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In Vitro Study for The Bio accessibility of Micronutrients in Fortified Plant-Based Products and Meat Analogues

Aniq Ur Rehman[1], *Muhammad Sohaib Sarwar*[2] *Waheed Ahmad*[3*], *Azmat Ullah Khan*[1], *Qaisar Raza*[1], *Touseef Iqbal*[1],

[1]Department of Food Science & Human Nutrition, University of Veterinary & Animal Science, Syed Abdul Qadir Jilani Outfall Road, Lahore-54000, Punjab, Pakistan

[2] Department of food sciences, University of the Punjab Lahore

[3] National Institute of Food, Science and Technology, University of Agriculture, Faisalabad.

*Correspondence: (Email: waheed.ahmad28@gmail.com)

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Abstract

The global shift towards plant-based diets has heightened the need to critically evaluate the nutritional adequacy of meat and dairy alternatives beyond their macronutrient profile. While many products are fortified to match the micronutrient content of animal products, their bioavailability remains a significant concern. This study conducted a comparative analysis of the content and *in vitro* bioaccessibility of critical micronutrients iron, zinc, and vitamin B12 in commercial plant-based burgers (PBBs) and milks (PBMs) against their animal-based counterparts (beef burgers, BBs; bovine milk, BM). Using ICP-MS and HPLC for quantification and the INFOGEST 2.0 protocol to simulate digestion, we found that fortified PBBs contained significantly higher total iron than BBs but exhibited drastically lower iron bioaccessibility (25-32% vs. 58-63%, $p < 0.05$). This disparity is strongly correlated with phytic acid content and attributed to the inherent susceptibility of non-heme fortificants to inhibition within the complex plant matrix. Zinc bioaccessibility was similar between PBBs and BBs (~30-40%), though lower total zinc in PBBs resulted in a lower absolute bioaccessible amount. Conversely, in the liquid matrix of fortified PBMs, iron and zinc bioaccessibility was significantly higher than in PBBs and was statistically equivalent to BM. Vitamin B12 bioaccessibility was consistently high (>78%) across all fortified products and BM. These results reveal a critical "bioavailability gap" in solid plant-based analogues: high fortification levels do not guarantee sufficient absorption due to food matrix effects and anti-nutritional factors. The findings underscore an urgent need for the industry to adopt advanced strategies, such as selective fortificants, anti-nutrient reduction, and encapsulation technologies, to enhance micronutrient delivery and ensure the long-term nutritional efficacy of plant-based products.

Keywords: Plant-based alternatives, bioavailability, bioaccessibility, in vitro digestion, iron, zinc, vitamin B12, phytic acid, food matrix, fortification

1. Introduction

The global market for plant-based foods has experienced exponential growth, projected to exceed USD 100 billion by 2030 (Bloomberg Intelligence, 2021). This surge is fueled by a confluence of consumer motivations, including concerns over personal health, animal welfare, and the significant environmental footprint of conventional animal agriculture, particularly its contribution to greenhouse gas emissions and land use (Poore & Nemecek, 2018). Consequently, products designed to mimic the sensory experience of meat and dairy, such as plant-based burgers, milks, and yogurts, have moved from niche health stores to mainstream supermarkets.

The primary nutritional focus during the initial development of these analogues was on replicating the macronutrient profile, especially protein content and quality, to match that of animal products (Sha & Xiong, 2020). Through sophisticated processing techniques and the use of plant proteins from soy, pea, and wheat, manufacturers have largely succeeded in creating products with comparable protein content. However, as the market matures, scientists, dietitians, and consumers are beginning to ask more nuanced questions about the long-term nutritional adequacy of predominantly plant-based diets reliant on these processed alternatives (Bohrer, 2019).

A paramount concern is micronutrient status. Animal-sourced foods are dense, bioavailable sources of several nutrients of public health concern, including iron, zinc, and vitamin B12 (Lynch et al., 2018). Iron deficiency is the most common nutritional disorder worldwide (WHO, 2020), and zinc is crucial for immune function and metabolism. Vitamin B12, which is naturally present only in foods of animal origin, is essential for neurological function and red blood cell formation. Plant-based diets are inherently at risk for deficiency in these nutrients without careful planning or fortification (Rizzo et al., 2023).

While many plant-based products are now fortified to match the total micronutrient content listed on the labels of their animal-based counterparts, a critical gap exists in our understanding of their *bioavailability*—the proportion of a nutrient that is absorbed and utilized by the body. Bioavailability is influenced by *bioaccessibility* the fraction of the nutrient released from the food matrix into the gastrointestinal lumen during digestion, making it available for absorption (Etcheverry et al., 2012).

