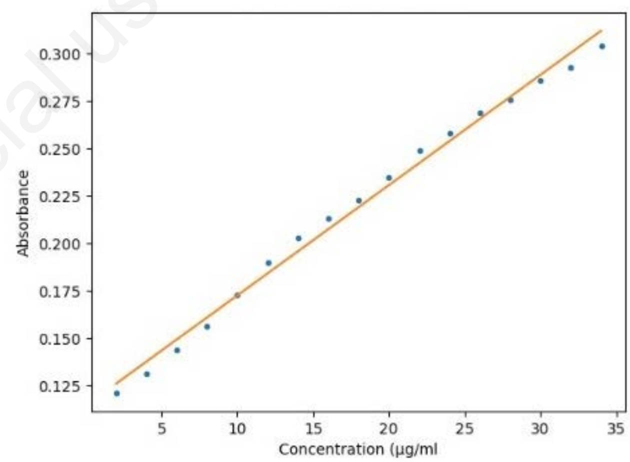


**Figure 3.** Standard curve of saccharin.


**Figure 4.** Average concentrations of saccharin in three batches of different brands.

## Discussion

Quantitative determination of the three specified artificial sweeteners from energy drinks and mango juice has not yet been documented in Bangladesh. In the current research, spectrophotometric methods were used to quantitatively determine the concentration of sucrose, saccharin, aspartame, and sodium cyclamate in energy drinks and mango juice commercially available in Dhaka. Beverages are the most frequently consumed non-nutritive sweetener (NNS)-containing product compared to NNS foods or packs of artificial sweeteners among male and female children and adults in the National Health and Nutrition Examination 2007-2008 (Gardner *et al*., 2012). So, more artificial sweeteners are being consumed by means of beverages (soft drinks and energy drinks). The findings of the aforementioned research work also show a positive correlation with Gardner *et al*. (2012), as the content of artificial sweeteners in experimental samples is high. Reid *et al.* (2017) state that global consumption of energy drinks doubled between 2006 and 2012, which can allude to a serious health hazard for young people due to their high sugar content. Similar to other countries, cold beverages (energy drinks and fruit juice) are popular among the youth and adults in Bangladesh.

