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# INFLUENCE OF ATMOSPHERIC COLD PLASMA TREATMENT ON MINERAL COMPOSITION AND BIOACCESSIBILITY IN CARBONATED MINERAL WATER

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## ABSTRACT

Atmospheric cold plasma is an emerging non-thermal technology that can induce physicochemical changes at the plasma-liquid interface; however, its influence on dissolved mineral fractions and gastrointestinally accessible minerals in carbonated mineral water remains poorly defined. This study investigated the effect of atmospheric-pressure plasma jet treatment on the dissolved elemental composition and in-vitro mineral bioaccessibility of a composite carbonated mineral water sample prepared by pooling equal volumes of commercially available brands in Türkiye. Plasma treatment was performed using dry air at 512-515 Hz and 3 bar inlet pressure for 60, 90, and 180 s. Element concentrations were determined by ICP-OES in the operationally defined dissolved fraction after 0.45 µm filtration. In-vitro mineral bioaccessibility was evaluated using a static gastrointestinal digestion model followed by centrifugation, filtration, and ICP-OES analysis. Plasma exposure significantly affected the dissolved mineral profile ($P<0.05$). Calcium and strontium decreased with increasing treatment time, whereas potassium and magnesium showed moderate but significant increases. Sodium increased after the shortest treatment but did not exhibit a consistent time-dependent pattern. In the digestion model, calcium bioaccessibility remained relatively stable (61.59-64.85%), while potassium (70.88-78.58%) and magnesium (77.95-83.57%) showed higher bioaccessible fractions after plasma treatment. Overall, the results showed that atmospheric cold plasma treatment reduced the dissolved Ca and Sr concentrations, increased the dissolved K and Mg concentrations, and enhanced the in vitro bioaccessible fractions of K and Mg in the carbonated mineral water sample.

**Key words:** Cold plasma, mineral bioaccessibility, functional beverages, in-vitro digestion, carbonated mineral water

# ATMOSFERİK SOĞUK PLAZMA UYGULAMASININ MADEN SUYUNDA MİNERAL KOMPOZİSYONU VE İN VİTRO BİYOERİŞİLEBİLİRLİK ÜZERİNE ETKİSİ

## ÖZ

Atmosferik soğuk plazma, plazma-sıvı ara yüzeyinde fizikokimyasal değişiklikler oluşturabilen yeni gelişen termal olmayan bir teknolojidir; ancak karbonatlı maden suyunda çözünmüş mineral fraksiyonları ve gastrointestinal olarak erişilebilir mineraller üzerindeki etkisi henüz yeterince tanımlanmamıştır. Bu çalışmada, atmosferik basınçlı plazma jet uygulamasının Türkiye'de ticari olarak temin edilebilen markalardan eşit hacimlerin birleştirilmesiyle hazırlanan kompozit karbonatlı maden suyu örneğinin çözünmüş elementel kompozisyonu ve in vitro mineral biyoerişilebilirliği üzerindeki etkisi araştırılmıştır. Plazma uygulaması, kuru hava kullanılarak 512–515 Hz frekansta ve 3 bar giriş basıncında 60, 90 ve 180 s süreyle gerçekleştirilmiştir. Element konsantrasyonları, 0.45 µm filtrasyon sonrasında operasyonel olarak tanımlanan çözünmüş fraksiyonda ICP-OES ile belirlenmiştir. İn vitro mineral biyoerişilebilirliği, statik gastrointestinal sindirim modeli sonrasında santrifüjleme, filtrasyon ve ICP-OES analizi kullanılarak değerlendirilmiştir. Plazma uygulaması çözünmüş mineral profilini anlamlı düzeyde etkilemiştir ($P<0.05$). Kalsiyum ve stronsiyum artan uygulama süresiyle azalırken, potasyum ve magnezyum orta düzeyde fakat anlamlı artışlar göstermiştir. Sodyum en kısa uygulama süresinden sonra artmış, ancak tutarlı bir zamana bağlı değişim göstermemiştir. Sindirim modelinde kalsiyum biyoerişilebilirliği nispeten stabil kalırken (%61.59-64.85), potasyum (%70.88-78.58) ve magnezyum (%77.95-83.57) plazma uygulamasından sonra daha yüksek biyoerişilebilir fraksiyonlar göstermiştir. Genel olarak elde edilen sonuçlar, atmosferik soğuk plazma uygulamasının karbonatlı maden suyu örneğinde çözünmüş Ca ve Sr konsantrasyonlarını azalttığını, çözünmüş K ve Mg konsantrasyonlarını artırdığını ve K ile Mg'nin in vitro biyoerişilebilir fraksiyonlarında artışa yol açtığını göstermiştir.