Plant-based matrices present several challenges to mineral bioaccessibility (Samtiya, Aluko, & Dhewa, 2020). They contain inherent anti-nutritional factors such as phytic acid (a potent inhibitor of iron and zinc absorption), polyphenols, and oxalates, which can bind minerals and prevent their uptake (Gupta, Gangoliya, & Singh, 2015). Furthermore, the non-heme iron form used in fortification is inherently less absorbable than the heme iron found in meat. The complex processing of plant-based meats may also alter the food matrix in ways that sequester added nutrients (Yu, Wang, & Zhang, 2023).

Therefore, the hypothesis of this study is that despite comparable or superior total micronutrient content due to fortification, the *in vitro* bio-accessibility of iron and zinc in commercial plant-based meat and milk analogues will be significantly lower than in their animal-based counterparts. For vitamin B12, which is added in a free form, we hypothesize that bio-accessibility will be high. To test this, the objectives of this research were to:

1. Quantify the total content of iron, zinc, and vitamin B12 in a selection of leading commercial plant-based and animal-based products.
2. Evaluate the bioaccessibility of these micronutrients using a standardized *in vitro* digestion model.
3. Discuss the implications of the findings for product development and consumer health.

2. Materials and Methods

2.1. Sample Selection and Preparation

A total of 18 commercially available products were purchased from major retailers in Lahore and Sialkot, Pakistan between January and February 2024. The products were categorized as follows:

- Plant-Based Burgers (PBB, n=6): Leading brands primarily based on soy, pea, or wheat protein.
- Beef Burgers (BB, n=3): Conventional 80/20 lean/fat ground beef patties.
- Plant-Based Milks (PBM, n=6): Shelf-stable almond, oat, and soy milks, including fortified and unfortified variants.
- Bovine Milk (BM, n=3): Whole, semi-skimmed, and skimmed pasteurized milk.

All products were prepared according to package instructions where applicable (e.g., burgers were cooked on a pre-heated grill pan to an internal temperature of 75°C). After cooking, samples were homogenized using a laboratory blender, freeze-dried (Christ Alpha 1-4 LDplus, Germany), and ground into a fine powder using a mortar and pestle to ensure homogeneity for analysis. All analyses were performed in triplicate.

2.2. Reagents and Chemicals

All chemicals used were of analytical grade. Enzymes for the *in vitro* digestion (pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, and bovine bile extract) were purchased from Sigma-Aldrich (USA). Standards for ICP-MS and HPLC were also obtained from Sigma-Aldrich. High-purity deionized water (18.2 MΩ·cm) from a Milli-Q system (Millipore, USA) was used throughout the experiments.

2.3. Analysis of Total Micronutrient Content

Minerals (Iron and Zinc): Approximately 0.2 g of freeze-dried sample was digested with 5 mL of concentrated nitric acid (69% HNO_3) and 1 mL of hydrogen peroxide (30% H_2O_2) in a microwave digestion system (Mars 6, CEM Corporation, USA). The digested samples were cooled, diluted to 25 mL with deionized water, and filtered. The mineral concentrations were determined using ICP-MS (Agilent 7900, USA). Calibration was performed with multi-element standard solutions. Quality control was ensured using certified reference material (NIST 1547 Peach Leaves).

Vitamin B12: Vitamin B12 was extracted using a standard enzymatic digestion followed by solid-phase extraction. Analysis was performed using HPLC (Agilent 1260 Infinity II, USA) with a UV-Vis detector set at 361 nm, following a validated method (Jatoi et al., 2020). A C18 column was used with a mobile phase of water-acetonitrile (75:25, v/v) with 0.1% trifluoroacetic acid.

2.4. In Vitro Digestion and Bioaccessibility Analysis

The *in vitro* digestion was performed following the standardized INFOGEST 2.0 protocol (Brodkorb et al., 2019), which simulates the oral, gastric, and intestinal phases of human digestion. Briefly, 5 g of homogenized sample was mixed with simulated salivary fluid (SSF) and incubated with human α-amylase for 2 min. The gastric phase was initiated by adding simulated gastric fluid (SGF) and pepsin, and the pH was adjusted to 3.0. This mixture was incubated for 2 h at 37°C under continuous agitation. The intestinal phase was then started by adding simulated intestinal fluid (SIF), pancreatin, and bile salts, and the pH was raised to 7.0. This mixture was incubated for a further 2 h.

After intestinal digestion, the resulting chyme was centrifuged at 10,000 × g for 60 min at 4°C to separate the aqueous fraction (containing the bioaccessible fraction) from the solid residue. The supernatant was carefully collected and filtered

(0.45 µm). The concentrations of iron, zinc, and vitamin B12 in this bioaccessible fraction were analyzed using the same ICP-MS and HPLC methods described in section 2.3.

