



Short communication

Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables

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ABSTRACT

Bioaccessibility of five essential micronutrients (iron, zinc, copper, manganese and molybdenum) from the Lebanese food basket including bread, different varieties of white cheese, fruit and vegetables was evaluated using the in vitro gastrointestinal digestion model. Only very small fraction of Fe and Zn (ca. 10%) was found bioaccessible from bread, squash and cucumber. Iron in apple was not bioaccessible either (<10%) but apples were found to be a good source of Zn (56%). Most of iron (>50%) in cheese was found to be bioaccessible but only one type of cheese, double crème, contained readily bioaccessible zinc. More than 50% of copper and molybdenum was found bioaccessible regardless of the investigated food. High bioaccessibility (>50%) was also observed for manganese in fruit and vegetables whereas that from bread and cheese was fair (25–30%).

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1. Introduction

Several trace elements, such as, iron, zinc, copper, manganese and molybdenum, are components of enzymes essential for life, and should be supplied to the human body, preferably from the diet. Information of the presence of mineral elements in the diet refers typically to their total concentrations, usually measured by atomic absorption spectrometry (AAS) [1], inductively coupled plasma emission spectrometry [2], or ICP mass spectrometry [3]. However, these methods do not allow the measurement of the fraction which can be bioaccessible to the human body [4]. This fraction is dependent on the speciation of the elements, on the behaviour of organometallic species and complexes in the gastrointestinal tract, and on interactions with the food matrix.

The determination of the potentially bioaccessible fraction from food and studies of factors enhancing (e.g. ascorbic acid, bovine serum albumin, and meat proteins) or inhibiting (e.g. dietary fibers, polyphenols, phosphates, phytic acids, oxalates, calcium salts and casein) [15] the bioaccessibility, both diet related (interactions from different foods) and physiological (sex, age, and nutritional status), are important to evaluate the real intake of essential micronutrients from the diet.

The bioaccessibility, which can be defined as the amount of metal converted to soluble forms in the gastrointestinal conditions, is typically evaluated using a sequential analysis with artificial gastric juice and intestinal juice, and the analysis of the soluble fractions [5]. In vitro methods were widely used to evaluate bioaccessibility of minerals and trace elements from different sort of food products [4–7].

The objective of this study was to evaluate the bioaccessibility of five essential micronutrients (Fe, Cu, Zn, Mn, and Mo) from the Lebanese diet including bread, vegetables, fruits and white cheese, which contribute by 136 g day⁻¹ for bread (10.3%), 9.8 g day⁻¹ for cheese (0.75%), 49.4 g day⁻¹ for cucumber or raw vegetables (3.7%), 13.7 g day⁻¹ for squash (1.03% presented by mixed vegetables stew) and 61 g day⁻¹ for apple (4.6%) to total diet of the Lebanese population [9], respectively, and to evaluate their contribution to the Recommended Daily Allowance (RDA). As the latter determines the quantity of elements needed by the body, it is of paramount importance to know the bioaccessible fraction of the consumed food.

2. Experimental

2.1. Samples

Cheese, bread, apple, cucumber and squash were selected to evaluate the bioaccessibility of nutrients from the different categories (cheese, cereals, fruit and vegetables, respectively). Two

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Table 1

Total concentrations and the bioaccessible fractions (BF%) (in $\mu\text{g g}^{-1}$) of, Cu, Fe, Mn, Mo, and Zn in bread, cheese, cucumber, squash and apple (duplicate average values \pm standard deviations).

