In-vitro Digestibility Methods and Factors Affecting Minerals Bioavailability: A Review

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ABSTRACT

Micronutrient deficiencies are known to affect many people worldwide. Among the factors contributing to this situation, the poor bioavailability of foods micronutrients is key. This review aims at highlighting the factors affecting minerals bioavailability and their assessment methods. The numeric approach was used during data collection. The results show that the bioavailability of minerals is key to assess the amount that is released for body metabolism. Different factors including inhibitors and promoters affect their bioavailability. There are different methods to assess the digestibility of minerals, which generally involve oral, gastric, and intestinal phases.

KEYWORDS

Bioaccessibility; bioavailability; dialysability; in vitro; promoter

Introduction

Minerals are required for maintaining normal metabolic functions in human body. These elements are classified as either major or trace elements that are needed in larger or fewer amounts, respectively.[1] They are known to have key roles in our body from building strong bones to transmitting nerve impulses for healthy and lengthy life.[2] According to De-Regil et al.,[3] more than two billion people worldwide were affected by minerals deficiencies, and the most vulnerable groups are under 5-year-old children and pregnant women.[4,5] Among these mineral deficiencies, iron and zinc deficiencies are the most prevalent for children. They are associated to poor growth, impaired cognitive development and poor health status.[6] According to FAO et al.,[7] 32.8% of women in reproductive age are worldwide anemic in 2016. Information on zinc deficiency prevalence is generally lacking. However, it is believed that where iron deficiency persists, zinc deficiency may possibly occur.[5] The long-term consequences of these mineral deficiencies are not only seen at the individual body level but also have unfortunately negative outcomes on the economic performance and human capital of the country.[5] The aforementioned mineral deficiencies may be due to not only the poor mineral contents of foods but also their poor bioavailability. Based on the total content of minerals of a food, it is well established that only a certain quantity is bioavailable after ingestion.[8] Thus, the supply of minerals to the human body should not only depend on the amount of the minerals in the food but also on their bioavailability. The bioavailability of food nutrients appears as a key factor for optimal health status.[9] However, there is no universally accepted definition of bioavailability. A definition that has widely accepted defines bioavailability as the quantity of a nutrient that is available for normal metabolic and physiologic processes.[10] This review aims at highlighting the importance of minerals and the assessment methods of minerals digestibility. It also targets the inhibitors and promoters of mineral bioavailability to

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optimize diet mineral absorption. The effects of various processing technologies on the mineral bioavailability are also reviewed.

Methods

The numeric approach was used during data collection from November 2019 to March 2020. Google (https://sites.google.com), Google Scholar (www.scholar.google.fr), Cab abstracts (https://www.cabi.org), PubMed (https://www.ncbi.nlm.nih.gov), Science Direct (https://www.sciencedirect.com) and Springer (https://www.springer.com) databases were especially consulted on the different review aspects. Combinations of the following keywords "minerals", "importance", "bioaccessibility", "bioavailability", "digestibility", "in vitro-solubility", "dialysability", "effect of processing", "absorption", "human body", "food sources", "methods", "inhibitors", "enhancers", "promoters", "bioaccessible minerals", "soluble minerals" were used. Google Scholar Alerts were created to receive any update papers on bioavailability of minerals. The terms used to create google alerts were "bioaccessibility", "bioavailability", "digestibility", "in vitro-solubility", "dialysability". No limit of year was used during the data collection. The bibliographies of the papers on hand were used to find other references. Of the 210 papers we found in the all consulted databases, 157 papers were selected after reading their abstracts and 130 papers were used in the review according to the relevance of their contents. Each article selected for inclusion in this review was critically analyzed.

Results and discussion

Minerals are believed to have beneficial effects in human body while their bioavailability is a key indicator to assess the amount that is released for body metabolism. Bioavailability minerals is affected by numerous dietary components, which include inhibitors and promoters of absorption.[11–14] It is also established that processing affects the bioavailability of minerals according to the processing parameters.[15–21]

Importance of minerals in human body

Minerals are inorganic nutrients, usually required in small amounts from less than 1 to 2500 mg/day according to the mineral.[22] They are required for maintaining normal metabolic functions in human body and are classified as macro (major) or micro (trace) elements.[1,22] They exist in specific food matrices and are vital for the health of bones, teeth and structural parts of enzymes.[1,2] The macro-minerals include magnesium, potassium, calcium, phosphorus, sodium and chloride while the micro-elements include iron, copper, cobalt, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium, and sulfur.[23,24]

Macro-minerals

The macro-minerals are minerals that are required in amounts greater than 100 mg/day.[25] Among them, calcium, sodium, magnesium, and potassium functions are developed in this section as they are considered as essential macro-minerals.[24]

Calcium

Calcium is an essential nutrient that is provided by the diet. The adult body contains approximately 1200 g (women) or 1 400 g (men), 99% of which is found in the skeleton and the remaining 1% is found in extracellular fluids, intracellular structures, and cell membranes.[26] Calcium (Ca) is known to play a structural role and also acts as an essential intracellular messenger in cells and tissues, with functions in blood coagulation, activation of enzyme reactions and hormone secretion.[27] If the calcium is insufficient in the diet, bones act as a reserve to maintain constant blood level.[28] In

blood coagulation, calcium activates the conversion of prothrombin to thrombin and takes part in milk clotting.[22]

Calcium deficiency is known to cause poor calcification and also affects the dentition of both children and adults.[22] If the dietary supply of calcium is insufficient to meet physiological requirements, calcium is resorbed from the skeleton which leads a reduction in bone mass associated with risk of fracture.[26] According to EFSA,[29] hypercalcemia is defined by serum calcium concentrations higher than 2.75 mmol/L (11 mg/dL). It may occur with high intake of calcium from the diet alone (i.e., milk) but can be caused by high-dose calcium supplements. The well-documented causes of hypercalcemia include hyperparathyroidism of different aetiologies and, less frequently, excessive calcium and/or vitamin D intakes.[26] Older people are sensitive to excess calcium intake because of the decrease of the renal function.[26] It appears then necessary for older people to reduce their calcium intake to avoid hypercalcemia as indicated in the suggested requirements. Indeed, the calcium estimate average requirements in mg/day (EARs) are respectively 390, 680, 960, 860 and 750 for 1–3 years, 4–10 years, 11–17 years, adults 18–24 years (pregnancy and lactation), adults ≥25 years (pregnancy and lactation) according to EFSA.[26]

Magnesium

Most of magnesium body content is bound to calcium and phosphorus as bone constituents, whereas the rest is distributed in organs and tissues. Cilla et al.[27] reported that magnesium deficiency is observed only in two situations: a secondary complication of primary disease such as cardiovascular or neuromuscular disorders and the result of rare genetic homeostatic disorders. It is also a constituent of bones, teeth, enzyme cofactor (kinases, etc.).[25] Magnesium has an impact on bone health through its role in the structure of hydroxyapatite crystals in bone.[30]

Acute magnesium deficiency results in vasodilation, vomiting and diarrhea, which appear a few days after consumption of poor magnesium diet.[22] According to EFSA,[30] magnesium deficiency can have many different causes, including renal and gastrointestinal disorders. Magnesium is known to interact with other minerals, vitamins or substances present in foods through different mechanisms such as during its absorption.[31] Magnesium adequate intake (mg/day) for 7–11 months, 1–<3 years, 3–<10 years, 10–<18 years, ≥18 years, are respectively 80, 170, 230, 300, 350 for males and 80, 170, 230, 250, 300 for females. The adequate intake for lactation and pregnancy females is 1.5 mg/day.[30] These adequate intakes could be met through the different foods intake of the different age groups.

Potassium

Potassium is an essential mineral in the human diet. It plays a role in cell metabolism, participating in energy transduction, hormone secretion and the regulation of protein and glycogen synthesis.[32] Potassium is believed to be the principal cation in intracellular fluid and have functions in acid–base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction, particularly the cardiac muscle and cell membrane function.[22]

Potassium deficiency also known as hypokalaemia is defined as a serum potassium concentration lower than 3.5 mmol/L.[33] In general, deficiency may be caused by increased potassium losses via diarrhea, vomiting, burns or excessive renal losses.[34,35] Hypokalaemia resulting from insufficient dietary intake is rare and may be associated with severe hypocaloric diets or appear as the result of an increased requirement needed for the formation of new tissue during recovery from malnutrition.[32] Hyperkalemia appears when serum potassium concentration is greater than approximately 5.5 mmol/L in adults.[33,36] Potassium adequate intakes (mg/day) for 7–11 months, 1–<3 years, 4–6 years, 7–10, 11–14 years, 15–17 years, ≥18 years, pregnancy and lactation women are respectively 750, 800, 1 100, 1 800, 2700, 3500, 3500, 3500, and 4000.[32]

Sodium

Sodium exists as the electrolyte Na^+ in body fluids; it is the dominant cation in the extracellular fluid.[37] The functions of sodium is recognized in its participation in the control of the volume and systemic distribution of total body water; enabling the cellular uptake of solutes; and the generation through interactions with potassium of transmembrane electrochemical potentials.[37,38]

The health consequences of both chronic and acute deficiencies and excesses of sodium are related to the distribution of total body water and sodium in the extracellular and intracellular fluid compartments.[37] The threshold of 135 mmol/L is believed to be the lower point of the reference range indicating potential sodium depletion and deficiency. However, because of the systemic interaction of sodium with water balance, the serum sodium concentrations at which symptoms of sodium deficiency become apparent are not well established.[37] Sodium adequate intake (mg/day) for children aged 7–11 months, 1–3 years, 4–6 years, 7–10 years, 11–17 years and ≥18 years are respectively 0.2, 1.1, 1.3, 1.7, 2.0, and 2.0.[37]