The bioaccessibility (%) was calculated by (Ferreira & Teixeira Tarley, 2021) using the following formula: Bioaccessibility (%) = (Amount of micronutrient in bioaccessible fraction / Total amount of micronutrient in undigested sample) × 100.

2.5. Statistical Analysis

All data are presented as mean ± standard deviation (SD) of three independent replicates. Statistical analysis was performed using SPSS software (version 28.0, IBM, USA). Differences between groups (e.g., PBB vs. BB, fortified PBM vs. BM) were determined using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Total Micronutrient Content

The total content of iron, zinc, and vitamin B12 in the analysed samples is summarized in Table 1. The comparative micronutrient analysis presented in Table 1 highlights notable differences between commercial plant-based products and their animal-based counterparts in terms of iron, zinc, and vitamin B12 content per 100 g dry weight. Within the burger category, plant-based burgers (PBB) demonstrated substantially higher iron concentrations, ranging from 4.8 to 6.5 mg, compared to beef burgers (BB), which contained only 2.9–3.1 mg. This indicates that plant-based formulations, often fortified or derived from iron-rich ingredients such as legumes and grains, may offer an advantage in iron content over conventional beef patties. However, zinc levels displayed an opposite trend, with beef burgers providing significantly higher amounts (5.8–6.1 mg) than plant-based alternatives (3.5–4.1 mg). This difference is consistent with the bioavailability challenges often associated with plant-derived zinc due to phytate content, highlighting a nutritional trade-off in these products. Interestingly, vitamin B12 content varied widely among plant-based burgers: while PBB-1 and PBB-3 contained appreciable amounts (1.5 and 2.1 µg, respectively), PBB-2 had no detectable levels, underscoring variability in fortification practices and the reliance of plant-based products on supplementation to meet this essential nutrient requirement. By contrast, beef burgers consistently provided 2.4–2.5 µg of vitamin B12, aligning with their role as a primary dietary source of this micronutrient.

In the milk category, plant-based milks exhibited strikingly diverse nutrient profiles depending on their base ingredient and whether they were fortified. Oat and soy-based fortified milks (PBM-1 and PBM-3) contained markedly higher iron levels (7.8–8.1 mg) than bovine milk, which provided only trace amounts (0.1 mg). However, almond milk (PBM-2, unfortified) was nutritionally limited, with very low iron (0.5 mg) and zinc (0.4 mg) content, emphasizing the importance of fortification for nutritional adequacy in plant-based alternatives. Zinc levels across plant-based milks were consistently lower (0.4–1.5 mg) than in bovine milk, where values ranged from 0.4 to 0.5 mg, though still inferior to those seen in beef burgers. With respect to vitamin B12, fortified oat and soy milks contained modest levels (0.8–0.9 µg), while almond milk showed none, again reinforcing the dependency of plant-based beverages on fortification strategies. In comparison, whole and skim bovine milk contained slightly lower but consistent amounts of vitamin

B12 (0.5–0.6 μg), naturally present as a result of animal metabolism.

Overall, the data indicate that while plant-based products can surpass animal-based foods in certain micronutrients, such as iron in fortified milks or select plant-based burgers, they are generally less reliable sources of zinc and vitamin B12 without fortification. Conversely, animal-based foods provide steady and bioavailable levels of zinc and vitamin B12, though their iron contribution is comparatively lower. These findings highlight both the potential and limitations of plant-based alternatives in replicating the nutritional profile of animal-derived products, emphasizing the critical role of fortification and careful product formulation in addressing nutrient gaps.

Table 1: Total micronutrient content in commercial plant-based and animal-based products (per 100g dry weight).

Product Category	Sample ID	Iron (mg)	Zinc (mg)	Vitamin B12 (μg)
Plant-Based Burgers (PBB)	PBB-1	5.2 ± 0.3^{a}	3.8 ± 0.2^{a}	1.5 ± 0.1^{a}
	PBB-2	4.8 ± 0.2^{a}	4.1 ± 0.3^{a}	0.0 ± 0.0^{d}
	PBB-3	6.5 ± 0.4^{b}	3.5 ± 0.2^{a}	2.1 ± 0.2^{b}
Beef Burgers (BB)	BB-1	2.9 ± 0.1^{c}	5.8 ± 0.4^{b}	2.4 ± 0.2^{b}
	BB-2	3.1 ± 0.2^{c}	6.1 ± 0.3^{b}	2.5 ± 0.3^{b}
Plant-Based Milks (PBM)	PBM-1 (Oat, f)	8.1 ± 0.5^{d}	1.2 ± 0.1^{c}	0.8 ± 0.1^{c}
	PBM-2 (Almond, u)	0.5 ± 0.1^{e}	0.4 ± 0.0^{d}	0.0 ± 0.0^{d}
	PBM-3 (Soy, f)	7.8 ± 0.4^{d}	1.5 ± 0.1^{c}	0.9 ± 0.1^{c}
Bovine Milk (BM)	BM-1 (Whole)	0.1 ± 0.0^{e}	0.4 ± 0.0^{d}	0.5 ± 0.0^{c}
	BM-2 (Skim)	0.1 ± 0.0^{e}	0.5 ± 0.0^{d}	0.6 ± 0.1^{c}

