Quantitative Analysis of Caffeine and Phosphoric Acid in Non-Alcoholic Beverages Marketed in Kosovo

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Abstract

Non-alcoholic beverages often contain various constituents and additives, influencing both their nutritional profiles and functional attributes. Given the ubiquity of these beverages and their potential health implications, rigorous quality checks are indispensable to ascertain compliance with health and safety standards. This research aimed to quantify caffeine and phosphoric acid levels in a diverse array of soft drinks from Kosovo's market. To achieve this, 41 distinct non-alcoholic beverage samples from the local market were scrutinized. Caffeine concentrations were determined via UV-Vis spectrophotometry, while potentiometric titration was employed to assess phosphoric acid levels. As expected, energy drinks in Kosovo contained markedly higher caffeine concentrations compared to carbonated soft drinks and ice-tea variants. On the other hand, based on the results, analysed energy drinks showed somewhat greater caffeine contents than those reported in packaging of the beverage samples. Our study showed that a significant portion of the samples (21.95% for caffeine and 9.76% for phosphoric acid) did not conform to the standards set by EU 1169/2011 regulation or the EU 1333/2008 regulation. These findings underscore the urgency for relevant food safety authorities to implement rigorous oversight and enact appropriate protective measures. It is also imperative for the Kosovo National Food Safety Authority to craft specific regulations stipulating permissible additive concentrations, especially for caffeine and phosphoric acid, in non-alcoholic beverages.

Keywords: Additives; Carbonated soft drinks; Energy drinks; Caffeine; Phosphoric acid

1 Introduction

The term 'soft drink' refers to beverages that contain flavourings and/or fruit juices, as well as other technological constituents designed to improve the product overall stability while keeping intact its sensory attributes during a reasonable shelf-life period. Although there are many definitions available on the meaning of soft drinks, it is generally agreed that the term soft drinks include non-alcoholic, water-based beverages, usually with a balancing acidity that are sweetened, flavoured and coloured by the use of natural or artificial materials. Regarding their categorisation, there are two main categories of soft drinks: ready to drink (RTD) and dilutable or concentrated products. The latter are usually 5-10 times more concentrated than RTD. RTD are further subcategorised into carbonated and noncarbonated soft drinks (Steen & Ashurst,

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2006). Shachman (2005) categorises soft drinks into: carbonates, still drinks, bottled water, dilutables and fruit juices/nectars. An important group of soft drinks are still or uncarbonated drinks and functional beverages such as sport and energy drinks. The Committee on Nutrition and the Council on Sports Medicine and Fitness in 2011 described sports drinks as those beverages designed to be consumed before or during exercise to prevent dehydration, provide electrolytes (such as sodium, potassium and calcium) and supply carbohydrates, and sometimes, vitamins or other nutrients, and they typically do not contain caffeine (Aron, 2019). Meanwhile, energy drinks are beverages that contain caffeine in addition to other ostensibly energy-boosting ingredients such as taurine, herbal extracts and B vitamins (Heckman et al., 2010).

Water is the primary component of soft drinks, accounting for approximately 87 to 92 % of the beverage volume. Water used in beverage formulations must meet the physicochemical and microbiological requirements of drinking water and be free of all contaminants, microorganisms and other unpleasant characteristics such as odours and turbidity. For this purpose, important attention in soft drinks production is given to water treatment (Shachman, 2005). Besides the water other ingredients involved in formulation of soft drinks are fruit materials; carbohydrate sweeteners; bulk or intensive sweeteners; flavouring, colouring, preservative, antioxidant and acidulant agents; and nutraceutical and functional nutrients such as vitamins, minerals, antioxidants and caffeine (Steen & Ashurst, 2006).

According to Global Industry Analysts, Inc., soft drinks share in the global market was estimated at US$ 994.7 Billion in 2020, with a projection of reaching US$ 1.3 Trillion by 2026, with a growth of 4.9% over the analysis period from 2020-2026. Soft and energy drink consumption has been increasing substantially over the last several decades, and the ingredients of these beverages are amongst the most important studies of this century because they are used all over the world (Aron, 2019). Although they offer significant energy and a pleasure of taste, several studies have shown that the consumption of soft drinks can contribute to the overall harmful effects on oral health in children and adolescents, obesity, increased risk of type 2 diabetes, dental caries and erosion (BaniHani & Tahmassebi, 2019). Vartanian et al. (2007), authors of a meta review of 88 research articles on the effects of soft drink consumption on nutrition and health, found association of soft drink consumption with increased body weight and a higher risk of development of diabetes by soft drinks' consumers.