Micro-minerals

The micro-minerals or trace elements are minerals that are required in amounts less than 100 mg/day.[25] The functions of iron, zinc, copper and manganese are developed as they are considered as essential micro-minerals according to Shar et al.[24]

Iron

Most of the iron in the human body is present in the erythrocytes as hemoglobin, where its main function is to carry oxygen from the lungs to the tissues.[28] It is stored in the liver as ferritin and as hemosiderin and its deficiency is associated with anemia, impaired cognitive and physical performance, and increased maternal and child mortality.[,39]Cilla et al[27]. reported that iron deficiency remains the most common nutritional deficiency in developing countries. There are two types of dietary iron such as heme-Fe which comes from hemoglobin and myoglobin (viscera, red meat, fish, and seafood) and non-heme form (inorganic iron) which is the most abundant type of dietary iron and is generally present in plant foods (vegetables, whole grains, nuts, and oil seeds).[40] Iron exists in the blood mainly as hemoglobin in the erythrocytes and as transferrin in the plasma.[25]

Iron deficiency leads to alterations in many metabolic functions that may impact brain functioning such as neurotransmitter metabolism, protein synthesis, organogenesis, etc.[22] Iron deficiency anemia, defined as the combination of iron deficiency and anemia (low hemoglobin), can be distinguished from that caused by other nutritional deficiencies, such as folate or cobalamin deficiency. However, the pathogenesis of iron deficiency may not be dietary. Non-dietary causes of iron deficiency and anemia include conditions that cause gastrointestinal blood loss or malabsorption.[41] Iron-deficient and anemic infants and children have delayed attention regarding its consequences mainly the poor recognition memory. Some studies have shown an association between iron deficiency anemia in early childhood and long-lasting poor cognitive and behavioral performance.[41] In women, anemia is declared apparent whether hemoglobin concentrations drop below 130 g/L, and the effect becomes greater with every 10 g/L fall in hemoglobin.[42] The risk of systemic iron overload from dietary sources is negligible with normal intestinal function.[41] The iron Estimate Average Requirements in mg/day (EARs) for 7–11 months, 1–6 years, 7–11 years, 12–17 years (male), 12–17 years (female), ≥18 years (male), ≥18 years (female premenopausal), ≥18 years (female postmenopausal) are respectively 8, 5, 8, 8, 7, 6, 7, 6.[41]

Copper

Copper (Cu) is essential as an enzymatic cofactor. It is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of iron in hemoglobin, assists in the absorption of iron from the gastrointestinal tract (GIT) and in the transfer of iron from

tissues to the plasma.[25,43] Most of the fecal copper is unabsorbed dietary copper, but some of it comes from the bile, which is the major pathway of copper excretion.[22] Copper serves as an electron donor and acceptor in a similar chemical reaction to that for iron.[44] Its deficiency is rare, but children with poor calcium diets are more susceptible, especially if they also have diarrhea or malnutrition.[44]

Clinical disorders associated with copper deficiencies include anemia, bone disorders, impaired growth and reproductive performance, heart failure, and gastrointestinal disturbances.[22] The effect of copper deficiency on hematological function was first recognized in the early 20th century, when anemia that was refractory to iron supplementation was shown to be cured by giving food containing copper.[44] A tolerable upper intake level (UL) of 5 mg/day was established for adults, but not for pregnant and lactating women, owing to the absence of adequate data, while for children, the UL of adults was extrapolated based on body weight.[44]EFSA[44] also reported that an excess of copper causes problems only under certain specific conditions, notably genetic disorders such as Wilson disease. Copper adequate intakes (mg/day) for 7–11 months, 1–<3 years, 3–<10 years, 10–<18 years, ≥18 years are respectively 0.4, 0.7, 1.0, 1.3, 1.6 for males and 0.4, 0.7, 1.0, 1.1, 1.3 for females. Lactation and pregnancy females have 1.5 mg/day as adequate intake.[44]

Zinc

Zinc is distributed widely in plant and animal tissues. It is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase and acts as cofactor.[22] Zinc has a structural or catalytic role or both, in each of the six classes of enzymes.[45] It is known to be essential for gene expression and the regulation of cellular growth and differentiation.[27,28]Drago reported that zinc is also involved in metabolism, as a component of metallo-protease enzymes. According to Allen et al.,[39] zinc deficiency is a significant public health problem and all population age groups are at risk of zinc deficiency, but infants and young children are probably the most vulnerable. In children, impaired growth (stunting) is one of the possible consequences of zinc deficiency.[39]

Zinc deficiency is also associated with hypogonadism, impaired wound healing, decreased taste and smell acuity, acrodermatitis enteropathica.[25,45] According to EFSA,[45] acute severe zinc deficiency results from genetic defects in zinc transporters involved in the intestinal absorption of zinc. Chronic high zinc intake can nevertheless result in severe neurological diseases attributable to copper deficiency.[46] The SCF[47] has set a tolerable upper intake level (UL) of 25 mg/day for adults, including pregnant and lactating women, based on studies of zinc supplementation for up to 14 weeks. EFSA[45] reported that to have high absorption level of zinc, we need dietary low in cereal fiber, low in phytic acid content and with a phytate–zinc molar ratio below 5; adequate protein content principally from non-vegetable sources, such as meat and fish. The zinc Estimate Average Requirements in mg/day (EARs) for 7–11 months, 1–3 years, 4–6 years, 7–10 years, 11–14 years, 15–17 years (male) and 15–17 years (female) are respectively 2.4, 3.6, 4.6, 6.2, 8.9, 11.8 and 9.9.[45] These EARs are difficult to meet especially among under 5-year-old children as zinc deficiency still a major issue in most of the developing countries. Many reasons could explain this situation such as the inadequate absorption of zinc due to inhibitory effect of phytate and polyphenols generally abundant in plant-based foods.[11,48]

Manganese

Manganese is an essential nutrient in humans and plays a role in bone mineralization, regulation of protein and energy metabolism and cellular protection from damaging free radical.[49] It is a cofactor of hydrolase, decarboxylase, and transferase enzymes[25] and is required for growth, development and maintenance of health.[50] Its role in enzyme activation and glycoprotein and proteoglycan synthesis is also recognized.[22,51] According to Wedler,[49] manganese has been shown to activate numerous enzymes involved with either a catalytic or a regulatory function (e.g., transferases, decarboxylases,

hydrolases). It plays also a role in mucopolysaccharides metabolism[52] and regulation of cellular energy, bone and connective tissue growth and blood clotting.[50]

Avila et al.[50] reported that few occurrences of manganese deficiencies have been recognized in humans. Nevertheless, the U.S. Food and Drug Administration (U.S. FDA) suggested a Reference Daily Intake (RDI) for manganese at 2 mg/day for adults (Federal Register 2007, 72 FR 62149) while Avila et al.[50] reported that there is no consensus regarding the safe and adequate levels of this nutrient for various age groups. Manganese and iron compete for absorption sites[51] meaning in foods with high manganese content, the bioavailability of iron could be low and then iron deficiency could occur. Fiber, phytate, calcium, phosphorus and magnesium may also interfere with manganese absorption, which underlined the complexity of mechanism that occur during the mineral absorption.[51] These interactions are either mineral–mineral or anti-nutritional factor-mineral. Manganese deficiency disease or symptoms are not well known in humans.[22]

Bioaccessibility and bioavailability of minerals

The supply of nutrients to the human body does not only depends on the amount of the nutrient in a food but also on its bioavailability.[14] Nevertheless, there is no universally accepted definition of bioavailability and different researchers have defined it in different ways. According to Hambidge [53] the bioavailability of a nutrient is defined as its accessibility to normal metabolic and physiologic processes. Gibson et al.[12] reported that the bioavailability is a proportion of an ingested trace element in food that is absorbed and utilized for normal metabolic and physiological functions or storage. The bioavailability of major and trace minerals can then be defined as the proportion of the ingested minerals, which is absorbed and available for metabolic functions. The bioavailability appears as the technical term used to underline the fact that not 100% of nutrients ingested will be absorbed. It aims to describe the effect of a sequence of metabolic events, including digestion, solubilization, absorption, organ uptake and release, enzymatic transformation, secretion and excretion and nutrient utilization.[14]Marze[9] qualified the bioavailability of food nutrients and micro-constituents as a determinant factor for optimal health status. It influences a nutrient beneficial effect.[10] Although bioavailability is only a partial measure of the body's ability to benefit from a nutrient, it quantifies the amount of a substance that successfully enters the bloodstream.[14] The bioavailability of minerals varied from less than 1% to more than 90%, and it is a key factor to assess the food effectiveness.[1] A definition that has gained wide acceptance qualifies the bioavailability as the amount of a nutrient that is available for normal metabolic and physiologic processes.[10]

Gharibzahedi and Jafari[2] reported three main steps in the bioavailability of nutrient including the nutrient absorption by improving its accessibility in the intestinal lumen, the maintenance and/or absorption/uptake in the body and the utilization by the body. Indeed, the bioavailability of nutrients is correlated with their bioaccessibility and bioactivity.[54] The bioaccessibility is the fraction of a compound that is released from food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption including absorption/assimilation into the cells of the intestinal epithelium and, lastly, pre-systemic intestinal and hepatic metabolism.[55]Khouzam et al.[56] reported that 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. The concept of bioactivity includes events linked to how the bioactive compound has reached systemic circulation and it is transported and reaches the target tissue interaction with biomolecules metabolism in these tissues, and all the cascade of physiological effects it generates.[54]

In vitro digestibility methods to assess mineral bioavailability: an appraisal

The bioaccessibility is evaluated using a sequential analysis with artificial gastric juice and intestinal juice, and the analysis of the soluble fractions as reported by Khouzam et al.[56] In vitro methods were designed as an alternative to in vivo methods for estimating mineral bioavailability as in vivo methods

are expensive, labor intensive and present ethical constraints according to Minekus et al.[57] Indeed, in vitro methods simulate the physiological conditions and the sequence of events that occur during digestion in the human gastrointestinal tract.[10] Many in vitro methods were developed by several authors.[57–60] These methods are mainly based on the simulation of gastrointestinal digestion involving two or three digestion steps such as oral digestion or not, gastric and intestinal digestion with some variations based on the amount and type of enzymes used (Table 1).

