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Iron complexes developed by peptides and hydrolysates derived from halal milks: an approach to enhance iron bioavailability

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Abstract

Background and objective: Iron deficiency is a global health concern. Fortification of foods with iron is a common strategy to solve the nutritional issue. However, traditional iron fortification methods lead to some challenges with regard to stability and bioavailability of the element. Therefore, finding alternative approaches have been developed. One approach is use of iron-chelating peptides derived from milk proteins. They have attracted a lot of interests in the scientific community due to their potential to enhance iron absorption and bioavailability. This article provides a review on iron-chelating peptides and hydrolysates derived from halal milks, shedding light on their underlying principles in iron complexation and the key binding sites involved.

Results and conclusion: The significance of iron-chelating peptides lies in their ability to address the critical issue of dietary iron deficiency. Halal milks' protein-iron complex offer several advantages such as superior bioavailability, minimal impact on taste, and excellent solubility. These characteristics introduce them as promising strategy in iron fortification of halal foods. These complexes are formed through specific chemical interactions between iron and the breakdown products of milk proteins, including hydrolysates or peptides, derived from both whey protein and casein. Several factors affect the efficacy of iron binding including pH, ionic strength, concentration of peptides to reach ideal ratio, temperature and time, appropriate enzyme (which impacts on the peptide characteristic), and potential interaction with other molecules. This multifaceted approach to enhance the iron fortification effectiveness underscores the importance of a deeper understanding of the intricate interplay between these factors in development of iron-fortified foods.

Keywords: Casein, Hydrolysates, Iron bioavailability, Peptide, Whey protein

1. Introduction

Iron is an essential nutrient and a critical cofactor for numerous proteins in vital systems [1]. Iron deficiency is the most common nutritional deficiency worldwide, affecting about 30% of the world's population, approximately 1.6 billion people [2]. Iron deficiency occurs by iron loss and/or its inadequate uptake [3].

Dietary iron exists in two forms: heme and non-heme. Nonheme iron is abundant in plant-based foods, while heme iron is found in animal sources [1,4,5]. Despite the abundance of iron-rich foods, iron bio-availability is low due to its low solubility at intestinal pH, presence of the inhibitors such as phytate in plant foods, and lack of promoters such as ascorbic acid [6-8].

Iron fortification of foods is a solution for the widespread anemia [9]. Multiple iron sources with different bioavailability are used for fortification. The first group are those with high bioavailability such as ferrous sulphate and ferrous fumarate. Nonetheless,

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these sources have some drawbacks such as short shelf-life, unsatisfactory taste, colour change, and reduction of minerals' and vitamins' absorption. The second group encompasses low-bioavailability sources such as iron (III) diphosphate and reduced iron. Thus, a challenge faced by the food scientists and the industry-men is to enhance the iron bioavailability while mitigating the associated problems [10-12]. For this, the researchers have either tried to encapsulate the iron within macromolecules, or use a chelated iron form [10].

Peptide complexes exhibit enhanced stability and reactivity in the environment. Amino and carboxyl groups, along with some of side chains of the amino acids in the peptides, interact with divalent cations, making the peptides a preferred ligand choice [13]. Iron complexation with peptides offers a potential solution to improve bioavailability, alleviate gastrointestinal symptoms, minimize iron's interference with other dietary factors, and even mitigate changes in taste and appearance of the food products [14]. Amino acid sequences of the proteins undergoing degradation by the enzymes from their parent protein, or those produced by digestive enzymes, whether *in vitro* or *in vivo*, are recognized as bioactive peptides [2].

Milk contains several proteins with distinct functional characteristic. An emerging trend is use of milk proteins as carrier for bioactive substances [15]. Casein (CN) and whey proteins (WP) are integral components of milk proteins, and play various physiological roles such as ion transport, lactose production, immunomodulation, and immunelogical defense. Both CN and WP yield bioactive peptides upon enzymatic degradation and display antihypertensive, antimicrobial, anticancer, immunostimulant, and mineral-binding activities [2,16].

This article aims to provide an in-depth overview on the iron-chelating peptides and hydrolysates derived from halal milks, their iron complexation principles, and the binding sites, as well as the conditions affecting their iron-binding capacity.