Caffeine is an important stimulant of the central nervous system (CNS) that belongs to the methylxanthine class (Fiani et al., 2021). Caffeine is widely used and can be found in over 60 different plants, including coffee beans, tea leaves, cola nuts and cocoa beans (Pray et al., 2014). Caffeine is easily absorbed and distributed evenly in all body fluids and reaches a maximum plasma level between 30 and 75 min (Nehlig, 2018; Preedy, 2012). Half the caffeine dose is excreted from the body of healthy people in approximately 4-5 hours but increases after higher intake levels or with impaired liver function (Mandel, 2002). Caffeine is widely consumed worldwide, particularly in coffee, tea, soft drinks and energy drinks. While moderate caffeine consumption is generally considered safe, high intake through caffeine-rich drinks has been associated with various health risks such as cardiovascular health (Mesas et al., 2011), anxiety and sleep disorders (Clark & Landolt, 2017), gastrointestinal issues (Boekema et al., 1999) and reproductive health (Ricci et al., 2017).

Individuals who have heart problems, high blood pressure or sleep problems or who are taking medication should be careful to limit the amount of caffeine they drink (Pray et al., 2014).

Phosphoric acid is an industrial acid derived from sedimentary and igneous phosphate rock (Schorr & Valdez, 2016). Pure phosphoric acid is a colourless crystalline solid (m.p. 42.35^{o}C) that is normally used in solution as a strong syrupy liquid that is miscible with water in all proportions. It is commercially available in solution concentrations of 75, 80 and 90% (Steen & Ashurst, 2006). This acid and its derived products constitute a very big market covering critical sectors of the global economy, e.g., fertilizers, metallurgy, food and beverage, medicine and chemicals (Schorr & Valdez, 2016). Acidulants such as phosphoric acid reduce the soft drinks' pH and thereby assist in beverage preservation for

long-term storage (Dionex, 2004). It is a relatively strong, dissociated acid, enabling it to easily acidify colas to the low desired pH (2.5) needed to establish proper carbonation, although its antimicrobial efficacy is far inferior to most organic acids, principally due to its dissociated state, which precludes ease of transport across the bacterial membrane (Pray et al., 2014).
The most frequent acidulants used in the beverage industry are phosphoric acid and citric acid. Phosphoric acid, which is typically added in colas, has a greater ability to lower pH than citric acid commonly used in fruit-based drinks (Dionex, 2004). Besides acidulant, food grade phosphoric acid has an important role in the flavour of different beverages. In this context, approximately 0.05 % phosphoric acid content provides a distinct taste of cola drink. Phosphoric acid is also a cheaper additive than food grade organic acids commonly used in the beverage industry (PotashCorp, 2012). Phosphoric acid, a common food additive found in many processed foods and beverages, particularly soft drinks, has been the subject of various health-related studies. The primary concerns relate to its potential impact on bone health and kidney function (Chang et al., 2014; Dhingra et al., 2007), cardiovascular disease (National Institutes of Health, 2023), and overall nutrient balance (Calvo & Uribarri, 2013). While phosphoric acid is widely used as a food additive and is generally considered safe in moderate amounts, excessive consumption, particularly through soft drinks and processed foods, may pose health risks. Further research is needed to fully understand the extent of these risks and the mechanisms involved.
Kosovo's market is characterized by a great variety of drinks, with carbonated soft drinks, energy drinks and ice-tea drinks being the most popular ones among children and young adults. Additives including psychotropic substances, acidulants and dyes are declared on the packaging for the majority of drinks sold in Kosovo. However, apart from some energy drinks, manufacturers typically do not disclose the quantities of additives present in their products. Up to date there are no studies which investigate the presence of additives in drinks available in Kosovo's market. The main goal of the current study was to determine the concentrations of a psychotropic substance such as caffeine and of an acidulant such as phosphoric acid in different samples of commercially available types of ice-teas, carbonated soft drinks and energy drinks in the supermarkets in Kosovo. The importance of this study lies in its focus on public health and safety, particularly in the context of Kosovo's market. Given the rising consumption of these beverages and the lack of prior studies in this area, the research provides valuable insights into the presence and levels of these additives. This information is crucial for consumers, health professionals and regulatory bodies to understand potential health risks and ensure that products comply with established safety standards.