	Cu		Fe		Mo		Mn		Zn	
	Conc. ($\mu\text{g g}^{-1}$)	BF%	Conc. ($\mu\text{g g}^{-1}$)	BF%	Conc. ($\mu\text{g g}^{-1}$)	BF%	Conc. ($\mu\text{g g}^{-1}$)	BF%	Conc. ($\mu\text{g g}^{-1}$)	BF%
Bread 1	4.1 \pm 0.05	62 \pm 4.2	47 \pm 0.5	12 \pm 0.2	0.49 \pm 0.011	82 \pm 9.1	51 \pm 0.8	21 \pm 0.4	34 \pm 0.6	5.9 \pm 0.04
Bread 2	3.6 \pm 0.21	53 \pm 4.7	43 \pm 0.99	12 \pm 0.1	0.43 \pm 0.004	97 \pm 3.4	43 \pm 0.7	27 \pm 0.5	28 \pm 1.3	8.9 \pm 0.2
Bread 3	4.1 \pm 0.95	49 \pm 10.6	41 \pm 0.03	11 \pm 0.2	0.51 \pm 0.009	98 \pm 3.9	33 \pm 0.5	27 \pm 0.4	24 \pm 1.4	9.5 \pm 1.2
Cheese 1	0.76 \pm 0.022	66 \pm 3.6	5.3 \pm 0.54	50 \pm 3.4	0.02 \pm 0.009	32 \pm 14.4	0.65 \pm 0.013	26 \pm 0.5	35 \pm 0.2	12 \pm 0.1
Cheese 2	0.54 \pm 0.024	51 \pm 7.5	3.3 \pm 0.34	84 \pm 2.9	0.03 \pm 0.006	53 \pm 39.4	0.34 \pm 0.009	30 \pm 0.8	43 \pm 1.7	12 \pm 0.6
Cheese 3	0.44 \pm 0.013	56 \pm 0.2	4.3 \pm 0.37	52 \pm 5.0	0.042 \pm 0.01	NA	0.15 \pm 0.001	NA	3.3 \pm 0.06	69 \pm 1.7
Cucumber	0.67 \pm 0.017	40 \pm 1.0	4.0 \pm 0.26	12 \pm 4.7	0.09 \pm 0.001	46 \pm 2.7	1.2 \pm 0.03	46 \pm 1.2	2.8 \pm 0.10	11 \pm 0.5
Squash	1.1 \pm 0.13	42 \pm 5.2	10.8 \pm 0.05	6.8 \pm 0.34	0.07 \pm 0.018	45 \pm 6.1	1.8 \pm 0.05	58 \pm 1.7	7.8 \pm 0.15	10 \pm 0.7
Apple	1.2 \pm 0.05	54 \pm 2.1	3.7 \pm 0.03	9.7 \pm 0.38	0.03 \pm 0.005	NA	0.82 \pm 0.017	92 \pm 1.9	0.47 \pm 0.03	55 \pm 3.5

NA, not available.

types of white cheese were tested: the Halloumi (semi-hard cheese traditionally made of unpasteurized sheep and goat's milk and stored in its natural juice with salt-water) and the double crème (made of cow's milk enriched with cream to contain at least 60% milk fat). In terms of bread, the double loaf and Saj Bread made from brown or full wheat flour were tested. These varieties from cheese and bread were selected as they showed the highest concentration of nutrient elements in comparison with other types of white cheese and white flour based bread [8].

2.2. Procedures

2.2.1. Total concentrations of elements

They were determined as described in detail elsewhere [10]. In brief, a 0.25 g sample was hot-plate digested at 85 °C with a mixture (10:3) of conc. HNO_3 and conc. H_2O_2 (Instra-analyzed, Baker, Deventer, The Netherlands) till a transparent solution was obtained. The solution was analyzed by ICP MS using a double-focusing sector-field mass spectrometer (Element XR, ThermoFisher, Bremen, Germany) fitted with a MicroMist glass concentric nebulizer (Glass Expansion, Melbourne, Australia) and a CETAC ASX-520 autosampler (Omaha, NE). ^{95}Mo was measured at $R = 300$ whereas the other elements (Cu, Fe, Mn, and Zn) at 4000. De-ionized water (18.3 M Ω cm) was used throughout. Calibration was done using an external calibration graph established with matrix matched standard solutions.