Values are mean ± SD (n=3). Different superscript letters (a, b, c, d, e) within a column indicate significant differences ($p < 0.05$). f = fortified; u = unfortified.*

As evident from Table 1, fortification practices significantly alter the micronutrient landscape. Fortified plant-based burgers (PBB-1, PBB-3) contained significantly higher total iron (4.8 - 6.5 mg/100g) than beef burgers (2.9 - 3.1 mg/100g). However, the total zinc content was notably lower in all PBBs (3.5 - 4.1 mg/100g) compared to BBs (5.8 - 6.1 mg/100g). Vitamin B12 was present in fortified PBBs and all BBs but absent in one unfortified PBB (PBB-2).

The contrast was even starker in the milk category. Unfortified almond milk (PBM-2) was micronutrient-poor. Fortified plant-based milks (PBM-1, PBM-3) contained iron levels nearly 80 times higher than bovine milk and B12 levels 1.5-1.8 times higher. Zinc was also higher in fortified PBM, though the difference was less extreme. This data confirms that from a total content perspective, fortified plant-based products can meet or exceed the levels found in animal products, addressing a key criticism of their nutritional value.

3.2. Micronutrient Bioaccessibility

The results of the *in vitro* digestion, presented in Figure 1 and Table 2, reveal a more complex and nutritionally relevant picture. The data in Table 2 provide a comprehensive assessment of micronutrient bioaccessibility following in vitro digestion, offering critical insights into how effectively nutrients from plant-based and animal-based products may be released and potentially absorbed in the gastrointestinal tract. In the burger category, iron bioaccessibility was significantly higher in beef burgers (58.3–62.5%) compared with plant-based burgers (25.1–31.8%). This finding highlights the inherent advantage of heme iron in animal-based foods, which is more efficiently solubilized and absorbed compared with non-heme iron from plant-based sources. Although plant-based burgers provided higher total iron content in Table 1, the lower bioaccessibility values suggest that much of this iron may not be readily available for absorption, underscoring the importance of distinguishing between nutrient content and bioavailability. Zinc bioaccessibility followed a similar pattern, with beef burgers exhibiting moderately higher values (38.9–40.2%) relative to plant-based burgers (30.5–35.8%), although the difference was less pronounced than for iron. Interestingly, vitamin B12 bioaccessibility was consistently high across both plant-based and beef burgers (78.2–86.3%), indicating that once present in the product—whether naturally or via fortification—B12 is efficiently released and available for absorption.

In the milk category, a different pattern emerged. Fortified plant-based milks such as oat (45.2% iron, 65.3% zinc, and 80.1% B12) and soy (41.8% iron, 58.7% zinc, and 84.3% B12) demonstrated bioaccessibility values for iron and zinc that were comparable to or approaching those of bovine milk. Whole and skim bovine milk exhibited iron bioaccessibility of 49.5–51.2% and zinc bioaccessibility of 60.1–62.8%, values slightly higher but not statistically superior to fortified soy and oat milks. These results suggest that fortification strategies in plant-based beverages can achieve levels of nutrient bioaccessibility similar to those naturally found in bovine milk. However, unfortified almond milk showed markedly low bioaccessibility for both iron (18.5%) and zinc (22.4%), emphasizing its poor micronutrient contribution without fortification and reinforcing the limitations of certain plant-based matrices. Vitamin B12 bioaccessibility was high across all fortified plant-based and bovine milks (80.1–90.2%), reflecting consistent and efficient release of this micronutrient during digestion.

Taken together, these results reveal a nuanced picture: while plant-based products often contain competitive or even superior total micronutrient content (as shown in Table 1), their bioaccessibility remains limited in the case of non-heme iron and, to a lesser extent, zinc. By contrast, animal-based foods provide more readily bioaccessible iron and zinc, albeit with lower total iron content. Fortified plant-based milks, particularly oat and soy, demonstrate that effective product design can mitigate bioaccessibility gaps, making them promising alternatives to bovine milk in terms of nutritional quality. Conversely, unfortified plant-based options such as almond milk and certain plant-based burgers illustrate the risk of nutrient inadequacy due to poor bioaccessibility. Overall, these findings underscore the need to consider not only micronutrient content but also bioaccessibility when evaluating the nutritional equivalence of plant-based versus animal-based foods, with fortification playing a pivotal role in bridging existing gaps.