2 Materials and methods

2.1 Materials and chemicals

In this research work forty-one samples were used which were divided in three different categories: eleven carbonated soft drinks, eighteen energy drinks and twelve ice-tea drinks. All samples were purchased from local grocery stores in Kosovo and were brought for analysis to the laboratories of the Department of Food Technology with Biotechnology, University of Prishtina. Prior to analysis, carbonated soft drinks were pre-treated with heat for 20 min to remove the carbon (IV) oxide. Caffeine was determined using UV/Vis spectrophotometry while phosphoric acid was determined using the potentiometric acid-base titration method. The analytical grade chemicals used in this study were caffeine standard ($C_8H_{10}N_4O_2$) and sodium hydroxide (NaOH) obtained from Sigma-Aldrich (Munich, Germany).

2.2 Preparation of caffeine standard solution

The caffeine standard solution was prepared by weighing 0.0264 g of pure caffeine and dissolving it in a 200 ml flask with distilled water to obtain the caffeine solution at a concentration of 132 mg/L.

Table 1: Determination of caffeine in drink sample 'X' by standard addition method

No. of volumetric flask	mL of drink sample 'X'	mL of caffeine standard solution	Absorbance
1	1	0	0.107
2	1	0.5	0.544
3	1	1	0.981

2.3 Caffeine determination

The method used for determination of caffeine in our research study was the standard addition method (Phan et al., 2012; Zellmer, 1998). According to several studies, the standard addition method presents a suitable method for determining caffeine concentrations in drinks because distilled water is used as the solvent. For each sample preparation, three 100 mL volumetric flasks were used by adding 1 mL of drink sample. Then to each volumetric flask was added 0, 0.5 and 1 mL of standard caffeine solution and distilled water to reach the volume of 100 ml as described in Table 1.

The quantification of caffeine in beverages in our study was performed using UV-Vis spectrophotometry. Absorbances of the three prepared samples in volumetric flasks were measured at a wavelength of 275 nm against a blank containing distilled water by an UV-Vis spectrophotometer (U2900 Hitachi Ltd, Tokyo, Japan) (Table 1). The three recorded absorbances of each sample were plotted against the added volume (mL) of caffeine standard solution into the sample solution and a linear curve was obtained (Figure 1). The correlation factor between the two variables was considered acceptable when <0.9. Caffeine was assessed from the linear equation of the curve for each individual sample and was expressed in mg caffeine per L of drink sample. Measurements were performed in triplicate.

2.4 Phosphoric acid determination

The method used for the determination of phosphoric acid content in drink samples was the potentiometric acid-base titration (Utange et al., 2015). Briefly, 40 mL of decarbonated drink sample was combined with 60 mL of distilled water using an erlenmeyer for sample preparation. The potentiometric titration was conducted by adding 0.1 M NaOH solution while monitoring the changes of the pH of the mixture with a pH meter (Testo 206, Testo SE & Co., Titisee-Neustadt, Germany). Hydrogen ions are formed due to the first dissociation of phosphoric acid during titration with sodium hydroxide, as shown in the equation (1) below:

$$H_3PO_4(aq) + OH^-(aq) \rightarrow H_2PO_4^-(aq) + H_2O(l) \quad (1)$$

A graph expressing the pH dependence on the volume of NaOH consumed is constructed to determine the first equivalent point, which represents the increased volume of mL NaOH that gives the greatest increase in pH of the sample. The volume of NaOH added at the first equivalent point is used to calculate the phosphoric acid concentration in the sample.

2.5 Statistical determination

The statistical data analysis was performed using IBM SPSS 27 software (Version 27.0, Armonk, NY, 2020). Normality of the data residuals was tested by the Kolmogorov-Smirnov test (P>0.05) and homogeneity of variances was checked by the Levene's test (P>0.05). One-way ANOVA was used for data analysis for caffeine content and phosphoric acid. The results obtained from ANOVA were significant, so Tukey's post hoc tests were used to separate significant groups.

3 Results and Discussion

The main objective of this scientific study was to examine the caffeine and phosphoric acid concentrations in soft drinks, energy drinks and ice-tea

Table 2: Caffeine and phosphoric acid contents of carbonated soft drinks, energy drinks and ice-tea drinks