2.2.2. Gastro-intestinal digestion

The study was carried out using the protocol previously developed in the laboratory [11]. In brief, a 0.3 g sample was incubated with 5.0 ml of a gastric solution (10 mg ml $^{-1}$ of pepsin in a 0.15 mol l $^{-1}$ NaCl solution, pH 2.5) for 4 h at 37 °C. Then, pH was brought to 7.4 by adding an adequate volume of a 1.0 mol l $^{-1}$ NaOH solution and 5.0 ml of an intestinal solution (30 mg ml $^{-1}$ of pancreatin, 10 mg ml $^{-1}$ of amylase and 1.5 mg ml $^{-1}$ of bile salts) was added. The mixture was incubated again for 4 h at 37 °C and centrifuged at 4000 rpm for 15 min (Hettich 16 centrifuge, Beverly, MA). The supernatant was made up to 25 ml and analyzed by DF-SP-ICP MS. Calibration was external using a matrix-matched solution.

2.2.3. Quality control and assurance

Samples were analyzed in duplicate. Procedural blanks were run in parallel. The accuracy of the measurement of total elements concentrations was verified by the analysis of CRMs (NIST SRM 1549 of non-fat milk powder, NIST SRM Wheat flour 1567a and IRMM Brown Bread BCR 191). The quality of the different extraction yield measurements was additionally made by checking the mass balance, analysis of the residue and comparing the sum of the concentrations measured with the supernatant and the residue

with that in the total sample. The agreement (98 \pm 12%) was judged satisfactory.

3. Results and discussion

The results obtained for the determination of total concentrations of Cu, Fe, Mn, Mo and Zn and bioaccessible fractions in the investigated products are summarized in Table 1. Contributions (in %) of bioaccessible fractions (BF) of elements of interest were calculated as follows:

$$\text{BF} = \frac{C_{\text{gid}}}{C_{\text{t}}} \times 100\%$$

where C_{gid} (in $\mu\text{g g}^{-1}$ of sample) denotes the concentration of an element determined in the supernatant resulted from gastro-intestinal digestion, while C_{t} is the total content of this element determined in the food sample [12]. Table 2 presents the data of total daily intake of elements from each food, comparing to their corresponding RDA. This daily intake values were computed by using the resulting concentration of elements $\mu\text{g g}^{-1}$ from this study and the daily intake of food commodities mg day $^{-1}$ reported elsewhere [9].

3.1. Total concentrations and RDA values

Results of elements concentrations showed that bread was the biggest contributor to total daily intakes of Cu (3.6–4.04 $\mu\text{g g}^{-1}$), Fe (41.9–47.0 $\mu\text{g g}^{-1}$) and Mn (33.15–51.07 $\mu\text{g g}^{-1}$). Zinc was present in similar amounts in bread and cheese (24.9–34.75 $\mu\text{g g}^{-1}$) and (35.09–43.93 $\mu\text{g g}^{-1}$), respectively, except for double crème cheese which showed a very low concentration 3.28 $\mu\text{g g}^{-1}$. Squash can be considered as a good source of Fe as it showed 10.8 $\mu\text{g g}^{-1}$ in comparison with other legumes and fruits (3.7–4.0 $\mu\text{g g}^{-1}$) and even cheese samples (3.3–5.3 $\mu\text{g g}^{-1}$).

The daily total intakes (Table 2) of elements from the analyzed food products consumed by the Lebanese population, permit to conclude that total consumption of nutrients as Cu (0.66 mg day $^{-1}$), Fe (6.6 mg day $^{-1}$), Mo (0.075 mg day $^{-1}$), Mn (5.9 mg day $^{-1}$) and Zn (4.5 mg day $^{-1}$) is mainly provided by bread. Indeed, the daily intake of bread is 136.8 mg day $^{-1}$ in comparison with 9.8, 49.4, 13.7 and 61 mg day $^{-1}$ for cheese, cucumber, squash and apple, respectively (cf. Total Diet Study [9]). In terms of vegetables, cucumber contributes more nutrients than squash, being responsible for the daily intake of 33.0 $\mu\text{g day}^{-1}$ Cu, 197.6 $\mu\text{g day}^{-1}$ Fe, 4.44 $\mu\text{g day}^{-1}$ Mo, 59.2 $\mu\text{g day}^{-1}$ Mn and 138.3 $\mu\text{g day}^{-1}$ Zn. From apple, the daily intake of Cu (73 $\mu\text{g day}^{-1}$) and Fe (225 $\mu\text{g day}^{-1}$) is relatively higher comparing to that from vegetables, while for Zn is much lower (28 $\mu\text{g day}^{-1}$). Almost similar daily intake for Mo (1.83 $\mu\text{g day}^{-1}$) and Mn (50 $\mu\text{g day}^{-1}$) has been evaluated between apple and other legumes.