Table 1 indicates that simulation of gastrointestinal digestion is performed under various conditions (temperature, pH, duration, agitation, enzymes and their chemical composition). It shows that the common temperature applied by authors is 37°C during the three digestion steps. As pH and digestion duration are concerned, it shows that pH 7 is applied during the oral phase with different incubation duration: 30[59] and 5 minutes.[77] This variation in the oral digestion duration could be certainly due to the food matrix composition. It is well known that the food composition and structure are two factors which influence the bioavailability and digestion predicting of food.[96] Indeed, Icard-Vernière et al.[77] measured the iron bioaccessibility in cereal grains (millet and sorghum grains) while the cooked soya bean, dehulled cooked cowpea (white maize dough) were analyzed by Kiers et al.[59] The pH and digestion duration during the both rest steps (gastric and intestinal digestion) vary according to authors and foods. During the gastric phase, the pH varies from 2[83,84] to 4[59] while the incubation duration varies from 1 to 4 hours. However, Moreno[97] reported that depending to some extent on the substrate, optimum pepsin activation can take place at a relatively broad pH range between 1.2 and 3.5 which broadly concord with the range of the pH used by the authors. Nevertheless, the aforementioned authors reported that at pH 4, the bovine α-lactalbumin and bovine casein were hardly hydrolyzed. Thereby, the pH 4 used by Kiers et al.[59] during the gastric digestion may certainly lead the poor hydrolyzation of some foods. It is then obvious to adjust the digestion pH according to the food matrices to be digested. As far as the intestinal phase is concerned, it is generally performed at pH range from 6[59] to 7.5[74] and between 2[77] to 4 hours.[79] It is well known that a short transit time of a food within the small intestine may limit the absorption of compounds, thereby reducing their bioavailability.[98] The duration of this phase must then take into account the food characteristics.

The most important factor in an in vitro digestion system is the enzyme characteristics.[96] Generally, the enzyme used during the food digestion are α-amylase (Sigma A-1031/Sigma A-3403-1MU), pepsin (porcine gastric mucosa, Sigma *P*-7000/P-6887 dissolved in HCl), pancretin solution (pancreatin Sigma *P*-1750 and bile salt (Sigma B-3883/Sigma B-8631/Sigma B-8756) dissolved in NaHCO3) for oral, gastric, and intestinal phase, respectively (Table 1). Hur et al.[96] reported that the choice of enzymes and incubation conditions and the need for equipment are dependent on the objectives of the study. Also, for the same enzyme, the quantity used and the incubation duration could vary according to the structure and the composition of the food matrix. For example, the gastric digestion of composite meal from cereals, legumes, milk, meat, fruits, and leguminous was performed using 3 g of freshly prepared pepsin solution (*P*-7000, from porcine stomach mucosa in HC1),[74] while Icard-Vernière et al. [77] used 1 mL of pepsin (Sigma *P*-7000, in HCl) solution for cereals (millet and sorghum grains). Indeed, it is not easy to choose an optimal enzyme:substrate ratio which reflects that found physiologically because humans show a wide variation in their gastric and pancreatic secretions and it also varies with type of food consumed.[97] However, the concentration and composition of enzymes are reported to be very important factors to consider when designing in vitro digestion models because at higher enzyme concentrations the digestion or degradation of food components accelerate.[96]

These different variations in the digestibility methods could lead to some differences in the production of bioavailabilty data, which could not allow objective comparison. To address this issue, an international consensus static method was developed by Minekus et al.[57] to produce reproducible data. This method provides at each digestion step parameters (enzyme used, pH, digestion duration, temperature) that are nearly close to the human conditions. The static character of this method may lead its global use, as most of the digestibility assays reported in the literature are the static ones.[96] The static digestibility methods are methods, which have a predefined ratio test

Table 1. In vitro digestibility methods.

