

Research Article

Optimal Food Matrix Model for Digestibility and Bioavailability of Calcium and Zinc

Muzna Khan,¹ Nazir Ahmad ,¹ Mahr Un Nisa,¹ and Aalia Jadaan²

¹Department of Nutritional Sciences, Government College University, Faisalabad, Pakistan

²Aziz Fatimah Medical and Dental College, Faisalabad, Pakistan

Correspondence should be addressed to Nazir Ahmad; drnazirahmad@gcuf.edu.pk

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The nutrient deficiency resulting from inappropriate dietary intake leads to major risk factors of malnutrition and poses many serious threats and challenges to human health and capabilities. Malnutrition can be prevented through efficient accessibility and bioavailability from different food matrices. The objective of this study was to assess the digestibility and bioavailability of calcium and zinc with food matrices such as casual food (bread curry mixture), yogurt (plain and fruited), juices (orange, apple, carrot, and tomato), coffee, water (water and sparkling water), and smoothies after digestion with saliva, gastric, duodenal, and small intestine juices. 20 mg calcium and 3 mg zinc were mixed with the above food matrices and digestibility and bioavailability were determined. The result showed that the highest calcium digestibility (49.75%) was observed with plain yogurt and the lowest digestibility (10.10%) was observed with sparkling water. The highest (22.80%) and lowest (6.20%) calcium bioavailability were observed with fruit yogurt and carrot juice, respectively. The highest (13.55%) and lowest (10.20%) zinc digestibility were observed with coffee and orange juices, respectively. The highest (4.85%) and lowest (1.05%) zinc bioavailability were observed with fruit yogurt and bread sauce, respectively. Thus, this study helps to determine the optimal food matrix model for the best digestibility and potential bioavailability of calcium and zinc from vitamin-mineral products.

1. Introduction

The nutrient deficiency resulting from inappropriate dietary intake leads to major risk factors of malnutrition. This results in many serious threats and challenges to human health and potential. Nutrient deficiencies can be rectified by making nutrients accessible and bioavailable through improved supplement formulations and nutrient-drug interaction [1]. Supplementation and fortification are the most cost-effective techniques to address nutrient deficiency in communities. Nutrient deficiencies occur as a result of reduction in calories and increase of calorie-dense foods in contemporary diets [2]. The availability of nutrients with the food matrix is a key element of fortification, determining the bioavailability of nutrients in the gut besides the nutrient composition and processing conditions. Besides the abovementioned conditions, the available fraction of nutrients from the food mixture has a major importance in the determination of the

bioavailability of nutrients. Bioavailability can be influenced by several factors such as the chemical state of the nutrient, its release from the food matrix, possible interactions with other food components, the presence of suppressors or cofactors, and the formation of stable compounds that are slowly metabolized *in vivo* [3]. Bioavailability or digestibility is the fraction of nutrients released from the food matrix after digestion and available for absorption. The fraction of nutrients in a food mixture that is absorbed and utilized by a body is called bioavailability [4]. The bioavailability of divalent cations is low with all food matrices. In the last two decades, despite a lot of awareness campaigns, calcium intake is still suboptimal and needs careful supplementation and intake models. Today, calcium is still singled out as a major public health concern due to its critical importance in bone health and other physiological functions [5].

The average digested available calcium from ingested calcium load ranges from 25% to 35% with net