Table 2

Daily intake of Cu, Fe, Mo, Mn and Zn from analyzed food commodities.

	Bread	Cheese	Cucumber	Squash	Apple	Total intake of elements from tested commodities (mg day ⁻¹)	RDA ^a (mg day ⁻¹)
Daily intake (g day ⁻¹)	136.8	9.8	49.4	13.7	61		
Daily intake of elements from each corresponding food commodity ^b (μg day ⁻¹)							
Cu	534	5.7	33.0	15.07	73	0.66	0.9
Fe	6046	42.1	197	148	225	6.6	8
Mo	65.2	0.43	4.44	0.9	1.83	0.07	0.05
Mn	5827	3.7	59.2	24.6	50	5.9	2.3
Zn	4008	268	138	106	28	4.5	11

^a RDA as published by Food and Drugs Administration-US.^b Value of daily intake of each elements were computed by reference to the element concentration in μg g⁻¹ and the mass of food commodity consumed per day.

This daily intake of nutrients calculated in this study was similar to the results of Nasreddine et al. [9] showing bread and cereals to be the main contributors to the intake of Cu (486.9 μg day⁻¹) and Zn (3.07 mg day⁻¹). Although both studies agreed that bread and cereals were the main contributors of Mn, a difference in the daily intake was observed (this study 5.8 mg day⁻¹; the former one 0.97 mg day⁻¹).

Results of total intake of each elements from the analyzed food varieties (bread, cheese, cucumber, squash and apple), showed that they contributed to 73% of the Cu RDA (0.66 mg day⁻¹ out of 0.9 mg day⁻¹ RDA), 82.6% of Fe RDA (6.61 mg day⁻¹ out of 8 mg day⁻¹ RDA) and 40.9% for Zn (4.5 mg day⁻¹ out of 11 mg day⁻¹ RDA). Mo and Mn are supplied in large excess (166% and 256% of their RDA values: 0.045 and 2.3 mg day⁻¹ respectively) which is partly explained by the high concentrations of Mo (0.42–0.51 μg g⁻¹) and Mn (33.15–51.07 μg g⁻¹) present in bread samples.

3.2. Bioaccessible fraction

A recent review of in vitro methods distinguishes the concept of bioaccessibility: the maximum soluble concentration of the target element in the simulated GI solution after filtration or centrifugation (used in this study) and bioavailability: the dialyzable fraction of the element which can pass through a semi-permeable membrane with a specified pore size at equilibrium and non-equilibrium conditions (Moreda-Pinero et al.).

The *sine qua non* condition for an element to be bioaccessible is to be present in forms soluble in the intestinal conditions. The experimental approach applied to verify this included two steps which imitated first extraction in an “acidic stomach” using a gastric fluid (pepsin) and then extraction in a “neutral small intestine” using an intestinal fluid (pancreatin, amylase and bile salts). Note that it should be kept in mind that some soluble species may not pass through the intestine membrane and thus not to be bioaccessible. From the analytical point of view, quality control by demonstrating the match of the sum of the soluble fraction and the residue and the total element concentration, is critical.

3.2.1. Iron

Iron is a component of haemoglobin and various enzymes. A deficiency of Fe causes anaemia while excess may increase the risk of developing cancer or heart attacks. Iron is present in both divalent and trivalent forms; the former is known to be readily absorbed [12].

