ORIGINAL PAPER

UDK: 613.292:549.752/.757
663.86:549.752/.757

DOI: 10.5937/hralsh2502026P

Spectrophotometric determination of phosphate content in samples of carbonated and energy beverages

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Received 26. 09. 2025.
Revised 28. 10. 2025.
Accepted 08. 12. 2025.

Abstract

Inorganic phosphates can be used in the food industry as additives, preservatives, acidifiers, or colour and flavour enhancers. In the soft and energy drink industry, the most commonly used acidifier is phosphoric acid. Excessive intake of phosphates may pose a potential health risk to humans. The aim of the conducted study was to determine the total phosphate content in samples of commonly consumed energy and soft drinks. The concentration of phosphates was determined using spectrophotometry in 15 different samples of soft and energy drinks available in Belgrade supermarkets. The spectrophotometric method applied was based on the formation of the blue-coloured complex "molybdenum blue" after adding ammonium-molybdate and a reducing agent to the samples, followed by measuring absorbance at the wavelength of 704.5 nm. The obtained results (0.28 mg/L – 539.32 mg/L) were compared with current Serbian and European regulations, and it was found that all 15 tested samples complied with the limits of 937.22 mg/L of phosphates for soft drinks and 669.45 mg/L for carbonated water. Furthermore, all tested samples were acidic, with pH values ranging from 2.75 to 6.50. Although the obtained results indicate that the tested beverages are safe in terms of phosphate content, it is important to note that they are not the only source of phosphates in the diet. Therefore, awareness should be raised regarding the harmful effects of their excessive intake.

Keywords: *phosphate; energy drinks; soft drinks; spectrophotometry.*

INTRODUCTION

Phosphorus is an essential element that plays a vital role in maintaining physiological functions and overall health. One of the most well-known roles of phosphate is its contribution to bone mineralization and health. Phosphates, together with calcium, form hydroxyapatite crystals, providing the structural integrity and support necessary for healthy bones and teeth [1]. They are also key components in intracellular signaling pathways, acting as modulators of enzyme activity, receptors, and transcription factors involved in cell growth and differentiation [2,3].

For healthy adults, the estimated average requirement for phosphorus intake is 580 mg/day (National Health and Nutrition Examination Survey), while the recommended daily intake is 700 mg/day (National Institutes of Health). However, the average phosphorus intake for individuals over 20 years of age is 1399 mg/day, which is almost 2.5 times higher than the estimated average requirement and twice the recommended daily intake [2,4].

Phosphates that occur naturally in food are organically bound, and only about 40% to 60% can be absorbed in the gastrointestinal tract. In contrast, phosphates added to food as additives are of inorganic origin, and these "free" phosphates can be absorbed much more efficiently, posing a potentially greater risk to human health when consumed in excessive amounts.

According to European Union regulations (Directive 2000/13/EC on the labeling and presentation of foodstuffs), phosphoric acid (E338), sodium phosphate (E339), potassium phosphate (E340), calcium phosphate (E341), and salts of orthophosphoric acid—diphosphates (E450), triphosphates (E451), and polyphosphates (E452)—may be added to food as preservatives, acidifying agents, acidity regulators, emulsifiers, stabilizers, or flavor enhancers. Carbonated beverages, especially carbonated fruit drinks, contain higher amounts of phosphoric acid (E338) to lower the pH value and thus prevent the growth of bacteria, fungi, and yeasts [5].

Increased dietary intake of phosphate can lead to the development of hyperphosphatemia, particularly in individuals with severe chronic kidney disease. However, it has also been shown that elevated phosphate intake may be harmful to the general population [5,6,7]. Hyperphosphatemia has been associated with neurological symptoms such as confusion, weakness, and seizures, as an imbalance of these ions can disrupt neurological function and neurotransmitter signaling in the brain [8].

In individuals whose diet consists largely of foods rich in phosphate additives, elevated phosphate concentrations in serum and urine have been observed, along with decreased calcium levels—changes associated with the development of secondary hyperparathyroidism [9]. The consumption of "cola" beverages (Coca-Cola, Pepsi), in particular, has been linked to altered bone metabolism and decreased bone density, as demonstrated in both human and animal studies [2,10,11].

The acidity of commercial non-alcoholic, non-dairy beverages ranges from 2.1 to 7.4. Acids are used in beverages to enhance their flavor and provide a unique aroma. Acidity contributes to a refreshing and intense taste while balancing the sweetness of the sugars present in the drink [12]. The consumption of carbonated beverages has also been linked to tooth erosion, and due to their acidity and the buffering effect of added phosphates, they are considered one of the most significant risk factors for human health [13-16].

