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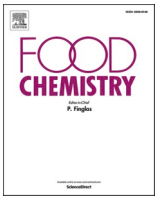

Review

# Metal-binding peptides and their potential to enhance the absorption and bioavailability of minerals

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ABSTRACT

Minerals including calcium, iron, zinc, magnesium, and copper have several human nutritional functions due to their metabolic activities. Body tissues require sufficient levels of a variety of micronutrients to maintain their health. To achieve these micronutrient needs, dietary consumption must be adequate. Dietary proteins may regulate the biological functions of the body in addition to acting as nutrients. Some peptides encoded in the native protein sequences are primarily responsible for the absorption and bioavailability of minerals in physiological functions. Metal-binding peptides (MBPs) were discovered as potential agents for mineral supplements. Nevertheless, sufficient studies on how MBPs affect the biological functions of minerals are lacking. The hypothesis is that the absorption and bioavailability of minerals are significantly influenced by peptides, and these properties are further enhanced by the configuration and attribute of the metal-peptide complex. In this review, the production of MBPs is discussed using various key parameters such as the protein sources and amino acid residues, enzymatic hydrolysis, purification, sequencing and synthesis and *in silico* analysis of MBPs. The mechanisms of metal-peptide complexes as functional food ingredients are elucidated, including metal-peptide ratio, precursors and ligands, complexation reaction, absorbability and bioavailability. Finally, the characteristics and application of different metal-peptide complexes are also described.

## 1. Introduction

The importance of metals in multifarious bioprocesses cannot be overemphasized as they are crucial to nutrition and the modulation of developmental pathways. The effectiveness of calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and other minerals are determined by their transport and physiological absorption. Likewise, the expression of divalent metal transporter in the outer mitochondrial membrane and its role in mitochondrial $Fe^{2+}$ and $Mn^{2+}$ absorption is very important (Wolff et al., 2018). However, excessive metal concentrations cause cell death, oxidative stress, and neurodegeneration by interfering with mitochondrial function (Mezzaroba et al., 2019). In this regard, the function of Zn, Cu, manganese (Mn), and Fe in Alzheimer's disease, Parkinson's disease, and multiple sclerosis have been reported (Mezzaroba et al., 2019).

Dietary absorption affects the bioavailability of metals. Dietary substances comprise nutrients like proteins which possess excellent functionalities, e.g. emulsifying, foaming, viscoelasticity, and oil/water-binding properties (Walters et al., 2018). When digested and absorbed, these proteins form the backbone of tissue building and energy sources in humans. Over the years, the study of protein hydrolysates and peptides showed that they are better alternatives to intact proteins as they are more readily absorbed and proffer enhanced physiologic functions, plus their beneficial use in food formulas targeting the required nutrition for people at different developmental and health stages (Ashaolu et al., 2017). These peptides reportedly demonstrate anticancer, antioxidant, hypotensive, anti-inflammatory, and mineral binding, among other bioactivities (Abdel-Hamid et al., 2017).

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For the mineral or metal binding peptides to be intact in food matrices, components like phytates pose an immense challenge to the uptake of metallic components due to their possible complexation reaction with the divalent metals, which makes them insoluble and unavailable, having no access to any gastrointestinal (GI) enzyme that could break down the complex. In this case, the recovery of metals from the formed complexes becomes difficult, leading to their deficiency in the individual. In terms of finding the right enzymes (e.g., phytase) that could aid the breakdown of these phytates-like components, or the employment of other modes of catalysis such as milling, germination, and fermentation, certain food matrices would disallow the intended outcomes (Rizwanuddin et al., 2023). Apart from that, other metals may interact with the complexes formed in a bid to compete exclusively for the carrier proteins and transporters (Żwierełło et al., 2020).

Many factors associated with the mechanisms and potent elements in metal-peptide complexes (MPCs) affect the bioavailability of metals in the human body. The chemical composition of the metal ion, including its charge, size, and reactivity, is a significant impact. It is possible to increase the bioavailability of peptides by forming complexes with metal ions that have a greater charge or smaller size. The development of MPCs and their bioavailability are both greatly influenced by the amino acid sequence of the peptide. Histidine, cysteine, and glutamate are three amino acids that have a high affinity for metal ions and may create strong chelation connections with them. In addition to pH, temperature, and the presence of other dietary ingredients, including phytates, calcium, and other minerals, other parameters can affect the bioavailability of MPCs. The absorbability of MPCs can also be impacted by the gastrointestinal environment. Metal ions can be released when peptides are hydrolyzed by proteolytic enzymes in the digestive system, and these metal ions can subsequently be absorbed into the circulation.

Any of the metals when deficient could create the malfunctioning of vital organs and functions, leading to critical disease conditions. For instance, Ca deficiency would create more transportation of Ca from the bone to create the conditions for the development of osteoporosis, and if Fe is deficient, the processes leading to impaired physical activity, anemia, and infantile cognitive impairment would commence (Vuralli, 2019; Means, 2020). Therefore, the use of peptides to bind these metals for enhanced absorption and availability is becoming popular because they can bind divalent minerals and improve their solubility, stability, and bioavailability as they possess several amino acid residues rich with ionic, electron side chains, and right conformations (Wu et al., 2020; Esfandi et al, 2019). It is the same capacity that improves the complexation reaction towards preventing and terminating free radicals and their reactions sponsored by other divalent metal ions like $Ca^{2+}$, $Fe^{2+}$, $Zn^{2+}$, and $Cu^{2+}$ (Dev et al., 2017; Chim-Chi et al., 2018). In individuals, this activity improves the absorption of major and required minerals like Ca and Fe in the digestive tract. Hence, this review aims to reveal the development of metal-binding peptides (MBPs) and their potential to enhance the absorption and bioavailability of minerals. The production of MBPs, the mechanisms and effective factors in MPCs, and different MPCs are also discussed.

## 2. Methodology

Electronic databases including PubMed, Scopus, and Web of Science were used in the literature search. The search comprised previously published publications (both original and review papers) that had concentrated on MPCs containing several metals with characteristics that might elevate the absorption and bioavailability of trace minerals. The entire search and selection procedure was carried out between November 2022 and January 2023, and the publishing period taken into account for the current review was from 2017 to 2022. To prevent losing any essential references, all of the chosen papers were evaluated. By examining the titles, abstracts, and keywords in each scientific literature, four authors independently determined the relevancy of the papers. The scientific articles were only included for examination after the screening.

## 3. Production of metal-binding peptides

### 3.1. Protein sources and their main amino acids residues for producing metal-binding peptides

The significance of metal-protein binding and their various metabolic and physiologic importance has been reported. Twelve metal ions including sodium (Na), potassium (K), Mg, Ca, manganese (Mn), Fe, cobalt (Co), Zn, nickel (Ni), vanadium, molybdenum, and tungsten have been identified to play vital and important roles in living organisms (Permyakov, 2021). To be fully functional and obtain a maximal biological potential of the metal ions and the associated proteins, the proteins associated with each metal ion are briefly examined, including their sources and the main amino acid residues involved.

A general overview of the natural sources of MBPs has been reported. For instance, Seregin and Kozhevnikova (2023) reported the availability of conserved domains present in some classes of plant proteins referred to as metallothioneins (MTs). These proteins can be divided into three types which include MT-1, II and III. The MT-III is referred to as phytochelatins, however, all the classes are rich in cysteine residues in their active sites. The phytochelatins are oligomers of glutathione, found in the cytosols of plant cells where they function as chelators, responsible for detoxification and improving metal tolerance (Yaashikaa et al., 2022). In addition, MBPs with bonded mineral ions were detected and extracted from the roots of plants, since plant uptake of minerals via their root system is germane to their growth and development (Nakayama et al., 2017).