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Standardized static in vitro method Minekus et al. (2014)	Mastication of solid food is simulated by mincing an appropriate amount of food using a commercially available manual or electric mincer. Simulated salivary fluid electrolyte stock solution is added to create a thin paste. A final ratio of food to SSF of 50:50 (w/v) is targeted.	Human salivary α-amylase (EC 3.2.1.1) is added to achieve 75 U mL^{-1} in the final mixture, followed by $CaCl_2$ to achieve 0.75 mM in the final mixture and the necessary amount of water to dilute the stock solution of simulated salivary fluid. The recommended time of contact with the enzyme is 2 minutes at 37°C, which requires pre-warming of all reagents to 37°C.	Porcine pepsin (EC 3.4.23.1) is added to achieve 2000 U mL^{*1} in the final digestion mixture, followed by $CaCl_2$ to achieve 0.075 mM in the final digestion. 1 M HCl is added to reduce the pH to 3.0. The necessary amount of water is added to the mixture to dilute the stock solution of simulated gastric fluid. The recommended time of digestion is 2 hours at 37°C. The pH may have to be re-adjusted with 1 M HCl during digestion	The gastric samples–chyme is mixed with SIF electrolyte stock solution. Addition of base (1 M NaOH) is required to neutralize the mixture to pH 7.0. Digestive enzymes can be added as either pancreatin from porcine pancreas or individual enzymes. $CaCl_2$ is added to reach 0.3 mM in the final digestion mixture. The pH may need re-adjustment before finally adding water to the mixture to dilute the stock solution of simulated intestinal fluid. The recommended time of intestinal digestion is 2 hours at 37°C. Alternatively, individual enzymes can be added to the digestion mixture to achieve the following activities in the final mixture: porcine trypsin (EC 3.4.21.4), bovine chymotrypsin (EC 3.4.21.1) (25 U mL-1), porcine pancreatic α-amylase (EC 3.2.1.1), porcine pancreatic lipase (EC3.1.1.3) and porcine pancreatic colipase (2:1 colipase to lipase molar excess). Bile extract: B8631 (porcine) or B3883 (bovine) from Sigma-Aldrich or fresh (frozen) porcine bile is added to the mixture.	• Fermented slurries and porridge from finger millet flour[61] • Fermented maize, sorghum and millets flours[62] • Fermented cereal porridges[63]	The dialysate in the dialysis bags will be transferred to new tubes while the digested mixture will be centrifuged and separated into the soluble non-dialyzable (SND) and insoluble fraction or pellet (P) until analysis.	[57]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Kiers et al. (2000) method	Samples are suspended distilled water and digested under simulated gastro-intestinal conditions with the use of artificial saliva containing human saliva α-amylase, artificial gastric juice containing lipase and pepsin, and artificial pancreatic solution consisting of pancreatin and bile. Sample and water in an Erlenmeyer flask was kept in a 37°C water bath while shaking for 5–10 minutes.	The α-amylase (Sigma A-1031) solution is added in the sample and incubated during 30 minutes at 37°C, pH 7 shaking, the Erlenmeyer flask is put on ice.	The pH was measured and adjusted to pH = 4 using 5 M HCl. 8 mL pepsin (Sigma *P*-6887) juice is added followed by 60 minutes of incubation while shaking at 37°C	The pH is noted and adjusted to pH 6 using 3 M $NaHCO_3$ and 10 mL pancreatic solution (pancreatin (Sigma *P*-1750), bile (Sigma B-3883), Na_2HPO_4, NaCl, $NaHCO_3$, KH_2PO_4) is added and they are incubated for 30 minutes while shaking at 37°C	• Cooked soya bean (Glycine max), dehulled cooked cowpea (Vigna unguiculata), white maize (*Zea mays*) dough[59] • Sorghum flour[15] • Baobab leaves[64] • Opaque sorghum beers[65] • Brown rice[66] • Rice products[67]	After digestion, the suspension is kept on ice and centrifuged for 15 minutes at 30,000× *g* at 4°C	[59]
Discrepancies between Kiers et al. (2000) method and consensus method	-	Incubation time (30 minutes against 2 minutes) and the type of enzymes (α-amylase Sigma A-1031 against α-amylase EC 3.2.1.1)	pH (4 against 3), incubation time (60 minutes against 120 minutes) and type of enzyme (pepsin Sigma *P*-6887 against Porcine pepsin EC 3.4.23.1)	pH (6 against 7), incubation time (30 minutes against 120 minutes)	-	-	[57,59]
Miller et al. (1981) method	The method involves simulated gastrointestinal digestion followed by measurement of soluble, low molecular weight iron. Iron from the digestion mixture, which diffused across a 6–8000 molecular weight cutoff semipermeable membrane is used as an indicator of available iron.	-	Samples of the frozen meals is thawed in a 37°C shaking water bath. Pepsin (hog stomach mucosa, Sigma Chemical Co., St. Louis, MO, suspended in HC1) is added in an amount that provided 0.5 g pepsin per 100 g of meal. The samples is incubated in a 37°C shaking water bath for 2 hours.	Aliquots of the pepsin digest is thawed and transferred to beakers. Segments of dialysis tubing containing water and an amount of NaHCO3 equivalent to the titratable acidity is placed in each beaker. The beakers is sealed with parafilm and incubated in a 37°C shaking water bath until the pH reached about 5 (approximately 30 mm). Pancreatin-bile (porcine pancreas, Sigma Chemical Co., St. Louis) extract mixture extract (porcine, Sigma Chemical Co., St. Louis, MO, is dispersed $NaHCO_3$) is added to each beaker and the incubation was continued.	• Meat, cereal products, legume, fruit, milk[60] • Fortified pearl millet porridge[68,69] • Enteral formulas + Rice pudding, Enteral formulas + Chocolate dessert, Banana smoothie+ Enteral formulas[70] • green leafy vegetables, fruits, meat, poultry, legumes, milk, curd and tea to cereal-based diets[71]	Bathophenanthroline reactive iron was measured in the dialysates immediately after the pancreatic incubation.	[60]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Discrepancies between Miller et al. (1981) method and consensus method	-	Miller et al. (1981) method do not apply the oral phase	Type of enzyme (Pepsin hog stomach mucosa, Sigma Chemical Co., St. Louis, against Porcine pepsin EC 3.4.23.1)	pH (5 against 7), incubation time (30 minutes against 120 minutes)	-	-	[57,60]
Luten et al. (1996) method	Simulation of gastrointestinal digestion as described by Miller et al. (1981) with some modifications. The dialyzable portion of the total mineral present in the sample represented the bioaccessible mineral.	-	The pH is adjusted to 2.0 by adding 6 M HCl. Freshly prepared pepsin solution (*P*-7000, from porcine stomach mucosa in HC1) is added in the sample. After mixing the sample is incubated at 37°C in a shaking water bath for 2 hours.	Titratable acidity is measured in an aliquot of the gastric digest and adjusting to the pH to 7.5. A homogeneous aliquot of pepsin digest was taken to 20°C and of freshly prepared pancreatic mixture (*P*-1750, from porcine pancreas and bile extract, B-8631, porcine dissolved in $NaHCO_3$) is added into wide-necked Erlenmeyer flasks, which is placed in a water bath at 37°C for 5 minutes. After 30 minutes, the pH was measured and the pancreatic mixture (*P*-1750, from porcine pancreas and bile extract, B-8631, porcine dissolved in $NaHCO_3$) was added to digest and was incubated in a shaking water bath for 2 hours at 37°C.	• Sorghum and wheat, pulses green gram (*Phaseolus aureus*) and red gram (*Cajanus cajan*), carrot, onion, dry mango powder[72] • Cereals (rice, finger millet, sorghum, wheat and maize), and pulses (chickpea, green gram, black gram, cowpea)[13,18] • Cereal (Pearl millet)[73] • Meal composite with the cereals, legumes, milk, meat, fruits, leguminous, etc.)[74]	The dialysates are pipetted into disposable tubes and mixed with protein precipitation solution. Each tube was covered with a watch glass and put into a boiling water bath for 10 minutes. The samples were then centrifuged for 10 minutes at 3500× *g*	[74]
Discrepancies between Luten et al. (1996) method and consensus method	-	Luten et al. (1981) method do not apply the oral phase	pH (2 against 3), type of enzyme (pepsin solution (*P*-7000,pH from porcine stomach mucosa against Porcine pepsin EC 3.4.23.1)	pH (7.5 against 7)	-	-	[57,74]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Miller et al., (1981) method modified by Wolfgor et al. (2002)	Conditions proposed by Miller et al. (1981) and a modification developed by Kapsokefalou and Miller (1991) were examined to estimate dialysable iron. Both methods involve digesting aliquots of homogenized foods adjusted to pH 2 and mixed with pepsin and pancreatin-bile at intestinal later.	-	The pH is adjusted to 2.0 by adding 6 N HCl. Freshly prepared pepsin suspension (Sigma *P*-7000 suspended in HCl.) is added and the mixture incubated in a shaking water bath at 37°C for 2 hours	The pH is adjusted to 7.5 before intestinal digestion. Pepsin digest aliquots plus dialysis bags containing one of the base systems were incubated for 30 minutes in a shaking water bath at 37°C. Pancreatin-bile mixture (Pancreatin, Sigma *P*-1750 and bile extract, Sigma B-8631 dissolved in $NaHCO_3$) is then added to flask and incubation continued another 2 hours. Erlenmeyer flasks are sealed with parafilm in order to reduce CO loss	● Jute leaves are cooked with meat and spices in a typical dark dish called Mloukhiya or Mulukhiyah[20]	On completion of pancreatin bile incubation, dialysis bags are removed and rinsed with water. Bag contents were transferred to tared flasks, insides are rinsed twice with 1 ml of water, then completed with water to 25 g and analyzed for their iron content.	[75]
Discrepancies between Wolfgor et al. (2002) method and consensus method	–	Wolfgor et al. (2002) method do not apply the oral phase	pH (2 against 3), type of enzyme (pepsin solution *P*-7000,pH against Porcine pepsin EC 3.4.23.1)	pH (7.5 against 7)	-	-	[57,75]
Icard-Vernière et al. (2013) method	Enzymatic in vitro digestions is carried out in two stages imitating the gastric step followed by the intestinal step.	Alpha-amylase from *Bacillus licheniformis* (Sigma A-3403-1MU) is added to sample in milliQ water and the mixture was incubated at 37°C for 5 minutes.	The pH is adjusted to 2.0 with 1 M HCl; 1 mL of pepsin (Sigma, *P*-7000, in HCl) solution is then added and the samples is incubated horizontally for 1 hour at 37°C in a shaking water bath.	Five milliliters of enzyme solution, containing pancreatin and bile extract (Sigma, P1750 and bile extract solution, Sigma B8631 in $NaHCO_3$) is then added, and the samples is incubated horizontally for 2 hours at 37°C, pH 6.7. in a shaking water bath.	● Cereals (Millet and Sorghum. grains) Product from wheat, red sorghum, teff, and white sorghum[76]	The digestion mixtures remaining in the tubes were centrifuged at 10,000× *g* for 15 minutes at 4°C to separate the insoluble and soluble iron fractions, respectively, in the pellet and supernatant.	[77]
Discrepancies between Icard-Vernière et al. (2013) method and consensus method		Incubation time (5 minutes against 2 minutes) and the type of enzymes (Alpha-amylase from *Bacillus licheniformis* (Sigma A-3403-1MU) against α-amylase EC 3.2.1.1)	pH (2 against 3), incubation time (60 minutes against 120 minutes) and type of enzyme (pepsin Sigma, *P*-7000 against Porcine pepsin EC 3.4.23.1)	pH (6.7 against 7)	-	-	[57,77]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Mounicou et al. (2002) method described by Khouzam et al.[56]	Simulated gastro-intestinal using a gastric solution and intestinal solution	-	Sample is incubated with gastric solution (pepsin in 1 NaCl solution, pH 2.5) for 4 hours at 37°C.	The pH is brought to 7.4 by adding an adequate volume of NaOH solution and an intestinal solution was added (pancreatin, amylase and bile salt) and incubated at 37°C about 4 hh.	• Lebanese diet including bread, vegetables, fruits, and white cheese[56] • Cocoa[78]	The mixture was centrifuged at 4000× *g* for 15 minutes.	[56,78]
Discrepancies between Mounicou et al. (2002) method and consensus method		Mounicou et al.(2002) method does not apply the oral phase	pH (2.5 against 3), incubation time (240 minutes against 120 minutes) and type of enzyme (pepsin against Porcine pepsin EC 3.4.23.1)	pH (7.4 against 7)	-	-	[57,78]
Cabanero et al. (2007)	Simulation of gastric digestion using gastric juice solution and pancreatic digestion using pancreatic digestion solution	–	The sample is placed in Erlenmeyer flask containing a gastric juice (pepsin (Porcine; catalogue no. *P*-7000) in NaCl, acidified with HCl) for 4 hours at 37°C, at pH adjusted to 3	The pH of the solution obtained after gastric digestion was adjusted to 6.8. The intestinal juice: pancreatin (Porcine; catalogue no. *P*-1750), bile salts (catalogue no. B-8756) and amylase (Darmstadt, Germany, catalogue no. 1329) in NaCl was added and the mixture was energetically shaken for 1 minute and left in a thermostatic water bath for 4 hours at 37°C	• Fish samples: Swordfish (*Aphanopus carbo*), sardine (*Sardina pilchardus*) and tuna (*Thunnus* spp.)[79] • Wheat seeds[80]	The digest is cold centrifuged 1575× *g* for 1 hour.	[79]
Discrepancies between Cabanero et al.[56] (2007) method and consensus method		Cabanero et al. (2007) method does not apply the oral phase	Incubation time (240 minutes against 120 minutes) and type of enzyme (pepsin (Porcine; catalogue no. *P*-7000 against Porcine pepsin EC 3.4.23.1)	pH (6.8 against 7) and incubation time (240 minutes against 120 minutes)	-	-	[57,79]
Skibniewska et al. (2002) method	Simulated gastric digestion using pepsin solution (0.5 ml/100 ml) and intestinal digestion using pancreatin solution (10 ml/40 ml of homogenate).	-	Sample is mixed with deionised water and supplemented with sodium azide. After, the solution pH is brought to 2.0 and pepsin Sigma (Pozna, Poland) is added and the mixture was incubated with shaking in a water bath at 37°C during 2 hours.	The pH was adjusted to 6.8–7.0 and a solution of pancreatin (Sigma) was added and incubated for another 4 hours.	• Gluten-free breads for-tified with milk and selected seed[81] • groats (buckwheat groats, barley groats, corn groats, couscous), rice (white rice and brown rice), legumi-nous grains (kidney bean, shell pea, green lentils) and nuts (brazil nuts, cashews, hazel-nuts, and walnuts)[82] • Oat products[83]	Once digestion was completed, the mineralized sample was centrifuged at 4000× *g* for 30 minutes and decanted by draining through medium-hardness filters.	[83]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Discrepancies between Skibniewska et al. (2002) method and consensus method		Skibniewska et al. (2002) method do not apply the oral phase	pH (2.0 against 3) and type of enzyme (pepsin Sigma (Pozna, Poland) against Porcine pepsin EC 3.4.23.1)	Incubation time (240 minutes against 120 minutes)	-	-	[57,83]
Mesías et al. (2009) method	The technique of Miller et al. (1981) with some modifications comprised gastric and intestinal digestion. Simulated gastric digestion using pepsin and intestinal digestion, a mixture of pancreatin and bile salts is used. After digestion, the digestive enzymes were inactivated by heat treatment for 4 minutes at 100°C in a polyethyleneglycol bath.	-	The sample is suspended in Milli-Qwater and its pH was adjusted to 2 and a pepsin Sigma solution was added. The mixture was incubated at 37°C in a shaking water bath at 110 oscillations/minute for 2 hours.	The pH of the digest was raised to pH 6 and a pancreatin bile salts (Sigma) mixture is added. The pH was then adjusted to pH 7.5 and the samples are incubated at 37°C at 110 oscillations/minute for 2 hours.	• Meals composite with legumes, salad of pasta, vegetable stew, russian salad (tuna, legumes, strawberry yogurt, bread without crust), boiled potatoes, boiled salad, …[84] • Milk samples[21]	The digestive enzymes are inactivated by heat treatment for 4 miutesn at 100°C in a polyethylene glycol bath. The samples were then cooled by immersion in an ice bath and centrifuged at 3200× *g* for 60 minutes at 4°C to separate the soluble and non-soluble fractions	[84]
Discrepancies between Mesías et al. (2009) method and consensus method		Mesías et al. (2009) method do not apply the oral phase	pH (2.0 against 3) and type of enzyme (pepsin Sigma against Porcine pepsin EC 3.4.23.1)	pH (7.5 against 7)	-	-	[57,84]
In vitro enzymatic method (Schwedt et al. 1998)	The simulation of gastric digestion with pepsin (Merck – EC 3.4.23.1) and intestinal digestion with bile salt (Sigma B8756) and pancreatin (Sigma P1750).	-	Homogenized sample and NaCl, HCl solution and pepsin (from porcine gastric mucosa, 0.7 FIP-U/mg, Merck) are extracted at 37°C and 100 rpm for 4 hoursours in the water bath. The pH is adjusted to 1–2.	pH of the samples was adjusted to 7.5 using concentrated $NaHCO_3$. A pancreatin solution (pancreatin and amylase from from porcine pancreas, P 1750, activity equivalent to 4*US. P, Sigma), amylase (from bacillus subtilis, 57.4 U/mg, Fluka); bile salt (B 8765, Lot 40H0333, approx. 50% sodium chelate and 50%, w/v, sodium dioxychelate, Sigma)) and bile salt solution are added to digestion solutions, simulation of intestinal digestion was continued for another 2 hours	• Tea biscuit from wheat flour[85] • Foodstuffs[86]	All extraction solutions were then centrifuged at 4500× *g* for 1 hour, decanted and filtered	[86]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Discrepancies between Schwedt et al. (1998) method and consensus method		Schwedt et al. (1998) method do not apply the oral phase	pH (1–2.0 against 3), incubation time (240 minutes against 120 minutes) and type of enzyme (pepsin (from porcine gastric mucosa, 0.7 FIP-U/mg, Merck) against Porcine pepsin EC 3.4.23.1)	pH (7.5 against 7)	-	-	[57,86]
In vitro digestion/Caco-2 cell method Glahn et al. (1998)	Simulation of in vitro gastric- and intestinal digestions. Aliquots of the digests are placed onto dialysis membranes positioned above monolayers of Caco-2 cells in six-well plates.		To start the peptic digestion, the pH sample is adjusted to pH 2.0 with 5.0 mol/L HCl. The sample is transferred to a 50-mL screw-cap culture tube, and 0.5 mL of the pepsin solution (800 ± 2500 units/mg protein purchased from Sigma Chemical dissolved in HCl) was added per 10 mL of sample. The tube was capped, placed horizontally and incubated for 60 minute on the rocking shaker at rocking speed (55 oscillations/ minute).	For the intestinal digestion step, the pH of the digest sample is raised to pH 6 by dropwise addition of 1 mol/L $NaHCO_3$. Then 2.5 mL of pancreatin-bile ((pancreatin (activity, 4*USP specifications) and bile extract (glycine and taurine conjugates of hyodeoxycholic and other bile salts) purchased from Sigma Chemical dissolved in $NaHCO_3$) extract mixture was added per 10 mL of original sample. The pH is adjusted to pH 7 with NaOH. After the preparation of the six-well culture plates with cell monolayers, the plate was covered and incubated on the rocking shaker at 6 oscillations/ minute for 120 minutes.	• Hawaiian seaweeds[87] • Insects and sirloin beef[88] • Complementary food formulated using cereals (corn, sorghum millet) + sweet potato + cassava + omena +groundnuts[89]	When the intestinal digestion was terminated, the insert ring and digest are removed. The solution in the bottom chamber is allowed to remain on the cell monolayer and an additional 1 mL of MEM is added to each well. The cell culture plate is then returned to the incubator for an additional 2 hours, after which the cells were harvested for analysis.	[90]
Discrepancies between Glahn et al. (1998) method and consensus method		Glahn et al. (1998) method do not apply the oral phase	pH (2.0 against 3), incubation time (60 minutes against 120 minutes) and type of enzyme (the pepsin solution (800 ± 2500 units/mg protein purchased from Sigma Chemical against Porcine pepsin EC 3.4.23.1)	-	-	-	[57,90]