2. Peptides/hydrolysates derived from whey proteins and casein as candidate for iron complexation

Iron-binding properties of WP and CN peptides/ hydrolysates have been interested due to their potential to increase iron stability and bioavailability in the gastrointestinal tract [8,17]. WP and CN, abundant in milk, have significant capabilities in binding to iron and influencing its fate during digestion [5].

CN, major protein of milk, is composed of several constituents including α-s1, α-s2, β, γ, and K, of them α-s1 and α-s2 are prominent. Bovine milk CN is primarily composed of α-s1 and β, comprising over 70% of total CN [16]. In contrast, WP mainly consist of β-lactoglobulins (β-Lg) and α-lactalbumins (α-La) [7], accounting as approximately 80% of total WP. In addition, WPs are included to serum albumin, immunoglobulins, glyco-macropeptide, lactoferrin, and various enzymes [16].

Within the spectrum of CN-derived peptides, casein phosphopeptides (CPPs) have attracted significant attention [3,18-21]. CPPs are bioactive peptides generated either *in vitro* or *in vivo* through enzymatic hydrolysis of whole CN or specific fractions from CN [16]. These peptides bind to dual-valency ions, including iron, owing to the presence of a polar acidic sequence composed of three phosphoseryl groups followed by two glutamic acid residues [5,18]. These residues serve as binding sites for minerals such as calcium, iron, and zinc, which introduce CPPs as booster of iron bioavailability in the fortified foods [4,13,20].

WPs, major by-product of cheese making, are cost-effective for industrial uses. These proteins serve as rich source of bioactive peptides with health-promoting characteristics. Furthermore, they possess noteworthy nutritional value due to their essential and branched-chain amino acids. Other than protein component, WPs are widely used as emulsifier in food products. Whey protein concentrates (WPC) and whey protein isolates (WPI) are industrially produced by membrane separation, that concentrates the protein while removes lactose and minerals [2,4,15]. WP-derived peptides particularly those with low molecular

weight have a great affinity to iron [14]. Unintended reaction of iron with other food components in the environment is reduced following its binding to WPs [12].

Both WP- and CN-derived peptides offer promising routes for improvement of iron bioavailability. These peptides form stable complex with iron, prevent its precipitation, and enhance its solubility, that is important in the various pH conditions of the digestive tract [4,18,22].

Figure 1 illustrates the steps in production of peptides and hydrolysates from whey proteins and casein, and their basic iron complexes. Each step is done under specific temperature, time, and pH, by using specialized equipment [2,7,8,13,14].