bioavailability of 10% to 12%. In general, the bioavailability of calcium is inversely proportional to the loaded quantity. For example, for a specific matrix, if the loaded dose is 20 mg, the bioavailability reaches 80% and if, the loaded dose is 1000 mg, the bioavailability decreases to 20% for the same matrix. Thus, from a nutritional point of view, the bioavailable contents are more important than the loaded fraction to understand the quantity and matrix model for nutritional and therapeutic purposes [6]. Most of the studies indicate that food matrix is the major factor in the bioavailability of calcium compared to an empty stomach and increases twofold when incorporated with various food, supplement, or beverage matrices [2, 7]. Calcium is less soluble and precipitates immediately in intestinal pH after making a complex with dietary phytate, and zinc remains soluble. Zinc is another important mineral that has a global deficiency impact affecting billions of people across the globe. It is normally observed that the bioavailability of zinc from fortified foods or supplements is higher in an empty stomach than in the fed state showing that food matrix and/or digestive processes can reduce zinc bioavailability [8, 9]. Besides that, the bioavailability of zinc depends on the metabolic quality of the natural composition of food. Zinc oxide is most commonly used as a fortificant or supplement, however, the bioavailability of zinc oxide in a fasted state is less as compared to zinc citrate or zinc gluconate and sometimes, observed undetectable absorption may be linked with stomach acid quantity [10]. Some animal studies show controversial opinions on zinc absorption with calcium and calcium-fortified foods. Calcium in the form of calcium carbonate with dietary phytate hinders the absorption of zinc, while Miller and his colleagues reported a significant favorable effect of calcium on zinc absorption [11, 12]. Thus, the discordances in calcium bioavailability highlight the reality that bioavailability cannot be predicted based on the current chemical knowledge of the source nor can the matrix be extrapolated to other untested matrices. Similarly, the bioavailability of zinc dynamics has not been clearly established in any study as per our knowledge. The objective of this study was to sort out the optimal food matrix model for digestibility and bioavailability of calcium and zinc from mineral products.

2. Materials and Methods

2.1. Procurement of Materials. Calcium and zinc supplements were purchased from the local market and brand selection was made after careful consideration of the reputation of the brand, peer recommendation, and annual consumption. Further selected food matrices i.e., casual food (bread curry mixture), yogurt (plain and fruited), juices (orange, apple, carrot, and tomato), coffee, water (mineral water and sparkling water), and smoothies were procured from the local market in highest possible quality standards. Analytical grade chemicals or reagents, enzymes, and secretions, i.e., pepsin, mucin, glucuronic acid, glucose, glucosamine, uric acid, and pancreatin along with lipase, bile salt, and bovine serum albumin, were also supplied by Chem-Tech, Pakistan, Aldrich Sigma Pakistan. The study

was conducted at the Department of Nutritional Sciences, Government College University, Faisalabad.

2.2. Food Matrix and Supplement Treatment. Two supplements of calcium and zinc having the highest annual sales in Pakistan from the previous five years were selected and subjected to be fortified with chosen food matrices as shown in Table 1. On the basis of recommendation and nutrient reference values (NRV) as per the recommendation of the manufacturer, calcium was recommended at 400 mg and zinc, at 6 mg. However, for easy handling and availability of reagents and chemicals, we reduced the recommended dose to half and took 200 mg of calcium powder, 3 mg of zinc, and half the quantity of each matrix to attain the recommended doses.

2.3. Sample Preparation for Digestion. Different food matrices such as bread sauce (100 g bread + 150 g sauce), coffee (120 mL), water (250 mL), plain and fruited yogurt (150 g), fruit juices (apple and orange, 150 mL), vegetable juices (carrot and tomato, 150 mL), and smoothie (carrot + orange) of fruit and vegetable (150 mL) were prepared as reported earlier [13]. The samples were mixed separately by employing a conventional mixer primarily to mimic the food fragmentation. The food matrices were homogenized with calcium and zinc separately. The samples were drawn as mentioned below and subjected to digestion by adding saliva, gastric, intestinal, and bile juices at successive steps at $37 \pm 0.3^\circ\text{C}$ [14].

2.4. Oral and Gastric Digestion. The samples from each of the above mixtures (5 g) were taken and mixed with 6 mL of simulated saliva solution (pH 6.8) and then stirred for 5 min at 37°C . Furthermore, 12 mL of simulated gastric juice (pH 1.5) was added and the mixture was stirred for 2 hr at 37°C .

