Screening for the Presence of Some Heavy Metals, Total Soluble Solids and Caffeine Contents in Some Energy Drinks Sold in Jordanian Market

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Energy drinks (EDs) are beverages that are devoid of alcohol containing high amounts of caffeine and sugar. Energy drinks are available in the Jordanian market and consumed by Jordanian youth. There is no Jordanian standard for EDs. Establishing a standardization for a certain contaminant requires assessing the heavy metal content in the EDs available in the market. This research aimed to screen the 10 most consumed energy drinks sold in the Jordan market for total soluble solids, caffeine contents, and the presence of some heavy metals. This study, thus, will serve as an initial step for setting a standard for heavy metals in EDs in Jordan. The analyzed EDs didn't contain detectable amounts of Pb. On the other hand, the analyzed samples contained 0.1 to 2.8, 1.8 to 6.5, 0.01, and 0.2 to 3.5 mg kg-1 Cu, Ni, Cd, and Fe respectively. The total soluble solids content of the studied samples ranged between 4.8 to 15.4 g/100 g. On the other hand, the caffeine content of the analyzed samples ranged between 24.7 to 32 mg/100 mL. It is recommended to establish a Jordanian standards specification for energy drinks including heavy metal, total dissolved solids, and caffeine.

Keywords: energy drinks, heavy metals, caffeine, total dissolved solids

Introduction

Energy drinks (EDs) are beverages that are devoid of alcohol and contain high amounts of caffeine, sugar [1], taurine, some vitamins such as B vitamins, and herbal extracts [2]. As their name indicates, EDs are claimed to boost energy levels and physical activity endurance [3]. Energy drinks are available in the Jordanian market and consumed by Jordanian youth [4].

Caffeine is a naturally occurring alkaloid occurring in plants, fruits, and seeds such as tea leaves, cocoa seeds, some berries, and kola nuts. In small amounts, it stimulates nervous system. In moderate amounts, it can enhance exercise performance in adults. On the other hand, in excessive doses, caffeine can cause gastrointestinal irritation, sleep restlessness, agitation, anxiety, as well as arrhythmia. The British Soft Drinks Association pertains the concern to consuming beverages containing more than 150 mg of caffeine per liter with a recommendation of moderate consumption of caffeine-containing beverages for children and adolescents [1].

Heavy metals (HMs) are metals of high specific weight [5] and density [6]. Examples of heavy metals are Pb, Cu, Zn, Fe, Cd, As, Ni, and Sn. HMs occur in the earth's crust and are not degraded easily from the environment [6, 7]. HMs are transferred to the environment specifically food *via* atmosphere, soil, water, plant food, workplace, and industry [5]. Some HMs have functions within the human body such as Fe, Cu, and Ni if present in minor amounts. Other HMs don't have any function in the human body. Rather, they are toxic and can't be recycled. For instance, Pb affects infants and children's nervous system. Arsenic is carcinogenic to the liver and the kidney [5]. The toxicity of heavy metals is well understood. However, the toxicity is highly affected by the accumulative concentrations [7]. Heavy metals find their way into energy drinks from the metal cans, water and/or raw materials, and manufacturing lines.

Despite the reported benefits for athletes when consumed in moderate amounts, the current evidence shows that excessive consumption of EDs may lead to hyperactivity, hyperthermia, cardiac palpitation, dizziness, disordered sleep, headache, and abdominal pain [1]. In addition, some energy drinks were found to be either fortified or contaminated with metals [2]. Because of this, it is recommended that governments establish standard specifications for EDs. In addition, governments should regulate marketing as well as consuming EDs.

Jordan Institution for Standardization and Meteorology (JISM) is the responsible party for setting standardized specifications for food and nonfood stuff. On the other hand, Jordan Food and Drug Administration (JFDA) is responsible for controlling and inspecting the application of standard specifications. Jordan doesn't have a standard specification of energy drinks. Energy drinks are referred to the Codex Alimentarius or European commission standard specifications. According to Codex [8], the permitted ranges or the maximum residues limits (MRLs) of Cadmium and Lead in food are 0.05-2 and 0.01-3 ppm, respectively, where the maximum amount of caffeine in EDs is 320 ppm.