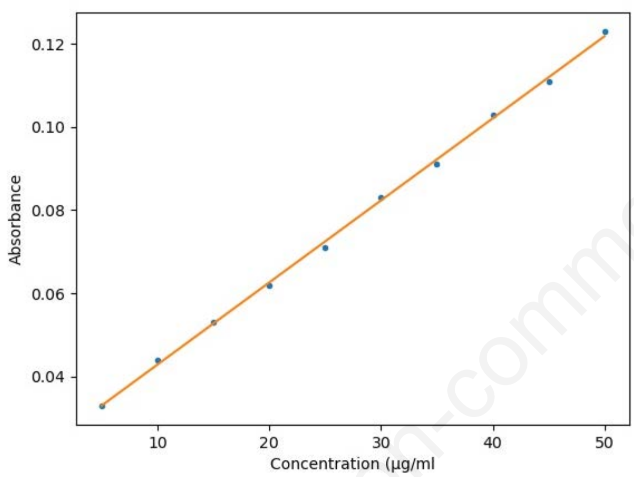


**Figure 5.** Standard curve of aspartame.

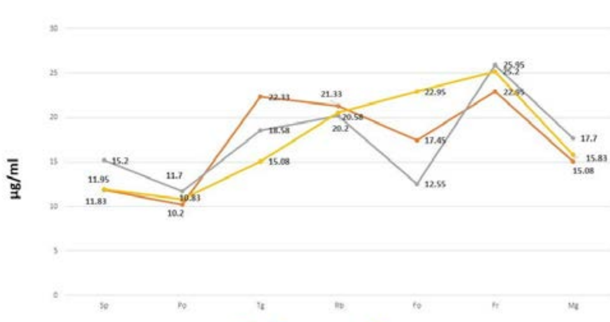


**Figure 6.** Average concentrations of aspartame in three batches of different brands.

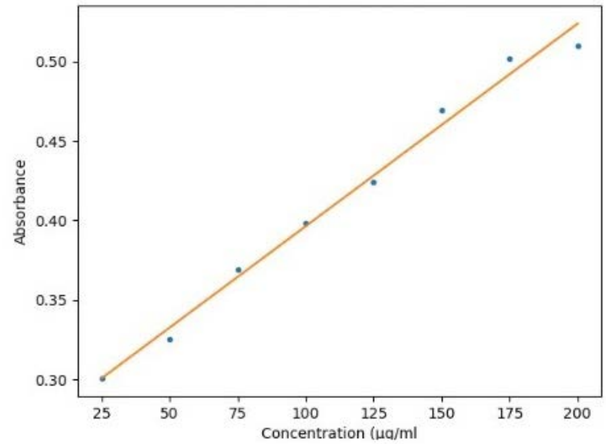


**Figure 7.** Standard curve of cyclamate.

**Figure 8.** Average concentrations of sodium cyclamate in three batches of different brands.

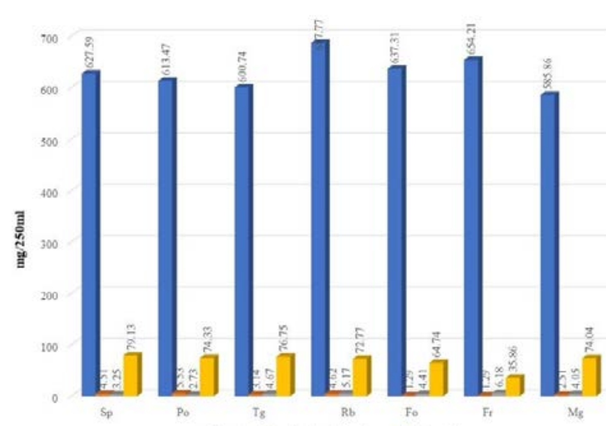


**Figure 9.** Approximate content of sucrose, saccharin, aspartame, and cyclamate in a 250 mL bottle of the samples available in the market.

In the present investigation, it was observed that the sugar (sucrose) concentration in the sampled energy drinks and mango juice ranged from 2353.89 $\mu$g/mL to 2784.44 $\mu$g/mL. Gimba *et al.* (2014) found a sugar (sucrose) concentration ranging from 91.05 $\mu$g/mL to 1686.73 $\mu$g/mL, by sampling energy drinks following a similar method. Rb had the highest concentration, and Tg had the lowest sucrose concentration. In the present study, the concentration of sucrose found was higher than that mentioned in the literature; this might be due to a change of country.

The results of the present research showed that the concentration of saccharin found in samples ranged from 2.42 $\mu$g/mL to 23.81 $\mu$g/mL. In addition, Fo had the least and Po had the highest amount of saccharin. Serdar and Knezevic (2011) reported that saccharin content ranged from 40.01 $\mu$g/mL to 55.24 $\mu$g/mL for fruit juice and from 65.77 $\mu$g/mL to 76.91 $\mu$g/mL for artificial and flavored drinks. Furthermore, the latest findings showed another range of saccharin concentration in liquid foods, where the maximum content was 145.20 $\mu$g/mL and the minimum was 9.46 $\mu$g/mL (Ramsurn *et al.*, 2015). Similar to Serder and Knezevic (2011), the present investigation also revealed that a lower amount of saccharin was present in mango juice and a bit higher amount was obtained in energy drinks. However, the results obtained here were much lower than those stated in the literature.