2.5. Intestinal Digestion. After completion of gastric digestion, 12 mL of duodenal juice, 6 mL of bile juice, and 2 mL of 70% bicarbonate solution (pH 8.0) were added and the mixture was stirred for 2 hr at 37°C . After small intestine digestion, incubated samples were subjected to targeted nutrient bioavailability.

2.6. Bioavailability of Nutrients. The dialysis tube of cutoff 50 kDa, flat (Membrane Filtration Products, Inc., Seguin, TX, USA) containing 10 mL of phosphate buffer (pH 7) was placed in the 250 mL flask containing the digested mixture and stirred for 2 hr at 37°C . The samples from dialysis tubes and flasks were taken to determine the calcium and zinc concentrations. Dialyzable nutrients will be available for absorption in the small intestine.

2.7. Mineral Analysis. Calcium and zinc levels in different food matrices were examined by the atomic absorption spectrophotometry technique at a high-tech lab facility at the

TABLE 1: Food matrix and supplement treatment.

Calcium (200 mg) and zinc (3 mg) supplement										
Coffee	Water		Yogurt		Bread sauce	Fruit		Juice		Smoothie
	Plain	Sparkling	Plain	Fruited		Apple	Orange	Carrot	Tomato	

University of Agriculture, Faisalabad, Pakistan. Wet digestion was carried out as described earlier [13]. Samples were filtered and subjected to calcium and zinc quantification by using a Hitachi Polarize Zeeman Atomic Absorption Spectrometer at 214 nm and 624 nm wavelength, respectively.

2.8. *Statistical Analysis.* A descriptive analysis of means was performed by using Microsoft Excel (Microsoft Corporation, Redmond, USA) and computed as mean \pm error and are presented in simple graphs. The digestibility/solubility and absorption/bioavailability of minerals were calculated by using the following equation:

$$\frac{\text{Digestibility}}{\text{solubility}} \% = \left(\text{Soluble contents of } \frac{\text{Calcium}}{\text{Total}} \text{ contents of the sample} \right) \times 100, \quad (1)$$

$$\frac{\text{Absorption}}{\text{Bioavailability}} \% = \left(\text{Calcium contents in } \frac{\text{dialysate}}{\text{Total}} \text{ calcium contents of the sample} \right) \times 100.$$

3. Results

3.1. *Digestibility and Bioavailability of Calcium with Different Food Matrices.* The calcium and zinc contents originally present in food matrices are shown in Table 2. The highest calcium contents were present in yogurt matrices (118.04 mg/100 g) and lowest, in water (4.58 mg/100 mL). The highest zinc contents were observed in bread + sauce (6.10 mg/100 g) and remained nondetected in coffee and water (see Table 3).

The digestibility and potential absorption/bioavailability of calcium with liquid matrices such as coffee and water (sparkling and plain) are presented in Figure 1. Calcium supplement (200 mg) was subjected to digestion with gastrointestinal enzymes. The results showed that calcium digestibility with coffee was 13.15% and potential bioavailability was 7.14%. Similarly, the digestibility of calcium with sparkling and simple water was 10.10% and 10.55%, respectively. The potential absorption of calcium with sparkling and plain water was 8.65% and 8.95%, respectively.

The digestibility and potential bioavailability of calcium with solid food matrices such as bread sauce, fruit yogurt, and plain yogurt are presented in Figure 2. The results showed that the digestibility of calcium with bread sauce was 31.85% and potential bioavailability was 10.35%. The digestibility and bioavailability with fruit yogurt were 34.60% and 22.80%, respectively. The calcium digestibility with plain yogurt was 49.75% and potential bioavailability was 22.20%.

The digestibility and bioavailability of calcium with fruit and vegetable matrices such as apple, carrot, orange, and tomato juices and smoothies (apple, orange, and carrot) are presented in Figure 3. The results showed that the digestibility and bioavailability of calcium with apple juices were 14.65% and 9.0%, respectively. Calcium digestibility

and bioavailability with carrot juice were 12.35% and 6.2%, respectively. The digestibility and bioavailability with orange juice were 24.15% and 11.55%, respectively. The digestibility and bioavailability with tomato juice were 21.40% and 14.30%, respectively. Smoothie matrix showed 13.50% and 7.6% digestible and bioavailable calcium, respectively.