Other sources such as microbial association with mineral ions have been identified besides plant sources. Microorganisms, adaptively, have been able to harness the energy and metabolic benefits needed for their survival through various mechanisms of association and interaction with these elements (Oomens et al., 2019). As a result, metallic ions have been identified and reported as major players in the evolutionary course of microbes (Morrison et al., 2020). Microbial communities with the unique characteristic of proximity to mineral sources are referred to as “mineral microbiomes”. Further extraction or modification of MBPs for the creation of synthetic or modified microbiomes can be accessed via genetic information richly acquired from such microbiome communities (Hadrich, 2018). This led to the exploitation of such communities to genetically engineer the organisms using the various MBPs that they naturally produced. For example, Using *E. coli* as a clone construct, a new peptide sequence (HARAERHHQ), which can uniquely bind Zn21 from the protein scaffold was expressed (Duan et al., 2023). Another instance revealed that glutathione S-transferase from the parasitic organism, *Schistosoma japonicum* (SjGST) can uniquely and with a very high affinity bind Ni21 as a result of muting Glu26 residue and the His residue activated, a potential mechanism for the use of *E. coli* in possible recombinant purifications of proteins (Kuan, Bergamini & Weil, 2018).

The major active amino acid residues present in the catalytic sites of MBPs are highly conserved. These were reported extensively in several articles. However, many advantages of metals concerning their chelating properties have made them a source of organic inspiration for both their chemical and biological production and function in plants, animals and other microorganisms.

### 3.2. Enzymatic hydrolysis and ultrafiltration

Proteins from various sources such as animals, fungi, and plants have been extracted using different methodologies. However, since there is an increasing demand for diets based on foods from both animal and non-animal-derived sources (such as vegetarian and vegan diets), there is a need for a better and more effective methodology of extraction and purification of proteins from plants, fungal and animal origin. Enzymatic hydrolysis (enzymolysis) of proteins is used in various procedures to

improve the nutritional values of food, as well as offer new opportunities to use undervalued parts of commercially low-valued food such as mushroom stems, fruits and vegetables (Permyakov, 2021). In comparison to chemical hydrolysis, Enzymatic production of protein hydrolysates can be a strategic alternative to improve the sensory properties and nutritional value of foods because, from the ecological point of view, it occurs under mild conditions and does not use non-ecological friendly solutions in its procedure. In addition, the use of enzymolysis preserves some amino acids such as tryptophan, that would have been damaged by the use of acid hydrolysis.

Recently, a more efficient methodology which combines enzymolysis and ultracentrifugation has been reported. For instance, after the hydrolysis of whey protein, the respective hydrolysates were separated using a spiral wound NF membrane (200 Da retention coefficient). The membranes were used to purify the reaction mixture and to separate the products (peptides) from the other proteins yet to be hydrolyzed (Dullius, Goettert & de Souza, 2018). Furthermore, membranes with coefficient retention of 8.0, 3.5 and 0.2 kDa were used to separate angiotensin-converting enzyme (ACE) in a mixture of whey protein hydrolysates from its inhibitors (Alizadeh & Aliakbarlu, 2020). The result of their work shows that the use of two steps membrane filtration can efficiently separate ACE inhibitors. Interestingly, enzymolysis ultracentrifugation can be integrated into a unit called the membrane bioreactor, characterized by the use of enzyme immobilization in the area where membrane interaction takes place. This procedure culminates in the reduction of cost and the separation of the final product from the unreacted substrate giving room to a purer product (Ewert, Eisele & Stressler, 2022). In another report, a 3.0 kDa membrane in a bioreactor was used to hydrolyze whey proteins and a stable conversion degree was obtained, which produced a permeable flux and several lengths of hydrolysates – 4 amino acids peptide chain on average leading to the production of bioactive peptides precursors (Mora & Toldrá, 2022). Another work reported the use of an ultrafiltration membrane of 8.0 kDa, establishing the proposal that a very high DH can be achieved with the use of membranes with very small pore sizes (Jakovetić Tanasković et al, 2018). However, the use of a bioreactor for the separation of MBPs between MBPs and the respective metal ions as well as MDPs from other hydrolysates should be a major area for future research considerations.

### 3.3. Purification, sequencing and synthesis of mineral-binding peptides

MBPs due to their origin and location, must undergo a series of purification steps to obtain the pure protein samples. At the baseline, a combination of column chromatography, affinity chromatography and the avidin–biotin method (Slatko, Gardner & Ausubel, 2018), was employed in the purification of Zn Responsive Factor (ZRF) proteins from a mixture of nuclear extracts of HeLa cells. Subsequent partial sequencing of the ZRF protein obtained shows that the ZRF amino acid sequences are highly homologous to those of a mouse MRE-binding protein, mMTF-1 (Marikar & Zi-Chun, 2022). However, the protein yield was very small and the procedure was very cumbersome. Furthermore, Bitter et al. (2022) expressed and purified a putative Cu-binding domain from the Cu-transporting ATPase implicated in Wilson disease as a fusion to glutathione S-transferase using immobilized metal ion affinity chromatography (IMAC). The fusion protein could bind to columns loaded with different transition metals with varying affinities in the following order: Cu(II) > Zn(II) > Ni(II) > Co(II). The IMAC was subsequently developed to purify functional heterologous proteins based on their ability to be modified with a *hexa*-histidine affinity tag (His-tag). However, the property of the protein can be altered because of this modification. As a result of the limitations that come with IMAC, taking advantage of the natural interaction of the Zn finger domains in Zn-binding proteins, similar functional MBPs in two retroviruses were purified (Ko, Ostermeier & Lin, 2018).

*In vivo* extraction and purification of MBPs associated with cadmium (Cd), Cu and lead (Pb) from the digestive tract of the marine bivalve molluscs, *Mizuhopecten yessoensis*, using gel filtration and anion exchange chromatography produced proteins with approximate molecular masses of 28, 37, and 42 kDa, respectively. Subsequent characterization and sequencing of the amino acids detected in these proteins revealed that they are highly similar to MBPs and ion transporters in order organisms (Berik, Çankırılıgil & Gül, 2017). Recently, *Lentinula edodes* Ca-binding proteins (LECBP) were purified using gel filtration and anion exchange chromatography. The 220 amino acid residues-containing LECBP peptide was subsequently identified by LC − MS/MS. The novel protein was found to be cysteine-residue free, with a high binding affinity, $K_d$, = 97.3 μM and with contents α-helix, uβ-sheet, β-turn, and a random coil of 15.7, 39.4, 8.0, and 37.1%, respectively (Dong et al., 2019). The characterization of the LECBP showed that this protein was significantly different from other Ca-binding proteins from different sources.

MBPs are organically synthesized in higher plants, algae and fungi in response to various heavy metals. HPLC and sequencing reveal that these peptides are mainly composed of three amino acids including cysteine, glycine and glutamic acid where they existed as phytochelatins, cadystins and γ-glutamyl peptides, respectively (Khalid et al., 2021). This was corroborated by (Hasn et al., 2017), that plants respond to metal ions in the environment by triggering the genes that code for proteins in stress response. In animals, however, the biosynthesis of MBPs can be induced by the oral or parenteral administration of metal ions such as Ca or Zn, respectively. A concomitant increase in the biosynthesis and metal binding activity of metallothioneins has been reported in the liver content of mRNA polysomes after the influx of minute quantities of metal ions in the hepatocytes of animals (Nordberg & Nordberg, 2022).