The prevalence of using energy drinks in Jordan necessitates establishing a Jordanian standard specification [4]. Establishing a food standardization for a certain contaminant requires assessing the risk for exposure. As well, identification of local situation serves as an integral part for identifying the human exposure assessment to the risk of heavy metals and reviewing neighbor countries standard specifications as well as international specifications [9]. To the best of the authors' knowledge, no studies in Jordan and the area assessed the heavy metal status of EDs sold in the market. Therefore, the aim of this research was to screen 10 samples of energy drinks sold in Jordanian market for total soluble solids, caffeine, as well as the presence of heavy metals: Pb, Cu, Cd, Fe and Ni identifying the current local situation. This study, thus, will serve as an initial step for setting a standard for heavy metals in EDs in Jordan.

Materials and Methods

The investigated samples. Ten samples of energy drinks were purchased from the local market representing the most 10 common brand names [4]. All samples were analyzed in triplicates with an accuracy value not less than 97%.

Chemical analysis. Heavy metal contents (HMCs). Twenty-five milliliters of each sample were prepared by digesting with 25.0 mL concentrated nitric acid (HNO_3) (Fluka, Germany). The acid-sample mixtures were evaporated under heat until brown fumes were converted into white. Thereafter, 50.0 mL of distilled water were added and the resultant solution mixture was concentrated under heat into 25.0 mL volume. Then, the final mixture was completed to a volume of 50.0 mL, filtered by vacuum, and the absorbance of each sample was measured by RayLEiGH (China) atomic absorption spectrophotometer. Standard curves were prepared to have correlation coefficient R^2 value of 0.99-1.00. The standard curves for each mineral (Pb, Cd, Fe, Cu, and Ni) were used to extrapolate the sample concentration [10, 11].

Caffeine content (CC). Caffeine content of samples was measured using high performance liquid chromatography (HPLC) with (AZURA)-UV detector where the column was used (Fortis C18, 100 Å, 5µm, 4.6 x 250 mm). The mobile phase consisted of water methanol mixture (60:40). The chromatographs were monitored at 273 nm and run time was set to be 10 min. [12]. Caffeine standard curves were prepared by dissolving 10 mg of caffeine in 100 mL mobile phase and dilution series (of 2, 5, 10, 25, 50, and 100 mg kg^{-1} concentrations). Standards and samples were sonicated (Branson / ultrasonic, Japan) for 10-15 minutes, filtered by 0.45 µm filter membrane, allowed in clean dry place to get rid of the most amount of gases, diluted with the mobile phase, and sonicated before injection.

Total Soluble Solids Content (TSSC). The analysis of TSSC readings were performed by Brix refractometry (Abbé refractometer, Germany). As the refractive index of a sugar-containing solution is also temperature-dependent, the refractometer was typically calibrated at 20 °C. The equipment was calibrated with deionized water (refraction index = 1.3330 and 0° Brix at 20 °C) and the readings of the samples were performed [13].

Sugar content. Sugar content of the collected samples was recorded as reported on the label. Data were expressed as average $\pm$ standard deviation.

Results and Discussion

Jordan has no standard for the presence of heavy metals in energy drinks. EDs sold in Jordan are, thus, referred to the Codex Alimentarius or European commission standard specifications. Other standards for EDs include TSS, sugar, and heavy metal contents [8, 14].

The Codex Alimentarius allows the presence of 150 mg/kg Sn in canned beverages [14]. On the other hand, the Gulf standard specification allows the presence of Pb, and Cu in concentrations of 0.2, and 2 mg kg^{-1} respectively in Eds [14]. Moreover, the World Health Organization (WHO) allows a maximum content of 0.05 mg kg^{-1} Pb, 2.00 mg kg^{-1} Cu in Eds [15]. Furthermore, Tanzanian standards allow maximum of 0.1 and 3 mg kg^{-1} of Pb and Cu respectively [16].

Table 1 shows the metal content of the analyzed energy drinks. The analyzed EDs didn't contain detectable amounts of Pb (knowing that the detection limit of the analysis instrument is 0.5 mg kg^{-1}). On the other hand, the analyzed samples contained 0.1 ± 0.05 to 2.8 ± 0.05, 1.8 ± 0.05 to 6.5 ± 0.05, 0.01 ± 0.05, and 0.2 ± 0.05 to 3.5 ± 0.05 mg kg^{-1} Cu, Ni, Cd, and Fe respectively. Nonetheless, some samples contained undetectable amounts of Cd and Fe (knowing that the detection limit is 0.5 mg kg^{-1}).