3.2. *Digestibility and Bioavailability of Zinc with Different Food Matrices.* The digestion and bioavailability of zinc with liquid matrices such as coffee, sparkling water, and plain water are presented in Figure 4. Zinc supplement (3 mg) was subjected to digestion with gastrointestinal enzymes by using the above liquids, and the results showed that the digestibility of zinc with coffee was 13.55% and its bioavailability was 4.45%. Zinc digestibility with sparkling water was 12.30% and its bioavailability was 1.45%. Zinc digestibility with simple water was 12.9% and bioavailability was 1.15%.

Zinc digestibility and bioavailability with solid matrices such as bread sauce, fruit yogurt, and plain yogurt are presented in Figure 5. The results showed that zinc digestibility with bread sauce was 12.95% and its bioavailability was 1.05%. Similarly, the amount of digested zinc with fruit yogurt was 13.15% and its bioavailability was 4.85%. The amount of digested zinc in plain yogurt was 13.70% and its bioavailability was 4.65%.

The digestibility and bioavailability of zinc with fruit and vegetable matrices such as apple, carrot, orange, and tomato juices and smoothies (apple, orange, and carrot) are presented in Figure 6. The results showed that the digestibility and bioavailability of zinc with apple juices were 13.55% and 1.20%, respectively. The digestibility and bioavailability with orange juice were 10.20% and 1.10%, respectively. Zinc digestibility and bioavailability with carrot juice were 11.05% and 1.45%, respectively. The digestibility and bioavailability

TABLE 2: Composition and concentrations of digestive juices.

Secretion	Saliva	Gastric juice	Duodenal juice	Small intestine juice
Reagents	3 M NaCl 0.41 M urea 0.09 mM uric acid	1.0 M HCl 0.15 M CaCl ₂ 1 g BSA*	0.83 M KCl 0.15 M CaCl ₂ 1.0 g BSA*	1.0 M NaHCO ₃ 0.15 M CaCl ₂ 1.8 g BSA* 30 g bile
Enzymes	90 mg α-amylase 25 mg mucin	2.5 g pepsin 3 g mucin	9 g pancreatin 1.5 g lipase	— —
pH	6.8 ± 0.2	1.50 ± 0.02	8.0 ± 0.2	7.0 ± 0.2

*BSA = bovine serum albumin.

TABLE 3: Calcium and zinc contents (mg/100 mL or 100 mg).

Food matrix	Coffee	Water		Yogurt		Bread sauce	Juice				Smoothie
		Plain	Sparkling	Plain	Fruited		Apple	Orange	Carrot	Tomato	
Calcium	7.78	4.58	4.69	118.04	105.30	11.31	5.33	10.01	49.66	32.91	31.28
Zinc	ND	ND	ND	0.57	0.54	6.10	0.04	0.03	0.30	0.12	0.04