However, Jaradat (2018) developed the concept of solid phase peptide synthesis (SPPS) which has been severally modified into a highly efficient set of techniques for the preparation of numerous peptides and even small proteins. An example is the alpha/beta scaffold of charybdotoxin used in the inorganic synthesis and engineering of metal binding sites. This procedure has enabled the substitution and introduction of nine amino acid residues into the original toxin sequence. The scorpion toxins have been adjudged as one of the best basic structures versatile and tolerant enough for substituting and presenting predetermined conformation and new sequences (Shafee et al., 2017). These chemical approaches provide a practical, rapid and effective way of designing and production of small novel peptides. Another adaptation of the SPPS is the Fmoc Solid-Phase Peptide Synthesis which has been used in the synthesis of drug compounds and other related areas of research (Jaradat, 2018). Subsequently, in an attempt to improve sustainability in green chemistry, the combination of SPPS with MBPs with the active amino acids bipyridylalanine, phenyl pyridylalanine and N, *N*-dimethylhistidine including peptide macrocylization making use of peptide cyclase 1 (PCY1) to yield cyclic peptides under mild conditions. This helps in the development of cyclic peptides as metal ligands with classic inorganic and organometallic ligands as side chains.

### 3.4. In silico analysis of mineral-binding peptides

Due to the therapeutic, nutritional and functional use of bioactive MBPs, there is a growing interest in the bioinformatics approach to predict the formation and also to analyze the protein structure and function relationship, which will help in amelioration of the expensive and time-consuming conventional methods which hitherto has been the practice (Peredo-Lovillo et al, 2021). *In silico* analysis is used extensively for the development of many other bioactive peptides. For instance, effective health-enhancing and potent bioactive peptides have been generated via a range of *in silico* techniques such as molecular docking, design of experiments (DOE), and the application of quantitative structure–activity relationship (QSAR) modelling approaches to optimize bioactive peptide production and identification (FitzGerald et al,

2020).

The use of *in silico* analysis for bioactive peptides is not limited to proteins of animal origin. Díaz-Gómez et al. (2020) reported the use of *in silico* analysis to elucidate the *in vitro* bioactive profile of 19 kDa α-zein sequences of maize via a developed structural model. The outcome revealed the three peptides, 19ZP1, 19ZP2 and 19ZP3, as α-helical structures having positive and negative electrostatic potential surfaces in the range of −1 to + 1. The *in silico* algorithm indicated that the three peptides displayed low probabilities for cytotoxicity (≤0.05%), cell penetration (10–33%) and antioxidant activities (9–12.5%). Similarly, the BIOPEP database was used to analyze 10 unique sequences of FAD3 from flaxseed protein for *in silico* proteolysis and releasing of various bioactive peptides using three plant proteases, namely ficin, papain and stem bromelain, evaluated with the help of BIOPEP database. Overall, 20 biological activities were identified from these proteins. The results showed that the FAD3 protein is a potential source of peptides with ACE inhibitory and dipeptidyl peptidase-IV (DPP-IV) activities (Brewster et al., 2021).

## 4. The mechanisms and effective factors in metal-peptide complexes

Metal peptides are a new class of materials created through the process of chemical bonding between metal ions and peptide chains. These materials have several uses, including drug delivery, nanotechnology, and more. The structure of the resulting metal peptides is relatively simple: it consists of a single peptide chain with one or more metal ions attached at intervals along its length. The most common metals used in this process are Fe and Zn, but other metals, such as Cu and Ni, can also be used. The stability of MPCs depends on a variety of factors, including the interactions between the peptide and metal ions, the structure of the peptide's metal-ion-binding site, and the environmental conditions in which the complex is formed. The structure of the metal-ion-binding site on a peptide determines the interactions between the peptide and the metal ions (Bahrami et al., 2022). The factors that influence the production and mechanism of MPCs are illustrated in Fig. 1. Peptides can bind to metal ions in a variety of ways, including the formation of ionic bonds, coordination of water, sharing of electron density, and coordination via hydrogen bonds. Each type of interaction has a different effect on the stability of the complex. Ion-dipole and cation–anion interactions increase the entropy of the complex making it less stable, while electrostatic interactions stabilize the complex by decreasing its entropy. The type of interaction between a peptide and metal ions is determined by the structure of the binding site on the peptide (Caetano-Silva et al., 2021).

### 4.1. Metal: Peptide ratio (MPR)

The MPCs may be produced using a variety of proteases to hydrolyze a group of peptides, or they can be produced using a synthesized peptide like the decapeptides GPAGPHGPPG from Alaska pollock skin (Chen et al., 2017) or the internal fragment of ovalbumin DKLPGFGDS($PO_4$) IEAQ from egg protein (Liu et al., 2018). The MPR might differ significantly between analyses. Peptides serve as ligands in the peptide-metal complexation process. Metal ions are stabilized with the electrons shared by peptides to consolidate the overall protein structure. Proteolytic enzyme-hydrolyzed peptides often have a lower average affinity than a particular synthesized peptide. Therefore, the ligand content is greater than the metal content to ensure the peptides can reach all metal ion binding sites. The MPR is exhibited as either a molar or mass basis. The MPR is usually expressed as a mass/mass ratio when the ligand is a combination of peptides derived from a hydrolysate with a huge spectrum of molecular mass. Besides, if the molecular mass of synthetic peptides or molecules is known during complex formation, MPR may be estimated on a molar basis. MPR during *in vitro* or *in vivo* tests can range from 1:1 to 40:1, according to several variables, including the intensity of the peptide bonds, the nature of the ligand and metal ions, and any prior separation procedures. However, due to the strong binding of the ligands, metal content is sometimes more than or equal to the ligand content.

### 4.2. Precursors and ligands

Ligands refer to protein molecules that can bond with a central atom of metal. A ligand generally donates at least one electron when participating in a bond. Meanwhile, the ligand acts as a Lewis base. But sometimes the ligand accepts electrons, acting as a Lewis acid. Ligands are often used to protect other functional groups or to stabilize some easily reactive compounds. The compound formed by the combination of a central metal atom and ligand is called a complex.

Metals can only exist in a gaseous state in a high vacuum environment without being bonded to other atoms. In addition, metals are bound to other atoms by coordination or covalent bonds. The ligands in the complex dominate the activity of the central metal, and the activity of the central metal is also affected by factors such as the speed at which the ligand itself is replaced and the activity of the ligand. Protein hydrolysates, ultrafiltered and isolated peptides, or synthesized peptides can serve as the ligands for complex formation. The complexes are