With regards to Pb, results of samples are consistent with the results found in EDs sold in Nigeria and Iraq [3, 16, 17]. Within the same context, the analyzed samples obey the standards of gulf and European commission as well as the maximum tolerable standards of WHO. The heavy metal Pb is found in some food and nonfood sources, replaces and competes utilizable minerals within the human body such as Fe, Zn, and Ca for their functionality.

Table 1. Pb, Cu, Cd, Fe and Ni concentration (ppm) in the studied samples of EDs[1].

Energy drink	Country of origin	Heavy metal concentration (ppm)				
		Pb	Cu	Ni	Cd	Fe
1	Jordan	ND[2]	0.2 ± 0.05	3.5 ± 0.05	ND[2]	ND[2]
2	Poland	ND[2]	0.3 ± 0.05	1.8 ± 0.05	ND[2]	0.6 ± 0.05
3	Austria	ND[2]	0.2 ± 0.05	6.5 ± 0.05	ND[2]	0.2 ± 0.05
4	Jordan	ND[2]	0.6 ± 0.05	2.0 ± 0.05	0.1 ± 0.05	2.8 ± 0.05
5	Saudi Arabia	ND[2]	0.7 ± 0.05	6.5 ± 0.05	0.1 ± 0.05	3.5 ± 0.05
6	Poland	ND[2]	2.8 ± 0.05	4.3 ± 0.05	0.1 ± 0.05	3.1 ± 0.05
7	Austria	ND[2]	0.3 ± 0.05	3.9 ± 0.05	ND[2]	0.2 ± 0.05
8	USA	ND[2]	0.2 ± 0.05	4.4 ± 0.05	ND[2]	1.9 ± 0.05
9	Jamaica	ND[2]	0.1 ± 0.05	3.0± 0.05	ND[2]	1.0 ± 0.05
10	Saudi Arabia	ND[2]	0.6 ± 0.05	2.7 ± 0.05	0.1 ± 0.05	4.1 ± 0.05

[1] value are average of triplicates ± standard deviation.

[2] not detected (<0.5 ppm).

It can't be destructed within the human body and replaces and competes utilizable minerals within the human body such as Fe, Zn, and Ca for their functionality [18].

Cu, on the other hand, is required for the human body in tiny amounts to help in activating certain enzymes. The toxicity of Cu causes liver damage [18]. Cu concentration of ED samples analyzed in this research falls within the concentration ranges reported in another research [7]. and much higher than those found by Gimba et al. [18]. On the other hand, Szymczycha-Madeja didn't detect Cu in energy drinks sold in Poland [19]. Nine of our samples obey the standard specifications [13]. A sample (number 6) which origin is Poland contained 2.8 ± 0.05 mg kg^{-1} Cu obeying the necessitating establishing a Jordanian standard for EDs.

Similar to Cu, Ni serves as a cofactor in many enzymes within the human body [18]. Ni finds its way to food *via* contamination of the water used, pipes, fittings, and stainless-steel utensils [20]. Ni toxicity is associated with ailments of thyroid, renal, and bone tissues [6]. Furthermore, metallic Ni is reported by the WHO as being potentially carcinogenic above 70 ppb in drinking water [21]. The studies analyzing Ni content in EDs are limited. For instance, Kilic found 35.98–303.97 ppb in Eds [21]. In the current study, Ni content of the analyzed samples are much higher than those found by Kilic [21] and disobey the European Commission (EC) standards (>0.003 mg kg^{-1}); and additional necessity for establishing a standard specification for EDs in terms of HMs [22].

Within the same context, Cd was reported by EC and WHO to be probably carcinogenic to humans and allowed to be not more than 3 ppb in drinking water. Concomitant with this, the European Union (EU) emphasized the reduction of Cd exposure to humans as minimum as possible due to its adverse effects [22]. On the other hand, it was reported that Cd has low potential for carcinogenicity [23]. Our samples contained 0.01±0.0.5; much higher than the standards of WHO, EC, as well as Bangladesh standards and most of those found by Ahmed FT [23].

The WHO [24], US Environmental Protection Agency (USEPA), and Bangladesh standards [23] for soft drinks allow the presence of 0.3, 1, and 0.3 mg kg^{-1} Fe respectively. Within the same line, the EC allow the presence of 200 Pb and Fe in energy drinks. Seven out of ten of our samples contained Fe higher than the above-mentioned standards for Fe. The samples analyzed in the current research contained 0.2 ± 0.05 to 3.5 ± 0.05 mg kg^{-1} Fe; much higher than those found in other reports [19, 20]. On the other hand, our values are consistent with those found by Attaha and Gimba [17, 18].