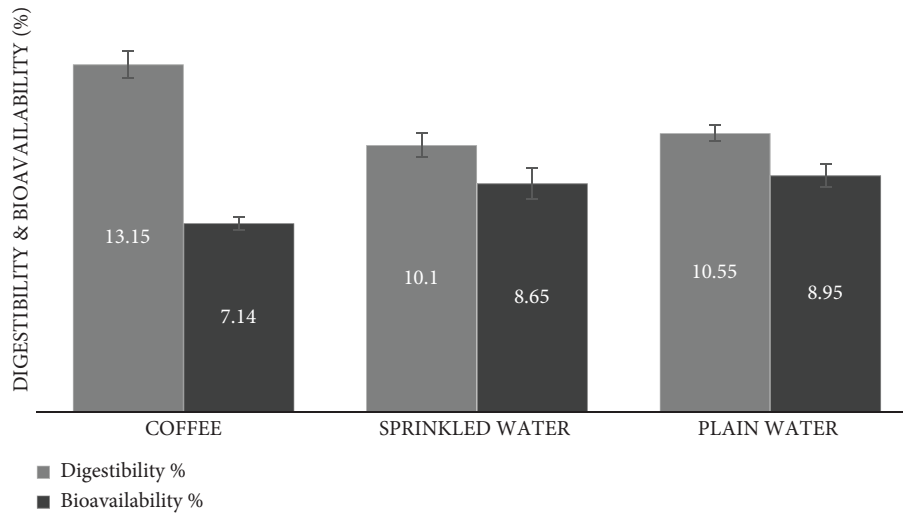


FIGURE 1: Digested and potentially absorbable calcium with liquid food matrices.

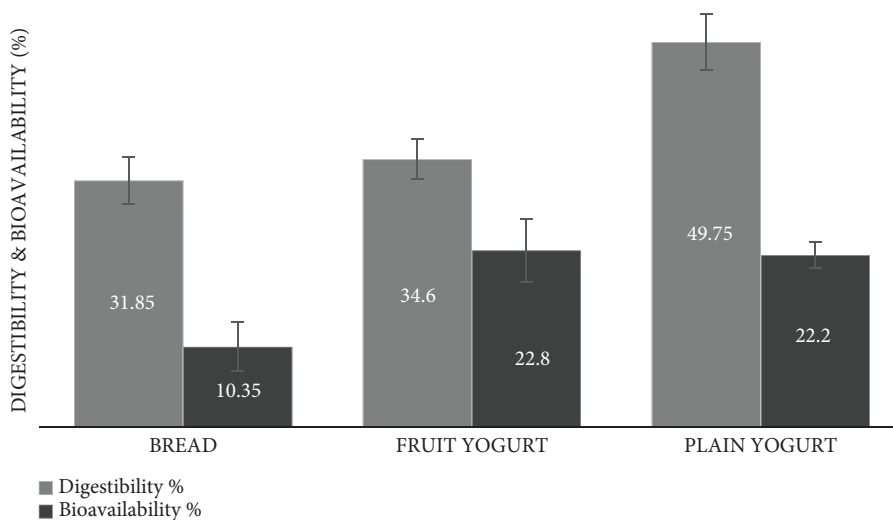


FIGURE 2: Digested and potentially absorbable calcium with solid food matrices.

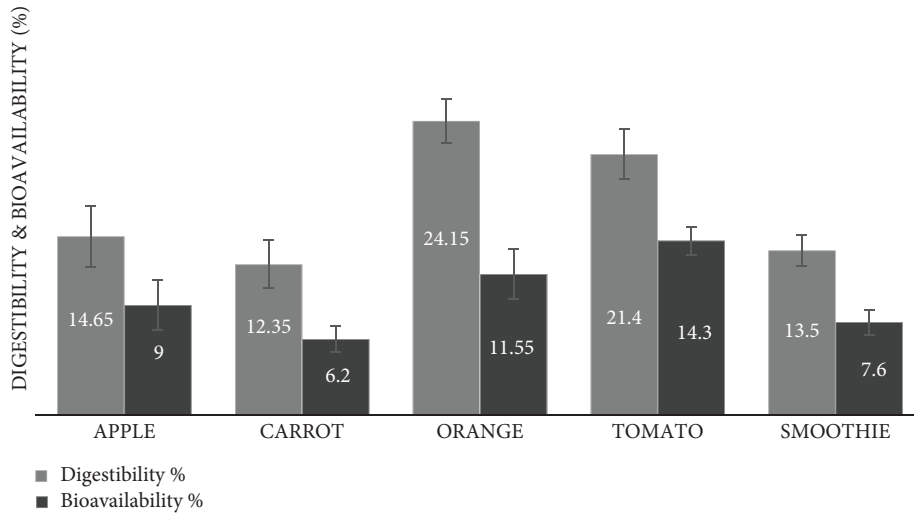


FIGURE 3: Digested and potentially absorbable calcium with fruit/vegetable food matrices.