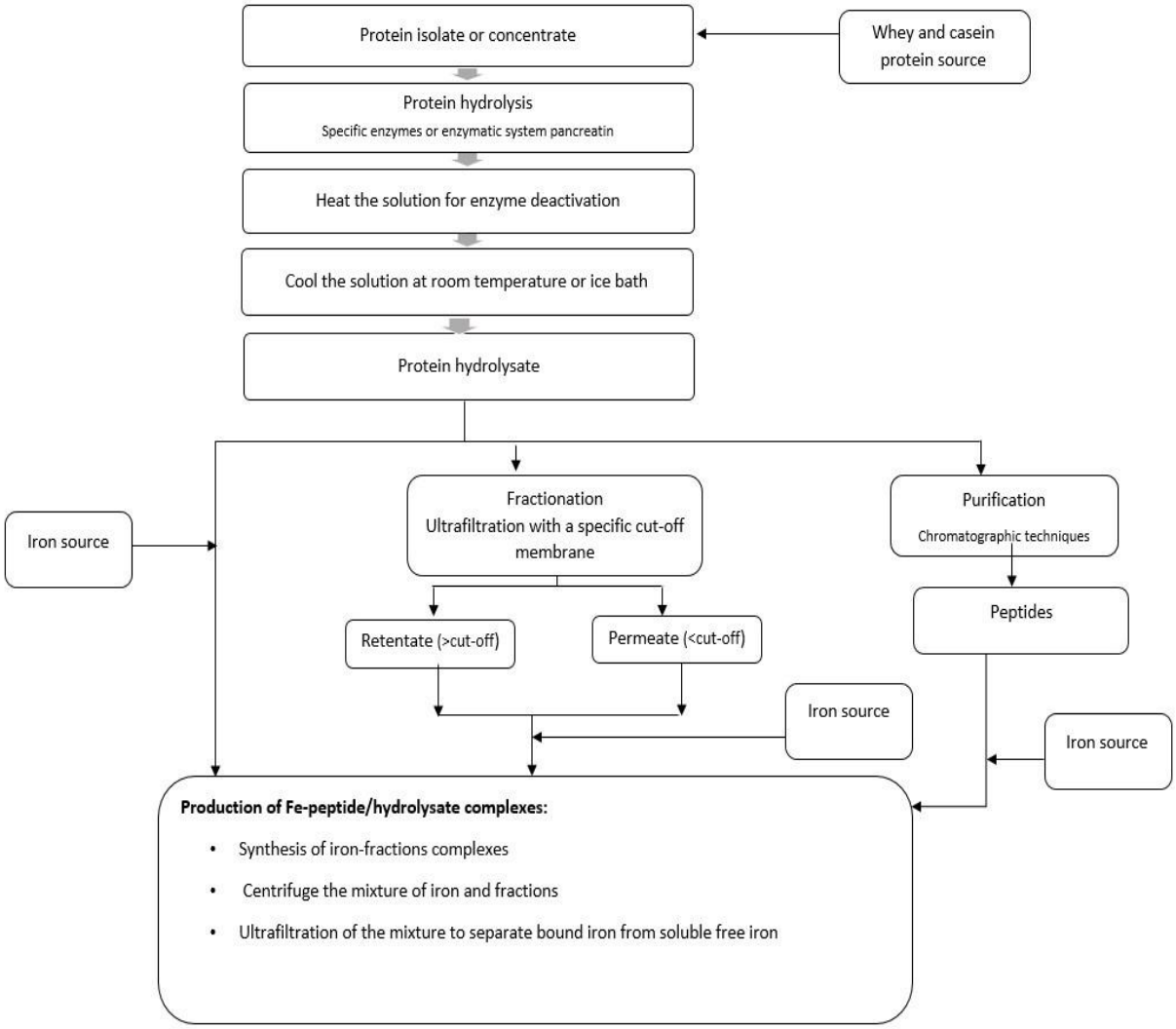

Figure 1- Fabrication of iron complex with peptides/hydrolysates derived from casein and whey protein

3. Complexation of iron with peptides/ hydrolysates

Binding of peptides to iron is conducted by interaction between an electron-donating group on the ligand surface (in this case, peptides and hydrolysates) and an electron receptor (iron). The peptides or hydrolysates may possess one or more accessible sites, ensuring that iron atom forms covalent bond, contributing to the stability of a physiologically enduring structure. Consequently,

reactivity of the iron within the complex with other components in the environment is reduced [13,14]. Recent studies have extensively investigated the positioning of binding sites on iron-chelating peptides [1,8,14,23,24]. The findings showed diverse binding sites for minerals in the peptides. These include terminal $–NH_2$ and –COOH groups, reactive side chains of some amino acids like aspartic acid and glutamic acid, and nitrogen atom of the peptide linkage [2]. According to Wang et al. [7], the amino acids containing free carboxyl groups complex with iron by carboxylate bonding. Furthermore, peptides and proteins can bind iron by peptide bond. Wu et al. [1] reported that primary Fe-binding site for whey protein, yak casein, and β-lactoglobulin were carboxylic groups (from histidine and lysine amino acids), both carboxylic groups (from glutamic acid and aspartic acid) and amide groups, and the nitrogen atom in amido bonds, respectively. Inclusion of carboxylic groups particularly in glutamic acid and aspartic acid in iron complexation was further reported by Caetano-Silva et al. In this regard, proline and glycine also tend to iron owing to their carboxyl group [13]. In other study, it was indicated that aside from the carboxyl groups serving as a primary binding site for iron, the ε-amino nitrogen of lysine, the guanidine nitrogen of arginine, and the imidazole nitrogen of histidine contribute to iron-peptide binding [8]. Moreover, it was shown that the peptides containing specific amino acids with size equal and below 10 kDa derived from neutralized-treated WPC may have promising potential for iron absorption in Caco-2 cell line. In this respect, aspartic acid, serine, glutamic acid, glycine, cysteine, histidine, and proline are reported [2,25].