**Anahtar kelimeler:** Soğuk plazma, mineral biyoerişilebilirliği, fonksiyonel içecekler, in vitro sindirim, karbonatlı maden suyu

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## INTRODUCTION

Mineral waters, particularly carbonated mineral waters, are a significant source of essential minerals such as calcium, magnesium, potassium, and sodium, contributing to bone health, cardiovascular function, and digestive processes (Gu et al., 2024). However, the potential health benefits of these minerals are not solely determined by their concentration but also by their bioaccessibility the extent to which they are released from the food matrix during digestion and become available for intestinal absorption (Cao et al., 2000; Heaney, 2006; Spears et al., 2004; Xu et al., 2024). Given the increasing interest in improving the nutritional quality of beverages, research on the bioaccessibility of minerals in carbonated mineral waters is crucial for understanding their true contribution to human health.

Recent advancements in food processing technologies have explored innovative methods to enhance nutrient bioaccessibility while ensuring product safety and quality. Among these, cold plasma technology has gained significant attention as a non-thermal, environmentally friendly processing technique capable of microbial inactivation, shelf-life extension, and modification of food components without compromising nutritional value (Harikrishna et al., 2023; Hossein pour et al., 2024). Cold plasma generates a range of reactive species, including oxygen and nitrogen-based radicals, which can interact with food components and influence physicochemical properties (Marotta & Paradisi, 2025). While extensive research has been conducted on the impact of cold plasma on microbial decontamination and food shelf-life, studies investigating its effects on mineral bioaccessibility in liquid food matrices, such as mineral waters, remain limited (Hossein pour et al., 2024; Nguyen et al., 2020; Rao et al., 2023). Given the role of mineral speciation and solubility in determining bioaccessibility, it is essential to examine how cold plasma treatment influences the mineral composition and digestibility of carbonated mineral waters.

Unlike complex food matrices, carbonated mineral water is an inorganic, low-solid beverage system in which minerals are present mainly in dissolved or readily soluble forms. Therefore, mineral bioaccessibility in this matrix is expected to be governed primarily by dissolved ion concentration, solubility, carbonate equilibria, pH transitions during digestion, and the proportion of elements remaining in the soluble fraction after gastrointestinal simulation rather than by the breakdown of macromolecular structures. Previous studies have shown that mineral waters may contribute to dietary calcium and magnesium intake; however, the nutritional relevance of these minerals depends not only on their total concentration but also on their soluble and bioaccessible fractions (Gu et al., 2024; Heaney, 2006). Static in vitro digestion therefore provides a practical screening approach for estimating the operationally defined bioaccessible fraction, although it should not be interpreted as a direct measure of in vivo bioavailability (Lucas-González et al., 2018; Minekus et al., 2014). In this context, the present study aimed to evaluate the effects of atmospheric cold plasma treatment on the dissolved mineral profile and in vitro mineral bioaccessibility of a composite carbonated mineral water sample prepared from commercially available brands in Turkiye. By focusing on both the operationally defined dissolved fraction and the post-digestion soluble fraction, the study provides preliminary evidence on how plasma treatment may influence mineral behavior in carbonated mineral water.

## MATERIAL AND METHODS

### Material

Carbonated mineral water samples were obtained from ten commercially available brands (coded as B1-B10) purchased from retail markets in Turkiye. The brands were selected to prepare a composite test matrix from products available to consumers, rather than to compare brand-specific mineral profiles. Unopened bottles were stored at 4 °C in the dark until analysis. Prior to plasma treatment, equal-volume composite sample was prepared from ten commercial brands to obtain a homogeneous composite sample. This approach was selected because the main objective of the study was to evaluate the effect of atmospheric cold plasma treatment under a standardized carbonated mineral water matrix, not to compare individual brands. Pooling provided sufficient and homogeneous sample volume for plasma treatment, in vitro digestion, and ICP-OES analyses. However, this design does not allow the evaluation of brand-specific responses to plasma treatment and may mask differences related to initial mineral composition, pH, alkalinity, $CO_2$ content, and buffering capacity among individual brands. Therefore, separate analysis of individual mineral water brands with different initial physicochemical properties should be considered in future studies.