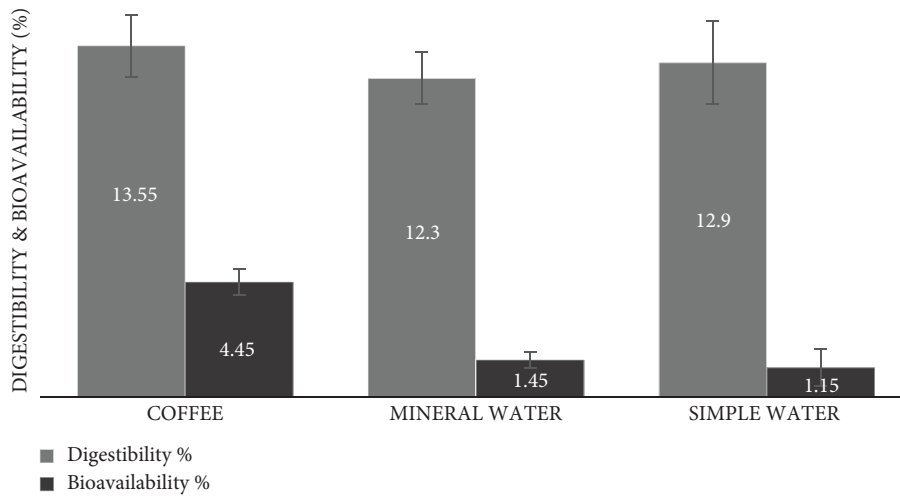


FIGURE 4: Digested and potentially absorbable zinc with liquid food matrices.

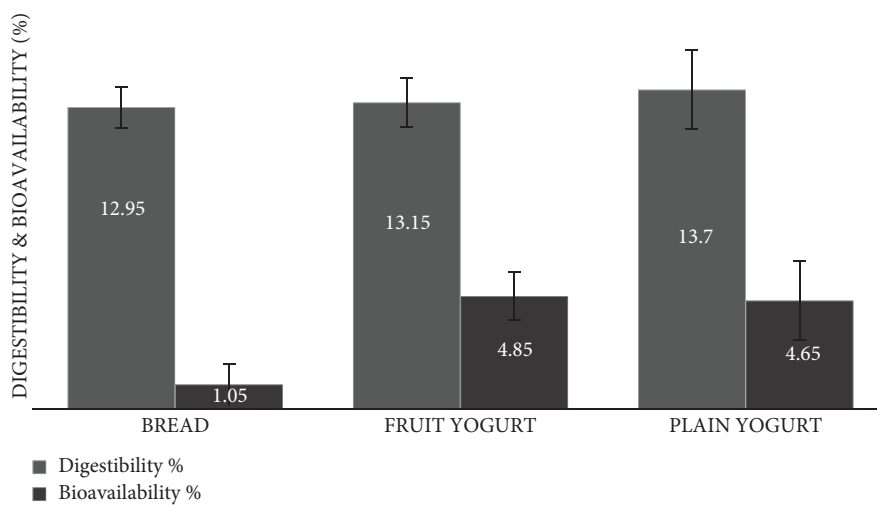


FIGURE 5: Digested and potentially absorbable zinc with solid food matrices.