Sample type	Sample name	Caffeine content (mg/L)	Max. allowed according to EU Regulation 1169/2011	Phosphoric acid content (mg/L)	Max. allowed according to EU Regulation 1333/2008
Carbonated soft drinks	S 1	102.975	↘	300	↘
	S 2	128.146	↘	620	↘
	S 3	122.426	↘	888	↗
	S 4	101.831	↘	340	↘
	S 5	117.849	↘	280	↘
	S 6	131.579	↘	200	↘
	S 7	137.3	↘	500	↘
	S 8	136.156	↘	344.8	↘
	S 9	97.254	↘	400	↘
	S 10	144.165	↘	588	↘
	S 11	125.56	↘	360	↘
Energy drinks	E 1	136.1556	↘	879.5	↗
	E 2	151.13	↗	400	↘
	E 3	180.778	↗	819.5	↗
	E 4	189.931	↗	348.57	↘
	E 5	143.021	↘	500	↘
	E 6	139.588	↘	500	↘
	E 7	132.723	↘	440	↘
	E 8	154.462	↗	400	↘
	E 9	148.74	↘	810	↗
	E 10	169.34	↗	500	↘
	E 11	171.62	↗	500	↘
	E 12	137.3	↘	700	↘
	E 13	155.61	↗	660	↘
	E 14	140.73	↘	580	↘
	E 15	146.45	↘	550	↘
	E 16	157.89	↗	510	↘
	E 17	162.47	↗	660	↘
	E 18	135.01	↘	690	↘
Ice-tea drinks	T 1	34.324	↘	120	↘
	T 2	85.812	↘	200	↘
	T 3	98.398	↘	280	↘
	T 4	113.27	↘	210	↘
	T 5	90.389	↘	240	↘
	T 6	117.848	↘	180	↘
	T 7	131.578	↘	347	↘
	T 8	114.416	↘	290	↘
	T 9	112.128	↘	450	↘
	T 10	120.137	↘	310	↘
	T 11	65.217	↘	313	↘
	T 12	56.064	↘	331	↘

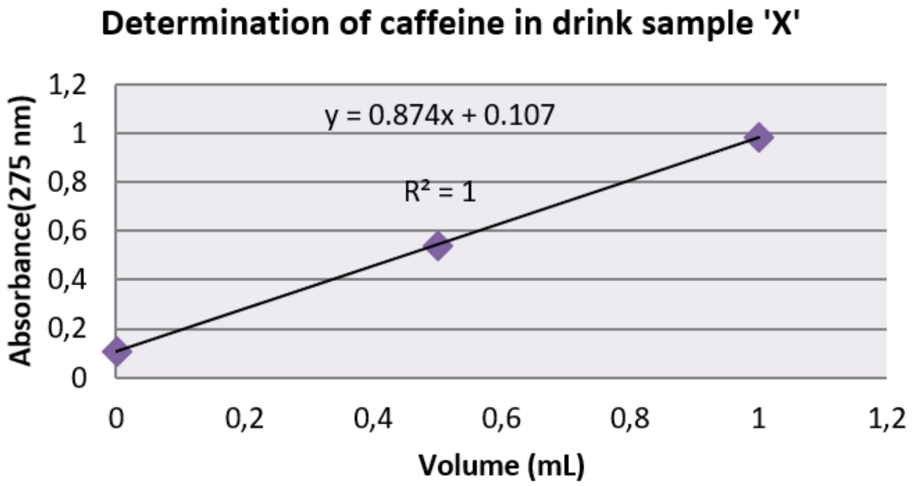

Figure 1: Determination of caffeine in drink sample 'X')

Table 3: Comparison of caffeine and phosphoric acid contents in mg/L between carbonated soft drinks, energy drinks and ice-tea drinks.

Sample type	Caffeine content (mg/L)	Phosphoric acid content (mg/L)
Carbonated soft drinks		
Mean	122.29^b	438.25^b
Range	97.25-144.17	200-888
Energy drinks		
Mean	152.94^c	580.42^b
Range	132.72-189.93	348.57- 879.5
Ice-tea drinks		
Mean	94.97^a	272.58^a
Range	34.32-131.58	120-450

Means with different superscript letters (a-c) in the same column are significantly different ($P \leq 0.05$)

Table 4: Comparison of caffeine and phosphoric acid contents in mg/L between carbonated soft drinks, energy drinks and ice-tea drinks.

Sample type	**Caffeine content mg/L** (Xhaferaj et al., 2019)	**Caffeine content mg/L** (Khalid et al., 2016)	**Caffeine content mg/L** (Vuletić et al., 2021)	**Caffeine content mg/L** (Amos-Tautua et al., 2014)
Soft drinks	49.55 58.04 48.21 56.70 79.46 24.55 26.25 56.25 60.71 39.29	37.62 12.34 10.69 19.11 42.17	102.252 95.12 86.486 136.036 49.7 48.198	44.22 45.83 43.71 44.31
Energy drinks	62.95 75.89 75.89 62.05 152.68	59.99 101.70 46.18 32.05	350.751 317.342 394.67 252.778 173.574	50.42 52.65 47.56 58.31

drinks available in the Kosovo market and see if they met the European regulation requirements for those substances (European Parliament & Council of the European Union, 2008, 2011). The results obtained for caffeine and phosphoric acid contents in carbonated soft drinks, energy drinks and ice-tea drinks are presented in Table 2.