In the present study, aspartame concentration ranged from 10.2 $\mu$g/mL to 25.95 $\mu$g/mL. Ramsurn *et al.* (2015) stated the concentration of aspartame in their sample was from 15.03 $\mu$g/mL to 844 $\mu$g/mL and Serder and Knezevic (2011) reported that aspartame content in fruit juice ranged from 80.29 $\mu$g/mL to 435.05 $\mu$g/mL and 198.22 $\mu$g/mL to 709.36 $\mu$g/mL for artificial and flavored drinks. The results obtained from the current research were much lower than reported in the aforementioned papers. According to the results found in this study, Fr had the highest and Po had the lowest concentration of aspartame in their respective samples. Rb had a more consistent aspartame content among its batches. The rest of the brands had variations among the batches.

Serder and Knezevic (2011) reported that the concentration of cyclamate ranged from 70.10 µg/mL to 583.94 µg/mL in fruit juice and 203.68 µg/mL to 621.75 µg/mç in artificial and flavored drinks. However, the results obtained in the present study ranged from 136.73 µg/mL to 320.1 µg/mL. Maximum sodium cyclamate was present in Sp and the minimum presence was found in Fr. The findings here remained within the range stated by Serder and Knezevic (2011).

In this investigation, the concentration range of aspartame and saccharin obtained is lower when compared with the mentioned papers, whereas, the concentration of cyclamate lies within the range reported, but the concentration of sucrose outlies the range reported in the literature. As three of these artificial sweeteners and sucrose were found in all the samples, their respective concentration was much lower. However, no possible reason can be put forward for the much higher concentration of sucrose present in all the variants.

The findings of the current analysis of both natural and artificial sugars can be summarized as follows, sucrose content varied in a range of 585.86 mg to 637.31 mg in a container of 250 mL, Rb contains the highest amount and Tg has the least amount. In addition, aspartame content is relatively low in all the samples (range is 2.73 mg to 6.18 mg, per 250 mL). Cyclamate had the highest content among artificial sweeteners, with the maximum concentration observed in Sp. However, the presence of sucrose was much higher than any other sugar, as expected.

The safety of artificial sweeteners has been considered by a range of regulatory organizations, their expert advisory groups, and interested scientists. The European Commission's Committee on Food set the acceptable daily intake (ADI) of aspartame, and saccharin at the same level as that set by the Joint Food and Agricultural Organization/World Health Organization Experts Committee on food and additives (JECFA). They had approved the ADI for aspartame, cyclamate, and saccharin as 40 mg/kg body weight (bw), 11 mg/kg bw, and 5mg/kg bw, respectively. Although the ADI of aspartame stated by the FDA was 50 mg/kg bw, for saccharin, it was the same as mentioned by the JECFA. No ADI level was mentioned for cyclamate by the FDA, as it was banned in the USA.

## Conclusions

The presence of three commonly used sweeteners in all the sampled brands indicates that manufacturers are using mixtures of sweeteners along with sucrose instead of adding one or two sweeteners. From the proportion of saccharin to cyclamate, it can be assumed that a blend of these is being used, like Sucaryl®, a common blend in another part of the world. More importantly, none of the manufacturers stated the presence of these artificial sweeteners in their products. Furthermore, there should be strict monitoring of foods and beverages that use non-caloric sweeteners. The regulatory bodies should be scrupulous about the usage of these sweeteners, as they show no signs of harm immediately or at present, but they harm the human body anonymously, and in most cases, they remain unrevealed and untraced. Moreover, none of the manufacturers of the samples maintained the code of conduct of the FDA, as there was no labeling for aspartame, or saccharin, which had been made mandatory.

## References

Arnold DL, Moodie CA, Grice MC, Charbonneau SM, Stavric B, Collins BT, McGuire PF, Zawidzka ZZ, Munro LC, 1980. Long-term toxicity of ortho-toluenesulfonamide and sodium-saccharin in the rat. Toxicol Appl Pharmacol 52:113-52.