According to European Union guidelines (Directive 2000/13/EC on the labeling and presentation of foodstuffs), the E-numbers of all food additives must be listed on product packaging. However, since the labeling requirement is qualitative rather than quantitative, consumers are not informed about the actual amount of phosphate they are ingesting by consuming such products [5].

Given the importance of controlling phosphate content in food products, the objective of this study was defined as follows: to determine the total phosphate content using a spectrophotometric method with molybdenum blue, as well as the acidity levels in commonly consumed carbonated and energy drinks available in supermarkets in Belgrade.

MATERIALS AND METHODS

Materials

To determine the phosphate content, 15 carbonated and energy drinks of different brands and manufacturers—commercially available in local markets in Belgrade—were collected using a random sampling method during February 2024. The samples were stored in a refrigerator at 4 °C until analysis.

The following equipment and instruments were used during the experiment: an analytical balance, volumetric flasks (100 mL and 250 mL), laboratory beakers, and automatic pipettes (Eppendorf Research Plus, Eppendorf, Hamburg, Germany), an ultrasonic bath (Bandelin Sonorex Digitec), a pH meter (InoLab® pH 7110), and a UV-VIS spectrophotometer with an external IBM™-compatible computer, model Cintra 20 (GBC Scientific Equipment, Perai, Malaysia), with a wavelength range of 190–900 nm.

For the preparation of samples for phosphate determination, deionized water and reagents of analytical (p.a.) grade purity were used: $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (PLIVA d.o.o., Zagreb, Croatia), H_2SO_4 (Merck Group, Darmstadt, Germany), $SnCl_2 \times 2H_2O$ (BDH Chemicals, Poole, United Kingdom), and HCl (Zorka, Šabac, Serbia). To prepare the standard series, $Na_2HPO_4 \times 2H_2O$ (Kemika, Zagreb, Croatia) was used.

Sample Preparation and Phosphate Determination

A volume of 50 mL of each beverage was transferred into glass beakers, and carbon dioxide was removed from the carbonated drinks using an ultrasonic bath for 30 minutes. The pH value was measured in all samples, after which they were prepared for spectrophotometric analysis. For the analysis, 10.00 mL of the sample was pipetted, followed by the addition of 400 μL of ammonium molybdate reagent and 2 drops of $SnCl_2$ solution. The mixtures were stirred and left to stand in the dark for 10 minutes, after which the spectra were recorded. A blank sample was prepared in the same manner, using deionized water instead of the sample.

Phosphate content in the samples was quantified using the standard calibration curve method. A series of phosphate standard solutions was prepared in the following concentrations: 0.005 mg/L; 0.01 mg/L; 0.05 mg/L; 0.10 mg/L; 0.50 mg/L; 0.75 mg/L; 1.50 mg/L; 3.00 mg/L; 6.00 mg/L.

The phosphate content was recorded in a continuous spectrum within the wavelength range of 600–800 nm, with the maximum absorbance observed at a wavelength of 704.5 nm. Standard solutions and samples were measured at the selected wavelength.

RESULTS AND DISCUSSION

To quantify the phosphate content in the analyzed samples within the concentration range of 0.005 mg/L to 6.00 mg/L, a calibration curve was constructed (**Figure 1**): A = 0.2058 C (mg/L) + 0.0007, with a correlation coefficient (r^2) of 0.9998, confirming the linearity of the method. The limit of detection (LOD) was calculated using the formula 3SD/a (where SD is the standard deviation of the y-intercept, and a is the slope of the

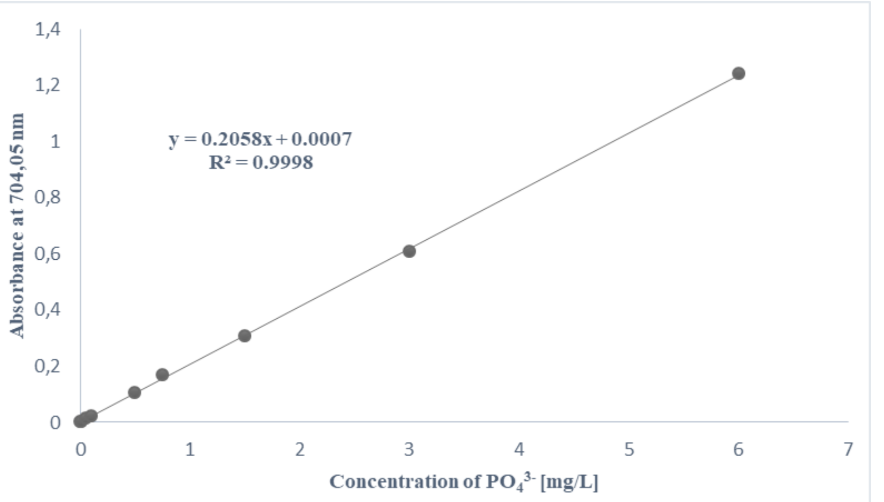

Figure 1. Calibration curve for the determination of phosphate content at λ = 704.5 nm.