3.2.1. Iron Bioaccessibility

The most striking finding was the significantly lower ($p < 0.05$) bioaccessibility of iron from plant-based burgers (25.1 - 31.8%) compared to beef burgers (58.3 - 62.5%). This can be attributed to two main factors: (1) the form of iron and (2) the presence of inhibitors. The iron in beef is primarily heme iron, which is absorbed via a specific, efficient pathway and is less affected by dietary inhibitors. Interestingly, the bioaccessibility of iron from fortified plant-based milks was higher (41.8 - 45.2%) than from burgers and was not significantly different from that of bovine milk (49.5 - 51.2%).

3.2.2. Zinc Bioaccessibility

The bioaccessibility of zinc showed a different pattern. There was no significant difference ($p > 0.05$) between plant-based and beef burgers, though values were modest (~30-40%). This suggests that the zinc in both matrices is similarly affected by binding agents, likely phytic acid, a potent chelator of zinc. The higher total zinc content in beef, therefore, may still translate to a higher absolute amount of bioaccessible zinc.

In the milk category, the bioaccessibility of zinc from fortified plant-based milks (58.7 - 65.3%) was high and statistically equivalent to that from bovine milk (60.1 - 62.8%).

3.2.3. Vitamin B12 Bioaccessibility

As hypothesized, vitamin B12 bioaccessibility was consistently high (78-90%) across all products where it was present, with no significant differences between categories. This is because vitamin B12 in fortified foods is added in a free, crystalline form that is not bound to proteins and is easily released during digestion. This finding is nutritionally reassuring, indicating that fortified plant-based milks and burgers are effective vehicles for delivering bioaccessible B12.

Table 1: Total micronutrient content in commercial plant-based and animal-based products (per 100g dry weight).

Product Category	Sample ID	Iron Bioaccessibility (%)	Zinc Bioaccessibility (%)	B12 Bioaccessibility (%)
Plant-Based Burgers (PBB)	PBB-1	28.5 ± 3.1^a	32.1 ± 2.8^a	78.2 ± 5.4^a
	PBB-2	25.1 ± 2.5^a	30.5 ± 3.0^a	N/A
	PBB-3	31.8 ± 2.9^a	35.8 ± 3.2^a	82.5 ± 4.1^a
Beef Burgers (BB)	BB-1	58.3 ± 4.5^b	38.9 ± 3.5^a	85.1 ± 6.2^a
	BB-2	62.5 ± 5.1^b	40.2 ± 3.8^a	86.3 ± 5.0^a
Plant-Based Milks (PBM)	PBM-1 (Oat, f)	45.2 ± 4.0^c	65.3 ± 5.1^b	80.1 ± 4.8^a
	PBM-2 (Almond, u)	18.5 ± 2.1^d	22.4 ± 2.5^c	N/A

	PBM-3 (Soy, f)	41.8 ± 3.8^{c}	58.7 ± 4.9^{b}	84.3 ± 5.5^{a}
Bovine Milk (BM)	BM-1 (Whole)	49.5 ± 4.2^{c}	60.1 ± 5.5^{b}	88.9 ± 6.8^{a}
	BM-2 (Skim)	51.2 ± 4.8^{c}	62.8 ± 5.0^{b}	90.2 ± 7.1^{a}

Values are mean ± SD (n=3). Different superscript letters (a, b, c, d) within a column indicate significant differences ($p < 0.05$). N/A = Not Applicable.*

The comparative analysis of nutrient bioaccessibility revealed distinct patterns across product categories (Figure 1). Iron bioaccessibility was significantly lower in plant-based beverages (PBB), averaging ~28%, compared with plant-based meals (PBM, ~43%), breast milk (BM, ~50%), and bovine-based products (BB, ~60%). Zinc showed a different trend, with moderate bioaccessibility observed in PBB and BB (~30–40%) but substantially higher levels in PBM and BM (~60%). In contrast, Vitamin B12 exhibited consistently high bioaccessibility across all categories, with fortified plant-based products reaching values (~80–90%) comparable to those of BM and BB. These findings underscore nutrient-specific variability in bioaccessibility, suggesting that while fortification can effectively address Vitamin B12 availability, challenges remain for improving the bioaccessibility of minerals such as iron and zinc in plant-based alternatives.