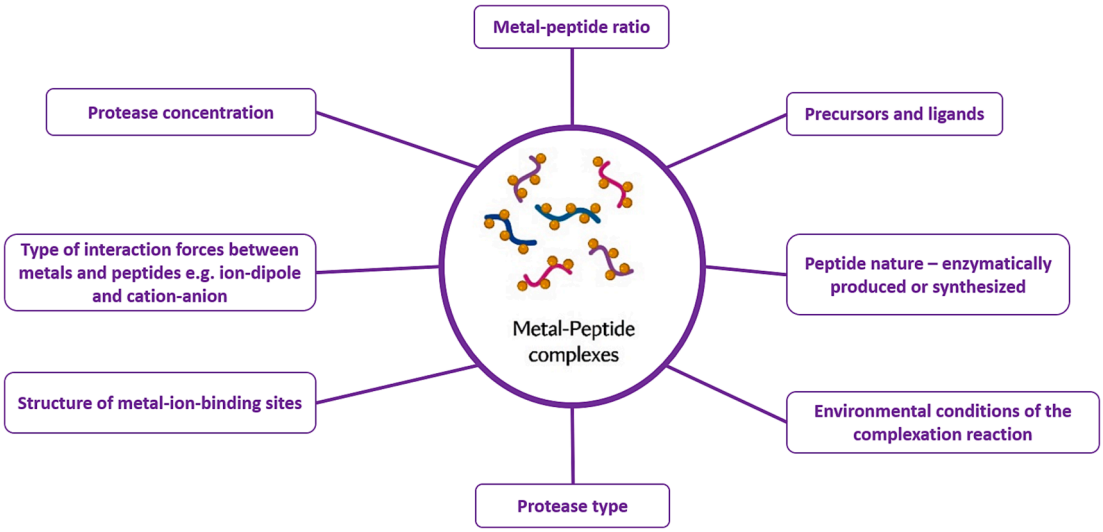


**Fig. 1.** Factors influencing the production and mechanisms of metal-peptide complexes.

constructed using a variety of metal precursors, such as the metallic salts employed as ion carriers to interact with peptides. Metallopeptides provide potential antimicrobial activity to modify bacterial behavior in the cell attachment (Alexander et al., 2018). Some circumstances wherein non-contiguous domains need even more stabilization through the tertiary stability of a protein provided by *van der Waals* forces, hydrogen bonds, salt bridges, and hydrophobic interactions. The stability of peptide structure can be achieved via complexation with metals or by creating covalent disulfide connections between cysteine residues. Fe, Cu, Zn, and Ca are the most prominent metals (Shalev, 2022). The hydrated metal salts are dissolved in an optimal amount of organic solvent while being stirred to produce the solutions of metal precursor salts. Solid complexes were developed by adding a base to peptide and Zn salt, such as Zn chloride, Zn iodide, and Zn perchlorate solutions in an equimolar ratio (Katimba, Wang & Cheng, 2021). Ferric chloride, anhydrous ferric nitrate, $FeCl_2$, and $FeSO_4$ are more frequently used for Fe (II or III) salts. Other salts, including NaCl, KCl, $CaCl_2$, and $MgSO_4$, also incorporate the multilayer peptide structure (Kang, 2017).

### 4.3. Reaction of complexation

MPCs are of great interest to scientists and engineers because they have the potential to be versatile and powerful tools for numerous applications in biochemistry, pharmacology, and material science. Although numerous peptides have been shown to form stable complexes with metal ions in aqueous solutions, not all complexes have desirable properties such as solubility and stability. Several factors affect the formation and stability of MPCs; including the nature of the peptide, the structure of the metal-ion-binding site on the peptide and the environmental conditions in which the complex is formed. These considerations are crucial in determining the structure of a stable complex and the suitability of the complex for a particular application. By changing the binding sites and environmental conditions under which the MPC forms, the stability of the complex can be improved. This has important implications for the design of MPCs with optimal stability. The large numbers of metal ions may establish stable complexes with peptides because of their versatility and strength as ligands. Furthermore, the cooperation between terminal amino acid and subsequently deprotonated amide nitrogen atoms in the peptide structure binding with meta ions is responsible for the exceptional stability of peptide complexes. The histidyl and cysteinyl residues are the most potent in terms of improving stability and drastically altering the protein structure, even though the complex interaction may be affected by any side chain. The most frequent instances of these complexes are those containing Zn (II), palladium (II), Fe (II or III), Cu (II), and Ni (II) ions.

There are several variables in the MPCs reaction, which have a significant impact on the effectiveness and efficiency of the complexation reaction. As presented above, MPR can range from 1:1 to 40:1 in mass/mass or molar. In general, the complexation reaction involves the addition of a specified concentration of metal salt into peptide solution under various incubation conditions, such as pH, temperature, time, and agitation. The pH value is usually controlled between 5.0 and 8.0 to enhance metal-binding with peptides by regulating electron transfer. After incubation time ranging from 30 to 120 min under room temperature (37 °C), the most common procedure is centrifugation for the suspension. The condition of centrifugation is approximately from 3000 to 12,000 × g for 10 or 20 min at 25 °C or 6.0 °C. The supernatant containing soluble complex is collected, and the precipitate is discarded. The supernatant is normally freeze-dried and stored at −20 °C for subsequent examination (Caetano-Silva et al., 2018). In some studies, 80 to 100% ethanol solution is added into suspension before centrifugation to precipitate the desired complexes and remove the free metal ions that are not bound to the target peptides (Sun et al. 2017; Li et al., 2019a). Besides, a penetrable membrane is also used in ultrafiltration before centrifugation to achieve a similar purpose for unbound metal ion removal with dialysis in several recent studies. To eliminate the free Fe ions, the residual solutions were dialyzed using a semipermeable membrane with a molecular weight cut-off ranging from 200 to 500 Da in most circumstances (Liu et al., 2020). In both free and complexed forms, including Fe salts or peptide-Fe complexes, Fe solubility and *in vitro* Fe absorption were assessed. A variety of ligands were used to create whey peptide-Fe complexes, including whey protein hydrolysate and its fractions >5 kDa and <5 kDa, which were produced by a 5-kDa cut-off membrane under ultrafiltration and diafiltration (Caetano-Silva et al., 2018).

### 4.4. Bioavailability

The bioavailability of the complexes following oral ingestion and GI digestion are crucial factors to take into account when assessing the effectiveness of peptide-mineral complexes as a strategy to mitigate the mineral shortage. Animal and *in vitro* cell models are frequently used to assess the bioavailability of peptide-mineral complexes, whereas human studies are less frequently employed due to the difficulty of human trials and possible safety issues (Udechukwu et al., 2018). To have an efficient delivery mechanism and to raise the concentration of minerals in an artery or intestine, MPCs must be soluble in the carrier. In Fig. 2, the bioavailability and accessibility of MPCs in the human digestive system are illustrated. The percentage of a molecule that is liberated from its food sources in the GI tract (GIT) to be available for intestinal absorption is known as bioaccessibility which is frequently used for the evaluation of mineral bioavailability (Caetano-Silva et al., 2021).

However, it is solely about breakdown and dissipation from the food product by *in vitro* techniques and ignores GI cell absorption. The approach of simulated GI digestion is used to produce soluble fractions in many studies. Metal bioaccessibility for luminal absorption can be considerably improved by the solubility of MPCs (Alboofetileh, Hamzeh & Abdollahi, 2021). The bioavailable fraction, as measured by *in vivo* tests, may be thought of from the perspective of nutrition as the part of a consumed substance that is used in regular physiological processes. Determining which portion of ingested nutrients, dietary contaminants, or medications may be utilized by the organism to express their beneficial or harmful effects is a critical concern in food and pharmaceutical studies related to the bioavailability of ingested components, which depends on the type of elements, dose type, food matrix or food processing, and GI characteristics (Shani-Levi et al., 2017).

Analysis of *in vitro* bioaccessibility and bioavailability is used for understanding potential interactions between food preparation and processing techniques, nutrients, the impact of luminal variables such as pH and enzymes, the composition of food products and so on. MPCs in foods could be influenced by different thermal processes (Lee and Taştemir, 2023; Demirci et al., 2020). Compared to human or animal investigations, *in vitro* techniques are more affordable, and efficient, and provide more excellent controls of experimental factors on micronutrient absorbability, a component of bioavailability, the capacity of a nutrient to be absorbed that is bioaccessibility (Mattar et al., 2022). Furthermore, because *in vitro* research cannot replace *in vivo* research, it

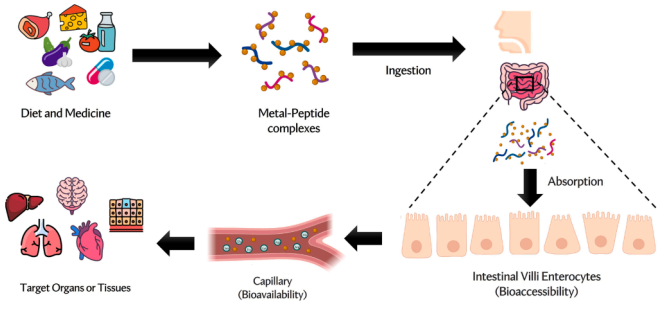


**Fig. 2.** Bioavailability and accessibility of metal-peptide complexes after human ingestion.

should be used as a screening, evaluation, or classification technique. Solubility, dialyzability, or a GI model for testing bioaccessibility, and the Caco-2 models for measuring bioavailability, are the four main approaches *in vitro*. Each approach utilizes a two- or three-step *in vitro* digestion that comprises both gastric and intestinal digestion to replicate the functioning of the human GI process (Ferruzzi, et al., 2020). Moreover, *in vitro* bioavailability is often used to describe the component that is taken up by intestinal cells or even transmitted across them and is then ready to be sent to the target location.