curve), resulting in 0.0974 mg/L. The limit of quantification (LOQ), calculated as 10SD/a, was 0.3248 mg/L, while the relative standard deviation (RSD) was 2.45%.

The phosphate content and pH values of the 15 tested samples of carbonated and energy drinks are presented in **Table 1**. The Regulation on Food Additives of the Republic of Serbia (Official Gazette 53/2018-22), which is in accordance with the European Union Regulation on Food Additives (Regulation (EU) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives), expresses the content of phosphorus-based additives as mg/L of phosphorus(V) oxide (P_2O_5).

According to these regulations, the maximum allowable concentration (MAC) of P_2O_5 is 500 mg/L for carbonated water and 700 mg/L for carbonated beverages,

Table 1. pH values, phosphate (PO_4^{3-}) content, and phosphorus(V) oxide (P_2O_5) content of the tested beverage samples.

	Sample name	**pH**	**Content of PO_4^{3-} (*mg/L*)**	**Content of P_2O_5 (*mg/L*)**
1	Mg Mivela	6.50	*< LOD*[a]	***UC***[b]
2	Kong strong, no sugar	3.67	*< LOD*	UC
3	Guarana, original	2.47	*< LOD*	UC
4	Kong strong, classic	3.67	*< LOD*	UC
5	Pepsi	2.57	470,40	351,52
6	Fanta orange	2.93	1,07	0,800
7	Schweppes bitter lemon	2.65	0,958	0,716
8	Coca-Cola zero	2.88	472,98	353,45
9	Cocta original	2.80	0,908	0,679
10	Coca-Cola original	2.64	539,32	403,028
11	Sprite	2.77	*< LOD*	UC
12	Saguaro lemon elder	3.32	0,698	0,522
13	Saguaro lemon flavoured sparkling soft drink	6.13	0,413	0,309
14	Saguaro sparkling water	6.41	0,279	0,208
15	Saguaro lemon flavoured soft drink	3.78	*< LOD*	UC

Legislative	**Concentration of PO_4^{3-} (*mg/L*)**	**Concentration of P_2O_5 (*mg/L*)**
Republic of Serbia[c]	669,45	218,31
	937,22	305,63
European Union[d]	669,45	218,31
	937,22	305,63

[a]LOD – limit of detection

[b]UC - unable to calculate

[c]SL GLASNIK 53/2018-22 - Pravilnik o prehrambenim aditivima

[d]Regulative (EU) br.1333/2008

which corresponds to phosphate concentrations of 669.45 mg/L and 937.22 mg/L, respectively.

Based on the presented results, it can be observed that phosphate content was quantified in 9 out of 15 tested samples, with concentrations ranging from 0.28 to 539.32 mg/L. Significantly higher phosphate concentrations were found in the so-called cola drinks: Pepsi 470.40; Coca-Cola Zero 472.98; and Coca-Cola Original 539.32 mg/L (**Figure 2**). The lowest concentration was determined in the Saguaro carbonated water sample (**Figure 3**). Phosphate concentrations in the samples of Mg Mivela carbonated water, Sprite, Saguaro lemon-flavored soft drink, as well as in the energy drinks Kong Strong (no sugar), Guarana Original, and Kong Strong Classic were below the detection limit (LOD) of the applied method.

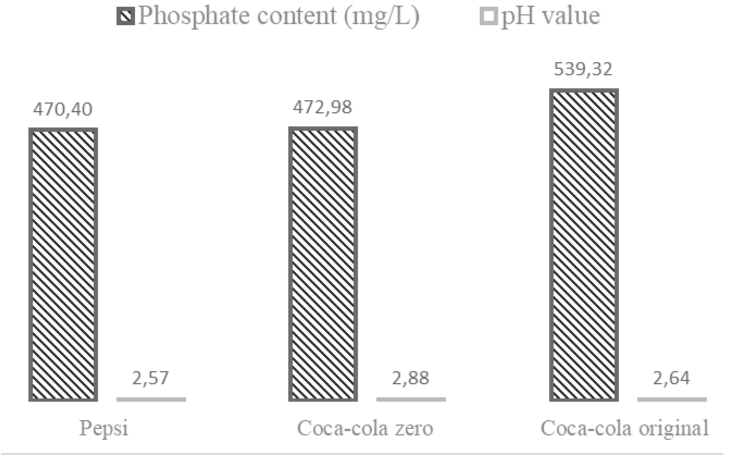

Figure 2. Phosphate content (mg/L) and pH values of "cola" carbonated beverage samples.

