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Evaluation of caffeine, aspartame and sugar contents in energy drinks

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ABSTRACT

The caffeine, aspartame and sugar contents of selected energy drinks were evaluated using HPLC and UV spectrophotometric procedures. The results obtained from this study indicated that the average concentration of caffeine, aspartame, and sugar in the analyzed beverages ranged from 1.11 ppm – 237.95 ppm, 283.84 ppm – 956.82 ppm and 91.05 ppm – 1686.73 ppm, respectively. None of the analyzed beverage samples was found to violate the current legal limits set by the US FDA food and beverages regulations.

Key words: Energy drinks, caffeine, aspartame and sugar.

INTRODUCTION

Energy drinks beverages often contain ingredients such as caffeine, guarana, glucuronolactone, methylxanthine, taurine, sugar, artificial sweeteners, physiological stimulants, preservatives, artificial flavors or colors, food acids and other food additives [1].

The majority of energy drinks contain caffeine as an active ingredient due to its stimulatory effect on the central nervous system [2]. Caffeine is also known to increase the secretion of epinephrine, which can lead to a variety of secondary metabolic changes that can positively affect physical or mental performance [3]. Once ingested, caffeine is rapidly absorbed from the gastrointestinal tract and undergoes demethylations which result in paraxanthine (84%), theobromine (12%), and theophylline (4%); with the xanthenes theobromine and theophylline having very similar chemical structures compared to caffeine [4].

Many studies also confirm its ability to enhance mood and alertness [5, 6, 7], exercise performance [3, 8], the speed at which information is processed, awareness, attention, and reaction time [9]. Caffeine has also been shown to reduce some of the negative side effects associated with sleep loss. Some studies suggest that caffeine can contribute to improved alertness and performance at doses of 75 to 150 mg after acute sleep loss and doses of 200 to 600 mg after a night or more without sleep [10]. Caffeine also has a diuretic effect regardless of its consumption as energy drink, tea, or coffee [11]. The cardiovascular effects of caffeine have also been studied and suggest that caffeine likely has an effect on hemodynamic parameters [12, 13, 14]. A review regarding caffeine consumption concluded that among the healthy adult population, a moderate daily caffeine intake of ≤400 mg (equivalent to 6 mg/kg/d for a 65 kg person) was not associated with any adverse effects [15]. Nonetheless, caution should be exercised in regard to the amount of caffeine consumed per day.

Aspartame is a white crystalline powder having no odour, but is intensely sweet. It is approximately 200 times sweeter than sucrose, the accepted standard for sweetness. It is a synthetic non nutritive sweetener (a methyl ester of a dipeptide) composed of aspartic acid and phenylalanine. There are two forms of aspartame, an alpha and a beta form. Only the alpha form is sweet. Although aspartame yields the same caloric intake as sugar on a weight to weight basis (4 kcal/g), it can be added at almost 200 times lower levels to achieve the same sweetness, thereby providing a far lower net caloric intake. This attribute has resulted in the use of aspartame as a low calorie or non nutritive sweetener in foods and beverages worldwide. Consumption of aspartame may cause brain damage [16]. Adverse neurological effects of aspartame include headache [17], insomnia and seizures after ingestion of aspartame, which are also accompanied by the alterations in regional concentrations of catecholamine [18]. Previous finding have shown that aspartate may lead to neurotoxicity through sustained contact with the receptors, such as glutamate producing an excitotoxic effect [19].

The term "sugars" is traditionally used to describe mono- and disaccharides [20]. Sugars are used as sweeteners to improve the palatability of foods and beverages and for food preservation [20]. Sugar-sweetened beverages (SSBs) include all sodas, fruit drinks, sport drinks, energy drinks, low-calorie drinks and other beverages that contain added caloric sweeteners, such as sweetened tea, rice drinks, bean beverages, sugar cane beverages, and non-alcoholic wines/malt beverages.

SSB has been shown to be a factor in the development of over weight [21, 22], dental caries [23] and obesity [24, 22] in children and adolescents, type 2 diabetes [25], bone fractures, tooth decay [26], pancreatic cancer [27], gastro esophageal reflux [28], cardiovascular diseases [29], Metabolic syndrome [30] and hypocalcaemia [22].

Reports on the level of these substances in energy drinks are scanty. Moreover, new energy drinks are being released into the market daily and the need for continuous monitoring of these substances in energy drinks is a necessity. This study aimed to determine the level of caffeine, aspartame and sugar in energy drinks.

EXPERIMENTAL SECTION

Ten **(**10) brands of energy drinks samples were randomly purchased from the market and the levels of caffeine, aspartame and sugar were determined. All reagents used in this study were of analytical or HPLC grade and all solutions were prepared by using demonized water.