Caco-2 cells have been more commonly utilized for *in vitro* bioavailability investigations because they resemble intestinal cells. Peptidomic analysis was used to determine a potential Ca-binding heptapeptide produced in trypsin hydrolysis from sea cucumber ovum. Ca uptake through Caco-2 cell monolayers may be facilitated by the heptapeptide-Ca complex (Cui et al., 2018). The free and complex forms of Fe, such as Fe salts or peptide-Fe complexes, were used for the evaluation of their solubility and *in vitro* Fe absorption. With a variety of ligands and Fe salts, the complex of Fe-binding whey peptide complexes was developed. While Fe absorption was explicitly quantified by measuring ferritin production in a Caco-2 cell model, Fe bioavailability was tested following *in vitro* GI digestion. Particularly complexes constructed with low-molecular-mass peptides with 5 kDa and Fe (II) chloride enhanced Fe intake by almost 70% in comparison to Fe (II) sulfate even though all complexes displayed >85% bioaccessibility (Caetano-Silva et al., 2018).

### 4.5. Absorbability

Many hypotheses have been revealed in relevant studies to elucidate the absorption of MPC in the human GIT. The internal binding force of MPCs must prevent metal binding with other peptides while also facilitating metal transport in the membranes of the enterocyte. It is preferable to absorb the bound metal freed from peptides in the small intestine. For the bound metals to be released at the absorption site in the intestine, they must be held in place by the internal binding forces between the metals and peptide when other competing species are involved, mainly in the stomach. On the brush boundary membrane, duodenal cytochrome *B* encoded by the cytochrome *B* reductase 1 gene is an important ferric reductase or absorbing Fe from the intestine. It has also been proposed that the six-transmembrane epithelial antigen of the prostate 2 family member is a duodenal ferric reductase (Rocha et al., 2021).

Transferrin binds to transferrin receptor protein 1(TfR1), allowing the protein complexation to be internalized by the cell through receptor-linked endocytosis. A proton-pumping ATPase then acidifies the subsequent endosome to a pH = 5.5 (Kleven, Jue & Enns, 2018). With the intervention of acidification and the conformational alteration brought on by transferrin binding to transferrin receptor protein 1, Fe may be detached from the transferrin molecule. Divalent metal-ion transporter 1 (DMT1) then moves Fe through the endosomal membrane into the cell (Reilley et al., 2020). DMT1 is a proton-coupled metal transporter that belongs to the natural resistance-associated macrophage protein family. DMT1 also moves reduced $Fe^{2+}$ (ferrous) over the brush boundary membrane and into the enterocyte. In intestinal tissue, the expression of DMT1 and Duodenal cytochrome *B* is strictly modulated, and both proteins are expressed more when the organism needs Fe (Yanatori and Kishi, 2019).

Phosphorylated peptides potentially regulate digestibility and absorbability by generating soluble compounds with minerals, including Ca, Fe, and Zn at a pH of GI. Casein phosphopeptides (CPPs) are a kind of phosphorylated peptides that can be produced by the proteolytic cleavage of dairy products *in vitro* or *in vivo* and can serve as carriers and emulsifiers of minerals (Chaudhari & Hati, 2022). The physiological impact of CCPs on metal digestion and absorption is influenced by the partial resistance of CPPs to certain hydrolysis in the digestive system due to the negative ionic charges on phosphate groups. The fact that CPPs have a strong affinity for divalent cations like Mg, Ca, Zn and Fe is one effect of the massive proportion of negative charges present in serine phosphate residues (Tenenbaum et al., 2022). The bioavailability of metal ions in tissues, which is reliant on GI absorption of metal ions in food consumption, is responsible for metal ions to perform their physiological roles. Various food preparation under high-thermal condition also affects the bioavailability of MPCs (Nazlı et al., 2022; Lee and Demirci, 2023). Dietary fiber and myo-inositol hexakisphosphate, known as phytate, can produce insoluble complexes with Zn and other divalent cations, reducing its bioavailability, and preventing Zn from being absorbed through the intestinal villi. The bioavailability and absorption of Zn can both be improved by peptides generated from dietary proteins. To produce soluble MPCs, Zn and other divalent metals can be chelated by peptides that include amino acid residues such as cysteine, histidine, serine, aspartate, and glutamate (Udechukwu et al., 2018).

Although resilient to simulated peptic digestion, the two peptides SM and NCS prepared from sesame protein hydrolysate were further degraded by pancreatin, which may free the bound Zn ions from peptide complexes to facilitate GI absorption (Wang et al., 2020a). Under *in vitro* simulated GI digestion, the MPCs with high molecular weight and high Zn-chelating ability still had approximately 85% of their original activity (Li et al., 2019b). Zn complexes made of protein hydrolysates exhibited higher bioavailability than Zn salts. According to the configuration correlation of Zn-binding peptides, the results of Zn-binding capabilities to peptides are displayed in descending order: Zn ion binding site, Zn ion binding strength, the number of Zn chelators, net charge, and complex molecular weight. Like many bioactive compounds, Zn-binding peptides can improve their stability, distribution, and absorption of Zn *in vivo*, *in vitro*, and *ex vivo* (Katimba, Wang & Cheng, 2021; Ashaolu et al., 2021).

A peptide transport pathway was used to carry the Fe-peptide complexes into enterocytes. Intestinal cells were able to absorb Fe-peptide complexes with a low molecular weight under various peptide transport pathways (Caetano-Silva et al., 2018). For intestinal peptide absorption, there are several peptide transport pathways, including the proton-coupled small peptide transporter 1 (PepT1), transcytosis, endocytosis, cell-penetrating peptides, vesicular and paracellular pathways (Ma et al., 2018). The ability of Ca and $Fe^{2+}$ ions chelating peptides to withstand enzymatic hydrolysis in the digestion system and reassemble with metal ions in GIT is a need for complex distribution into intestinal epithelium. The anti-digestibility is desired to increase the intestinal bioavailability and absorption of metal ions in Ca and $Fe^{2+}$ binding peptide complexes (Caetano-Silva et al. 2021).

### 4.6. Confirming metal-peptide complexation and bioavailability

Confirming the formation of an MPC and determining its bioavailability involves several techniques, such as spectroscopy, mass spectrometry, and *in vivo* studies. To investigate the chelating activity and absorption mechanism of peptides that chelate metal ions, the MPC analysis is required on affinity attributes, binding mechanism, binding site, intermolecular forces, and the confirmational structure (Tian et al., 2021). Spectroscopy techniques, such as X-ray diffraction (XRD), Fourier transform infrared (FTIR), $^{1}H$ nuclear magnetic resonance (NMR), and ultraviolet (UV)-visible spectroscopy, can be used to confirm the formation of MPC by identifying characteristic structural changes and absorption or scattering peaks associated with the metal and peptide components. Mass spectrometry techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), liquid chromatography-mass spectrometry (LC-MS), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), can be used to confirm the presence of the metal and peptide components in the complex and to determine their relative ratios. Energy dispersive spectroscopy (EDS) and scanning electron microscopy (SEM) are also commonly used to examine polypeptide structures. The ability of peptides to chelate metal ions is frequently assessed using colorimetric assays. Zn, Ca, and Fe are the

most prevalent metal ions in many investigations of MPCs, particularly $Fe^{2+}$ ions. Heightened bioavailability is determined by the metal-binding affinity or even by the potential to maintain the solubility of MPCs. The differences in infrared absorption peaks before and after metal chelating allowed researchers to determine the binding groups of complexations.