## Methods

### Cold plasma technology

Cold plasma treatment was applied to mineral water samples using an atmospheric pressure plasma jet (APPJ) system (Palabiyik et al., 2023). The plasma system operated at a frequency of 512-515 Hz, with a fixed distance of 5 cm between the plasma nozzle and the sample surface. This nozzle-to-liquid surface distance was selected based on the operating conditions previously established for the APPJ system and preliminary setup stability, as it allowed stable plasma exposure of the liquid surface while avoiding direct contact between the nozzle and sample, excessive splashing, and localized overheating. The power supply was set to approximately 1 kVA (~1 kW), and dry air at an inlet pressure of 3 bar was used as the working gas. The plasma treatment was conducted for 60, 90, and 180 seconds on the mineral water samples. For each experiment, 50 mL of mineral water was placed in a glass beaker and subjected to plasma treatment under identical conditions while being continuously stirred. The discharge was applied continuously for the specified duration under the same nozzle-to-liquid surface distance. Dry air was used as the working gas, with a constant air velocity of 5 m/s. To monitor temperature variations during plasma exposure, real-time infrared temperature measurements were conducted using a non-contact infrared thermometer (SM580, ISISO). The recorded temperature ranges were 30.1-30.7°C for the 60 s treatment, 32.1-32.5°C for the 90 s treatment, and 39.1-39.7°C for the 180 s treatment. To ensure the repeatability and reliability of the results, each plasma treatment was performed twice under identical conditions, and measurements were taken in triplicate to enhance statistical accuracy. The steps performed in the study are outlined in Figure 1.

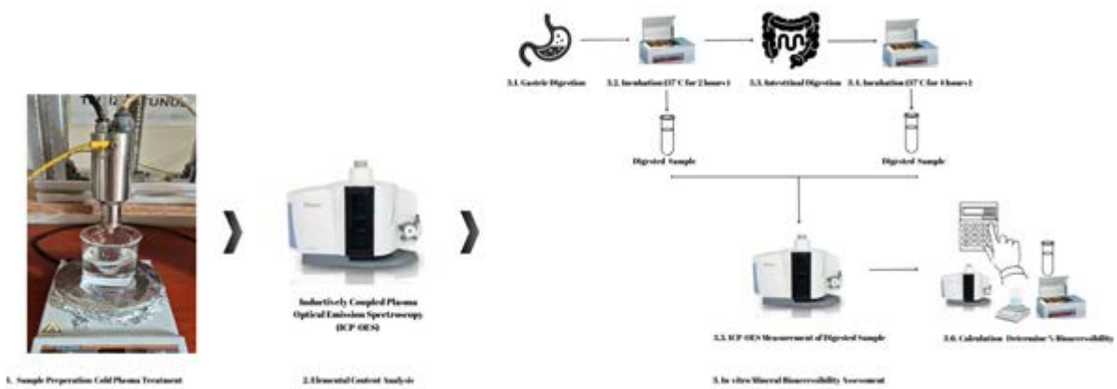


Figure 1. Experimental setup used for atmospheric-pressure plasma jet treatment of carbonated mineral water samples, including the APPJ configuration and analytical workflow

### Elemental Content Analysis

The elemental composition of carbonated mineral water samples, including Ca, K, Li, Mg, Na, Sr, Ag, Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn, was analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Thermo Scientific iCap 6000 Dual View, Cambridge, UK). Before injection into the instrument, samples were filtered through 0.45 μm syringe filters. Accordingly, all concentrations reported in this study correspond to the operationally defined dissolved fraction (<0.45 μm). No total digestion was performed; therefore, the results should not be interpreted as total elemental content (dissolved + particulate). Any plasma-induced precipitation or particle formation would be removed during filtration and could appear as an apparent decrease

in the measured dissolved concentration. A multi-element standard solution (Merck, Product No: 111355, Germany) was used to prepare the calibration curves. Dilution of samples was performed using 5% $HNO_3$ solution when necessary to ensure analysis within the calibration range (Apaydın, 2026). The determination coefficient ($r^2$) for calibration curves was maintained above 0.99.

To determine the Limit of Detection (LOD) and Limit of Quantification (LOQ) for the analyzed elements, calculations were performed following internationally recognized guidelines (EURACHEM) (Magnusson, 2014). The LOD and LOQ values were obtained based on repeated measurements of blank samples (n=10) using a standard deviation-based approach. The equations used for these calculations are as follows:

$$LOD=3\times\sigma \quad \text{(Eq. 1)}$$

$$LOQ=10\times\sigma \quad \text{(Eq. 2)}$$

where σ represents the standard deviation of blank measurements. LOD defines the lowest concentration at which an element can be detected with statistical significance, whereas LOQ represents the lowest concentration that can be quantified with acceptable precision and accuracy. All measurements below the LOD were reported as "ND" (Not Detected), while values below LOQ but above LOD were considered detectable but not quantifiable with high accuracy.