4. Conditions affecting the iron-binding capacity

Development of iron complexes with milk-derived peptides and hydrolysates involves intricate interactions influenced by various conditions. These conditions play a pivotal role in effectiveness of the process, and also iron bioavailability. Understanding these deterministic factors is crucial for optimizing the formation of iron complexes. Furthermore, comprehending iron-binding capacity offers valuable insights for predicting its effectiveness in safeguarding iron from inhibitors and minimizing its precipitation in the intestinal tract [3,7]. When iron binds to the peptides, it forms stable and soluble complex in the harsh acidic environment of the stomach and also alkaline condition of the intestine [18,26-27]. However, there are conflicting studies that have not clearly demonstrated these effects [28-30]. It should be noted that an increased binding of iron to the bioactive peptides does not necessarily correlate with higher bioavailability [19]. Typically, there is an optimum level of binding that leads to maximum bioavailability [1,26-27]. The optimum level specific to each peptide should be determined in the laboratory. Table 1 shows the ligands, the peptide-iron ratios, and other conditions involved in synthesis of peptide-iron complexes, as well as effects of the complexes on bioavailability of iron. Further details about the internal and the external factors are presented as follows.

4.1. Peptide structure

Structure of the peptides and the hydrolysates derived from CN and WP in term of amino acid composition, their sequence, three-dimensional structure, steric hindrance, charge distribution, and secondary structure significantly influences their capacity in iron binding.

Peptides with specific amino acid residues such as histidine, cysteine, tyrosine, and aspartic acid form strong iron complex due to their coordination capabilities [27]. Sulfur-containing amino acids like cysteine and methionine can interact with iron, and affect its solubility and absorption [31]. Cysteine, in particular, has been shown to form complex with iron, making it more soluble and possibly more bioavailable for absorption in the intestine [32].

Table 1- General conditions of iron complexation

Ligand	Iron precursor	Peptide: iron ratio	General steps in complexation	Main conditions of complexation	Separation steps in peptide-iron complexation	Ref.
Whey protein concentrate by ultrafiltration (cut-off 30 kDa)	$FeSO_4$	40:0.5 40:1, 40:1.5 40:2	Previous hydrolysis with alcalase and complexation	Mixing the ligand and $FeSO_4$ (pH 3.0, 5.0, and 7.0, 40 °C, 2 and 4 h)	Ultrafiltering the complex➔ Centrifugation (1300 ×g, 20 °C) ➔ Collecting the retentate and permeate ➔Freeze-drying the complex	[2]
Whey protein concentrate hydrolysate obtained by ultrafiltration (cut-off 10kDa)	$FeCl_3$	-	*In vitro*: complexation *in vivo*: complexation	Mixing the ligands and 1 M ferric chloride	-	[4]
CPPs	$FeSO_4$	Not specified	Previous hydrolysis with *in vitro* digestion (gastric and intestinal) + complexation	Not specified	Centrifugation (3500 ×g, 1 h, 4 °C) ➔ Supernatant was used in the Caco-2 cell ferritin assays	[3]
α-lactalbumin (α-LAH) and β-lactoglobulin (β-LGH) hydrolysate obtained by ultrafiltration (cut-off 10 kDa)	$FeCl_3$	40:1	Previous with alcalase and complexation	Mixing the ligands and ferric chloride (pH 7.0, 25 °C, 3 min)	Centrifugation (3000 ×g, 20 min, 25 °C) ➔Collecting the complexes in the supernatant	[7]
Whey protein isolate, and whey protein isolate hydrolysate and its fractions obtained by ultrafiltration (cut-off 5 kDa)	$FeCl_2$	40:1 (w/w)	Previous hydrolysis with alcalase, pancreatin, and flavourzyme separately+ complexation	0.1% w/v Fe and 4% w/v ligand (pH 7.0, 25 ±2 °C, 1 h) under stirring	Centrifugation (5000 ×g, 20 min, 25 °C) ➔ Freeze-drying of supernatant	[8]
Whey protein isolate, and whey protein isolate hydrolysate and its fractions obtained by ultrafiltration (cut-off 5 kDa)	$FeCl_2$ or $FeSO_4$	40:1 (w/w) or 5:1 (w/w)	Previous hydrolysis with pancreatin, and complexation	0.1% w/v Fe and 4% w/v or 0.5% w/v ligand (pH 7.0, 25 ± 2 °C, 1 h) under stirring	Centrifugation (5000 ×g, 20 min) ➔ Supernatant freeze-drying ➔ storage at -18 °C	[14]
Whey protein isolate, and whey protein isolate hydrolysate and its fractions obtained by ultrafiltration/ diafiltration (cut-off	$FeCl_2$ or $FeSO_4$	40:1 (w/w)	Not mentioned	0.1% w/v Fe and 4% w/v ligand (pH 7.0; 25 ± 2 °C, 1 h) under stirring	Centrifugation (5000 ×g, 20 min) ➔ Passed through a common filter paper➔ Freeze-drying the supernatant ➔ analysis	[23]

