

EVALUATION OF IN-VITRO MINERAL BIOACCESSIBILITY OF COMMONLY CONSUMED VEGETABLES

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ABSTRACT

Vegetables are vital sources of dietary minerals, particularly for individuals following plant-based diets. However, the total mineral content does not necessarily reflect its bioaccessibility, which determines the proportion available for absorption in the gastrointestinal tract. This study evaluates the elemental composition and in-vitro mineral bioaccessibility of commonly consumed vegetables, including spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), broccoli (*Brassica oleracea*), and parsley (*Petroselinum crispum*). Mineral concentrations of K, Mg, Ca, Na, Fe, Mn, and Zn were quantified using ICP-OES following microwave-assisted digestion. An in-vitro digestion model simulated gastric and intestinal conditions. The highest Fe bioaccessibility was 18.82%, while K, Mg, and Ca were most bioaccessible in cucumber (21.9%), lettuce (25.09%), and parsley (23.42%), respectively. These findings highlight the importance of considering bioaccessibility in nutritional assessments. Future studies should investigate the effects of cooking methods and soil composition on mineral bioavailability to improve dietary recommendations.

Keywords: Mineral bioaccessibility, In-vitro digestion, Nutritional bioavailability, Dietary minerals

YAYGIN OLARAK TÜKETİLEN SEBZELERİN *İN-VİTRO* MİNERAL BİYOERİŞİLEBİLİRLİĞİNİN DEĞERLENDİRİLMESİ

ÖZ

Sebzeler, özellikle bitkisel bazlı diyetleri takip eden bireyler için önemli mineral kaynaklarıdır. Ancak toplam mineral içeriği, minerallerin mide-bağırsak sisteminde emilim için ne kadar erişilebilir olduğunu ifade eden biyoerişilebilirliği doğrudan yansıtmaz. Bu çalışma, yaygın olarak tüketilen ıspanak (*Spinacia oleracea*), marul (*Lactuca sativa*), salatalık (*Cucumis sativus*), brokoli (*Brassica oleracea*), ve maydanoz (*Petroselinum crispum*) gibi sebzelerin elementel kompozisyonu ve in-vitro mineral biyoerişilebilirliğini değerlendirmektedir. K, Mg, Ca, Na, Fe, Mn ve Zn konsantrasyonları, mikrodalga destekli sindirim sonrası ICP-OES kullanılarak analiz edilmiştir. *İn-vitro* sindirim modeli, mide ve bağırsak koşullarını simüle etmiştir. En yüksek Fe biyoerişilebilirliği %18.82, en yüksek K, Mg ve Ca biyoerişilebilirlikleri sırasıyla salatalık (%21.9), marul (%25.09) ve maydanozda (%23.42) gözlemlenmiştir. Bu sonuçlar, beslenme değerlendirmelerinde biyoerişilebilirliğin dikkate alınması

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gerektiğini vurgulamaktadır. Gelecek çalışmalar, pişirme yöntemleri ve toprak bileşiminin mineral biyoyararlanımı üzerindeki etkilerini incelemelidir.

Anahtar kelimeler: Mineral biyoerişilebilirliği, İn-vitro sindirim, Besinsel biyoyararlanım, Diyet mineralleri

INTRODUCTION

Nutrition plays a fundamental role in maintaining overall health, supporting physiological functions, and enhancing quality of life. Adequate intake of vitamins and minerals is essential for sustaining metabolic activities and preventing nutritional deficiencies (Song et al., 2020; Tokaloğlu, 2023). In recent years, vegan and vegetarian dietary patterns have gained increasing attention due to their potential health benefits, environmental sustainability, and ethical considerations (Song et al., 2020). These diets primarily rely on plant-based foods, which are rich in bioactive compounds and essential nutrients (Shi et al., 2022).

Among these, spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), broccoli (*Brassica oleracea*), and parsley (*Petroselinum crispum*) are widely consumed due to their high concentrations of essential minerals, including calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K), and zinc (Zn) (Aghili et al., 2009; Atta-Aly, 1999; do Nascimento da Silva Cadore, 2019; Ismail et al., 2024; Lafarga et al., 2019). These minerals are crucial for various biological processes, such as bone development, enzymatic reactions, oxygen transport, and immune system regulation. However, despite their high mineral content, not all of these nutrients are fully absorbed by the human body (Liu et al., 2018).

Bioaccessibility, defined as the fraction of a nutrient released from the food matrix during digestion and made available for intestinal absorption, is a key factor in determining the actual nutritional value of plant-based foods (Liu et al., 2018). Several factors influence the bioaccessibility of minerals, including the presence of antinutritional compounds such as oxalates, phytates, tannins, and dietary fibers, which can bind minerals and reduce their intestinal absorption (Shi et al., 2022). Additionally, cooking methods, soil composition, and cultivation practices significantly impact

mineral bioavailability. Therefore, measuring the total mineral content of foods alone does not accurately reflect their true nutritional contribution (Ololade et al., 2025).