The calculated LOD and LOQ values for each element in this study were as follows: Ca (LOQ: 0.019 ppm, LOD: 0.006 ppm), K (LOQ: 0.040 ppm, LOD: 0.012 ppm), Li (LOQ: 0.075 ppm, LOD: 0.022 ppm), Mg (LOQ: 0.065 ppm, LOD: 0.020 ppm), Na (LOQ: 0.065 ppm, LOD: 0.019 ppm), Sr (LOQ: 0.094 ppm, LOD: 0.028 ppm), Ag (LOQ: 0.046 ppm, LOD: 0.014 ppm), Al (LOQ: 0.041 ppm, LOD: 0.012 ppm), Cd (LOQ: 0.086 ppm, LOD: 0.026 ppm), Co (LOQ: 0.047 ppm, LOD: 0.014 ppm), Cu (LOQ: 0.085 ppm, LOD: 0.025 ppm), Fe (LOQ: 0.099 ppm, LOD: 0.030 ppm), Mn (LOQ: 0.017 ppm, LOD: 0.005 ppm), Ni (LOQ: 0.085 ppm, LOD: 0.025 ppm), Pb (LOQ: 0.105 ppm, LOD: 0.031 ppm), and Zn (LOQ: 0.049 ppm, LOD: 0.015 ppm).

These values were used to determine the significance of the measured trace element concentrations in carbonated mineral water samples, ensuring accurate detection and quantification of elements in the analyzed samples. The application of LOD and LOQ calculations enhances the reliability of analytical results, ensuring that the reported values accurately reflect the trace element composition of carbonated mineral waters.

**Static in-vitro gastrointestinal digestion (modified INFOGEST approach)**

Static in vitro gastrointestinal digestion was conducted based on the INFOGEST consensus protocol (Apaydın, 2026) with adaptations for a low-solid, inorganic beverage matrix. Because carbonated mineral water does not contain starch or protein requiring oral enzymatic breakdown, the oral phase was omitted and the digestion was initiated directly with the gastric phase. Briefly, 2.5 mL of each sample was transferred into polypropylene tubes and diluted with ultrapure water to obtain a workable volume. The gastric phase was initiated by adjusting the pH to 2.0 ± 0.1 with 0.1 N HCl. Fresh pepsin solution was prepared immediately before use by dissolving 0.2 g of pepsin (Sigma-Aldrich, P-7000; declared activity ≥250 U/mg protein) in 5 mL of 0.1 N HCl, and 0.5 mL of this solution was added to each tube. The samples were incubated at 37 °C for 2 h in a shaking water bath.

After the gastric phase, the pH was gradually adjusted to 6.8-7.0 using 1 M $NaHCO_3$. Fresh pancreatin solution was prepared by dissolving 0.4 g of pancreatin (Sigma-Aldrich, P-1750) in 10 mL of ultrapure water, and 1.0 mL was added to each tube. In addition, bile extract solution was prepared by dissolving 0.25 g of bile extract (Sigma-Aldrich, B-8631) in 10 mL of ultrapure water, and 1.0 mL was added to each tube. The samples were then incubated at 37 °C for 4 h in a shaking water bath. Enzyme activities and product specifications were reported according to the manufacturers' declarations. Because the declared specific activity of pancreatin was not available in the records used for this study, the supplier, catalogue number, preparation concentration, and added volume were provided to improve methodological transparency.

After digestion, samples were diluted to a fixed final volume, centrifuged (15100 × g, 20 min), and the supernatant was sequentially filtered using filter paper and 0.45 μm syringe filters. ICP-OES was performed on the filtered supernatant. In vitro mineral bioaccessibility (%) was calculated as the ratio of the element concentration in the digested, centrifuged, and 0.45 μm-filtered supernatant to the corresponding concentration in the undigested 0.45 μm-filtered sample, multiplied by 100. Therefore, the reported bioaccessibility represents an operationally defined soluble/bioaccessible fraction rather than in-vivo bioavailability.

**Statistical Analysis**

Statistical analyses were performed using IBM SPSS Statistics (Version 22). The ten commercial brands were used to prepare a pooled composite carbonated mineral water sample and were not treated as independent statistical units. Therefore, statistical comparisons were interpreted within the composite-sample design and should not be considered as brand-level inference. For each treatment condition, replicate analytical measurements were obtained from the treated composite sample and used to evaluate treatment-related differences. For ICP-OES, analytical readings were obtained in triplicate for each treatment condition and averaged prior to statistical evaluation. Treatment effects were evaluated using one-way ANOVA separately for each element. When ANOVA indicated significance ($p < 0.05$), post-hoc pairwise comparisons were performed using an appropriate procedure consistent with variance assumptions (e.g., Tukey HSD for homogeneous variances or Games-Howell otherwise) (Apaydın et al., 2024).