5kDa)						
Succinylated sodium caseinate	$FeSO_4$	Not specified	Complexation+ ultrafiltering (10 kDa) the complexes; Use of UF membrane processing system for large-scale production of the complexes	Adding Fe to the protein solution (pH 6.6, 20 °C, 2 h) under constant stirring (magnetic stirrer)	Centrifugation (12000 ×g, 30 min, 20 °C) ➔ Passed supernatant through a special filter paper ➔ Ultrafiltering (10 kDa) the filtered supernatants ➔ Freeze-drying the supernatants➔ Analysis	[51]
Whey protein concentrate	$FeSO_4$	Not specified	Not mentioned	3.0 mmol/L iron and WP solution with 0.01 g protein/mL (pH 6.6, 20 °C, 2 h) under stirring	Centrifugation (12000 ×g, 30 min) ➔ Supernatant decanting and concentrating (4-fold) using UF membrane ➔ Lyophilizing the concentrate ➔ Storage in airtight container	[52]
CPPs	$FeSO_4$	Not mentioned	Fruit beverages with $FeSO_4$ (with or without Zn) subjected to SGID+ skimmed milk was subjected to SGID yielding CPPs+ adding the CPPs to fruit beverage fractions ➔Complexation	Adding the CPPs to the fruit beverage fractions	-	[17]
Casein	$FeCl_3$ (Fe^{57} isotope)	Not mentioned	Not mentioned	Not specified	Not specified	[53]
Succinylated sodium caseinate	$FeSO_4$	-	Not mentioned	7.4 mmol/L Fe and 1% g/mL protein solution (pH 6.6, 20 °C, 2 h) under constant stirring (magnetic stirrer)	Centrifugation (12000 ×g, 30 min, 20 °C) ➔ Separation of the supernatant ➔ Putting the supernatant under ultrafiltration ➔ Lyophilizing and powdering the concentrated retentate ➔ Storage in airtight container	[54]
Whey protein concentrate	$FeSO_4$	Not mentioned	Not mentioned	Not specified	Not specified	[55]

Amino acid composition of peptides/hydrolysates varies depending on the hydrolysis process and the enzymes used [33]. However, they generally retain a substantial amount of the original amino acids. The peptides derived from CN and WP may have various structures including helical, beta-sheet, and random coil conformations [34-35]. These structures expose different functional groups such as amine and carboxyl groups, which are capable of forming coordination bonds with metal ions like iron [36].

CN is relatively rich in proline, glutamine, and leucine [37], but it has sulfur-containing amino acids like cysteine and methionine at lower concentration [38]. CN peptides/hydrolysates may still have low levels of sulfur-containing amino acids, which are important for improvement of iron absorption [39]. Nonetheless, the presence of CPPs in the structure of these peptides plays additional role in iron interaction and absorption [36]. CPPs contain phosphorylated amino acids such as serine, threonine, and tyrosine. They allow CPPs to strongly bind to minerals like iron [28]. CPPs enhance iron solubility, protect it from the inhibitors, and remain it stable during digestion [19,39].

WP is rich in branched-chain amino acids such as leucine, isoleucine, and valine. It also contains significant amounts of other essential amino acids including lysine, threonine, and methionine [31,37]. In addition, WP has relatively high concentration of cysteine compared to other protein sources [32]. As mentioned above, cysteine forms complex with iron, keeping it in a soluble and absorbable form [40]. Therefore, the high content of cysteine in WP and its derived peptides/ hydrolysates positively affects iron affinity, and leads to increased iron absorption [36].