To address this issue, in-vitro digestion models have been developed to simulate gastrointestinal conditions and assess the extent to which minerals are released and absorbed in the intestines (Fernández-García et al., 2009). These models provide valuable insights into the gastric and intestinal solubility of minerals under controlled conditions. The Unified Bioaccessibility Method (UBM) and other in-vitro digestion techniques have been widely employed to evaluate mineral bioaccessibility in various fruits, vegetables, and food products (Tokaloğlu, 2023). Previous studies suggest that certain minerals, such as Mn and Zn, exhibit higher bioaccessibility, whereas Fe and Cu tend to form insoluble complexes with dietary fibers and polyphenols, resulting in lower absorption rates (Buturi et al., 2021; Fernández-García et al., 2009).

In-vitro digestion models have demonstrated that the bioaccessibility of minerals in leafy green vegetables varies depending on multiple factors. A study on spinach revealed that the bioaccessibility of iron (Fe), zinc (Zn), and calcium (Ca) decreased with plant maturation, whereas lactic acid fermentation significantly enhanced their bioaccessibility. Following fermentation, Fe bioaccessibility increased from 19.6% to 26.7%, Zn from 32.4% to 41.6%, and Ca from 19% to 33.9% (Ismail et al., 2024). Similarly, a study on lettuce reported Fe bioaccessibility at approximately 10%, while Zn bioaccessibility reached up to 80%. The bioaccessibility of Mn was determined as 52%, whereas Cu and Mo exhibited bioaccessibility levels exceeding 70%. Selenium (Se) bioaccessibility varied depending on the form of supplementation, reaching 22% in selenite-enriched samples, whereas it was as low as 6% in selenate-containing lettuce (do Nascimento da Silva Cadore, 2019).

Furthermore, a study conducted by Amalraj Pius, (2015) investigated Ca bioaccessibility in raw and cooked leafy vegetables and identified oxalate as the most significant inhibitor of Ca absorption. The same study demonstrated that phytates (PA), tannins (TA), and dietary fiber also negatively impact mineral absorption. These findings highlight the necessity of considering fermentation and cooking processes as effective strategies to enhance mineral bioaccessibility, emphasizing that the nutritional value of vegetables should not be assessed solely based on their total mineral content.

Given the rising prevalence of plant-based diets and the significant role of green vegetables in human nutrition, it is essential to determine the bioaccessibility of key minerals in commonly consumed vegetables. This study aims to evaluate the in-vitro bioaccessibility of Ca, Fe, Mg, Mn, Na, K, and Zn in frequently consumed vegetables, including spinach, lettuce, cucumber, broccoli, and parsley. The findings will provide a comprehensive assessment of how these vegetables contribute to daily mineral intake. Additionally, the study will explore the impact of soil composition and agricultural practices on mineral bioavailability, offering insights into how environmental factors influence nutrient retention in fresh produce. Therefore, region-specific studies gain considerable importance when evaluating mineral bioaccessibility.

Also, this research aims to provide valuable insights for dietitians, nutritionists, and consumers regarding the actual mineral contribution of leafy greens in plant-based diets. The results will help optimize dietary recommendations, ensuring adequate mineral intake for individuals following vegan, vegetarian, or predominantly plant-based diets.

MATERIALS AND METHODS

Materials

This study utilized spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), broccoli (*Brassica oleracea*), and parsley as representative green vegetables. To ensure a diverse mineral composition, samples were

obtained from three different locations in distinct agricultural areas within Çorum province, Türkiye. Specifically, lettuce, parsley, and cucumber were sourced from organic greenhouse farms in the central district, while broccoli and spinach were obtained from semi-controlled field cultivation areas in the central and Mecitözü districts. This sampling strategy was designed to reflect variations in cultivation practices and soil characteristics. All vegetables were harvested at the beginning of the winter season (December 2023), ensuring consistency in growth conditions and nutrient accumulation. Prior to mineral analysis, the samples were dried, and their moisture content was recorded for subsequent bioaccessibility calculations. Since spinach is commonly consumed after boiling, in-vitro mineral bioaccessibility analysis for spinach was performed using boiled (with ultra-pure water) samples, prepared following traditional cooking methods (100 °C, 20 min). The soil analysis results for the cultivation of lettuce, parsley, and cucumber were provided by the producers, who had the analyses performed in accredited agricultural laboratories prior to planting. According to the laboratory reports, potassium (K₂O) content was 57.7614 kg/da, phosphorus (P₂O₅) was 0.19 kg/da, lime content was 6.675%, organic matter content was 1.2573%, total salt content was 0.0158%, pH level was 8.15, and soil saturation was 38.83%.