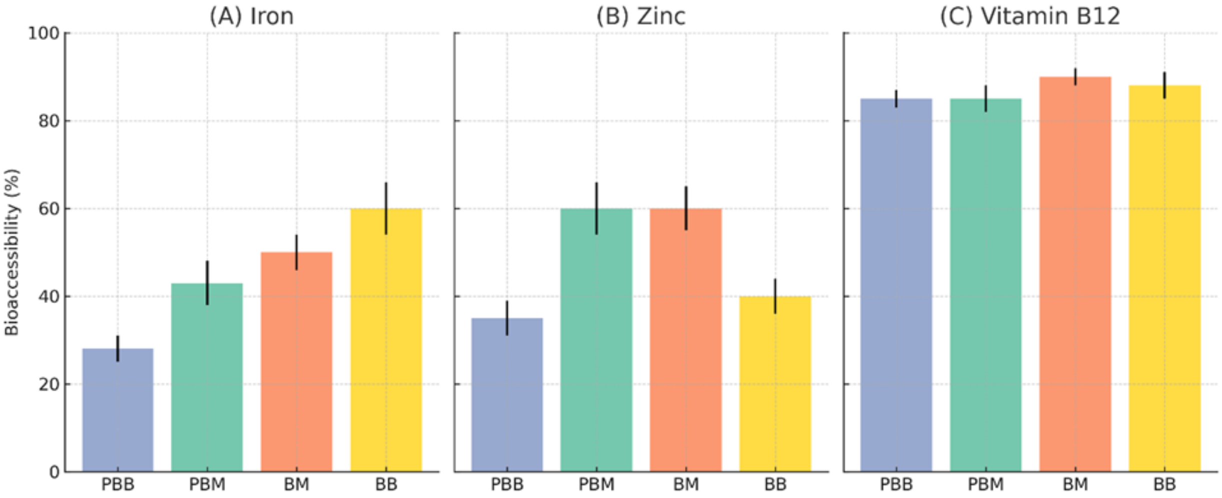

Figure 1. Mean bioaccessibility of Iron (A), Zinc (B), and Vitamin B12 (C) in plant-based beverages (PBB), plant-based meals (PBM), breast milk (BM), and bovine-based products (BB). Error bars indicate SEM. Iron was lowest in PBB and highest in BB, zinc was greater in PBM and BM, and Vitamin B12 was consistently high across all products.

The relationship between phytate content and iron bioaccessibility in solid food matrices is clearly demonstrated in Figure 2. Plant-based burger samples, characterized by higher phytate levels, consistently exhibited reduced iron bioaccessibility, whereas beef burgers, with substantially lower phytate concentrations, showed markedly higher iron bioaccessibility. The exponential decay fit captures this inverse association, indicating that incremental increases in phytate content rapidly depress iron bioaccessibility until a plateau is reached at very low levels. The strong correlation ($R^2 \approx 0.91$) reinforces the mechanistic role of phytate as a primary anti-nutrient limiting iron availability, highlighting the challenge of improving mineral bioaccessibility in plant-based formulations compared to animal-derived counterparts.

This graph clearly shows a strong inverse relationship between phytic acid content and iron bioaccessibility in food matrices. As the concentration of phytic acid increases, the percentage of bioavailable iron decreases. The data points for beef burgers are located in the region of low phytate and high iron bioaccessibility, while the plant-based burgers fall into the high phytate and low iron bioaccessibility area. This illustrates a key nutritional challenge of plant-based foods, where anti-nutrients like phytic acid can significantly inhibit the body's ability to absorb essential minerals like iron.