Sequence of amino acids in a protein determines the overall conformation and spatial arrangement of the functional groups, which provides appropriate environment for metal-binding interactions [13,23]. Based on degree of hydrolysis, the peptides and the hydrolysates may lose their conformation and spatial structure or may retain it to some extent [3,41]. However, the primary structure (amino acid type and sequence) significantly determines the conditions for metal binding [36,42].

Arrangement of the amino acids in CPPs results in a net negative charge. It ensures the solubility at alkaline pH. This property enhances iron bioavailability, making CPPs valuable for use in iron supplementation and fortification [5,18,19,42]. Furthermore, folding and conformational flexibility of the peptides may impact on their iron-binding properties [34]. The flexible and the open structure of CN and its hydrolysates due to the presence of proline-rich regions and the phosphorylated amino acids allows them to accommodate metal ions in their binding sites. Iron, being a transition metal, can interact with these phosphorylated residues and form stable complexes [34,35,42-44].

4.2. Peptide molecular mass

Some studies have suggested the ultrafiltration as alternative to extract the peptides with low molecular mass (MM). It has been reported that MM affects iron-binding capacity of the peptides [13,14,23,45]. Miao et al. observed that among four CN-derived peptides with MM of 830.6120 Da,1012.5280 Da, 873.4440 Da, and 829.4570 Da, the peptide with the lowest MM (829.4570 Da) had the highest iron-chelating rate (59.76%) [21]. Similarly, O'Loughlin et al. showed that iron-binding capacity of WPI hydrolysate with 1 kDa MM was much higher than the retentate with >30 kDa MM [46]. In agreement, Liu et al. reported that more than 70% of iron ions bound to the CPPs with MM of 0.5-1.4 kDa, while only 30% of iron ions bound to the CPPs with MM of 1.4-4.5 kDa [47].

4.3. Peptide concentration

Concentration of the peptides and the hydrolysates in solution plays a critical role in determining the saturation point for iron binding. High concentration of the peptide may lead to oversaturation and formation of unstable complexes. Therefore, finding the appropriate concentration is important to ensure the formation of well-defined and stable iron complex [14,27,48]. To provide accurate definition of peptide

concentration, the term "peptide-iron ratio" in the reaction solution could be helpful.

Protein-iron ratio affects the iron-chelating ability [2,14,19,22]. Athira et al. showed a remarkable increase in the content of bound iron by changing the WP-iron mass ratio from 40:0.5 to 40:1. In their study, additional increase in iron content had no significant change in complexation [2]. It is assumed that ferrous ions occupied all the binding sites at ratio of 40:1, and additional mass ratio over the optimal level did not increase the iron-binding capacity. This is in line with the study of Zhou et al. who showed that the highest iron-binding capacity was achieved when the mass ratio of β-Lg hydrolysates to iron reached 40:1, and additional increase in mass ratio (45:1) did not improve the iron binding capacity because ferric ions entirely occupied the binding sites at hydrolysate-iron mass ratio of 40:1 [22]. In accordance, Caetano-Silva et al. observed the most iron solubility in whey peptide-iron complex at ratio of 40:1. Iron solubility is an indicator of proteins, hydrolysates, and peptides binding to iron, and is obtained by dividing the iron content in the supernatant by the initial iron [14]. Moreover, Wang et al. observed the highest binding capacity of CN hydrolysate-iron at mass ratio of 15:1 among the five ratios of 30:1, 15:1, 10:1, 1:1, and 1:5 [48].

4.4. pH

Iron-binding capacity of milk-derived peptides and hydrolysates is notably affected by pH of the solution. Different pH levels can influence the charge distribution of these peptides, affecting their ability to chelate iron ions [22,36]. Certain pH ranges promote strong interactions between the peptides and iron, leading to the formation of stable complexes. El-Sayed et al. showed that the binding ability of WP to iron decreased by pH reduction [15]. In their opinion, it was due to the competition between hydrogen atoms and metal ions in protein binding. At low pH, the amino groups of protein are protonated, by which their tendency to cations decreases. A similar result was observed in the study of Singh Banjare et al. that used different pH (3, 4, 5, 6, and 7) for complexation of spray-dried WPC-iron. In their study, amount of bound iron in the complex increased at higher pH [12]. In agreement, Athira et al. said that acidification reduces the ability of peptides to bind iron, and neutral pH facilitates the chelation process [2]. Delshadian et al. [19] showed that CCPs have low solubility at acidic pH, through which lower iron bind to CPPs at low pH. Likewise, iron-binding capacity of β-Lg hydrolysates diminished considerably when pH increased or decreased beyond the appropriate range in study of Zhou et al. Indeed, neutral or near neutral pH (6.5-7) could improve the process of chelation. They concluded that complexation is related to ability of the ligands to provide electrons. On the one hand, the functional groups (i.e., COOH, CONH, and NH_2) have negative charge as pH exceeds 7, that is appropriate to bind ferric ions. On the other hand, ferric solubility decreased at pH higher than 7 followed by binding capability reduction [22]. In summary, low pH reduces binding due to the competition between hydrogen and metal ion. In comparison, solubility of iron decreases at high pH. Therefore, neutral or near neutral pH would be appropriate for chelation.