## RESULTS AND DISCUSSION

**Effect of cold plasma treatment on dissolved mineral composition**

This study evaluated the influence of atmospheric cold plasma on (i) the dissolved elemental composition (operationally defined as the <0.45 μm-filtered fraction) of a composite carbonated mineral water sample prepared by pooling equal volumes from commercially available brands, and (ii) the in-vitro bioaccessibility of selected minerals following static gastrointestinal digestion.

Table 1 summarizes the dissolved elemental concentrations and reports both absolute and relative changes compared with the untreated control (Δ, mg/L and Δ, %). Cold plasma exposure produced clear, element-dependent shifts in the dissolved mineral profile. Relative to the control, dissolved calcium (Ca) decreased markedly at all treatment durations (-61.5% to -80.4%), while strontium (Sr) also declined substantially (-66.4% to -87.6%). In contrast, potassium (K) and magnesium (Mg) increased after treatment, reaching maximum relative increases of +88.1% and +76.7%, respectively. Sodium (Na) exhibited the largest increase at 60 s (+52.3%), followed by smaller changes at 90-180 s (+7.3-12.6%). Figure 2 visualizes these relative shifts (Δ%) and facilitates comparison of the direction and magnitude of changes across exposure durations. It should also be noted that the longest plasma exposure resulted in a higher sample temperature than the shorter treatments. The temperature increased to 39.1-39.7°C during the 180 s treatment, whereas the 60 and 90 s treatments remained within approximately 30-32°C. Although this temperature range is still relatively mild and does not correspond to conventional thermal processing, it may have contributed to some of the observed changes in the dissolved mineral fraction. In carbonated mineral water, even moderate heating may influence $CO_2$ retention, carbonate equilibria, and the tendency of calcium- and strontium-containing phases to precipitate or shift from the dissolved fraction to forms removed by 0.45 μm filtration. Therefore, the stronger decreases in dissolved Ca and Sr observed at 180 s should not be attributed solely to plasma-generated reactive species. They may reflect the combined effect of longer plasma exposure, mild temperature increase, and changes in carbonate-related equilibria. Since pH, CO content, alkalinity, conductivity, and mineral speciation were not measured during treatment, the specific contribution of temperature cannot be separated from the plasma effect in the present study. The observed treatment-related changes should be interpreted with strict attention to the operational definition used in this study, namely the dissolved fraction defined as the <0.45 μm-filtered portion, which does not represent total element content and does not resolve chemical speciation or particle-associated forms (Kiran et al., 2022).

Table 1. Dissolved elemental concentrations (mg/L; 0.45 µm-filtered fraction) of carbonated mineral water following cold plasma treatment, and absolute (Δ, mg/L) and relative (Δ, %) changes compared with the control

| Element | Control (mean±SD) | 60 s (mean±SD) | Δ 60 s (mg/L) | Δ 60 s (%) | 90 s (mean±SD) | Δ 90 s (mg/L) | Δ 90 s (%) | 180 s (mean±SD) | Δ 180 s (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| Ca | 31.97±0.17[a] | 12.32±0.06[b] | -19.65 | -61.5 | 10.32±0.06[c] | -21.65 | -67.7 | 6.26±0.03[d] | -25.71 |
| K | 10.05±0.01[d] | 15.58±0.01[b] | 5.53 | 55.0 | 12.61±0.09[c] | 2.56 | 25.5 | 18.90±0.01[a] | 8.85 |
| Mg | 6.00±0.02[d] | 8.96±0.06[b] | 2.96 | 49.3 | 7.59±0.01[c] | 1.59 | 26.5 | 10.60±0.06[a] | 4.60 |
| Na | 1208.25±5.80[c] | 1839.95±44.54[a] | 631.70 | 52.3 | 1360.80±1.85[b] | 152.55 | 12.6 | 1296.70±12.99[b] | 88.45 |
| Sr | 0.411±0.002[a] | 0.103±0.001[c] | -0.31 | -74.9 | 0.138±0.001[b] | -0.27 | -66.4 | 0.051±0.010[d] | -0.36 |
| Ag | ND | ND | - | - | ND | - | - | ND | - |
| Al | ND | ND | - | - | ND | - | - | ND | - |
| Cd | ND | ND | - | - | ND | - | - | ND | - |
| Co | ND | ND | - | - | ND | - | - | ND | - |
| Cu | ND | ND | - | - | ND | - | - | ND | - |
| Fe | ND | ND | - | - | ND | - | - | ND | - |
| Mn | ND | ND | - | - | ND | - | - | ND | - |
| Ni | ND | ND | - | - | ND | - | - | ND | - |
| Pb | ND | ND | - | - | ND | - | - | ND | - |
| Zn | ND | ND | - | - | ND | - | - | ND | - |

Values are mean ± SD of replicate analytical measurements obtained from the composite sample. Analytical readings were performed in triplicate and averaged prior to statistical analysis. Δ (mg/L) and Δ (%) were calculated using group means relative to the control. Different lowercase superscript letters (a, b, c, …) within the same row indicate statistically significant differences among treatment groups for each element ($p < 0.05$).