Methods

Moisture Content Determination

Moisture content was determined following the methodology (International et al., 2012). Petri dishes were pre-dried in an oven at 105 °C for 1 hour, cooled in a desiccator for 30 minutes, and weighed using a precision balance (± 0.1 mg sensitivity). Approximately 4–5 g of homogenized vegetable samples were placed in the pre-weighed petri dishes, and their initial weights were recorded. The samples were then dried at 105 °C for 5–6 hours until a constant weight was achieved. After cooling to room temperature in a desiccator, the final weights were recorded, and the moisture content was calculated using

Equation 1:

$$\% \text{Moisture} = \frac{((\text{Initial Weight (g)} - \text{Final Weight (g)}) / \text{Initial Weight (g)}) \times 100}{\text{Eq. 1.}}$$

Elemental Composition Analysis

Quantification of Ca, Fe, Mg, Mn, Na, K, and Zn. The concentrations of calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), potassium (K), and zinc (Zn) were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Prior to analysis, vegetable samples were digested using a microwave-assisted acid digestion system.

Table 1. Microwave Digestion Conditions

Stage	Temperature (°C)	Pressure (bar)	Time (min)	Power (%)
1	170	30	5	80
2	200	35	15	90
3	50	25	10	0

Upon completion of digestion, the clear supernatant solutions were transferred into polypropylene tubes for elemental analysis.

Instrument Settings

ICP-OES analysis was performed at specific emission wavelengths for each mineral: Ca: 317.9 nm, Fe: 259.9 nm, Mg: 279.5 nm, Mn: 257.6 nm, Na: 588.9 nm, K: 766.5 nm and Zn: 213.8 nm.

A multi-element standard solution was used to generate calibration curves, and necessary dilutions were prepared using a 5% HNO₃ solution (Sezer et al., 2018). The elemental concentrations in vegetable samples were calculated using Equation 2:

$$A \left(\frac{\text{mg element}}{\text{kg vegetable}} \right) = \frac{\left(B \left(\frac{\text{mg element}}{\text{L solution}} \right) * C \text{ ml solution} \right)}{D \text{ g vegetable}} * \frac{1 \text{ L}}{1000 \text{ ml}} * \frac{1000 \text{ g}}{1 \text{ kg}} * \text{DF}$$

A: Mineral concentration in vegetable samples (mg/kg)

B: Measured ICP-OES reading (mg/L)

C: Volume of digestion solvent used (6 mL HNO₃ + 2 mL H₂O₂)

D: Sample weight (g)

DF: Dilution factor

Eq. 2.

Microwave Digestion Protocol

Vegetable samples were homogenized and oven-dried at 105 °C for 1 hour. A precisely weighed portion (0.3 g, ±0.01 g accuracy) of the dried sample was transferred into Teflon digestion vessels. A digestion mixture of 6 mL of 65% (m/V) HNO₃ and 2 mL of 30% (m/V) H₂O₂ was added, and the vessels were left undisturbed for 10 minutes to allow gas release before initiating microwave digestion. The digestion process was carried out in three sequential stages (Table 1), following the procedure (Sezer et al., 2018).

In-Vitro Mineral Bioaccessibility Assessment

The experimental procedure for in-vitro mineral bioaccessibility assessment is illustrated in Figure 1.

To assess mineral bioaccessibility, 2.5 g of dried vegetable samples were accurately weighed (±0.01 g sensitivity) and placed in polypropylene Falcon tubes. A total of 25 mL of deionized water was added. To simulate gastric conditions, the pH was adjusted to 2.0 using 0.1 N HCl before adding pepsin enzyme. Pepsin (Sigma-Aldrich, Product No: P-7000, USA) was prepared by dissolving 0.2 g in 5 mL of 0.1 N HCl (Etcheverry et al., 2004). A 0.125 mL aliquot of prepared pepsin solution was introduced into each sample, followed by incubation in a shaking water bath at 37 °C for 2 hours.

Subsequently, the pH was adjusted to 6.8–7.0 using 1 M NaHCO₃ solution, and 0.35 mL of pancreatin enzyme solution (Sigma-Aldrich, Product No: P-1750, USA) was added. The samples were further incubated at 37 °C for 4 hours to simulate intestinal digestion (Aribas et al., 2020).