Bhupathiraju S, Pan A, Malik V, Manson J, Willett W, van Dam R, Hu F, 2013. Caffeinated and caffeine-free beverages and risk of type 2 diabetes. Am J Clin Nutr 97:155-66.

Celik E, Demirhan BE, Demirhan B, Yentur G, 2014. Determination of aspartame levels in soft drinks consumed in Ankara, Turkey. J Food Res 3:156-9.

Duffey KJ, Steffen LM, Van Horn L, Jacobs DR, Popkin BM, 2012. Dietary patterns matter: diet beverages and cardiometabolic risks in the longitudinal coronary artery risk development in young adults (CARDIA) study. Am J Clin Nutr 95:909-15.

Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP, 2008. Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. Obesity 16:1894-900.

Gardner C, Wylie-Rosett J, Gidding SS, Steffen LM, Johnson RK, Reader D, Lichtenstein AH, 2012. Non-nutritive sweeteners: current use and health prospectives. Circulation 126:509-19.

Gimba CE, Abechi SE, Abbas NS, Gerald IU, 2014. Evaluation of caffeine, aspartame and sugar content in energy drinks. J Chem Paharm Res 6:39-43.

Hashemi M, Zohrabi P, Abdolhosseini S, 2015. Spectrophotometric determination of cyclamate in artificial sweeteners and beverages after ultrasound-assisted emulsifica-

tion microextraction. Anal Methods 7:2594-602.
Kojima S, Ichibagase H, 1966. Studies on synthetic sweetening agents. 8. Cyclohexylamine, a metabolite of sodium cyclamate. Chem Pharm Bull 14:971-4.
Lau OW, Luk SF, Chan WM, 1988. Spectrophotometric determination of aspartame in soft drinks with ninhydrin as reagent. Analyst 113:765-8.
Mathew SB, Pillai AK, Gupta VK, 2006. Spectrophotometric method for the determination of saccharin in food and pharmaceutical products. Indian J Pharm Sci 68:821-3.
Medline Plus, 2017. Phenylketonuria. Available from: https://ghr.nlm.nih.gov/condition/phenylketonuria.
Mortensen A, 2006. Sweeteners permitted in the European Union: safety aspects. Scandinavian J Food Nutr 50:104-16.
Price JM, Biava CG, Oser BL, Vogin EE, Steinfeld J, Ley HL, 1970. Bladder tumors in rats fed cyclohexylamine or high doses of a mixture of cyclamate and saccharin. Science 167:1131-2.
Ramsurn D, Jhaumeer Lauloo S, Bhowon MG, 2015. Determination of artificial sweeteners in liquid foods by high performance chromatography. Int J Pharm Drug Anal 3:311-21.
Reid JL, McCrory C, White CM, Martineau C, Vanderkooy P, Fenton N, Hammond D, 2017. Consumption of caffeinated energy drinks among youth and young adults in Canada. Prev Med Rep 5:65-70.
Sardesai VM, Waldshan TH, 1991. Natural and synthetic intense sweeteners. J Nutr Biochem 2:236-44.
Schernhammer ES, Bertrand KA, Birmann BM, Sampson L, Willet WC, Feskanich D, 2012. Consumption of artificial sweetener and sugar-containing soda and risk of lymphoma and leukemia in men and women. Am J Clin Nutr 96:1419-28.
Serdar M, Knezevic Z, 2011. Determination of artificial sweeteners in beverages and special nutritional products using high performance liquid chromatography. Arh Hig Rada Toksikol 62:169-73.
Singh M, Kumar A, Tarannum N, 2013. Water-compatible 'aspartame'- imprinted polymer grafted on silica surface for selective recognition in aqueous solution. Anal Bioanal Chem 405:4245-52.
Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss C, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A, Kuperman Y, Harmelin A, Kolodkin-Gal I, Shapiro H, Halpern Z, Segal E, Elinav E, 2014. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature 514:181-6.
Swithers SE, 2013. Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. Trends Endocrinol Metab 24:431-41.