(*Continued*)

Table 1. (Continued).

Methods	Principles	First step of digestion (Oral phase)	Second step of digestion(Gastric phase)	Third step ofdigestion (Intestinal phase)	Type of matrix analyzed	Intestinal absorption simulation	References
Caco-2 cell Culture Model: Modification of the Original Method[91]	The original Caco-2 method was modified by using ferrous sulfate tablet as a standard and also including blank well in the cell culture plate to subtract the baseline ferritin concentration from the ferritin concentration of other wells.	-	One milliler of sample is mixed with 0.05 mL freshly prepared pepsin (Sigma-Aldrich, porcine: cat. No. *P*-7000) in 0.1 M HCl (800–2500 units/mg protein). The tubes are then kept on horizontal mixer at 55 oscillations/minute for 60 minutes at room temperature (RT)	pH was adjusted to 7 using 1 M $NaHCO_3$ and2.5 mL of fresh pancreatin bile extract ((Sigma-Aldrich, porcine: cat. No. *P*-1750) and bile extract (deoxycholin-glycine, taurine and other bile salts conjugates) (Sigma-Aldrich, porcine: cat. No. B-8631)) is added to each 10 mL sample. The pH of this preparation is adjusted to 7.4 using 1 M NaOH, and the final volume is made to 15 mL using 120 mM NaCl and 5 mM KCl (1:1).	• Iranian traditional flat breads[91]	1.5 mL of the digest is transferred to the upper chamber containing a prepared dialysis and Caco-2 cells in passages 26–30	[91]
Discrepancies between modified original Caco-2 method and consensus method		Modified original Caco-2 method do not apply the oral phase	Incubation time (60 minutes against 120 minutes) and type of enzyme (pepsin (Sigma-Aldrich, porcine: cat no. *P*-7000) against Porcine pepsin EC 3.4.23.1)	pH (7.4 against 7)	-	-	[57,91]
Glahn et al. (2000) TC7 Caco-2 cell clone (INSERM U505)	Simulation of in vitro gastric- and intestinal digestions. Aliquots (1.5 mL) of the digests were placed onto dialysis membranes positioned above monolayers of Caco-2 cells in six-well plates.	-	To start the peptic digestion, a sample of each solubilized iron supplement is placed in a screw cap culture tube containing NaCl, KCl at pH 2.0 (adjusted with HCl). Then, pepsin solution (Sigma #P-7000, 800–2500 units/mg protein) is added and the tube is capped, placed horizontally, and incubated for 60 minutes on the rocking shaker and rocked at speed (55 oscillations/minute).	For the intestinal digestion step, the pH of the sample was raised to pH 6 by adding $NaHCO_3$ dropwise. The pancreatin-bile extract ((Sigma # *P*-1750, activity 5 4 3 U.S. P. specifications), and bile extract (Sigma # B-8631, glycine and taurine conjugates of hyodeoxycholic and other bile salts)) mixture was added. The pH was adjusted to pH 7 with NaOH. After the preparation of the six-well culture plates with cell monolayers, the plate was covered and incubated on the rocking shaker at 6 oscillations/minute for 120 minutes.	• Commercial preparations of $FeSO_4$, ferrous gluconate, ferrous fumarate, and a polysaccharide–iron complex[92] • Dark green leafy vegetables[93] • Ferrous sulfate tablets with alternative commercial iron products[94] • Sweet potato- and cereal-based complementary foods[95]	When the intestinal digestion was terminated, the insert ring and digest were removed. The solution in the bottom chamber was allowed to remain on the cell monolayer and an additional 1 mL of Minimum Essential Media as added to each well. The cell culture plate was then returned to the incubator for an additional 2 hours, after which the cells were harvested for analysis	[92]
Discrepancies between Glahn et al. (2000) TC7 Caco-2 cell clone (INSERM U505) and consensus method		Glahn et al. (2000) TC7 Caco-2 cell clone (INSERM U505) do not apply the oral phase	pH (2.0 against 3), incubation time (60 minutes against 120 minutes) and type of enzyme (pepsin solution (Sigma #P-7000, 800–2,500 units/mg protein) against Porcine pepsin EC 3.4.23.1)	-	-	-	[57,92]

meal-enzymes at each digestion step. This method developed by Minekus et al.[57] apart from being an international consensus method, took into the three digestion steps and then mimic as much as possible the in vivo digestibilty assay. Nevertheless, despite it proposes specific treatment for solid and liquid food, some issues need to be addressed by further researches. Indeed, researchers should adapt this method to specific composition of the food matrices to be digested. Varieties of this method could then be developed for specific food groups (i.e., cereals, root and tubers, fruits, etc.). It appears obvious that the specific composition of the foods to be digested could lead some modifications of the original digestion process. This could justify the large variety of the modifications of original digestibility methods in the literature. For overall approval of the consensus method, it will be necessary to assess its efficacy (capacity of the method to provide results similar to in vivo method) using different food matrices.