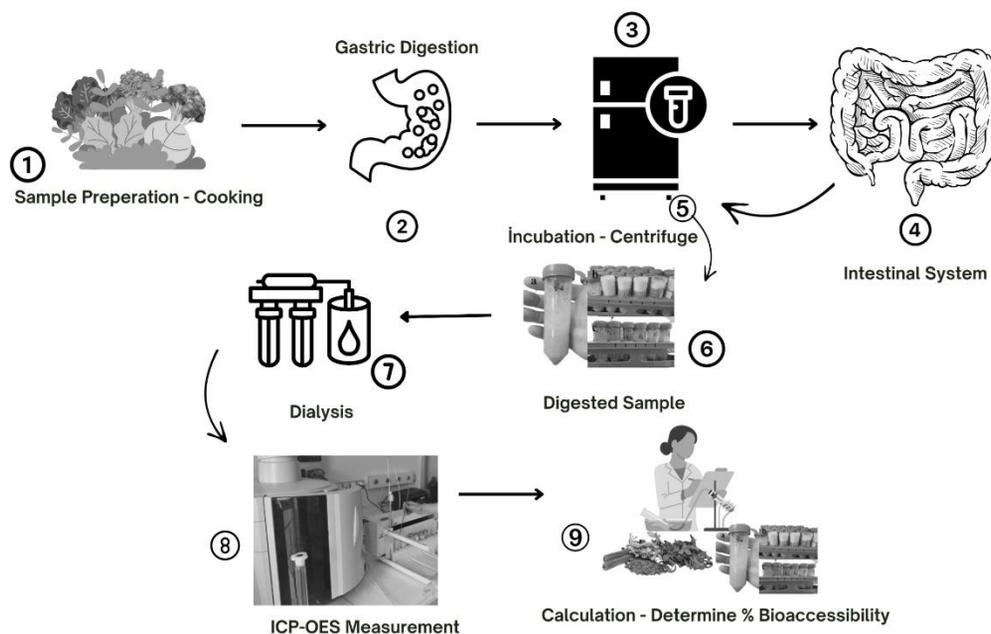


Figure 1. The experimental procedure for *in vitro* mineral bioaccessibility

After digestion, samples were diluted to 50 mL with ultrapure water, centrifuged at $15,100 \times g$ for 20 minutes, and filtered sequentially through Whatman filter paper and $0.45 \mu\text{m}$ syringe filters before ICP-OES analysis. Mineral bioaccessibility was calculated using Equation 3 (Klepacka et al., 2020):

$$\% \text{ Mineral Bioaccessibility} = \frac{\text{Dialyzable mineral concentration (ppm)}}{\text{Total mineral concentration in sample (ppm)}} \times 100$$

Eq. 3.

Statistical Analysis

All data were analyzed using IBM SPSS Statistics 22.0 software. One-way analysis of variance (ANOVA) was performed to determine significant differences among sample groups, with statistical significance set at $P < 0.05$. Duncan's Multiple Range Test was applied for post-hoc comparisons between group means (Apaydın et al., 2024).

RESULTS AND DISCUSSION

In this study, the elemental composition and *in vitro* mineral bioaccessibility of lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), broccoli (*Brassica oleracea*), parsley (*Petroselinum crispum*), and spinach (*Spinacia oleracea*) samples were evaluated.

The mineral concentrations of potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn), and zinc (Zn) were quantified. Additionally, soil analysis results from the gardens where lettuce, parsley, and cucumber were cultivated were assessed to gain a better understanding of the soil composition.

Elemental Composition and Moisture Content of Vegetables

Analysis of the elemental composition of vegetables revealed that spinach had the highest potassium content (72,944.49 ppm), followed by cucumber, lettuce, and parsley. The lowest potassium content was found in broccoli (3,607.28 ppm). Statistical comparison showed that all vegetables differed significantly in their potassium levels, with spinach (a) and broccoli (d) representing the extremes of the group ($P < 0.05$). These findings are consistent with those reported by Lafarga et al. (2019). In terms of magnesium content, spinach exhibited the highest concentration (6,930.38 ppm), followed by cucumber (3,448.51 ppm), parsley (733.58 ppm), and broccoli (481.95 ppm). Lettuce contained the lowest magnesium concentration (202.42 ppm), with statistically significant differences observed among all samples ($P < 0.05$). This is in agreement

with the results of Hayes et al., (2020) and Lafarga et al., (2019).

Regarding calcium content, cucumber contained the highest concentration (9,277.48 ppm), whereas lettuce and broccoli exhibited lower levels. Although spinach (4,230.85 ppm), broccoli (3,949.98 ppm), and parsley (3,608.66 ppm) formed a statistically similar group (b–cd), cucumber (a) differed significantly from all other samples ($P < 0.05$).

Sodium concentrations also showed significant variation among vegetables. Lettuce (397.38 ppm) had the highest Na content, followed by parsley (324.09 ppm), broccoli (291.7 ppm), and cucumber (255.06 ppm), while spinach had the lowest value (97.65 ppm) ($P < 0.05$).

For iron content, parsley (113.17 ppm) exhibited by far the highest Fe concentration, significantly exceeding all other vegetables ($P < 0.05$).