Sample Preparations:

The samples were shaken before opening. 2 ml of the liquid samples were measured into 10 ml volumetric flask. 5 ml of demonized water added and shaken for 5mins using vortex mixer/sonnicator. They were made up to mark with demonized water and filtered with whatman filter paper (2). The filtrate were transferred into the auto sampler vials, cork and load into auto sampler tray and thereafter injected into HPLC column using the auto sampler. The relative peak areas were determined for three replicates of each dilute sample. The concentration of each dilute sample and finally the concentration of caffeine and aspartame in energy drinks samples were calculated from a calibration curve. The HPLC system used in this study was the Hitachi Auto Sampler HPLC L-2200, which consist of a Hitachi pump L-2130, Hitachi UV-VIS detector L-2400, Column Oven L-2300 and a Dell monitor and laser Jet O1006 Printer. Chromatographic analysis was carried out at wavelength of 214 nm, using Waters Spherisorb C18, 5µm ODS2, 4.6 x 250mm column at a flow rate of 1.0 ml/minute. The mobile used was 125 cm^3 methanol 225 cm^3 Acetonitile in 650 cm^3 buffer.

Determination of Sugar [31]

Sucrose standards solutions were prepared by suitable dilution of the stock solution. 20.00 ml of the stock solution was pipetted into a clean 100-ml volumetric flask. Distilled water was added to a point about 1 cm below the calibration mark. A Pasteur pipette was carefully used to add water until the bottom of the meniscus is exactly on the line. The flask was covered with Parafilm and shake well to mix. In a similar fashion, 40:100, 60:100, and 80:100 dilutions were prepared. 2.00 ml of each sucrose standard was pipetted into a large test tube; also 2.00 ml of distilled water was pipetted into a test tube for the blank solution. 2.00 ml of 6 *M* HCl was pipetted into each test tube and place in a boiling water bath for 10 minutes. The test tubes were removed and 8.00 ml of 2.5 *M* NaOH was carefully pipetted into each, then 2.00 ml of 0.050 *M* 3, 5-dinitrosalicylic acid (DNSA) into each. As soon as the DNSA was added, it was shaken to mix the solution thoroughly, and the tubes were placed in a boiling water bath for 5 minutes. Each tube was made to remain in the boiling water for the same duration of time. After removal from the boiling-water bath at the proper time, the test tubes were quickly placed in an ice-water bath for 10 minutes. The blank solution was poured into a clean, dry cuvette and placed in a spectrometer and the absorbance readings were taken. 2.00 ml of each of the energy drinks was pipetted into a clean 100-mL volumetric flask. Distilled water was added to a point about 1 cm below the calibration mark. A Pasteur pipette was carefully used to add water until the bottom of the meniscus is exactly on the line. 2.00-ml aliquots of the diluted energy drink samples were treated in the same manner as the standards. The determination was carried out in triplicate for each dilution. The absorbance for the diluted samples was recorded (in triplicate).

RESULTS AND DISCUSSION

The mean concentrations of caffeine as determined in each of the sampled energy drinks are shown in Table 1. The results obtained showed that caffeine concentrations ranged from 1.11 ppm – 237.95 ppm. These were lower than 170 ppm – 324 ppm for caffeine concentrations in energy drink reported in literature [32], and lower than the ranged of 440 ppm – 473 ppm for caffeine concentration in tea samples reported in literature [32]. The values were also lower than 1.41 mg/serving reported in literature [33, 34]. Sample EV had the least concentration while sample PH had the highest concentration of caffeine. The safety of caffeine intake has been assessed by several national regulatory scientific committees for use at the levels of consumption estimated by their respective populations. According to the Food Standards Agency UK, drinks containing more than 150 mg/L of caffeine must be labeled with the term 'high caffeine content' in the same field of vision as the name of the food. This must be accompanied by an indication of the amount of caffeine per 100 ml in the product. No other labeling is currently required by law and this labeling does not apply to drinks such as tea and coffee [35].

Table 1: Concentration of Sugar, Caffeine and Aspartame

Samples	Sugar (ppm)	Caffeine (ppm)	Aspartame (ppm)
SO	942.90^{g}	67.08^{c}	624.84^{e}
BU	936.73^{f}	30.94^{b}	532.23^{d}
HC	938.27^{f}	200.10^{h}	788.13^{f}
LB	1686.73^{i}	190.22^{g}	876.06^{g}
EV	845.68^{c}	1.11^{a}	956.82^{h}
PH	868.83^{d}	237.95^{i}	876.21^{g}
XL	922.84^{e}	62.84^{c}	332.64^{b}
JW	91.05^{a}	130.58^{f}	423.52^{c}
WD	1098.76^{h}	103.97^{d}	283.84^{a}
KM	660.50^{b}	118.63^{e}	599.93^{e}

a-k: Increasing order of sugar and aspartame. a-j: Increasing order of caffeine.
Values numbers with the same letters in the column, by treatment, are not significantly different while those with different letters are significantly different at p<0.05% from each other.