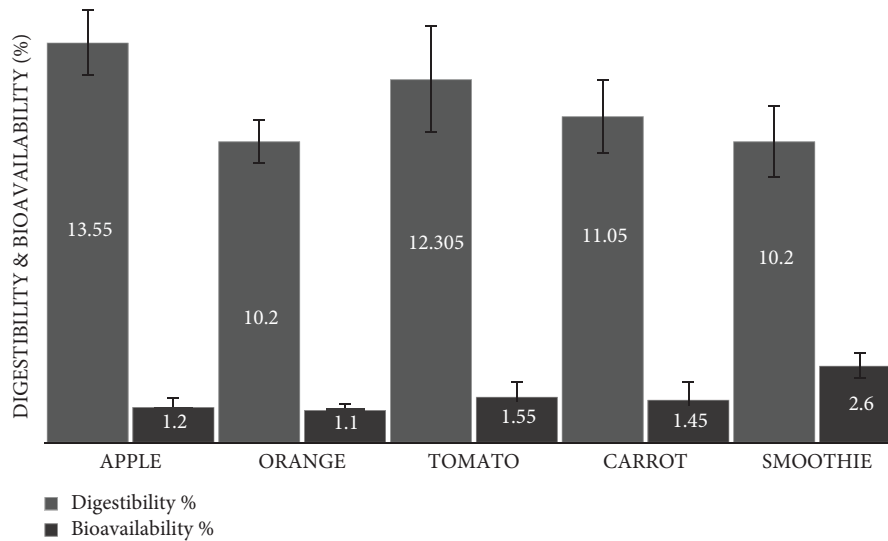


FIGURE 6: Digestibility and bioavailability of zinc with fruit and vegetable matrices.

with tomato juice were 12.31% and 1.55%, respectively. Similarly, the smoothie matrix showed 10.20% and 2.60% digestible and bioavailable zinc, respectively.

4. Discussion

The fortification and supplementation is one of the main strategies to meet the recommended level of calcium; however, the food matrix and nature of calcium salt is the key consideration in the processing and bioavailability. It has been shown that milk and milk products serve as the best food matrix for absorption of calcium with most of the salt except tricalcium phosphate. However, information is lacking regarding the provision of calcium from pure salt and supplements and food/beverage vehicles posing a challenge in the identification of suitable vehicles for the absorption of calcium [16]. We observed that among eleven tested food matrices, yogurt was the best matrix for digestibility (49.75%) and bioavailability (22.80%) of calcium. It was reported earlier that all available formulations of calcium in the form of excipients, encapsulation, and granulation showed 7.5%–39% calcium availability [17]. The natural composition synchronizing with salt ingredients can strongly influence the bioavailability of that mineral as yogurt has naturally the highest content of calcium which may influence the bioavailability and help in determining the precise additional dose of calcium for fortification because above a certain quantity, additional calcium fortification leads to a reduced bioavailability of calcium [6]. This required a precise extrapolation of salt, supplement, and food matrix/beverage without generalization of only calcium sources for pharmaceutical preparation.

Besides the original calcium present in the food matrix, several other factors can enhance or inhibit the bioavailability. For example, citrus juice can enhance the bioavailability of calcium and bread can reduce it [17]. We observed that the digestibility of calcium was the highest with yogurt but the bioavailability was low. In contrast,

higher dietary intake increases the intestinal absorption of zinc from foods [18] but we did not observe that higher concentration increases the bioavailability of zinc. The digested zinc contents were similar in all food matrices but coffee and yogurt have 4 times higher bioavailability of zinc than the other eight tested matrices. This indicates that food matrix and nutritional contents are the key factors in determining the bioavailability of zinc. The higher phytate and phosphorus contents inhibit zinc absorption, while the protein matrix enhances the intestinal intake of zinc [19]. Similarly, the other minerals may influence the absorption of zinc. Calcium and cadmium are considered major inhibitors of zinc [20].

5. Conclusion

Thus, the main challenge for better digestibility and bioavailability is associated with the prevention of interaction with other minerals of food matrix and processing additives. We observed that high protein content in food matrices such as yogurt can be better for the absorption of calcium and zinc. This study will help to fortify the products intended for mass or specific consumption, including cereals, fruits, and dairy-based products.

Data Availability

The presented data are complete and will be available on publication.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

The supplementary data information of food matrices with original mineral contents used in this study are presented in the supplementary data file. (*Supplementary Materials*)

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