It is important to emphasize that the phosphate content in all tested samples complies with Serbian and European regulations. Comparing the obtained results with a similar study conducted in Australia [14], it can be observed that the phosphate concentrations in cola samples are similar or slightly higher (Coca-Cola – 568.87 mg/L; Pepsi – 517.59 mg/L; Coca-Cola Zero – 503.34 mg/L) than in this study, while phosphates in Sprite and Fanta Orange were below the detection limit. Similar results were also reported for the U.S. market (Coca-Cola – 604 mg/L and Pepsi – 494 mg/L) [19].

On the other hand, a study conducted on samples from the Brazilian market showed significantly higher phosphate concentrations in Coca-Cola, Pepsi, and Sprite samples (1593, 1537, and 21 mg/L, respectively) [17]. Seatear and colleagues [18] determined the phosphate content in Coca-Cola samples in Thailand using both Flow Injection Analysis and spectrophotometric methods, with the average detected phosphate content in this beverage being 348 mg/L, which is somewhat lower compared to the content found in this study.

Additionally, results published by Larsen and collaborators show that phosphate content in the Danish market for Pepsi, Fanta Orange, Schweppes Bitter Lemon, and carbonated waters (519.00; 12.35; 17.09; 0.95 mg/L) is higher compared to the results obtained in this study, while for the Coca-Cola Original sample, it was lower (519.98 mg/L) [16]. These differences can be explained by variations in formulation and production methods of beverages in different countries.

In addition to phosphate content, it was interesting to compare the pH values of the analyzed samples. This parameter is important because beverages with significant acidity are closely associated with dental erosion in humans, as well as potential other problems that can disrupt the normal functioning of some metabolic pathways involved in the incorporation/consumption of this inorganic form of phosphorus.

The acidity of the tested samples ranged from 2.47 to 6.50, and as many as 13 samples had a pH below 4.00 (**Figures 2** and **3**). Based on relative zones of beverage erosiveness, derived from studies of apatite solubility in acid, it can be concluded that 53.3% (8/15) of the samples fall into the zone of extremely erosive beverages (pH < 3.00), 26.7% (4/15) of the samples fall

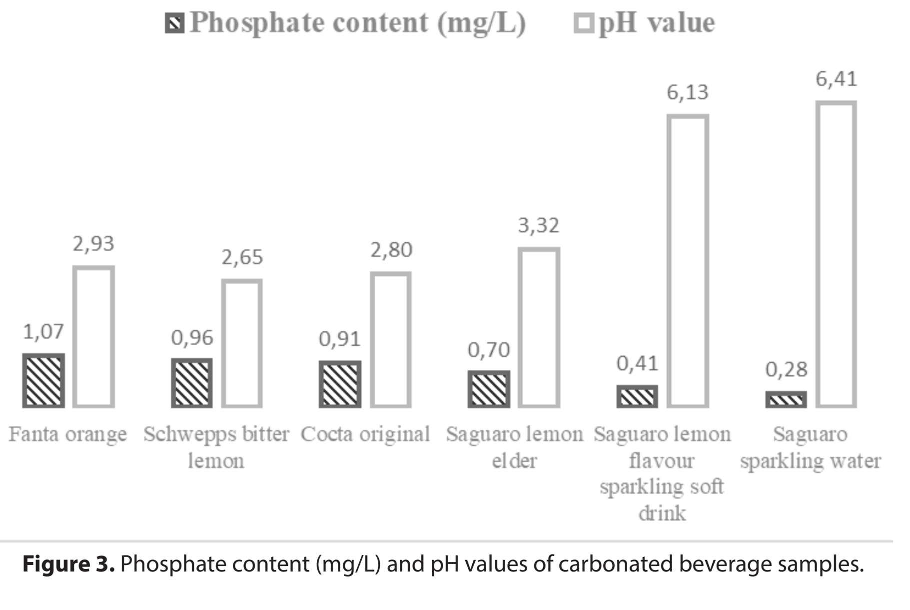

Figure 3. Phosphate content (mg/L) and pH values of carbonated beverage samples.

into the zone of erosive beverages (pH = 3.00 – 3.99), while 20% (3/15) of the samples fall into the zone of minimally erosive beverages (pH > 4.00) [12].