Cucumber (58.29 ppm) also had a notably higher Fe level compared to spinach, lettuce, and broccoli, which showed similarly low values ranging from 5.55 to 10.11 ppm. Manganese concentrations differed markedly across the vegetable types. Broccoli (99.39 ppm) had the highest Mn content, followed by parsley (64.91 ppm) and cucumber (29.88 ppm), whereas spinach (9.73 ppm) and lettuce (4.03 ppm) had the lowest values ($P < 0.05$). Zinc content was highest in spinach (102.92 ppm), followed by cucumber (53.26 ppm), parsley (50.11 ppm), broccoli (39.86 ppm), and lettuce (2.96 ppm). Spinach formed a distinct group (a), statistically higher than all other vegetables in Zn content ($P < 0.05$). These patterns are in general agreement with previously published findings (Shi et al., (2022) and highlight the diversity in elemental uptake among green vegetables. The element content analysis results are presented in Table 2.

Table 2. Element Contents of Vegetables (ppm)

	Spinach	Lettuce	Cucumber	Broccoli	Parsley
K	72944.49±1274.75 ^a	32740.96±400.23 ^c	57913.02±1239.56 ^b	3607.28±137.89 ^d	34080.94±503.66 ^c
Mg	6930.38±3.08 ^a	202.42±8.43 ^c	3448.51±137.79 ^b	481.95±13.56 ^d	733.58±11.68 ^c
Ca	4230.85±7.32 ^b	3458.31±72.15 ^d	9277.48±304.99 ^a	3949.98±48.81 ^{bc}	3608.66±64.27 ^{cd}
Na	97.65±3.68 ^c	397.38±6.77 ^a	255.06±3.55 ^d	291.7±6.81 ^c	324.09±14.61 ^b
Fe	10.11±0.12 ^c	6.58±0.15 ^c	58.29±0.06 ^b	5.55±0.02 ^c	113.17±4.44 ^a
Mn	9.73±0.36 ^d	4.03±0.03 ^c	29.88±1.3c	99.39±0.53 ^a	64.91±1.07 ^b
Zn	102.92±2.38 ^a	2.96±0.06 ^d	53.26±0.27 ^b	39.86±0.3 ^c	50.11±1.01 ^b

The differences between results indicated by different letters in the same row are statistically significant ($P < 0.05$; $n = 3$)

The similarity of elemental composition values across different geographical locations suggests that, despite variations in cultivation conditions, the elemental profiles of vegetables remain relatively stable. Consequently, the in-vitro mineral bioaccessibility results obtained in this study may be applicable beyond the regional scope and could have broader global relevance.

Also, moisture content plays a crucial role in determining mineral bioaccessibility and overall elemental composition in vegetables. The measured moisture content values in this study were consistent with previously reported data (Liu et al., 2018).

Spinach had the highest moisture content (92.83%), while parsley exhibited the lowest (85.27%) (Table 3). Moisture levels influence the solubility and bioavailability of minerals during digestion, impacting their overall absorption efficiency in the human gastrointestinal tract (Liu et al., 2018).

Table 3. The Moisture Content of Vegetables

Spinach	92.83% ± 1.14
Lettuce	92.14% ± 0.83
Cucumber	92.14% ± 0.82
Broccoli	91.23% ± 1.82
Parsley	85.27% ± 0.56

In-Vitro Mineral Bioaccessibility of Vegetables

Table 4. In-Vitro Mineral Bioaccessibility (%)

	Spinach	Lettuce	Cucumber	Broccoli	Parsley
K	21.41±0.63 ^{aB}	17.65±0.03 ^{bC}	21.9±1.46 ^{aC}	21.71±0.2 ^{aDE}	21.11±0.46 ^{aB}
Mg	22.81±1.22 ^{a^bB}	25.09±1.06 ^{aB}	20.63±0.96 ^{bC}	24.14±0.04 ^{aCD}	23.84±0.33 ^{aB}
Ca	20.54±0.63 ^{bcBC}	19.26±1.19 ^{cC}	22.84±0 ^{abC}	20.07±0.25 ^{cE}	23.42±1.05 ^{aB}
Na	24.09±2.95 ^{aB}	22.84±0.63 ^{aB}	22.07±0.7 ^{aC}	25.6±0.03 ^{aC}	25.52±0.19 ^{aAB}
Fe	15.4±0.64 ^{aC}	16.58±1.6 ^{aC}	15.15±0.6 ^{aD}	18.82±2.34 ^{aE}	16.3±1.14 ^{aC}
Mn	31.16±3.09 ^{abA}	25.13±0.02 ^{bB}	32.78±0.76 ^{aA}	30±1.34 ^{a^bA}	29.97±2.8 ^{abA}
Zn	26.3±1.18 ^{aAB}	28.83±0.03 ^{aA}	26.59±1.47 ^{aB}	27.5±1.06 ^{aAB}	25.67±2.21 ^{aAB}

Differences in the same row are indicated by lowercase letters (a, b, c...), while differences in the same column are represented by uppercase letters (A, B, C...). Differences between results marked with different letters are statistically significant ($P < 0.05$; $n = 3$).