Table 2 shows the TSS in the investigated samples of EDs. The TSS of the studied samples ranged between 4.8 ± 0.05 to 15.4 ± 0.05 g/100 g. Samples analyzed in this investigation contain much higher values than the WHO as well as Bangladesh standards for TSS in EDs (500 and 1000 mg kg^{-1} respectively). Within the same line, only one of our samples (sample number 1) obey the Tanzanian standards [16] (2-4.3 mg kg^{-1} (. Compared to previous reports, our samples contain values that are lower than those published by Gimba [18]. TSS represent sugars, caffeine, amino acids, vitamins, flavorings, non-carbohydrate, and other additive contents of drinks [1].

Concomitant with this, Table 3 shows the reported sugar content of the collected samples (as reported on the cans). As reported, the collected ED samples claimed to contain 4.2-14.5 g sugar/100 g; a value which corresponds to ~1 teaspoon to ~1 table spoon of sugar per 100 g. Increased sugar intake is known as risk factor for obesity especially abdominal obesity [1]. Thus, governmental and non-governmental organizations are called to control the use of energy drinks specially in children and non-athletes.

Table 2. TSS of the investigated samples of EDs[1].

Energy drink	Total soluble solids (g/100 ml)
1	4.8 ± 0.05
2	14.2 ± 0.05
3	11.7 ± 0.05
4	14.6 ± 0.05
5	12.7 ± 0.05
6	11.1 ± 0.05
7	12.4 ± 0.05
8	15.4 ± 0.05
9	12.9 ± 0.05
10	14.1 ± 0.05

[1] values are average of triplicates ± standard deviation.

Table 3. Reported sugar content of the investigated samples of EDs[1].

Energy drink	Total sugar content (g/100 ml)
1	4.2
2	11
3	10.5
4	13.6
5	11
6	11
7	11.7
8	14.5
9	10.8
10	10.5

[1] as reported on the can label.

Table 4 shows the reported and analyzed caffeine content (mg/100 ml) of the collected samples. The values found in this investigation ranged between 24.7 and 32 mg caffeine/100g. Some of our values are lower while others fall within the range found by Mohammed [25] i,e 31.1-130 mg/100 ml caffeine in 6 different energy drinks in Poland. What is noteworthy is that the found amount of caffeine in the current investigation is little bit lower than the reported value on the nutrition facts panel. In addition, the manufacturers reported caffeine content of EDs; an obligation of the food law [26].

Caffeine values found in this investigation whether reported or analyzed fall within those recommended by the Gulf [14] as well as Tanzanian standards [16] which allow the presence of 32 and 20-32 mg caffeine /100 ml ED respectively.

Literature suggests that moderate intake of caffeine is about 300 mg/day. Higher intake (~400 mg) is associated with adverse health effects [26]. Hospitalizations from energy drinks are related to caffeine content with symptoms of gastrointestinal upset, restlessness/agitation, tachycardia/palpitations. The government should, thus, restrict the consumption of caffeine [1]in common with many other countries, has seen a rapid rise in the per capita consumption of energy drinks in the last few years. Energy drinks (soft drinks containing more than 150 mg of caffeine per litre and before that, should obligate certain amount of caffeine in EDs and other beverages.

Table 4. Reported[1] and analyzed[2] caffeine content (mg/100 mL) of the investigated samples.

Energy drink	Caffeine content (mg/100 ml)	
	Reported	Analyzed
1	31	26.5±0.5
2	32	25.2±0.5
3	30	27.6±0.5
4	36.3	24.7±0.5
5	30	25.0±0.5
6	32	24.3±0.5
7	32	26.3±0.5
8	32	24.0±0.5
9	29	29.0±0.5
10	32	32.0±0.5

[1] as reported on the can label.
[2] values are average of triplicates ± standard deviation.

Conclusions

This is the first study of its kind analyzing HMs, caffeine, as well as TSS of the 10 most common energy drinks brands sold in Jordan market. Values were compared against WHO, EU, and standards of other countries available in the literature. The found values were comparable for certain analytes. On the other hand, they are incomparable for others. Establishing a standard specification is a daunting process encompassing reviewing the functions and requirements and upper tolerable limits of the metals, current status of the food item, risk assessment and risk exposure of the contaminant under interest, and standard specifications of other countries and international organizations. The current report, thus, will serve as the basis for initiating the establishing of standard specification for EDs in Jordan.

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Conflict of Interest

The authors declare no conflict of interest.

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