Within this operational framework, the pronounced decreases in dissolved Ca and Sr after plasma treatment may be associated with changes in carbonate-related equilibria and/or the formation of less soluble mineral phases that were subsequently removed by 0.45 µm filtration. In carbonate-rich aqueous systems, changes in $CO_2$ retention, pH and carbonate speciation can influence the tendency of calcium carbonate phases to form, thereby reducing the concentration of calcium measured in the filtered dissolved fraction (de P. Cosmo et al., 2019). A similar explanation may be relevant for Sr, since Sr can be incorporated into or co-precipitated with calcium carbonate phases during crystallization processes (Hodkin et al., 2018; Littlewood et al., 2017). However, this interpretation should be considered tentative because pH, $CO_2$ content, alkalinity, conductivity, ionic strength, particle formation and mineral speciation were not directly measured in the present study.

The increases observed for dissolved K and Mg may likewise reflect plasma-induced changes in the aqueous environment, including possible shifts in ion distribution between soluble and filter-retained fractions. Reactive oxygen and nitrogen species generated during plasma-liquid interactions can modify the physicochemical properties of water-based systems, but their specific contribution to the mineral changes observed here cannot be resolved from the available data (Simon et al., 2022). Therefore, the present results demonstrate treatment-associated changes in the operationally defined dissolved mineral fraction, but they do not allow a definitive mechanistic explanation. Accordingly, the carbonate-equilibrium and precipitation-based explanation proposed here should be regarded as a plausible hypothesis derived from the observed changes in the filtered dissolved fraction, rather than as a confirmed mechanism. Future studies should include

direct monitoring of pH, $CO_2$ content, alkalinity, conductivity, carbonate speciation and particle-associated mineral fractions to clarify the mechanisms underlying these element-specific responses.

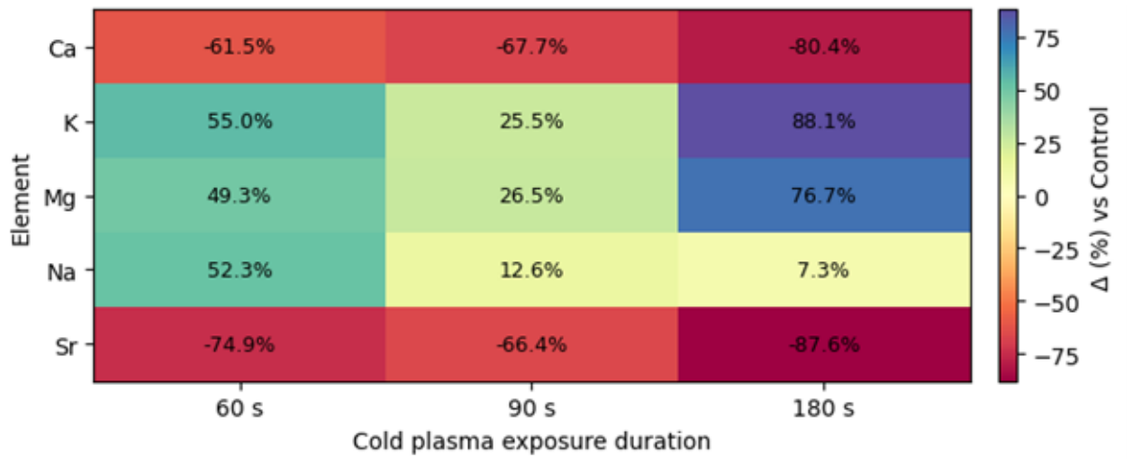


Figure 2. Heatmap of relative changes (Δ, %) in dissolved elemental concentrations (0.45 μm-filtered fraction) following cold plasma treatment (60, 90, 180 s) compared with the control

**Effect of cold plasma treatment on in-vitro mineral bioaccessibility**

Table 2 and Figure 3 present in-vitro mineral bioaccessibility values obtained after static digestion. Calcium bioaccessibility remained relatively stable across treatments (61.59-64.85%), despite the pronounced decrease in dissolved Ca concentration measured prior to digestion. Potassium and magnesium showed higher bioaccessible fractions in plasma-treated samples than in the control, with ranges of 70.88-78.58% for K and 77.95-83.57% for Mg. Sodium bioaccessibility increased modestly from 72.28% in the control to 75.16-78.46% after plasma exposure. Overall, the results indicate that cold plasma treatment can modify both the dissolved mineral concentrations measured in the filtered fraction and the extent to which selected minerals are released into the soluble fraction under simulated gastrointestinal conditions.