A key objective of this study was to assess the in-vitro bioaccessibility of minerals, which represents the proportion of minerals available for absorption after digestion (Table 4). The findings indicated that K and Mg exhibited the highest bioaccessibility rates, with cucumber demonstrating the highest K bioaccessibility (21.9%) and lettuce displaying the highest Mg bioaccessibility (25.09%). Among the analyzed vegetables, parsley exhibited the highest Ca bioaccessibility (23.42%).

Post-digestion Fe bioaccessibility ranged between 15.15% and 18.82%, highlighting the limited bioavailability of iron from plant-based sources. These values are consistent with earlier in-vitro studies on spinach and broccoli, which reported iron bioaccessibility typically below 20% (Rodríguez-Ramiro et al., 2019). The highest Mn bioaccessibility was observed in cucumber (32.78%), while Zn bioaccessibility was highest in lettuce (28.83%). These results are in agreement with the findings of Ismail et al., (2024). Similar high Mn availability was also reported in green vegetables by Choleva et al., (2023), while Zn bioaccessibility exceeding 25% is considered favorable and has been associated with low oxalate and phytate content, as seen in lettuce (do Nascimento da Silva Cadore, 2019).

The observed variations in mineral bioaccessibility are largely influenced by antinutritional factors such as oxalates, phytates, and tannins, which are known inhibitors of

essential mineral absorption, particularly Fe and Zn (do Nascimento da Silva et al., 2015). In particular, the poor Fe and Ca bioaccessibility of spinach has been previously attributed to its high oxalate content, which forms insoluble complexes with divalent minerals (Singh et al., 2016). Additionally, differences in soil composition, fertilization practices, and agricultural techniques may contribute to these variations (Lafarga et al., 2019).

One of the most significant aspects of this study is that it evaluates not only the total mineral content of vegetables but also their in-vitro bioaccessibility, providing a more accurate assessment of the fraction of minerals available for absorption. While many nutritional studies focus solely on total mineral content, these values do not necessarily reflect the bioavailable fraction, as some minerals are bound to indigestible compounds and thus remain inaccessible for absorption (Liu et al., 2018). For instance, Ceccanti et al., (2022) reported that while potassium is generally highly bioaccessible in vegetables due to its ionic form, the actual K bioaccessibility can still vary widely depending on tissue water content and structure. The present finding of ~22% K bioaccessibility in cucumber is within the expected range.

Research on in-vitro mineral bioaccessibility in vegetables remains limited, making this study particularly valuable. The present study offers one of the most comprehensive evaluations of mineral

bioaccessibility in commonly consumed vegetables, considering the effects of different soil compositions on elemental content. A meta-review by Muleya et al., (2024) showed that calcium bioaccessibility in green vegetables can range from <10% (in spinach) to >70% (in low-oxalate crucifers), confirming the importance of matrix-specific evaluation. This is especially relevant for plant-based diets, where vegetables serve as primary mineral sources, yet their bioaccessibility is often lower than that of animal-based foods (Doniec et al., 2022; Tokaloğlu, 2023).

Moreover, by analyzing vegetables obtained from different producers, this study ensures greater variability and enhances the generalizability of the results. The findings provide a critical reference for dietary planning, particularly in plant-based nutrition, underscoring the need to consider both mineral content and bioaccessibility when evaluating the nutritional value of vegetables (Doniec et al., 2022; Hayes et al., 2020; Khouzam et al., 2011). In this context, the current study contributes a novel regional dataset on in-vitro bioaccessibility from Türkiye, which may serve as a reference for further dietary optimization efforts.

CONCLUSION

This study provides a comprehensive assessment of the elemental composition and in-vitro mineral bioaccessibility of frequently consumed green vegetables, including spinach, lettuce, cucumber, broccoli, and parsley. The findings emphasize the importance of evaluating mineral bioaccessibility rather than relying solely on total mineral content, as the actual fraction of minerals available for absorption varies significantly among different vegetables.

Spinach exhibited the highest potassium and magnesium concentrations, whereas cucumber contained the highest calcium levels. However, despite their high mineral content, not all minerals were equally bioaccessible. Iron bioaccessibility was relatively low, consistent with well-documented challenges in Fe absorption from plant-based sources. Manganese and zinc

demonstrated moderate bioaccessibility, with cucumber and lettuce showing the highest values, respectively. These results underscore the necessity of evaluating mineral bioaccessibility in plant-based diets, as total mineral content alone does not accurately reflect the nutritional contribution of vegetables.

From a nutritional perspective, mineral bioaccessibility should be considered when formulating dietary recommendations, particularly for individuals following plant-based diets. This study highlights the importance of selecting vegetables not only based on their total elemental composition but also on their bioaccessibility levels.

Furthermore, this research contributes to the growing body of literature on the impact of agricultural conditions, soil composition, and food processing techniques on mineral bioavailability. Future studies should explore the influence of cooking methods and soil variations on mineral bioaccessibility to optimize dietary strategies and enhance mineral intake, particularly in populations reliant on plant-based nutrition.