The methods of Luten et al.,[74]Mesias et al.,[84] and Icard-Vernière et al.[77] are very near to the consensus method based on these digestion parameters (pH, incubation duration, and enzymes used). Indeed, pH, incubation duration, and enzyme characteristics are known to be important for the digestion process.[57,96]Luten et al.,[74] and Mesias et al.[84] methods could be then used as alternative methods whether the oral phase is added as described in the consensus method.[57] The substitution of Alpha-amylase from *Bacillus licheniformis* (Sigma A-3403-1MU) by α-amylase EC 3.2.1.1 during the oral phase of Icard-Vernière et al.[77] method could allow using this method as an alternative method to the consensus one.

One of the issue to be addressed after digestion is the analysis of the digested samples. Before the analysis of the digested samples, a centrifugation is generally applied to separate pellet and the supernatant,[59] which have the limit to separate only undigested material with high density.[57] In this framework, Miller et al.[60] have suggested the measure of the soluble minerals after simulating the gastro-intestinal digestion while Minekus et al.[57] considered ultrafiltration or dialysis as the best choice.

Factors affecting mineral bioavailability

The bioavailability of nutrients can be influenced by various factors such as chemical binding form; the food vehicle in which the nutrient is incorporated or presented; the presence or absence of other food components that promote or inhibit absorption; metabolization after absorption; host-related factors as well as other isolated individual factors.[14] The inhibiting factors are known to reduce nutrient bioavailability by binding the nutrient in consideration into a form that is not recognized by the uptake systems while enhancing factors can act in different ways such as keeping a nutrient soluble or protecting it from interaction with inhibitors.[14] Different nutrients (including protein, iron and vitamin A) and the forms, in which they exist in the ingested medium, will react in different ways to inhibit or enhance the minerals absorption depending on their ingested quantity. In addition, the bioavailability can be affected by the concentration of a nutrient, dietary factors, chemical form, supplements taken separately from meals, nutrition and health of the individual, excretory losses and nutrient–nutrient interactions.[10] Bioavailability of iron for example is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption.[13] In the case of minerals, the amount available for absorption (bioaccessible or soluble) depends on the composition and physical characteristics of the diet, the content and the chemical nature of the mineral, the presence of ligand promoters or inhibitors of the absorption, luminal gastrointestinal secretions, and interactions that occur as a result of the interplay of these factors.[28]

Inhibitors of mineral bioavailability

Diet-related factors in plant foods that negatively affect bioavailability include the chemical form of the nutrient in food and/or nature of the food matrix; interactions between nutrients and other food components such as phytate, polyphenols, and dietary fiber.[12]

Table 2. Effect of processing on bioaccessibility of minerals.

Minerals	Food	Processing	Description	Effect on mineral bioaccessibility	References
iron and zinc	Sorghum	Soaking and germination	Soaking and germination of three white sorghum varieties (*Sorghum bicolor* L. Moench). Sorghum varieties were soaked for 20 hours and germinated for 72 hours	The in vitro bioavailability of iron and zinc were significantly improved	[15]
Iron and zinc	Fermented porridges prepared with finger millet with and without peanut butter	Fermentation	Fermentation flour water for 24 hours at 25–30°C + cooking for 20 minutes at 80–100°C	There was no improvement in iron and zinc bioaccessibility due to fermentation and cooking.	[61]
Iron and zinc	Opaque sorghum beer	Soaking, germination, mashing as well as fermentation	On average, grain were soaked overnight at room temperature, germinated, sun-dried at ambient temperature of about 30–37°C, ground in a disc mill, mashed in water by gradually heating until the boiling point was reached after 2 hours, soured during an overnight rest, filtered, boiled, cooled, inoculated and fermented overnight	The total soluble Fe increased from 13.7 mg kg^{-1} in the raw grain to 35.4 mg kg^{-1} (dm) in the finished beer.	[65]
Iron, zinc, calcium, magnesium, and potassium	Mloukhiya, a Mediterranean dish based on jute leaves and meat	Cooking	The preparation was simmered on a hot plate for 5 hours at boiling temperature. Mloukhiya samples were collected every 30 minutes during the 5 hours of cooking	Iron bioavailability decreased. After 5 hours of cooking, non-heme iron showed poor bioaccessibility (1.2%).The bioaccessibility of other minerals (zinc, calcium, magnesium and potassium) was high, indicating that the food matrix had no or little effect	[20]
Calcium	Milk	Overheating	Thermally damaged milk (overheated; 3 cycles of sterilization at 116°C, 16 minutes) was compared with UHT milk (150°C, 6 seconds)	A negative effect on calcium solubility was observed after in vitro digestion of milk compared with UHT milk.	[21]
Calcium	Green leafy vegetables (GLVs)	Cooking	GLVs were cooked using Millipore – MilliQ distilled water in microwave oven until the water was evaporated and marked	Cooking did not affect significantly calcium bioavailability in any GLVs	[16]
Calcium, iron and zinc	Granny Smith apple	High hydrostatic pressure (HHP)	The apple was pressurized at 500 MPa during 2, 4, 8, and 10 minutes	HHP increased the mineral contents availability by 2.11–303.00% for calcium, 4.63–10.93% for iron and 8.68–28.93% for zinc	[121]
Calcium, iron, and zinc	Prosopis chilensis seed	High hydrostatic pressure (HHP)	Seed was pressurized at 500 MPa during 2, 4, 8, and 10 minutes	Bioaccessibility of calcium, iron, and zinc in the treated sample for 500 MPA at 10 minutes was several-fold higher (three, three and five times, respectively) than in the untreated sample.	[122]
Calcium, copper, iron, potassium, magnesium, and zinc	Bovine liver	Cooking in water and using the sous vide procedure	For cooking the sample in water (conventional cooking method), 6 cubes (2.0 cm× 2.0 cm) of liver samples were boiled in for 20 minutes at 180°C. For sous vide cooking, 6 cubes (2.0 cm ×2.0 cm) of liver samples were packed under vacuum using a vacuum system and cooked at 65°C in a heating bath for 2 hours.	All heat treatments of bovine liver promoted the increase of the bioaccessibility of Ca, Cu, Fe, K, and Mg, except for Zn when the effect was the opposite. The sous vide method provided higher bioaccessibility of these minerals than cooking in boiling water, except for K when both methods presented equivalent values.	[17]

(*Continued*)

Table 2. (Continued).

Minerals	Food	Processing	Description	Effect on mineral bioaccessibility	References
Iron and zinc	Cereals – rice (*Oryza sativa*), finger millet (*Eleusine oracana*), Sorghum (*Sorghum vulgare*), wheat (*Triticum aestivum*), and maize (*Zea mays*), and pulses – chickpea (*Cicer arietinum*) – whole and decorticated, green gram (*Phaseolus aureus*) – whole and decorticated, decorticated black gram (*Phaseolus mungo*), decorticated red gram (*Cajanus cajan*), cowpea (*Vigna catjang*), and French bean (*Phaseolus vulgaris*)	Pressure-cooking and microwave heating	Pressure-cooking and microwave cooking were employed. Ten grams of the food grains was pressure-cooked in 30 mL of triple distilled water for 10 minutes. In the case of French bean and cowpea, the grains were soaked in triple distilled water overnight and then pressure cooked as above. For microwave cooking, 10 g of the food grains were cooked in 150 mL of triple distilled water at 360 W for 30 minutes in the case of whole grains (pre-soaked overnight) and 20 minutes for cereals and decorticated pulses (pre-soaked for 4 hours).	Among cereals, pressure-cooking decreased zinc bioaccessibility by 63% and 57% in finger millet and rice, respectively. All the pressure-cooked cereals showed similar percent zinc bioaccessibility with the exception of finger millet. Bioaccessibility of zinc from pulses was generally lower as a result of pressure-cooking or microwave heating. The decrease in bioaccessibility of zinc caused by microwave heating ranged from 11.4% in chickpea (whole) to 63% in cowpea. Decrease in zinc bioaccessibility was 48% in pressure-cooked whole chickpea, 45% and 55% in pressure-cooked or microwave-heated whole green gram, 32% and 22% in pressurecooked or microwave-heated decorticated green gram, and 45% in microwave-heated black gram.	[18]
Calcium, copper, iron, magnesium, and zinc	Beef, pork, and chicken	Cooking	(1) Cooked in water (CW): with 500 ml of cold water for 30 minutes; (2) Baked in microwave oven (MW): at 650 W power for 6 minutes; (3) Grilled (GR): grilled for 10 minutes on a preheated grill; (4) Baked in conventional oven (B1): for 45 minutes at 180°C in a preheated conventional oven; and (5) Baked in conventional oven (B2): for 60 minutes at 180°C in a preheated conventional oven.	The decreased of bioaccessibility of minerals mainly at higher temperatures. The heat processing named B2, which consisted of baking in a conventional oven for 60 minutes at 180°C, presented the lowest bioaccessibility of minerals	[19]
Zinc and iron	Bred cowpea (*Vigna unguiculata* L. Walp)	Home-cooking	Two cooked methods were used: pressure cooking and cooking in a regular pan, with and without previous soaking in water using five bean cultivars.	In all cultivars, cooking of cowpea beans in both pressure cooker and in a regular pan yielded higher percentages of bioaccessible zinc compared with availability of bioaccessible iron.	[123]
Calcium	Cladodes of prickly pear cactus (*Opuntia ficus-indica* L. Miller)	Cooking	The spines were removed and 1.0 kg of cladodes were chopped and boiled in 1.0 L distilled water during 20 minutes	The cooking process did not affect significantly intestinal calcium bioaccessibility in any of the cultivars studied	[124]
Iron	Pork meat *Musculus longissimus* dorci)	Porcine meat was heated-treated at 60°C, 80°C, 100°C, and 120°C for 1 hour	Pork meat heated at 60°C, 80°C, 100°C, and 120°C for 1 hour(1	Heat-treatment of pork meat at 80–120°C for 1 hour was found to enhance iron availability measured as Fe^{2+}-dialyzability after in vitro pepsin digestion.	[125]
Calcium, iron, and zinc	Beans, chickpeas, and lentils	Cooking	Legumes were cooked following a previously described treatment usually found in traditional culinary practices	Traditional cooking significantly ($p < .05$) increased the uptake (%) of calcium, iron and zinc from white beans, and of calcium from lentils	[126]
Calcium, iron, and zinc	White beans	Cooking	White beans were cooked following a previously described treatment as usual in traditional culinary practice	Cooking increases the Caco-2 cells' uptake percentages (calcium, 18.8 versus 3.6; iron, 33.7 versus 1.7; and zinc, 17.2 versus 2.1)	[127]

(*Continued*)

Table 2. (Continued).