Table 2. In-vitro mineral bioaccessibility rates

| Element | Control | 60 s plasma-treated | 90 s plasma-treated | 180 s plasma-treated |
|---|---|---|---|---|
| Ca | $61.59\pm0.80^{aD}$ | $61.60\pm2.96^{aB}$ | $64.12\pm1.22^{aC}$ | $64.85\pm5.29^{aB}$ |
| K | $70.88\pm2.16^{bC}$ | $71.78\pm2.77^{bAB}$ | $77.45\pm1.84^{abB}$ | $78.58\pm0.34^{aA}$ |
| Mg | $77.95\pm2.83^{aA}$ | $78.03\pm4.90^{aA}$ | $81.64\pm0.63^{aA}$ | $83.57\pm1.58^{aA}$ |
| Na | $72.28\pm1.25^{bB}$ | $75.16\pm0.71^{abA}$ | $76.05\pm0.64^{abB}$ | $78.46\pm1.66^{aA}$ |

Values are expressed as mean ± SD of replicate analytical measurements obtained from the composite sample. Lowercase superscript letters (a, b, c…) indicate row-wise comparisons among treatment durations within the same mineral; different lowercase letters in the same row indicate statistically significant differences in the bioaccessibility of that mineral as affected by plasma treatment time ($P<0.05$). Uppercase superscript letters (A, B, C…) indicate column-wise comparisons among different minerals within the same treatment group; different uppercase letters in the same column indicate statistically significant differences among minerals under the same treatment condition ($P<0.05$). This dual-letter notation was used to present both the effect of plasma treatment duration on each mineral and the relative differences in bioaccessibility among minerals within each treatment group.

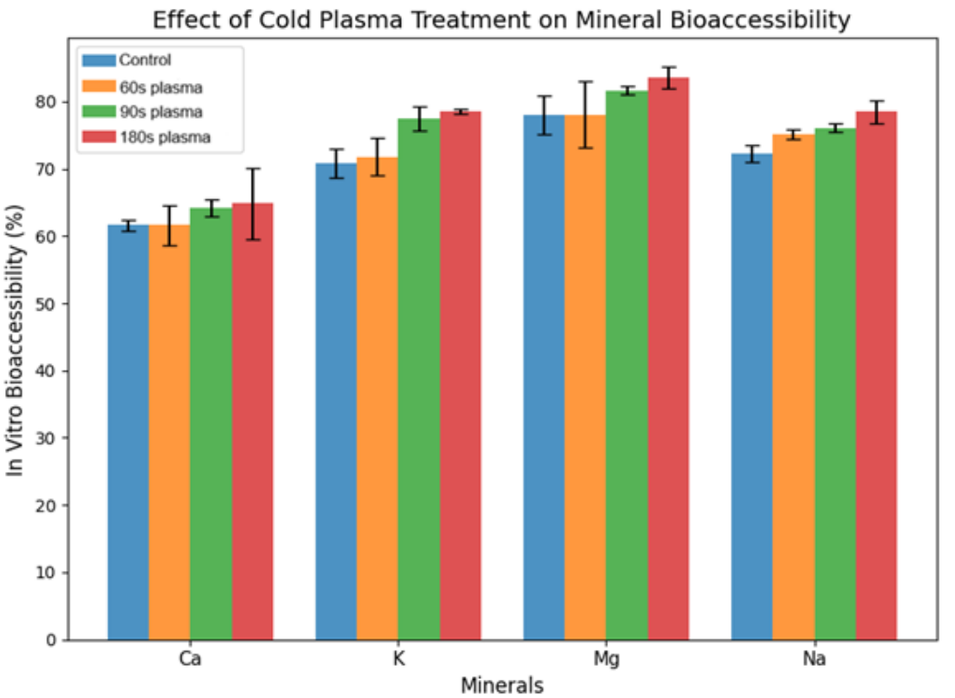


Figure 3. Effect of atmospheric cold plasma treatment on in vitro mineral bioaccessibility of carbonated mineral water samples