4.5. Ionic strength

Ionic strength of solutions that is governed by concentration of salts can influence affinity of the peptides to iron [27]. High ionic strength may hinder or facilitate the binding process by altering the electrostatic interactions between the molecules [5,14]. Thus, optimizing the salts' concentration is crucial to achieve optimum iron complexation.

4.6. Temperature and time

Temperature and duration of complexation process affect the rate and the extent of iron binding to the peptides. Higher temperatures may accelerate the reaction rate [48-49], while longer incubation time allows more interactions between the peptides and iron [2]. Finding the appropriate point of time and temperature is essential to achieve optimum complexation.

4.7. Type of enzyme

Different enzymes can generate various peptides with different sequences, that may enhance or hinder iron binding [33]. Therefore, choose of appropriate enzyme is important to reach the best effectiveness in the process. Athira et al. reported that WP hydrolysates produced by alcalase had the most iron binding capacity than pancreatin, flavourzyme, esperase, neutrase, papain, pepsin, and trypsin [2]. In agreement, Zhou et al. found that the best iron binding capacity for β-Lg hydrolysates was achieved by alcalase rather than trypsin and neutrase [22]. To the contrary, Caetano-Silva et al. found that the fractions obtained by pancreatin from WP hydrolysate were superior to those obtained by alcalase and flavourzyme. They assumed that it might be due to endogenous enzymatic activity that released small amounts of large peptides and considerable amounts of di- and tripeptides, which are more absorbable than longer peptides [8]. Mia et al. reported that due to the considerable hydrolysis ability and similar situation in the organism, trypsin has strong ability to hydrolyze CN [21].

4.8. Interaction with other molecules

Presence of other molecules such as minerals and polyphenols can impact on iron complexation. Some molecules may compete with the peptides for iron binding [5,36,50]. Understanding these interactions is vital to assess the compatibility of various components and their impact on iron bioavailability.

5. Conclusion

This article addressed the global problem of iron deficiency, that affects about 30% of the population. We reviewed the factors effective in iron absorption and highlighted the milk-derived peptides and hydrolysates to enhance iron uptake. Iron, essential element for health, has poor bioavailability, particularly in plant-based foods due to its low solubility and presence of the inhibitors. Fortification of foods by bioavailable iron is a challenge. The scientists explored some techniques like encapsulation and chelation to enhance iron bioavailability and mitigate the associated problems. Peptides are promising candidate to improve iron absorption. Iron-binding peptides form stable complexes, prevent its precipitation, and enhance iron solubility. Thus, they are a potential route to solve the iron deficiency. The bioactive peptides derived from milk proteins such as CN and WP show various health-promoting activities like antihypertensive, antimicrobial, and immunostimulant effects. These peptides specially CPPs have strong iron-binding capability. Interaction between iron and the peptides occurs at different binding sites including terminal groups, side chains of amino acids (e.g., histidine, cysteine, and aspartic acid), and peptide bonds. Various conditions influence the iron-binding capacity of the milk-derived peptides and hydro-lysates. They include peptide structure, molecular mass, concentration, pH, ionic strength, temperature, time, enzyme type, and interaction with other molecules. Understanding these intricacies offers valuable insight into development of effective strategies to enhance iron bioavailability, and mitigate iron deficiency-related concerns. Further research about exploration of other factors will undoubtedly contribute to better improve the effectiveness of iron fortification efforts.

6. Conflict of interest

The authors declare that they have no conflict of interest.

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