Overall, this study provides a valuable reference for dietitians, nutritionists, and food scientists, offering insights into the actual nutritional value of commonly consumed green vegetables and reinforcing the significance of bioaccessibility studies in food science.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest

AUTHOR CONTRIBUTIONS

Hakan APAYDIN: Investigation, writing – original draft, review editing, Methodology, validation; Ahmet Serhat AFŞAR: Writing, investigation, visualisation

REFERENCES

- Aghili, F., Khoshgoftarmanesh, A. H., Afyuni, M., Mobli, M. (2009). Relationships between fruit mineral nutrients concentrations and some fruit quality attributes in greenhouse cucumber. *Journal of Plant Nutrition*, 32(12), 1994–2007. <https://doi.org/10.1080/01904160903308119>
- Amalraj, A., Pius, A. (2015). In vitro study on the bioavailability of calcium and its absorption inhibitors in raw and cooked pulses commonly consumed in India. *International Food Research Journal*, 22(4), 1525–1532.
- Apaydın, H., Demirci, M., Bölük, E., Kopuk, B., Palabiyik, I. (2024). Effect of different roasting conditions on the physicochemical properties, acrylamide concentration, and mineral bioaccessibility of nuts. *Food Bioscience*, 58, 103646. <https://doi.org/10.1016/j.fbio.2024.103646>
- Aribas, M., Kahraman, K., Koksel, H. (2020). Effects of resistant starch type 4 supplementation of bread on in vitro glycemic index value, bile acid-binding capacity, and mineral bioavailability. *Cereal Chemistry*, 97(2), 163–171. <https://doi.org/10.1002/cche.10229>
- Atta-Aly, M. A. (1999). Effect of nickel addition on the yield and quality of parsley leaves. *Scientia Horticulturae*, 82(1–2), 9–24. [https://doi.org/10.1016/S0304-4238\(99\)00032-1](https://doi.org/10.1016/S0304-4238(99)00032-1)
- Buturi, C. V., Mauro, R. P., Fogliano, V., Leonardi, C., Giuffrida, F. (2021). Mineral biofortification of vegetables as a tool to improve human diet. *Foods*, 10(2), 1–23. <https://doi.org/10.3390/foods10020223>
- Ceccanti, C., Guidi, L., D'Alessandro, C., Cupisti, A. (2022). Potassium Bioaccessibility in Uncooked and Cooked Plant Foods: Results from a Static In Vitro Digestion Methodology. *Toxins*, 14(10). <https://doi.org/10.3390/toxins14100668>
- Choleva, T. G., Tziasiou, C., Gouma, V., Vlessidis, A. G., Giokas, D. L. (2023). In Vitro Assessment of the Physiologically Relevant Oral Bioaccessibility of Metallic Elements in Edible Herbs Using the Unified Bioaccessibility Protocol. *Molecules*, 28(14). <https://doi.org/10.3390/molecules28145396>
- do Nascimento da Silva, E., Cadore, S. (2019). Bioavailability Assessment of Copper, Iron, Manganese, Molybdenum, Selenium, and Zinc from Selenium-Enriched Lettuce. *Journal of Food Science*, 84(10), 2840–2846. <https://doi.org/10.1111/1750-3841.14785>
- do Nascimento da Silva, E., Heerdt, G., Cidade, M., Pereira, C. D., Morgon, N. H., Cadore, S. (2015). Use of in vitro digestion method and theoretical calculations to evaluate the bioaccessibility of Al, Cd, Fe and Zn in lettuce and cole by inductively coupled plasma mass spectrometry. *Microchemical Journal*, 119, 152–158. <https://doi.org/10.1016/j.microc.2014.12.002>
- Doniec, J., Florkiewicz, A., Duliński, R., Filipiak-Florkiewicz, A. (2022). Impact of Hydrothermal Treatments on Nutritional Value and Mineral Bioaccessibility of Brussels Sprouts (*Brassica oleracea* var. *gemmifera*). *Molecules*, 27(6). <https://doi.org/10.3390/molecules27061861>
- Etcheverry, P., Wallingford, J. C., Miller, D. D., Glahn, R. P. (2004). Calcium, zinc, and iron bioavailabilities from a commercial human milk fortifier: A comparison study. *Journal of Dairy Science*, 87(11), 3629–3637. [https://doi.org/10.3168/jds.S0022-0302\(04\)73501-8](https://doi.org/10.3168/jds.S0022-0302(04)73501-8)
- Fernández-García, E., Carvajal-Lérida, I., Pérez-Gálvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research*, 29(11), 751–760. <https://doi.org/10.1016/j.nutres.2009.09.016>
- Hayes, M., Pottorff, M., Kay, C., Van Deynze, A., Osorio-Marin, J., Lila, M. A., Iorizzo, M., Ferruzzi, M. G. (2020). In Vitro Bioaccessibility of Carotenoids and Chlorophylls in a Diverse Collection of Spinach Accessions and Commercial Cultivars. *Journal of Agricultural and Food Chemistry*, 68(11), 3495–3505. <https://doi.org/10.1021/acs.jafc.0c00158>
- International, A., of official analytical chemists international, A., Horwitz, W., Latimer, ed,