Bread: Concentrations of Fe in bread samples were established to be within 41.9–47.0 μg g⁻¹. Tokalioglu and Gurbuz [13] reported mean concentration values of Fe in some Turkish cereals to be 19.5 ± 0.25 mg kg⁻¹ in wheat and 12.5 ± 0.8 mg kg⁻¹ in flour. Another study from Turkey reported a mean concentration of Fe as 18.2 ± 8.1 mg kg⁻¹ (dry weight) in different bakery products [14]. The bioaccessible fraction was very low (11.8–12.8%).

This is very likely due the high contents of phytates [high chelating power of Fe(II) to form insoluble compounds or precipitation as soluble Fe–Ca–phytate complex] and myo-inositol hexaphosphates, that has same chemical formula as phytic acid or its salts, phytates present in plants. This reason was evoked to explain similarly low bioaccessibility of Fe from beans [15,16], lentils [17], cereals and wheat [12] and bran [15], although the evaluation of the bioaccessible/bioavailable fraction was assessed by different in vitro approaches such as measuring the water soluble fraction [15], evaluation by the dialyzable fraction after gastrointestinal digestion [12,17] or the soluble fraction after the gastrointestinal digestion [16,17,25] like in this study.

Cheese: The total concentration range was 3.3–5.3 μg g⁻¹ of which a large fraction 50.8–84.4% was bioaccessible. Differences in solubility of chelates that are formed between peptides and trace elements were proposed as a primary factor accounting for the enhancing effect on non-heme Fe absorption of whey and casein proteins [18]. On the other hand, the presence of milk-derived caseino-phospho-peptides (CPPs) formed during gastro-intestinal digestion in dairy samples decreases the Fe bio-accessibility [6].

Vegetables: The total iron content in the vegetables and fruit is relatively high (3.7–10.8 μg g⁻¹) but only a small fraction is bioaccessible (6.7–12.7%). This is likely to be due to the presence of phytate, oxalic acid, carbonate and polyphenols which form insoluble salts and impair absorption of Fe [12,15,17,19,20]. The bioaccessibility of iron from vegetables is also hampered by the relatively low concentrations of proteins and aminoacids (especially cysteine) which are known to increase the bioaccessibility by reducing and chelating iron [15,19].

3.2.2. Zinc

Zinc helps to regulate the activities of certain metalloenzymes. It is also involved in maintaining the structure and function of biomembranes, by inhibiting oxidative damage by binding to membranes at sites which might be occupied by metals with redox potential [18].

Bread: Phytates are known to bind strongly Zn [15,21] which is reflected by the very low bioaccessibility of this element (<10%) from bread despite its fairly high concentrations 24.9–34.8 μg g⁻¹. As in contrast to iron, Zn is exclusively present in the divalent state; this effect should be less pronounced [12]. Phytates present in cereals and seeds can bind Zn²⁺, Ca²⁺, Mg²⁺, Mn²⁺, Cu²⁺ and other divalent cations, whereas bioaccessibility of Zn²⁺ was reported to be the most impaired in humans because of the formation of insoluble salts or even co-precipitation of Zn as a Zn–Ca–phytate complex, which is enhanced by high Ca intake from milk [18,22].

Cheese: Halloum cheese contains the highest total Zn concentration (35.1–43.9 μg g⁻¹) which shows low bio-availability (12.4%). This is due to the presence of casein and casein phosphopeptides which make Zn unavailable for absorption [6]. Although in the acid medium of the stomach, dietary Zn can be released from casein, a considerable proportion of this casein is not digested, making Zn

less available in this form [6]. On the other hand, in double crème cheese the total Zn concentration is 10 times lower but 70% of the Zn present is bioaccessible. This is likely due to the high percentage of fat in double crème (60%) in comparison with Halloum.