Numerous findings indicate that particular amino acids in peptides may involve in Ca-binging complexation, e.g. histidine, glutamate, threonine, phosphoserine, aspartate, arginine, and lysine. Besides, it has been observed that Ca chelation and promoting Ca uptake is associated with various functional groups and atoms, such as the nitrogen atom in imino, amino, imidazole, and amide groups, as well as oxygen atoms in carboxyl, carbonyl, and hydroxyl groups (Wang et al., 2020a; Tian et al., 2021; Zhang et al., 2021a). According to the result of the N—H band, amide-I, and amide-II absorption peaks, the carboxylic group and nitrogen atoms of amino acid histidine and glutamate were confirmed to be in the Ca-binding sites of the peptide, as demonstrated by FTIR and mass spectrometry (Chen et al. 2019). By analyzing the distribution of the electron cloud surrounding the hydrogen proton, $^1$H NMR can show the structural confirmation of peptides and metal ions (Sun et al., 2020).

The primary binding sites for $Fe^{2+}$ ions, in contrast to Ca ions, are nitrogen atoms in different functional groups, e.g. the nitrogen atom of the guanidine group in arginine, the nitrogen atom of the imidazole group in histidine, and the nitrogen atom of the ε-amino group in lysine (Wu et al. 2017a). Additionally, the chelation of peptide and bioavailability of $Fe^{2+}$ ions is linked to histidine, serine, arginine, cysteine, lysine, and the carboxylic groups of glutamate and aspartate (Li et al., 2019a). By investigating the $^1$H NMR spectra of nonapeptide Fe-chelating DTDSEEEIR from Antarctic krill, the results concluded that the carboxyl groups of the glutamate and aspartate, as well as the hydroxyl groups of the threonine and serine, are associated with the binding process (Sun et al., 2020). $Fe^{2+}$-peptides chelation in desalted duck egg white indicated that lysine, aspartate, glutamate, and histidine are crucial in the enhancement of $Fe^{2+}$ absorption and Fe-binding (Li et al., 2019a). Under SEM investigation, the mung bean peptide formed a polymeric complex by chelating $Fe^{2+}$ ions. The findings suggested that peptide-Fe chelates were primarily synthesized when $Fe^{2+}$ ions interacted with the amino, carboxyl, and imidazole groups in mung bean peptides to influence $Fe^{2+}$ bioavailability (Zhang et al., 2021b).

The frequently used technique for evaluating the level of free Zn and determining the Zn-chelating ability of peptides is atomic absorption spectrometry (ABS). Determination of Zn ion concentration in the Zn-chelating protein hydrolysates before and after treatment is essential to measure the Zn-binding capacity of yak casein hydrolysate under ABS (Liu et al., 2020). Moreover, a reddish compound formed by 4-(2-pyridylazo) resorcinol and free Zn ions can be detected in its absorbance peak at 500 nm under colorimetric assays. Several studies have applied this method to determine the Zn-binding ability in peptide complexation, such as in rye secalin-derived tripeptides and their analogs (Udechukwu et al., 2017), and in whey-derived peptides (Udechukwu et al., 2018). The metal-chelating titration with ethylenediaminetetraacetic acid (EDTA) was also designed to determine the Zn level. It involves the addition of xylenol orange and hexamethyl-enetetramine following the precipitation of the Zn-peptide complexes with ethanol to analyze the bound Zn content. Besides, six Zn-binding peptides were discovered using reversed phase-HPLC and LC-MS/MS (Udechukwu et al., 2021).

Simulated GI *in vitro* digestion (SGID) is a lab technique to mimic the human digestive process and study the breakdown and absorption of food and food components. The process typically involves a series of reactors that simulate different stages of digestion, such as the stomach and small intestine, and the addition of enzymes and other digestive secretions to mimic the actions of the human digestive system. The end products of SGID are usually analyzed to understand the changes in the food during digestion and how it is absorbed and metabolized by the body. This technique is used in food science, nutrition, and medicine to study the effects of food and food components on health and to develop functional foods and dietary supplements (Yao et al., 2021). Biopeptides from vegetable proteins and cell survival could be influenced by different antimicrobial treatments during the post-harvesting of vegetables (Montesano et al., 2020). A protein hydrolysate with Zn ion binding was prepared from oysters (*Crassostrea rivularis*) to evaluate this Zn bioavailability in MPCs. In comparison to Zn sulfate, the Zn-peptide complex demonstrated higher Zn bioavailability both under particular pH during SGID. Additionally, OPH which had been chelated with Zn during SGID maintained its antioxidant activity effectively (Zhang et al., 2018). Also, a protein hydrolysate from Manchurian walnut was produced, and these peptides binding with Ca ion were prepared to obtain the Ca-peptide complexes. The bioavailability of Ca ions may be impacted by serum peptidase and protease in the digestive system during SGID. Using peptides to bind metal ions might potentially be a promising strategy for increasing their solubility. The high solubility of Ca might be anticipated to promote Ca absorption in the intestines with the low pH in SGID (Fang et al., 2020).

*In vivo* studies, such as pharmacokinetic and pharmacodynamic studies, can determine the bioavailability of MPCs in the body, including their absorption, distribution, metabolism, and excretion. For bioavailability assay, two types of human colon cancer cell lines, Caco-2 and HT-29 cell lines, are commonly used (Ferraretto et al., 2018). Investigations on *in vitro* absorption involving cell culture models following SGID intending to test the absorption *in vivo* have received prominence in recent years. Even though SGID displays certain limitations in its ability to accurately simulate the complexities of the GI system, they are highly beneficial in determining how digestion would behave *in vivo*. In some conditions, greater bioaccessibility may not lead to better bioavailability (Bohn et al., 2018).

The Ca absorption of the complexes of Ca-chelating soybean protein hydrolysates with various molecular weights *in vitro* was measured by using Caco-2 cells (Liu et al., 2017). Peptides that exhibit specific properties, as indicated by their amino acid sequences during interaction with cells, can significantly raise the amount of free Ca ions. CPPs with the sequence PPPEE can activate specific Ca ion channels and enhance Ca absorption in the colorectal cancer cell line HT-29 (Ferraretto et al., 2018). Theoretically, the creation of Ca peptide products might be supported by information about which peptides have improved Ca transport, their amino acid structure, and their method of transport. Through cytological investigation in the Caco-2 cell model, the relationship of Ca-peptide complexes with the cell membrane was examined and the route of Ca transport in specific peptides was determined. To evaluate two kinds of peptides with varied Ca chelating capabilities and their impacts on the quantity of Ca transported, several examinations were developed, including quenching assay, Caco-2 monolayer cell model test, zeta potential assay, and tryptophan fluorescence emission chromatography (Liu et al., 2017).

The cytotoxicity of MPCs should be assessed by a technique such as the MTT (3-[4,5-dimethylthiazol-2-yl]-2,3-diphenyl tetrazolium bromide) assay to determine if the compounds do not deteriorate the cell function. Many cytotoxicity results of MPCs demonstrated no evidence of a substantial impact on cell survival (Caetano-Silva et al., 2018). Overall, a combination of these techniques is typically used to confirm the formation of MPCs and determine their bioavailability.