- Latimer, G. W. (2012). *Official Methods of Analysis of AOAC International* (Issue 1. c.). AOAC International. <https://books.google.com.tr/books?id=kPe4NAEACAAJ>
- Ismail, T., Akhtar, S., Qamar, M., Esatbeyoglu, T., Sestili, P., Saeed, W., Lazarte, C. E. (2024). Effect of crop maturity stages and lactic acid fermentation on nutrient absorption properties, and bioaccessibility of Fe, Zn and Ca in *Spinacia oleracea* L. *Journal of Food Composition and Analysis*, 133(June), 106427. <https://doi.org/10.1016/j.jfca.2024.106427>
- Khouzam, R. B., Pohl, P., Lobinski, R. (2011). Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables. *Talanta*, 86(1), 425–428. <https://doi.org/10.1016/j.talanta.2011.08.049>
- Klepacka, J., Najda, A., Klimek, K. (2020). Effect of buckwheat groats processing on the content and bioaccessibility of selected minerals. *Foods*, 9(6). <https://doi.org/10.3390/foods9060832>
- Lafarga, T., Gallagher, E., Bademunt, A., Viñas, I., Bobo, G., Villaró, S., Aguiló-Aguayo, I. (2019). Bioaccessibility, physicochemical, sensorial, and nutritional characteristics of bread containing broccoli co-products. *Journal of Food Processing and Preservation*, 43(2), 1–11. <https://doi.org/10.1111/jfpp.13861>
- Liu, C., Wu, L., Xue, Y., Liu, F., Sun, S., Wang, L. (2018). Effect of cooking methods on bioaccessibility of Zn, Se, Cd, Cu in sea cucumber (*Apostichopus japonicus*). *Food Science and Biotechnology*, 27(3), 899–904. <https://doi.org/10.1007/s10068-017-0298-5>
- Muleya, M., F. Bailey, E., H. Bailey, E. (2024). A comparison of the bioaccessible calcium supplies of various plant-based products relative to bovine milk. *Food Research International*, 175, 113795. <https://doi.org/10.1016/j.foodres.2023.113795>
- Ololade, I. A., Akindumila, O. I., Oloyede, O. J., Asanga, O. P., Ololade, O. O., Oladoja, N. A. (2025). Exploring the influence of household cooking energy sources on trace metal bioavailability in cobwebs: A health risk assessment. *Journal of Environmental Chemical Engineering*, 13(2), 115673. <https://doi.org/10.1016/j.jece.2025.115673>
- Rodriguez-Ramiro, I., Dell'Aquila, C., Ward, J. L., Neal, A. L., Bruggaber, S. F. A., Shewry, P. R., Fairweather-Tait, S. (2019). Estimation of the iron bioavailability in green vegetables using an in vitro digestion/Caco-2 cell model. *Food Chemistry*, 301, 125292. <https://doi.org/10.1016/j.foodchem.2019.125292>
- Sezer, B., Apaydin, H., Bilge, G., Boyaci, I. H. (2018). Coffee arabica adulteration: Detection of wheat, corn and chickpea. *Food Chemistry*, 264(January), 142–148. <https://doi.org/10.1016/j.foodchem.2018.05.037>
- Shi, M., Gu, J., Wu, H., Rauf, A., Emran, T. Bin, Khan, Z., Mitra, S., Aljohani, A. S. M., Alhumaydhi, F. A., Al-awthan, Y. S., Bahattab, O. (2022). Health Benefits in Lettuce — A Comprehensive Review. *Antioxidants*, 11(1158), 23.
- Singh, A., Bains, K., Kaur, H. (2016). Effect of inclusion of key foods on in vitro iron bioaccessibility in composite meals. *Journal of Food Science and Technology*, 53(4), 2033–2039. <https://doi.org/10.1007/s13197-015-2154-z>
- Song, J., Huang, H., Hao, Y., Song, S., Zhang, Y., Su, W., Liu, H. (2020). Nutritional quality, mineral and antioxidant content in lettuce affected by interaction of light intensity and nutrient solution concentration. *Scientific Reports*, 10(1), 1–9. <https://doi.org/10.1038/s41598-020-59574-3>
- Tokalioğlu, Ş. (2023). Bioaccessibility of Cu, Mn, Fe, and Zn in Fruit and Vegetables by the In Vitro UBM and Statistical Evaluation of the Results. *Biological Trace Element Research*, 201(3), 1538–1546. <https://doi.org/10.1007/s12011-022-03253-z>