US FDA has cited 400 mg/day as an amount not generally associated with dangerous, negative effects. It has however not set a level for children. The American Academy of Pediatrics discourages the consumption of caffeine and other stimulants by children and adolescents.

Caffeine concentrations in all the energy drink samples were below the FDA set standards. This implies that a daily consumption of one can of any of the sampled energy drink may not have any adverse effect on the consumer. However, a daily consumption of two or more cans of the energy drink may have adverse effects especially on children and pregnant women.

The concentration of aspartame as determined in each of the sampled energy drink is given in Table 1. The results obtained shows that aspartame concentration ranged from 283.84 ppm – 956.82 ppm. These values were with in the range of 153.69 ppm – 876.42 ppm and 198.22 ppm – 709.36 ppm reported by [36] for soft drinks and artificial flavored drinks respectively but lower compare to 80.29 – 435.05ppm reported for fruit juices and 156.98 ppm – 554.35 ppm reported for powdered drinks. The values were also lower compared to 40.25 ppm – 507.75 ppm reported in literature [37] and 127.2 ppm – 344.5 ppm reported in literature [34]. Sample WD had the least caffeine concentration while sample EV had the highest caffeine concentration.

As shown in Table 1, mean concentrations of caffeine are labeled a-i which shows the order of increasing caffeine concentrations, and represents the ANOVA analysis of the sampled energy drinks. There was no significant difference between concentrations of caffeine in samples SO and XL. The concentration of caffeine in all other samples differs significantly from each other at $p<0.05\%$ confidence limit.

The safety of aspartame has been considered by a range of regulatory organizations, their expert advisory groups and interested scientists. The European Commission’s Scientific Committee on Food (SCF) set the acceptable daily intake (ADI) of aspartame at the same level as those set by Joint FAO/WHO Experts Committee on Food and Additives (JECFA). The US Food and Drug Administration approved aspartame for use in 1984 and have subsequent reaffirmed its safety in 1996. The FDA established a slightly higher ADI for aspartame (50 mg/kg body weight). The acceptable daily intake of aspartame range between 40 - 50 mg/kg/body weight which is equivalent to 2400 – 3000 mg/day for a 60 kg adult. Aspartame concentrations in all the energy drink samples analyzed were below the FDA set standard. This is an indication that they will not have adverse effects on the consumers except multiples of the drinks are consumed.

As shown in Table 1, the mean concentrations of aspartame are labeled a-i which shows the order of increasing concentration of aspartame in the sampled energy drinks and represents the ANOVA analysis of the sampled energy drinks. There was no significant difference in the aspartame concentrations of samples PH and LB, and samples KM and SO. All other sampled energy drinks differ significantly in their aspartame concentration at $p<0.05\%$ confidence limit.

The concentration of sugar as determined in each of the sampled energy drink is given in Table 1. The results obtained shows that the sugar concentration in the sampled energy drinks ranged from 91.05 ppm – 1686.73 ppm. Sample JW had the least concentration while sample LB had the highest concentration. Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when prophylactic measures, e.g. oral hygiene and fluoride prophylaxis, are insufficient. However, available data do not allow the setting of an upper limit (UL) for (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cryogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption, and various other factors [35].

As shown in Table 1, the mean concentrations of sugar are labeled a-k which shows the order of increasing concentration of sugar in the sampled energy drinks and represents the ANOVA analysis of the sampled energy drinks. There was no significant difference in the sugar concentration of samples BU and HC. All other sampled energy drinks differ significantly from each other in their concentration of sugar at $p<0.05\%$ confidence limit.

CONCLUSION

Debate regarding the overall risks and benefits of energy drinks has gained momentum in recent times. Health researchers agree that caffeine consumption can have adverse health consequences, particularly at high doses. The concentration of caffeine and aspartame in the energy drinks were observed to be lower than the acceptable daily intake (ADI) set by the WHO/FDA.