## 5. Different metal-peptide complexes

Considering that metal chelating peptides can be suitable carriers for rare metals and micronutrients and increase their absorption (Feng et al., 2019), and on the other hand, according to FDA regulations, bioactive peptides obtained from protein hydrolysates are known as safe nutrients (Aguilar-Toalá et al., 2019), therefore, MPCs have been studied by many researchers (Table 1). Usually, peptides that contain some amino acids, e.g. histidine, cysteine, glutamic and aspartic acids have a high potential to bind to metal ions (Bingtong et al., 2020). Until now, a variety of these peptides have been found in diverse sources. In this

**Table 1**
Metal binding proteins, main amino acid residue for binding and their sources.

| S/n | Metal ion | Metal-Binding Proteins (MBPs) | Source of MBPs | Major Active Amino Acid residues | References |
|---|---|---|---|---|---|
| 1. | Calcium | EF-hand proteins: Calmodulin, Pervalbumin. Troponin C, S100 proteins, Annexins. C2 Domain Containing Proteins: cytoplasmic Phospholipase A2, phosphoinositide-specific phospholipase C, phosphatidylinositol 3-kinases, etc Calcium ATPases | *in vivo* synthesis in vertebrates and higher eukaryotes. | Asp, Glu | Permyakov, 2021; Permyakov et al, 2017; Dürvanger & Harmat, 2019; Haiech et al, 2019; Gonzalez et al, 2020; Permyakov, 2021. |
| 2. | Magnesium | Protein CorA | Eubacteria and Archea eg. S. typhimurium and E. coli | Asp, Glu | Lunin et al, 2006 |
| 3. | Zinc | Carboxypeptidase A, Superoxide Dismutase | Produced in the Pancreas, SOD is produced in the chloroplast and cytoplasm. | Glu, Asp, Cys, and His residues (His 63). | Lee, 2018. |
| 4. | Copper | Multicopper blue proteins e.g Nitrite reductase and Multicopper oxidases. | *In vivo* synthesis of plant and animal tissues | His, Cys, Met, Leu, Phe, Tyr. | Pérez-Henarejos et al. (2015) |
| 5. | Iron | Transferrin, Ferritin, haemoglobin, Myoglobin, Cytochromes | Liver, Foods e.g. legume seeds | Asn413, Asn611, Ser32, Tyr, Asp, His | Fernandes et al. (2019), Zhang et al. (2021c), González-Arzola et al. (2019) |
| 6 | Molybdenum and Tungsten | Pterin, xanthine oxidase, sulphite oxidase, nitrate reductases. xanthine dehydrogenase, aldehyde oxidase, | Cow milk, Chicken and bacteria | Ser, Cys, selenocysteine, | Krompholz et al., 2012 |
| 7. | Nickel and Cobalt | urease, carbon monoxide dehydrogenase, S-methyl coenzyme-M reductase and hydrogenase, Superoxide dismutase and Methionine aminopeptidase. | Bacteria eg Salmonella typhimurium, Streptomyces seoulensis | His, Cys, Asp97, Asp108, His171, Glu204, and Glu235 | Zambelli et al., 2016 |
| 8. | Manganese | cytochrome *c* oxidase, Mn-superoxide dismutase, Mn-catalase, Arginase, 3-phosphoglycerate mutase, | Chloroplast and Mitochondria, gram-positive bacteria. prokaryotes such as hyperthermophiles, | Asp and Glu, His. | Christianson & Cox, 1999 |
| 9. | Sodium and Potassium | Diol and glycerol dehydratases, trypsin –like enzymes, Kinases | All kingdoms of life | No conserved active domain residues. | Zhorov & Tikhonov, 2004; Page & Di Cera, 2006. |

section, we will introduce more precisely the types of MPCs related to some metals and their benefits and importance for people.

### 5.1. Calcium

Ca is the richest element in the human body, which generally makes up about 1.5 to 2% of the body weight of each person. Most of this element in the human body is in the form of phosphate, present in bones and teeth, and the rest is in the form of ions in soft tissues, cells, and blood (Kheeree et al., 2022). Ca is an essential element, especially for bone development (Erfanian et al., 2017), because its deficiency causes diseases and problems such as destruction of bone microstructure, reduction of bone mass, osteoporosis, rickets, cramps, and even hypertension. However, Ca deficiency is common worldwide, especially among elderly people (Erfanian et al., 2017, Wang et al., 2017, Zhao et al., 2022, Zhang et al., 2022, Nuti et al., 2019,). Hence, several Ca supplements, for instance, amino acid chelate Ca and Ca salts have been developed to prevent Ca deficiency (Guo et al., 2020). But these supplements, in addition to having a poor effect, cause problems, e.g. disruption to amino acid absorption, adverse reactions, and intense irritation (Aditya, Stephen & Radhakrishnan, 2021). Consequently, a new form of Ca supplement is needed to provide Ca with fewer complications and better effectiveness. Especially peptide–Ca chelate because some peptides can form such complexes by connecting to Ca ions (Wu et al., 2017b, Katimba, Wang & Cheng, 2021).

Peptides derived from the wheat germ (Wang et al., 2018), pig-bone collagen (Wu et al., 2019), Alaska pollock skin (Luo et al., 2020), cucumber seeds (Wang et al., 2017), tilapia (Bingtong et al., 2020), and chicken-foot broth byproduct (Malison et al., 2021) are types of food source Ca-binding peptides described so far. Peptide–Ca chelate reduces the construction of insoluble Ca compounds and increases Ca absorption through the intestine (Katimba, Wang & Cheng, 2021). Ca-binding peptides can be isolated from two general sources, animal and plant sources (Fig. 3). Soy protein (Yu et al., 2023), mung bean protein (Budseekoad et al., 2018), lemon basil seed protein (Kheeree et al., 2022), *Chlorella pyrenoidosa* protein (a type of green algae) (Hua et al., 2019) and *Schizochytrium* sp. protein (a marine fungus) (Cai et al., 2017) are vegetable proteins; and milk CPPs), cattle and sheep bone collagen (Wang et al., 2020b, Zhang et al., 2021a), and pig plasma proteins (Sun et al., 2021) are animal proteins from which peptides with the ability to

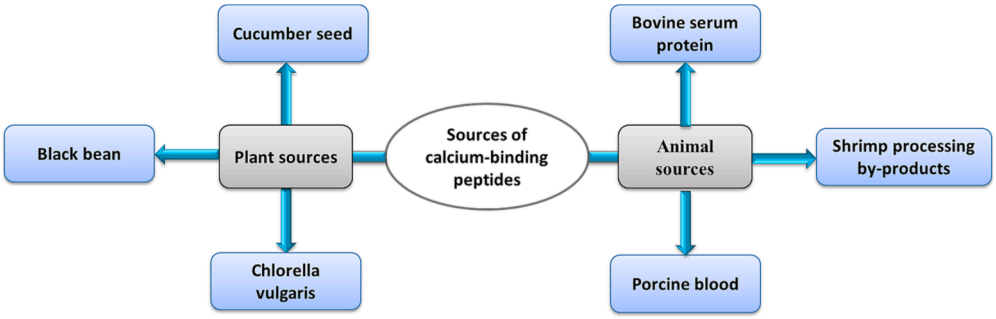


**Fig. 3.** Some plant and animal sources of calcium-binding peptide.

form Ca chelate peptides have been extracted. Therefore, many peptides have shown the ability to bind to Ca ions. However, the search for peptides with higher chelation rates for commercial-scale applications can be continued in future studies.