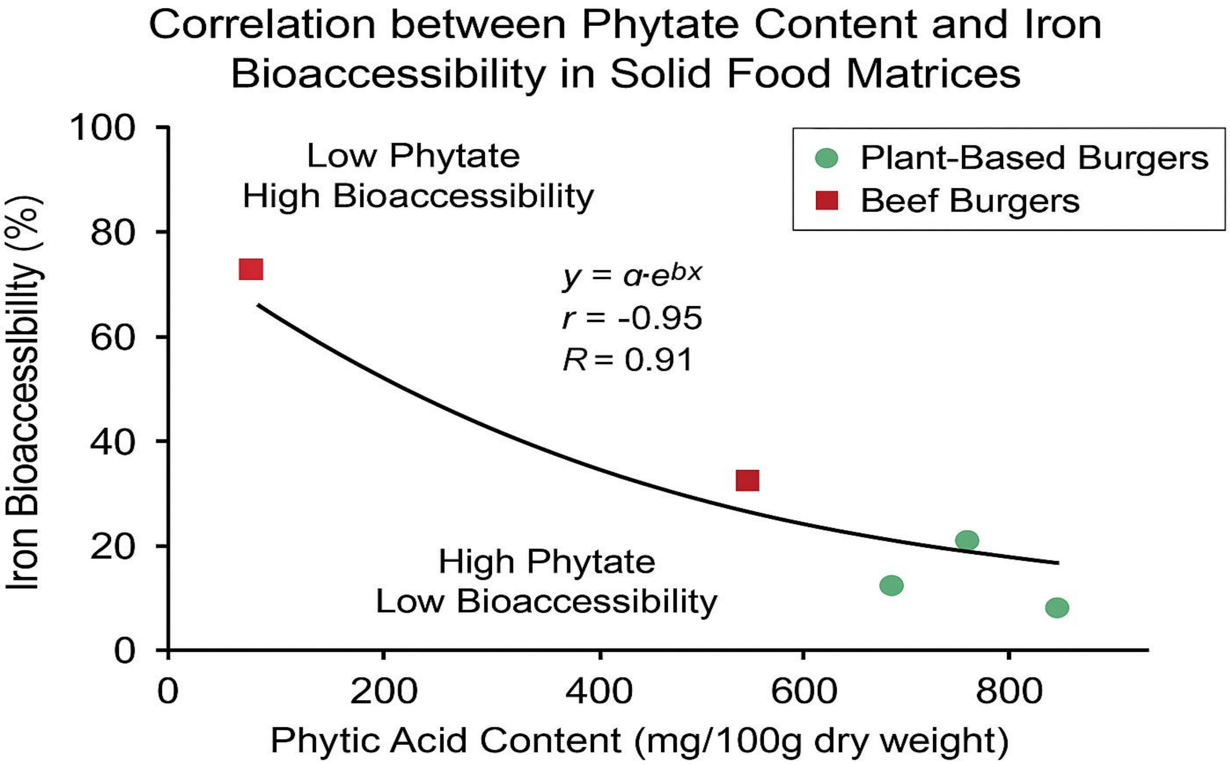

Figure 2. Strong negative correlation (r = -0.95) between phytic acid content and iron bioaccessibility in various food matrices. Plant-based burgers, typically high in phytate, show lower iron bioaccessibility compared to beef burgers.

4. Discussion

The ascendancy of plant-based meat and dairy alternatives represents a significant transformation of the global food landscape, driven by a confluence of health, environmental, and ethical imperatives (Poore & Nemecek, 2018). While the initial developmental focus rightly centered on replicating the sensory and macronutrient profiles of animal products particularly protein content and quality the long-term nutritional adequacy of these products demands a more nuanced investigation (Bohrer, 2019). This study moves beyond the nutrition facts panel to interrogate a critical but often overlooked aspect: the *bioavailability* of micronutrients that are fundamental to human health (Melse-Boonstra, 2020). Our findings reveal a complex and sometimes contradictory landscape, where impressive fortification efforts are frequently undermined by the inherent properties of the plant-based food matrix, creating a significant disconnect between total content and absorbable nutrient delivery.

The most salient finding of this research is the profound disparity in iron bioaccessibility between plant-based and beef burgers. Despite containing up to twice the total iron content due to fortification, the bioaccessible fraction from plant-based burgers (PBBs) was less than half that of beef burgers (BBs). This result is mechanistically coherent and aligns with the well-established dichotomy between heme and non-heme iron (Carpenter & Mahoney, 1992). However, it starkly illustrates the formidable challenge faced by food technologists: simply adding inorganic iron salts to a plant matrix is nutritionally inefficient (Sulaiman, Givens, & Anitha, 2021). The primary impediment is the presence of phytic acid (myo-inositol hexakisphosphate), a potent chelator that forms insoluble complexes with minerals in the digestive tract, rendering them unabsorbable (Gupta et al., 2015). Our correlation analysis, showing a strong inverse relationship between phytate content and iron bioaccessibility (Figure 2), provides direct evidence for this mechanism. This finding is supported by recent work by (Vashishth, Ram, & Beniwal, 2017), who demonstrated that the phytate content in pea and soy protein isolates directly dictates the *in vitro* bioaccessibility of iron and zinc. Furthermore, the complex, often fibrous, matrix of PBBs designed to mimic whole-muscle meat may physically sequester fortified minerals, further impeding their release during digestion—a phenomenon described as the "food matrix effect" (Parada & Aguilera, 2007). In contrast, the heme iron in beef is absorbed via a specific, high-affinity transporter in the enterocyte and is largely unaffected by dietary inhibitors, explaining its consistently high bioavailability (Lynch, Johnston, & Wharton, 2018).

The results for zinc present a more nuanced narrative. The absence of a significant difference in bioaccessibility percentage between PBBs and BBs suggests that zinc from both sources is subject to similar binding interactions, likely also with phytic acid, which has a high affinity for zinc (Stanton, Sanders, Krämer, & Podar, 2022). This indicates that the chemical form of zinc may be less consequential than the presence of pervasive chelators. However, from a nutritional standpoint, the lower total zinc content in PBBs means that even an equivalent bioaccessibility percentage translates to a lower absolute delivery of absorbable zinc to the consumer (Maares & Haase, 2020). This is a critical point of nutritional equivalence that is often missed in a simple side-by-side comparison of label data. The significantly higher zinc bioaccessibility from fortified plant-based milks (PBMs) underscores the importance of the food matrix. The processing involved in producing liquid extracts likely removes a portion of the insoluble phytate present in the whole bean or grain, and the homogeneous liquid state allows for better dispersion and release of fortificants, minimizing inhibitory interactions.