Minerals	Food	Processing	Description	Effect on mineral bioaccessibility	References
Iron, calcium, zinc, and copper	Brown rice (*Oryza sativa* L.)	High hydrostatic pressure	HHP treatments at 0.1, 100, 300, and 500 MPa for 10 minutes	The in vitro bioaccessibility of calcium and copper was increased by 12.59–52.17% and 2.87–23.06% after HHP, respectively, but bioaccessible iron was decreased.	[128]
Manganese, medium, and phosphorus	Brown rice (*Oryza sativa* L.)	Ultra-high pressure	UHP treatments were applied at 100, 300, and 500 MPa for 10 minutes, and 0.1 MPa as control.	The mineral bioaccessibility, measured by percentage solubility, were the highest when at 300 MPa whereas it decreased at 500 MPa.	[129]
Calcium, magnesium, and zinc	Landrace carrots (*Daucus carota*, L.)	Cooking	Carrots were cooked in a domestic steamer for 20 minutes. The temperature inside of the carrot increased from 45°C to 50°C toward the end of the cooking process	Cooking increased the in vitro bioaccessibility of Ca and Zn but had no effect on Mg	[130]

Phytate

Phytate is known as one of the strongest inhibitory predictors of calcium, iron and zinc bioavailability.[11] Phytate intake was found to inhibit the bioavailability of iron and calcium from the diets.[11] In plant-based diets, phytate (myo-inositol hexakisphosphate) is the main inhibitor of iron absorption. Its negative effect on iron absorption has been shown to be dose dependent and starts at very low concentrations of 2–10 mg/meal.[99,100] It has six reactive phosphate groups and acts as a chelating agent. A cation can bind to one or more phosphate groups of one single phytic acid molecule or it can even bridge two or more phytic acid molecules.[101] The stability and solubility of these complexes depend on the type of binding mineral, the pH, the molar ratio (phytate: mineral) and the presence of other compounds in solution.[101] The molar ratio of phytate to Fe or Zn was suggested as an index to estimate the bioavailability of Fe and Zn in foods.[100,102,103] Indeed, a [phytate]/[Fe] molar ratio above 10–14 was believed to strongly impair iron bioavailability in rats fed with wheat-based diets.[99,103]Hallberg et al. showed that phytate to iron molar ratio >1 is critical for iron absorption. Hurrell and Egli[100] reported that this molar ratio should be <1:1 or preferably <0.4:1 to significantly improve iron absorption in plain cereal or legume-based meals that do not contain any enhancers of iron absorption, or 6:1 in composite meals with certain vegetables that contain ascorbic acid and meat as enhancers. Value of [phytate]/[Zn] molar ratio ranged between 10 and 15 is believed as the critical value above which Zn bioavailability decreases in humans.[104–106] For calcium, the critical value of phytate to calcium molar ratio is >0.24.[107]

Fiber and lignin

Vegetables and grains contain starches, various fibers, lignin, and polyphenolic compounds, which may bind minerals and inhibit bioavailability under certain conditions. Indeed, foods rich in fiber reduce absorption when containing high contents of phytic acid[28] due to their electrostatic interactions with minerals.[101] The minerals that are most often cited as being negatively affected by fiber are iron, zinc and calcium.[108] The lignin, which is a high molecular-weight polymer containing aromatic phenolic residues, is often found in the food matrix associated with other fiber constituents and may be responsible for many of the inhibitory effects assigned to fibers.[108] In addition, insoluble dietary fiber interfered with iron dialyzability, while both the insoluble and soluble fractions of dietary fiber had negative effect on zinc dialyzability.[13]

Interaction between minerals

Calcium has been shown to have a negative effect on non-heme iron absorption.[14] Calcium content can also affect zinc absorption if the diet contains phytate.[28] According to Hemalatha et al.,[13] a decreasing trend in zinc bioaccessibility from cereals was observed with an increase in inherent calcium content of grains. However, the aforementioned authors added that this trend was not significant and it was inferred that the calcium levels normally found in mixed diets are unlikely to bring about a phytate-induced decrease in zinc bioaccessibility. Thus, when zinc bioaccessibility from various food grains was pointed out in terms of molar ratio: [phytate] × [calcium]/[zinc], a slight decrease in zinc bioaccessibility from cereals and pulses was generally evidenced if this ratio increased. The phytate × Ca/Zn molar ratio can then be used to predict zinc bioavailability.[109] Indeed, a significant reduction of zinc absorption was observed at phytate × Ca/Zn molar ratios of 0.13 and higher.[103] According to Gibson et al.,[109] a millimolar ratio (phytate × calcium)/zinc above 200 is critical for the combined effect of phytate and calcium on zinc absorption. This possibly underlined the negative interaction with calcium and zinc on zinc absorption. Furthermore, Krishnan and Meera[110] underlined that the iron bioaccessibility appeared to be influenced by iron:zinc ratio. Indeed, according to Drago,[28] iron can also alter zinc absorption, since they have many similar absorption and transport mechanisms and may therefore compete for absorption. The same authors also reported that interaction and competitive inhibition of zinc's uptake by iron occurs when the Fe/Zn ratio is 2:1 or greater. All this constitute some evidences, which could help better understanding the bioaccessibility mechanism while considering a food matrix with multi-micronutrients. Indeed, new trends in the development of Complementary Food Supplement (CFS) or complementary food is to provide a food with multi-micronutrients properties as micronutrients deficiencies rarely occur alone; often, multiple micronutrients deficiencies coexist.[5]

Enhancers of mineral bioavailability

Incorporation of enhancers (i.e., vitamin A, citric and ascorbic acids) to food can enhance the mineral bioaccessibility by releasing minerals in solubilized form and protecting them from interacting with inhibitory factors. Organic acid (ie ascorbic acid) and vitamins (vitamin C; β-carotene, provitamin A) and certain amino acid like cysteine compete for the same transport carriers.[110]

Organic acids

Organic acids, including ascorbic, citric and lactic acids can enhance the mineral bioavailability. Their effect is dependent on the food composition and concentrations of mineral and organic acids.[1] They are well known to have a significant enhancing effect on iron bioavailability.[111] The aforementioned authors also demonstrated that acidulants such as lime from *Citrus aurantifolia*, amchur from dry *Mangifera indica* mango powder, tamarind from *Tamarindus indica*, and kokum from *Garcinia indica*, amchur and citric acid (the major acid present in lime) generally enhanced the bioaccessibility of zinc and iron from food grains.

Organic acids, such as citrate, malate, and lactate, may chelate minerals, but none have been found to be as effective as ascorbic acid in enhancing iron absorption. However, the other organic acids may be more effective than ascorbic acid when considering zinc and calcium absorption.[108] Ascorbic acid appears as a remarkable enhancer by improving absorption through ferric ion reduction and forming stable chelates with iron in the stomach.[28] However, it has convincingly been shown to enhance iron bioavailability in a dose-dependent manner. This effect is largely due to its ability to reduce ferric to ferrous iron.[14] Nevertheless, its effect is much less pronounced if added to high-iron bioavailability foods.[28]Zimmermann et al.[112] reported that a molar ratio of ≥2:1 ascorbic acid: Fe is needed for foods with low-medium levels of inhibitors while a molar ratio excess of ≥4:1 is required for foods with high level of inhibitors. Even if the baobab fruit pulp contained higher ascorbic acid content as reported in literature, the recommended molar ratio will still be difficult to attain according to Gabaza

et al.[63] when they test baobab fruit pulp and mopane worm as potential functional ingredients to improve the iron and zinc content and bioaccessibility of fermented cereals. Nevertheless, they recognized that baobab fruit pulp generally had a positive effect on the bioaccessibility of iron and zinc but not necessary on their content. Van der Merwe[113] showed that in the presence of provitamin A-rich plant sources, baobab fruit pulp, moringa leaves powder and *Roselle calyces*, bioaccessibility of iron in pearl millet-based porridge is improved. They correlated this positive effect on bioaccessibility to baobab fruit pulp due probably primarily to its high contents in citric and ascorbic acids.[68] According to Christides et al., [95] the high iron bioavailability from Cerelac® was associated with the highest levels of ascorbic acid. Ascorbic acid has also been shown to, at least partially counteract the inhibitory effect of both phytate and polyphenols on non-heme iron absorption.[14]