Beverages with a pH lower than 3.00 have been shown to pose a risk of esophageal injury, which means that as many as 53.3% (8/15) of the tested samples could lead to such problems if consumed continuously and uncontrollably over a long period of time [20]. Considering that the lowest pH value does not correspond to the highest phosphate concentration (**Figure 2**), it can be said that no correlation between these two parameters was observed. This can be explained by the fact that beverages, in addition to phosphoric acid, may contain other substances that affect acidity, such as citric, lactic, fumaric, acetic acids, and others, which vary in concentration and thus contribute to differences in pH values [21].

To assess the risk to dental health and the buffering capacity of non-alcoholic carbonated beverages, scientists from Denmark measured the pH values of beverage samples (Schweppes Bitter Lemon, Fanta Orange, Sprite, Coca-Cola Original, Pepsi, flavored mineral waters) and found that the results (2.40–5.01) were consistent with those obtained in this study [16].

Interestingly, despite the significantly higher phosphate concentration in beverages from the Brazilian market compared to the Serbian market, the measured acidity for Pepsi and Sprite was lower, while for Coca-Cola it was equal to that in the tested samples [17]. The acidity of beverages produced for the Australian market is consistent with the obtained results [14]. Taking the average pH value of energy drinks produced in the United States (2.47–3.97), it was shown that none of the tested samples from the domestic market fell outside the expected range [12].

CONCLUSION

The phosphate content in all 15 tested samples of carbonated and energy drinks complies with both domestic and European regulations. The results showed that the highest phosphate content was found in cola carbonated beverages, while in some carbonated waters and energy drinks, the concentrations were below the detection limit of the method. The measured pH values were also consistent with previous studies.

This study highlights the importance of determining phosphate content in beverages to ensure consumer safety and health concerning excessive phosphate intake. It is also important to emphasize that, although the obtained results characterize these drinks as safe, consumers should moderate their consumption, especially since food products are not the only source of phosphate; phosphates can also be found in medications, dietary supplements, and oral hygiene products. The combination of these factors could easily lead to exceeding the allowable daily phosphate intake.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, through two Grant Agreements with the University of Belgrade-Faculty of Pharmacy No 451-03-136/2025-03/ 200161 and No 451-03-137/2025-03/ 200161.

AUTHORS' CONTRIBUTION

MS and SDjM designed the experiments. JP and MS performed the experiments. JP and MS did analyses of experiments. JP and MS wrote the manuscript; SDjM and MS reviewed and edited the manuscript.

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Spektrofotometrijsko određivanje sadržaja fosfata u uzorcima gaziranih i energetskih pića

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Kratak sadržaj

Neorganski fosfati se koriste u prehrambenoj industriji kao aditivi, konzervansi, regulatori kiselosti ili pojačivači boje i ukusa. U industriji gaziranih i energetskih pića, najčešće korišćen regulator kiselosti je upravo fosforna kiselina. Preterani unos fosfata može predstavljati potencijalni rizik po zdravlje ljudi. Cilj ovog rada bio je određivanje sadržaja ukupnih fosfata u uzorcima često konzumiranih energetskih i gaziranih pića. Sadržaj fosfata određivan je standardnom spektrofotometrijskom metodom u 15 različitih uzoraka gaziranih i energetskih pića dostupnih u beogradskim marketima. Metoda se zasniva na formiranju plavo obojenog kompleksnog jedinjenja „molibdenskog plavog" nakon dodatka amonijum-molibdata i redukujućeg sredstva u uzorke i očitavanju apsorbancije na talasnoj dužini od 704,5 nm. Dobijeni rezultati (0,28 mg/L – 539,32 mg/L) poređeni su sa važećim srpskim i evropskim regulativama i utvrđeno je da je svih 15 ispitivanih uzoraka u skladu sa preporukama od 937,22 mg/L fosfata za gazirana pića i 669,45 mg/L za gazirane vode. Takođe, svi ispitivani uzorci bili su kiseli, sa pH vrednostima u opsegu od 2,75 – 6,50. Iako dobijeni rezultati pokazuju da su ispitivana pića, po pitanju sadržaja fosfata, bezbedna za ljudsko zdravlje, važno je napomenuti da ona nisu jedini izvor fosfata u ishrani, pa bi trebalo podizati svest o štetnosti njihovog prekomernog unosa.

Ključne reči: *fosfati; energetska pića; gazirana pića; spektrofotometrija.*