REFERENCES

[1]MF Jacobson. Liquid Candy-How Soft Drinks are harming America's Health, 2nd Edition, Blackwell Scientific Publication Washington DC, **2005**, 464.
[2]A Pennay; ID Lubman; P Miller. *Australian Family Physician*. **2011**, 40: 104-105
[3]TE Graham. Sports Med., **2001**, 31:785–807.
[4]K Safranow; Z Machoy. *Clinical Chem*., **2005**, 51: 1493–8.
[5]GB Kaplan; DJ Greenblatt; BL Ehrenberg; JE Goddard; MM Cotreau; JS Harmatz; RI Shade. Dose-dependent, pharmacokinetics and psychomotor effects of caffeine in humans. **1997**
[6] HJ Smith; PJ Rogers. *Food Qual Pref*. **2002**, 13: 317–26.
[7]M Lorist; MM Tops. *Brain Cogn.*, **2003**, 53: 82–94.
[8]M Doherty; PM Smith. *Int. J Sport Nutr Exerc Metab*., **2004**, 14: 626–46.
[9]RM Cysneiros; D Farkas; JS Harmatz; LL von Moltke; DJ Greenblatt. *Clin Pharmacol Ther*. **2007**, 82: 54–62.
[10]MH Bonnet; TJ Balkin; DF Dinges; T Roehrs; NL Rogers; NJ Wesensten. *Sleep*, **2005**, 28: 1163–87.
[11]A Riesenhuber, M Boehm, M Posch, C Aufricht. *Amino Acids.* **2006**, 31: 81–3.
[12]E Karatzis; TG Papaioannou; K Aznaouridis. *Int J Cardiol*, **2005**, 98: 425-30.
[13]TR Hartley; WR Lovallo; TL Whitsett. *Am J Cardiol*, **2004**, 93: 1022-6.
[14]JM Hodgson; V Burke; IB Puddey. *J Hypertens*. **2005**, 23: 47-54.
[15]P Nawrot; S Jordan; J Eastwood; J Rotstein; A Hugenholtz; M Feeley. *Food Addit Contam*., **2003**, 20(1): 1-30.
[16]RH Haschemeyer; AEV Haschemeyer. Proteins: A guide to study by physical and chemical methods vol I (New York: John Wiley and Sons), **1973**, 1–30
[17]DR Johns. Aspartame and headache. In Dietary Phenylalanine and Brain Function (R. J. Wurtman and E. Ritter-Walker, Eds.). Birkh¨auser, Boston, **1988**, 303–312.
[18]RA Coulombe; RP Sharma. *Toxicol Appl Pharm*., **1986**, 83(1): 79-85.
[19]JW Olney; J Labruyere; T De Gubareff. *Neurobehav. Toxicol*., **1980**, 2: 125–129.
[20]FAO/WHO, Carbohydrates in Human Nutrition. Rome, **1998**, FAO.
[21]CS Berkey; HR Rockett; AE Field; MW Gillman; GA Colditz. *Obesity research*. **2004**, 12(5): 778-88.
[22]LR Vartanian; MB Schwartz; KD Brownell. *Am. J. Public Health*. **2007**, 97: 667 - 675.
[23]AM Rangan; D Randall; DJ Hector; TP Gill; KL Webb. *European Journal of clinical Nutrition*. **2008**, 62(3): 356-64
[24]C Ebbeling; H Feldman; S Osganian. *Pediatrics*, **2006**, 117(3): 673–680.
[25]MB Schulze; JE Manson; DS Ludwig; GA Colditz; MJ Stampfer; WC Willett; FB Hu. (**2004**). *JAMA*, 2004, 292(8): 927 - 934.

[26]JD Shenkin; KE Heller; JJ Warren; TA Marshal. *General dentistry* .**2003**, 51(1): 30-6.
[28]T Jensdorttir; B Arnadottir; I Thordottir; A Bardow; K Gudmundsson; A Theodor; WK Holbrook. *Clinical oral investigation.* **2004**, 8(2): 91-6.
[29]A Astrup; J Dyerberg; M Selleck; S Stender. *Obesity review*. **2008**, 9: 48-52.
[29]ES Schernhammer; FB Hu; E Giovannuci; DS Michaud; GA Colditdz; MJ Stampfer; CS Fuchus. Cancer *epidemiology, Biomarkers and Prevention*, **2005**, 14(7): 2098-105.
[30]R Dhingra; L Sullivan; PF Jacques; TJ Wang; CS Fox; JB Meigs; RB D'Agostino; JM Gaziano; RS Vasan. *Circulation*, **2007**, 116(5): 480 - 488.
[31]GL Miller. *Annal Chem*., **1959**, 31: 427- 431.
[32]MA Mei; E Mawahib; IT Mohammed; AZ Badawi; AE Abdalla. *Pakistan Journal of Nutrition*. **2012**, 11(4): 336-342.
[33]RM Rachel; AG Bruce; JC Edward. *Journal of analytical toxicology*. **2006**, Vol. 30
[34]AH Alghamdi; AF Alghamdi; AA Alwarthan. *J. King Saud Univ*., **2005**, 18(2): 99-109.
[35]EFSA *Journal.* **2010**, 8(3):1462.
[36]M Serdar; Z Knežević. *Arh Hig Rada Toksikol*; **2011**, 62: 169-173.
[37]R Mackenzie; S Erik. *Concordia College Journal of Analytical Chemistry* I, **2011**, 73-77