Vitamin A and β-carotene

Pure β-carotene added at an equivalent level enhanced the bioaccessibility of iron (from 19.6% to 102% increase) and zinc (from 16.5% to 118.0% increase) from cereal foods.[114] This positive effect of β-carotene-rich sources on the bioaccessibility of either iron or zinc was generally greater in the cooked grains than the raw grains.[114] According to Schönfeldt et al.,[14] vitamin A and β-carotene can enhance non-heme iron absorption and improve hemoglobin levels, although several studies suggest that it is only observed in iron-deficient individuals. Addition of carrot (*Daucus carota*) and amaranth (*Amaranthus gangeticus*), rich sources of β-carotene at two levels (2.5 g and 5 g per 10 g of grain) significantly enhanced the bioaccessibility of iron and zinc from the food grains. This positive effect was maximal with the level of vegetable sources corresponding to 200 μg β-carotene per 10 g of grain.[114]

Foods enhancers

Food acidulants, β-carotene-rich vegetables and allium spices are understood to promote mineral bioaccessibility from food grains. The negative effect of the inhibitors was not only annulled but also the positive influence of the promoters was fully retained.[72] Novel enhancers of micronutrients bioaccessibility have recently been identified, and these include sulfur-rich compound spices, such as onion and garlic, which also possess antioxidant properties; β-carotene-rich vegetables such as yellow-orange or green leafy and pungent spices red pepper, black pepper as well as ginger.[111] Both garlic and onion were evidenced to have a promoting influence on the bioaccessibility of iron and zinc from food grains. There are characterized by a high content of thiosulfinates, sulfides, polysulfides, mercaptans, and other odoriferous sulfur compounds.[115]Clydesdale et al.[108] also found the evidence of dietary protein in increasing zinc bioavailability in the presence of phytate and showed that it may be due to the desorption of zinc from phytate complexes by histidine, cysteine, and methionine, which also desorb other minerals.

Improving mineral bioaccessibility of foods using local food ingredients

The bioaccessibility or in vitro solubility of minerals indicates the portion of minerals that is available for metabolic function in our body and expressed as the percentage of the mineral in the dialysate (i.e., of dialysability assay) relative to the total mineral content in the samples. The amount of bioaccessible minerals (soluble minerals) is generally computed based on the mineral content of the samples and the percentage of bioaccessible minerals.[64,69,116,117] The soluble minerals is due both by the in vitro solubility (IVS in %) and the nutritional value of the foods. Indeed, a low mineral IVS food, [64,69,116,117] could have a high soluble mineral due to its high mineral content.[116] Due to the capital role of bioaccessibility, it is important to consider it when estimating minerals cover rate of foods.[69,118] In the frame of affordable and sustainable food-based strategies in alleviating mineral deficiencies, interest on mineral bioaccessibility improving is growing using different approaches such

as food-to-food fortification. Food to-food fortification is an approach that uses an interesting (contain useful amounts of micronutrients), available, and accessible local resource (plant or animal) to fortify another food.[119] This approach targets the food enhancer potential as a pathway to increase mineral bioaccessibility of the food vehicles. Baobab fruit pulp is one of the most reported food enhancers believed to be most effective in mineral bioaccessibility improving.[63,68,69] According to van der Merwe et al.,[69] the inclusion of baobab fruit pulp improves the iron and zinc bioaccessibility of cereal porridge containing a provitamin A source. This improvement in iron and zinc bioaccessibility is probably due to the high content of mineral bioaccessibility-enhancing of baobab fruit pulp. Indeed, organic acids are known to improve mineral bioaccessibility depending on their quantity in foods matrices.[1] [111]Gabaza et al.[63] also confirmed this role of mineral bioaccessibility improvement of baobab fruit pulp when they underlined general positive effect on iron and zinc bioaccessibility baobab fruit pulp-enriched cereal slurries. Aside from ascorbic acid, the positive effect of baobab fruit pulp on iron and zinc bioaccessibility could have resulted from the presence of other organic acids such as citric, tartaric, malic acid, which give the baobab fruit pulp an acidic nature. As baobab fruit pulp generally had a positive effect on the bioaccessibility of iron and zinc but not on the content of iron and zinc, [63] it will be necessary to promote its combination with another micronutrients rich food ingredients as a strategy to optimize mineral bioaccessibility of foods. In that framework, very recently, some alternatives were proposed by authors to challenge this issue. Affonfere[116] proposed a dual food-to-food fortification for sorghum porridge using moringa leaf powder and baobab fruit pulp. van der Merwe et al.[69] also investigated the combination of moringa leaves, roselle calyces and baobab fruit pulp to improve the iron and zinc bioaccessibility of cereal porridges containing a provitamin A source. Gabaza et al.[63] explored the incorporation of baobab fruit pulp and mopane worm as functional ingredients in complementary porridges. Food-to-fortification using sustainable food sources appears very useful not only for mineral improving of foods but also a good alternative for bioaccessibility improvement mainly for population with cereal-based diets as it is the case in most of the African countries. Other food ingredients were also reported to enhance mineral bioaccessibility. Rich sources of β-carotene such as carrot (*Daucus carota*), yellow-orange or green leafy and amaranth (*Amaranthus gangeticus*) were known to enhance the bioaccessibility of iron and zinc from the food grains.[111,114] The sulfur-rich compound spices such as onion garlic and pungent spices red pepper, black pepper as well as ginger were evidenced to enhance mineral solubility in food grains.[111] Further research needs to be carried out to identify the optimal incorporation level of these local ingredients to the food matrices in order to obtain an optimal bioaccessibility and maintain the improved food sensory acceptance.

Effect of processing on mineral bioaccessibility of foods

Food processing technologies are known to affect mineral bioaccessibility of foods. Fermentation, cleaning and soaking increase significantly iron and zinc bioaccessibility.[15,62,65] This increase in mineral bioaccessibility can possibly be due to the elimination of mineral binders and the increase in mineral enhancers such as organic acids. Indeed, these operations eliminate phytates which are believed to decrease mineral bioaccessibility.[65,117] Also, during fermentation, some organic acids could be developed and enhance minerals solubility. Lestienne[40] underlined that the effects of treatments (soaking, dehulling/soaking, and dehulling/milling/soaking) were different according to the kind of seeds and the concerned mineral. The dehulling removes phytate and consequently increases mineral bioavailability, while fermentation and germination increase the activity of endogenous phytase in cereals.[27]Kayodé et al.[117] also remarked that milling, sieving of sorghum flour had no significant effect on phytate. According to Gabaza et al.[61] fermentation generally increase the soluble phenolic compounds and decrease the bound phenolic compounds, which will possibly increase the mineral solubility. In general, the effect of food processing on the bioaccessibility of minerals is linked with numerous factors mainly (i) type of processing, (ii) type of mineral considered, (iii) food matrix composition and structure, and (iv) presence of factor components that affect

absorption efficiency.[120] In the case of foods of animal origin, it seems that in general, shorter times and lower temperatures increase mineral bioaccessibility.[27] Table 2 shows that the effect of processing on mineral bioaccessibility is dependent on the processing parameters and the foods processed. Indeed, the reviewed processing techniques such as soaking and germination, fermentation, mashing, cooking, overheating, high hydrostatic pressure (HHP), cooking in water and using vacuum procedure showed to have different effects on mineral bioaccessibility. For example, fermentation of finger millet flour at 25–30°C during 24 hours following by cooking (20 minutes/80–100°C) did not improve iron and zinc bioaccessibility of porridge,[61] while Afify et al.[15] noticed a significant increase in iron and zinc bioavailability after a 20 hours soaking and germination of sorghum for 72 hours. Moreover, high hydrostatic pressure (HHP) is believed to have positive effect on mineral bioaccessibility especially calcium due to microstructural modification.[121,122] As cooking is concerned, the effect on mineral bioaccessibility is also variable. Ramírez-Moreno et al.[124] found that cooking process did not significantly affect the intestinal calcium bioaccessibility of cladodes, while Njoumi et al.[20] observed an increase in minerals (zinc, calcium, magnesium and potassium) bioaccessibility after cooking mloukhiya, a Mediterranean dish based on jute leaves and meat. Nevertheless, it is important to underline that the cooking parameters applied by the authors are not the same, which could lead to the observed difference. Also, the improvement of mineral bioaccessibility of this Mediterranean dish could be due to the protein content of meat, a constituent of this diet as protein is known to improve mineral solubility.[108]

It is then clear that the effect of cooking on mineral bioaccessibility is very variable depending on the cooking parameters, mainly the temperature and the time of cooking and the type of mineral. Further study is needed to better elucidate this issue. According to Kayodé et al.[117] cooking was found to be the main unit operation that restricts the iron and zinc availability in sorghum porridge. Many complementary porridges in Africa are based on spontaneously fermented cereals as reported by Gabaza et al.[61] and consequently will be cooked before consumption. As cooking restricts the mineral bioaccessibility, it will be necessary to find the way to optimize the solubility of minerals during the cooking process as a pathway to deliver to population minerals bioaccessible-based porridges. Indeed, iron and zinc deficiencies are a major public health concern, particularly in developing countries where many people consume monotonous, predominantly cereal-based diets.[62,113] The most affected by these mineral deficiencies are women of reproductive age and children under 5 years.[4] Processes optimization could be key actions to deliver high bioaccessible minerals to these vulnerable groups coupled with the use of natural resources with bioaccessibility enhancers potential.

Conclusion

The present review allows highlighting the health benefit of minerals in human body. The bioavailability of these minerals is a key indicator, which quantifies the amount of minerals that is released in the intestinal tract and then becomes available for metabolic functions. Many in vitro methods are widely used for mineral digestibility. The international consensus method developed by Minekus et al[57] will help produce reproductive data even if some limits were pointed out. Some components known as promoters such as organic acids and inhibitors, i.e., phytic acid influences the mineral bioavailability through different interactions. Finally, the processing operations have various and different effects on mineral bioavailability depending on the processing parameters applied. This review constitutes a baseline to make assumptions for diet mineral bioavailability optimization.

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Disclosure of potential conflicts of interest

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