Caffeine contents (mg/L) in carbonated soft drinks and ice-tea drinks were below the maximum limits (150 mg/L) according to the EU Regulation 1169/2011 requirements (European Parliament & Council of the European Union, 2011). Out of eleven carbonated soft drinks, the highest caffeine content was found in brand S10 (144.165 mg/L), while the lowest content was found in brand S9 (97.254 mg/L). The average caffeine content in carbonated soft drinks was 122.29 mg/L.

Out of eighteen analysed energy drinks, nine (or 50%) of them had higher caffeine content than the maximum amount allowed by the EU Regulation 1169/2011. The highest content in energy drinks was encountered in brand E4 which is 189.931 mg/L, while the lowest amount was encountered in brand E7 which is 132.723 mg/L. Meanwhile, out of the 12 ice-tea drinks analysed, the highest caffeine content was observed in brand T7 (131.578 mg/L), while the lowest caffeine content was observed in brand T1 (34.324 mg/L).

The present study shows that from a total of 41 analysed drinks' samples, 37 samples meet the criteria set for the maximum allowed amount of phosphoric acid (700 mg/L) in these products (European Parliament & Council of the European Union, 2008). Meanwhile a total of 4 samples, one carbonated soft drink (S3) and three energy drinks (E1, E3 and E9), exceed the maximum for phosphoric acid content.

A comparison of caffeine and phosphoric acid contents in mg/L between carbonated soft drinks, energy drinks and ice-tea drinks are shown in Table 3. It has been observed that caffeine concentrations in energy drinks were significantly higher ($P < 0.05$) than caffeine concentrations in carbonated beverages and ice-tea drinks. Rehman and Ashraf (2017) also reported lower caffeine contents in carbonated soft drinks compared to energy drinks marketed in Pakistan. The average caffeine content in energy drinks was 152.941 mg/L which is higher than the maximum limit (150 mg/L) according to European Parliament and Council of the European Union (2011). Jagim et al. (2022) also reported a high prevalence of caffeine in energy drinks marketed in United States. Meanwhile, Rehman and Ashraf (2017) reported that levels of caffeine concentrations in beverages marketed in Pakistan were between 38.1 to 222.3 mg/L.

Caffeine concentrations in analysed energy drinks were slightly higher than those declared on packaging of the beverage samples. In contrast, Athanasiadis et al. (2023) reported that caffeine contents were similar to the values shown on labels for beverages marketed in Greece. However, no comparison could be made between the declared and analysed caffeine contents in ice teas and carbonated soft drinks because the caffeine concentrations were not disclosed on the packaging label.

Phosphoric acid contents in carbonated soft drinks and energy drinks were significantly higher than in ice-tea drinks ($P<0.05$). On the other hand, no significant difference was observed between phosphoric acid contents of carbonated soft drinks and energy drinks ($P>0.05$). No comparison between the phosphoric acid claimed and tested is available since the phosphoric acid contents were not disclosed on the drink samples' packaging.

Table 4 shows that caffeine contents of carbonated soft drinks in our study were higher than those obtained by other authors (Amos-Tautua et al., 2014; Khalid et al., 2016; Xhaferaj et al., 2019). On the other hand, caffeine content of energy drinks in our study was lower than those reported by Vuletić et al. (2021) but higher than those reported by Xhaferaj et al. (2019) and Khalid et al. (2016). Phosphoric acid contents in the carbonated soft drinks measured by Utange et al. (2015) using potentiometric titration had a range of 131.8 to 187.6 mg/L. In contrast to this result, the present study showed higher phosphoric acid contents in carbonated soft drinks, energy drinks and ice-tea drinks marketed in Kosovo.

4 Conclusions

This study revealed that energy drinks available in Kosovo's market exhibit slightly elevated caffeine concentration in comparison to the declared amounts on the label of packaged product. Out of 41 analysed samples, 9 (21.95%) exceeded the EU Regulation 1169/2011 recommended threshold for caffeine in soft and energy drinks, set at 150 mg/L. Conversely, 37 out of the 41 samples complied with the established criteria for phosphoric acid, according to EU Regulation 1333/2008, leaving 9.76% of the samples non-compliant. The considerable proportion of samples that fail to align with the established standards for caffeine and phosphoric acid content is troubling. As such, vigilant oversight by the pertinent food control authorities in Kosovo is crucial to guarantee the safety and integrity of these beverages.

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