Fruit and vegetables: Zinc in cucumber and squash show a low bioaccessibility (10.9%). Total concentration in apples is 5–15 times lower but the bioaccessibility 5 times higher. Note that food processing, such as heat treatment, malting and soaking was reported to increase bioaccessibility of Zn by altering its complexes with phytates and dietary fibers [6,18,20,23,24].

3.2.3. Copper, manganese and molybdenum

Copper is a component of enzymes required for Fe metabolism, its essentiality is mediated through specific copper proteins. The Cu deficiency leads to high blood pressure and infertility [25]. Manganese is involved in bone formation and as a required cofactor for enzymes responsible for amino acids, cholesterol and carbohydrate metabolism as oxidoreductase, transferase, and hydrolases [26]. Molybdenum is an active cofactor for many enzymes as aldehyde oxidase, sulphide oxidase and xanthine oxidase which activity is directly proportional to the amount of Mo in body. High concentration of Mo can act as inhibitor of these enzymes, as it also affects protein synthesis, metabolism and growth [27]. Literature data on the bioaccessibility of these elements from foods are scarce, and for Mo non-existent.

Bread: Copper, Mo, and Mn show, in general, much higher bioaccessibility than iron and zinc. The bioaccessible fraction of Cu was between 49.2 and 62.4% from bread samples. This is in reasonably good agreement with bioaccessibility of Cu from tea-biscuit (46.7–69.1%) determined as in this study [25]. Note that the Cu water-soluble fraction from cooked beans did not exceed 15% which could be due to the formation of an insoluble complex of polyphenol–proteins under heat treatment [15]. In contrast to Fe and Zn, copper–phytate complexes remain soluble at the pH in the intestinal lumen [15,17,23] and inhibitors of copper absorption are sugars, animal proteins, S-amino acids and histidine rather than dietary fibers [25]. The manganese bioaccessible fraction in bread was between 21.71 and 27.66%. This result is similar to the bioaccessibility of Mn from tea-biscuit (18.1–59.9%). Mn bioaccessibility correlated negatively with the phytic acid content. According to Vitali et al. [25], this correlation was significant only in biscuits samples that contained exclusively wheat flour, while addition of other raw materials, as inulin or oat decreased the importance of phytate as major chelating compound. Regarding Mo, the entirety of this element present in bread is well bioaccessible (82.16–98.05%).

Cheese: The bioaccessibility of Cu from cheese samples was relatively high (51.9–66.5%). Regarding the Mo and Mn bioaccessibility, its range varied with the type of analyzed cheese. Indeed, the Halloum cheese showed a bioaccessibility range of 26.9–30.69% for Mn and 32.0–53.6% for Mo, while both elements were not detected after the gastrointestinal digestion in the double crème cheese, probably because fat materials (60% in double crème) to which these elements were bound, were not well digested.

Fruit and vegetables: The bioaccessibility of Cu, Mo and Mn from fruit and vegetables showed almost similar results (40.7–58.9%) with the exception of Mo which showed no bioaccessibility from

apple (probably because of chelation with polyphenols). The opposite was observed for Mn which was twice more available from apple than from cucumber and squash. Manganese present in apple was found to be almost totally bioaccessible (92.9%). Note that total bioaccessibility of this element was also reported from aquatic plants recently [4].

4. Conclusion

The application of the in vitro digestion model using simulated gastro-intestinal juice allowed the demonstration that, in some cases, only a very small fraction of the total quantity of element present in food is potentially bioaccessible. In addition, the results of nutrients bioaccessibility (Fe, Zn, Cu, Mo and Mn) show that it varies largely as a function of food matrix components and elements of interest. This is especially true for two essential elements, such as Fe and Zn which showed low bioaccessibility from bread (<10%) and vegetables (5–12%) in comparison with Cu (50–60% and 40–50% for the two matrices, respectively), and the higher bioaccessible fraction of Fe from cheese ($\geq 50\%$) compared to Zn (12%). Bioaccessibility studies should therefore complete the total element survey campaigns in order to obtain a realistic picture of the human intake of essential and toxic elements.

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