### 5.2. Zinc

There are about 2 to 3 g Zn in the human body (Trame et al., 2018), which makes it the second most abundant inorganic micronutrient (Sunuwar, Gilad & Hershfinkel, 2017). Zn is an essential element for human nutrition because it is effective in immune functions, protein synthesis, DNA synthesis, and cell growth and differentiation, and its deficiency can cause neurological disorders, cognitive disorders, anorexia, and slow growth, especially in children (Wang et al., 2020a). Nevertheless, billions of populaces around the world struggle with Zn deficiency (Khan et al., 2021), especially in developing countries, among infants, children, pregnant women, and the elderly. Since the storage of Zn in the body is limited and the required amounts of Zn are obtained through diet, it must be supplied continuously (Sunuwar, Gilad & Hershfinkel, 2017). Mineral salts are one of the available forms of Zn supplements (Hurrell, 2022), but these mineral salts are inaccessible due to the construction of insoluble complexes and the inhibitory effects of food compounds such as phytate, tannins, and dietary fibers(Gibson, Raboy & King, 2018). In addition, long-term consumption of Zn salts can be undesirable because of the possibility of GI irritation (Syam et al., 2020).

Peptides derived from food can be considered Zn carriers due to their ability to bind with Zn ions (Udechukwu et al., 2018). The impacts of peptides on increasing Zn absorption have been previously reported (Feng et al., 2019)) and evidence suggests that the absorption of Zn from Zn-peptide complexes is better than mineral Zn salts (Udechukwu et al., 2018). Until now, peptides and hydrolyzed proteins derived from several sources showed the ability to bind with Zn ions. These sources include oysters (Li et al., 2019a), chickpeas (Sun et al., 2018), sesame (Wang et al., 2012), milk (Feng et al., 2019, Udechukwu et al., 2018, wheat germ (Katimba, Wang & Cheng, 2021), rapeseed, silver carp, walnut, and casein (Katimba, Wang & Cheng, 2021). Despite the benefits of peptide-Zn complexes, some factors may affect their stability. Factors such as acid or alkali treatment and thermal processing of food, and reaction with sugar, salt, or other nutrients can diminish the solubility of Zn and the absorption rate of peptides-Zn chelate (Singh and Vij, 2018). Various GI proteases destroy the amino acid sequence of peptides and terminate the peptide-Zn complex construction (Wang et al., 2021). Therefore, it seems that in future studies, in addition to the preparation and absorption of peptide-Zn complexes, focusing on their stability will also help to develop the goals related to Zn supply and increasing its bioavailability.

### 5.3. Iron

Iron is an essential element for human nutrition and the main component of cytochromes, myoglobin, and hemoglobin (Rahate, Madhumita & Prabhakar, 2021). This mineral plays a role in many biochemical processes of the human body and acts as an activator, controller, and regulator of several enzyme reactions. Among the functions of Fe, we can mention the formation of red blood cells, electron transfer, oxygen, and $CO_2$ transport, cell growth regulation, cellular energy production, gene regulation, and enzyme operation (Gharibzahedi and Jafari, 2017). Therefore, insufficient intake of Fe will lead to adverse health effects, e.g. fatigue, weakness, and lethargy; reduced performance in work and sports, anemia, and even death; its excessive increase is also undesirable (Aisen et al., 2001, Vogt et al., 2021, Gowan and Roller, 2022, Safiri et al., 2021). Considering these nutritional needs and especially the fact that a significant population around the world suffers from anemia (WHO, 2022), so far many efforts have been made to prevent Fe deficiency in various ways, including the use of Fe salts (Verna et al., 2021). However, there are challenges such as the lack of Fe bioavailability in this way (Zhang et al., 2021b, Athira et al., 2021).

Peptide-Fe complexes are one of the proposed alternatives to deal with such challenges. The formation of a complex by binding between Fe and an organic compound can protect it in the conditions of the digestive tract (Shi et al., 2022). CPPs derived from milk are metal-chelating peptides with a high Fe-chelating activity and can be used as a mineral supplement to increase Fe absorption (Cao et al., 2017). It has been described that Glu., Pro., and Asp. were the most abundant amino acids in Fe-binding peptides derived from whey protein (Corrochano et al., 2018). The construction of peptides and the composition of amino acids are effective in determining Fe chelating activity (Irankunda et al., 2022). Different types of proteins have unique properties that can be used to prepare peptides with high affinity to metal ions. However, studies on Fe-chelating peptides are restricted and more sources should be sought for the fabrication of such peptides.

### 5.4. Magnesium

Mg, as one of the vital elements for the human, has many health effects. Mg can play a beneficial role in a wide range of diseases comprising asthma, heart disease, diabetes, depression, and anxiety. Mg deficiency can also lead to joint pain and muscle stiffness (Szymoniak et al., 2022). Despite such important effects on human health, it is estimated that more than half of people in the US do not consume the recommended daily intake of Mg, which is 320 mg for women and 420 mg for men (Mazidi, Rezaie & Banach, 2018). One of the reasons for this deficiency is that around 80 to 90% of Mg is missing throughout food processing (Szymoniak et al., 2022). Therefore, finding a way to protect Mg in the diet would be an important achievement. Since the connection of Mg with protein is weak connection, it is thought that the connection of Mg with peptides is also very weak (Shalev, 2022). Considering that the studies on such binding are limited, therefore, studying the stability of peptide-Mg complexes to find the best binding peptide can be important. Especially, it has already been established that the peptide-Mg complex may be even more beneficial in terms of health properties than some other MPCs. As in a study by Zhang et al. (2023), the antioxidant property of Mg complex with bovine bone collagen peptides was significantly higher than other divalent metal ions for instance Fe, Ca, Zn, and Cu (Zhang et al., 2023).

### 5.5. Copper

Cu is another vital element; the lack of it can be associated with problems and diseases such as cardiovascular risk, osteoporosis, immune system dysfunction, and cholesterol metabolism disorder (Tsang, Davis & Brady, 2021). On the other hand, the high concentration of Cu ions also leads to its deposition and induction of cellular toxicity (Zhang et al., 2018). Therefore, using a method for the controlled release of Cu ions in the body can be an appropriate solution. The binding of some ligands to Cu ions can lead to the creation of a complex with therapeutic effects (Denoyer, Clatworthy & Cater,2018). It has been reported that various Cu-peptide complexes sometimes have positive and sometimes negative effects on health. While the binding between Cu and some peptides can lead to anticancer or antibacterial effects or act as a carrier for drugs, on the contrary, the binding of Cu to some peptides can cause cancer, bacterial diseases or, neurodegenerative diseases. These positive and negative effects depend on the effects of reactive oxygen species (ROS) produced by peptide-Cu complexes (Komarnicka et al., 2022). Tripeptide glycyl-l-histidyl-l-lysine has shown a high affinity of connecting with Cu ions with good therapeutic applications, including skin repair, damaged tissue repair and bone healing (Wehbe et al., 2017). The main locations for connecting Cu ions to peptides are histidine residues, and the histidine location in the peptide sequence is a significant factor in determining the characteristics of the peptide-Cu complexes (Liu et al., 2022).

## 6. Conclusions

In this review, we have established the functions of essential metals along with their bioavailability and absorption. The production of peptides that could bind to these essential metals was also discussed by explaining key parameters such as the parental protein sources and amino acid residues, and the enzymolysis, purification and *in silico* analysis of MBPs. The MPCs and their bioavailability could be confirmed when the effective factors and mechanisms of MPR, precursors and ligands, reaction conditions and absorbability are understood. The different MPCs studied so far include Ca, Fe, Zn, Mg and Cu metal components. It is concluded that MPCs could contribute to the absorption and bioavailability of essential minerals.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors are unable or have chosen not to specify which data has been used.

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