The digestion results emphasize that pre-digestion dissolved concentration and post-digestion bioaccessibility do not necessarily change in parallel in static gastrointestinal simulations (Grundy et al., 2024; Minekus et al., 2014). The stability of Ca bioaccessibility (61.59-64.85%) despite substantial reductions in pre-digestion dissolved Ca suggests that digestion conditions (acidification, ionic strength, and the presence of bile salts and enzymes) may re-solubilize Ca-containing forms that were not present as dissolved species prior to digestion or were removed by filtration (Minekus et al., 2014). This aligns with the broader evidence that apparent mineral bioaccessibility is strongly influenced by gastrointestinal pH and model conditions, and therefore should be interpreted as an operational endpoint rather than a direct proxy of in vivo absorption (Grundy et al., 2024)

The comparatively higher bioaccessible fractions observed for K and Mg in treated samples are consistent with the generally high solubility of these ions under simulated gastrointestinal conditions; however, the present study cannot attribute these outcomes to a specific physicochemical mechanism without speciation and buffering measurements (Grundy et al., 2024).

Two limitations are central. First, bioaccessibility estimated from a static in vitro digestion provides a standardized, practical screening endpoint but does not equate to in vivo bioavailability (Grundy et al., 2024; Minekus et al., 2014). Second, the lack of concurrent monitoring (e.g., pH, $CO_3$ content/alkalinity, conductivity, and speciation/particle-associated fraction) restricts mechanistic inference and limits the transferability of the findings to technological optimization (Simon et al., 2022). Accordingly, the present results should be framed as a preliminary assessment of process-induced shifts in the dissolved mineral profile and in vitro release behavior, rather than a definitive evaluation of nutritional impact.

**Trace metals (heavy metals)**

All monitored trace metals (Ag, Al, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) were below the method detection/quantification limits in all treatments (Table 1). Accordingly, within the analytical sensitivity of the present ICP-OES method and for the dissolved (<0.45 µm) fraction, no evidence was found that cold plasma increased these metals in carbonated mineral water. However, "non-detected" should be interpreted as <LOD (or <LOQ) rather than absolute absence.

Although all monitored trace metals were below the method LOD/LOQ for the dissolved fraction, "non-detected" should

be interpreted as <LOD (or <LOQ) and not as absolute absence (EPA, 2022). Demonstrating compliance with very low maximum limits would require method-specific LOQs aligned with the relevant standards and, where necessary, more sensitive instrumentation. The conclusion that is supported here is therefore limited but clear: within the analytical sensitivity of the current ICP-OES method, cold plasma treatment did not measurably increase dissolved trace metal concentrations in the tested carbonated mineral water.

## CONCLUSION

This study examined the influence of atmospheric cold plasma treatment on the dissolved mineral composition (0.45 µm-filtered fraction) and in-vitro bioaccessibility of a composite sample representing commercially available carbonated mineral water brands in Turkiye. Plasma processing at different exposure times led to clear, element-specific changes in the mineral profile, with pronounced reductions in calcium and strontium concentrations and moderate increases in potassium and magnesium levels. In the static in-vitro digestion model, calcium bioaccessibility remained relatively stable, whereas potassium and magnesium exhibited higher bioaccessible fractions in plasma-treated samples compared with the untreated control. Sodium showed a less consistent pattern, indicating that cold plasma treatment can induce time-dependent and mineral-specific responses rather than a uniform trend across all elements.

Taken together, these findings indicate that cold plasma can modify both the concentration and in-vitro bioaccessibility of selected minerals in carbonated mineral water, but the nutritional consequences of these changes are not straightforward. The observed decrease in calcium and strontium may be unfavourable from a dietary perspective, while the increased bioaccessible fractions of potassium and magnesium could be regarded as potentially beneficial. Without detailed information on pH shifts, $CO_2$ loss, precipitation phenomena and mineral speciation during treatment, however, the mechanisms behind these shifts remain speculative. In addition, the use of a simplified static digestion model, the focus on a single product type and processing configuration, and the lack of in vivo data limit the extent to which the present results can be directly extrapolated to human mineral bioavailability or to other beverage matrices. The present work should therefore be considered a preliminary assessment of process-induced changes rather than definitive evidence of improved mineral delivery.

From a technological standpoint, cold plasma remains a promising non-thermal option for beverage processing, but its application to mineral waters requires cautious optimisation. Future research should include a more comprehensive physicochemical characterisation of plasma-treated systems, including pH, carbonate equilibrium, potential precipitation and the formation of reactive oxygen and nitrogen species, as well as more detailed speciation and stability studies during storage. Extending the investigation to different mineral water brands and compositions, and integrating more advanced in-vitro digestion models, would help to clarify the robustness and generalisability of the observed effects. Ultimately, in vivo studies will be necessary to determine whether plasma-induced modifications in mineral composition and in-vitro bioaccessibility translate into meaningful nutritional and functional benefits in commercial mineral water products.

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## CONFLICT OF INTEREST

The authors declare no competing interests.

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