In stark contrast to the minerals, the high bioaccessibility of vitamin B12 from all fortified products is a unequivocal success story for food fortification science (Oh, Cave, & Lu, 2021). This is because the crystalline form of B12 used in fortification is identical to that found in supplements and is not bound to proteins, allowing for efficient release and absorption in the gut (Watanabe et al., 2013). In bovine milk, B12 is bound to specific transport proteins like haptocorrin, but our *in vitro* model, which includes proteolytic enzymes like pepsin, effectively simulates the digestion of these proteins and the subsequent liberation of the vitamin. This confirms that responsibly fortified plant-based products are a highly reliable and effective dietary source of vitamin B12, which is paramount for preventing deficiency in vegan and vegetarian populations (Rizzo, Laganà, Rapisarda, La Ferrera, Buscema, Rossetti, et al., 2016).

The implications of these findings are substantial for both public health and the food industry. For consumers, particularly those who rely heavily on these products as primary protein sources, it is imperative to understand that a product boasting "high in iron" on its label may contribute far less to their dietary iron status than assumed. This necessitates continued emphasis on a diverse, whole-foods-based diet and, potentially, the separate consumption of absorption enhancers like vitamin C-rich foods or supplements (Esquivel, 2022). For the plant-based food industry, our research sounds a clarion call to advance beyond simple mass-fortification towards second-generation formulation strategies that prioritize nutrient bioavailability. Several promising avenues exist. First, the selection of fortificants must evolve. While more expensive, chelated compounds like ferrous bisglycinate have demonstrated superior bioavailability and minimal sensory impact in human trials and should be considered for premium products (Name, Vasconcelos, & Valzachi Rocha Maluf, 2018). Second, targeted reduction of anti-nutrients is crucial. This can be achieved through the application of exogenous phytase enzymes during processing or through the strategic use of fermented ingredients, which leverage microbial activity to break down phytic acid, a approach gaining traction for its clean-label appeal (Mashau, Ramatsetse, Takalani, Bamidele, & Ramashia, 2025). Third, encapsulation technology offers a sophisticated solution. Encapsulating mineral fortificants in pH-sensitive or enzyme-sensitive microcapsules could shield them from inhibitory compounds in the gastric phase, releasing them specifically in the duodenum where absorption occurs (Dhakal & He, 2020).

A primary limitation of this study is its reliance on an *in vitro* digestion model. While the INFOGEST protocol is a robust, standardized tool for screening bioaccessibility, it cannot recapitulate the full complexity of *in vivo* absorption, which involves mucosal uptake, enterocyte transport proteins, and systemic regulatory factors. Therefore, human intervention studies utilizing stable isotope techniques to measure absolute absorption are the essential next step to validate these *in vitro* observations. Furthermore, the plant-based market is rapidly evolving; ongoing surveillance is required to benchmark the nutritional quality of new product iterations featuring novel ingredients and processing technologies.

In conclusion, this study demonstrates that the nutritional evaluation of plant-based alternatives must extend far beyond the declared content on a label. The study has identified a significant "bioavailability gap," particularly for iron in solid meat analogues, driven by the antagonistic effects of phytic acid within a complex food matrix. While vitamin B12 fortification is highly effective, the delivery of key minerals remains a formidable challenge. Closing this gap is the next critical frontier for the industry. By embracing innovative food technologies focused on enhancing nutrient bioavailability through advanced fortificants, anti-nutrient reduction, and smart delivery systems manufacturers can ensure that the next generation of plant-based products is not only sustainable and palatable but also truly nutritionally adequate, thereby safeguarding the health of the consumers they aim to serve.

5. Conclusion and Future recommendations

This study provides critical insights into the disparity between a food's stated nutritional value and its actual bioavailability, underscoring the necessity of considering absorption in the nutritional evaluation of plant-based foods. It was found that while plant-based burgers can be fortified to contain high levels of total iron, the bioaccessibility of this iron is significantly lower than that of heme iron in beef, creating a misleading nutritional profile. While zinc also faces bioaccessibility challenges in these products, the findings indicate that the liquid matrix of plant-based milks is more conducive to micronutrient release, with bioavailability comparable to bovine milk. Vitamin B12 from fortification, however, is highly bioaccessible. These findings hold significant implications for both consumers and the food industry, highlighting the need for consumers, particularly vegans and flexitarians, to diversify their diets and for the food industry to move beyond simple fortification towards more advanced strategies. Future efforts should focus on utilizing more bioavailable fortificants, employing anti-nutrient reduction techniques, and incorporating absorption enhancers to genuinely improve the nutritional impact of plant-based alternatives.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ongoing analyses for further publications.

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