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Structural properties of food proteins underlying stability or susceptibility to human gastrointestinal digestion

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Is any specific structural trait that makes food proteins resistant or sensitive to proteolytic degradation in the gastrointestinal tract? In principle, elements at various hierarchical levels of protein structure may confer stability or susceptibility to hydrolysis. However, based on a critical revision of the recent literature, we emphasize the impossibility of providing absolute answers because protein digestion is affected by a complex series of factors ascribable to the food micro-/macrostructure and the intricacy of human digestion. Taking into consideration the uncertainty associated with the current *in vitro* models of food digestion/absorption and the capability offered by recent analytical advancements, we emphasize the need for suitable *in vivo* readouts for improving the comprehension of the fate of dietary proteins in our body and their effects on human health.

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Introduction

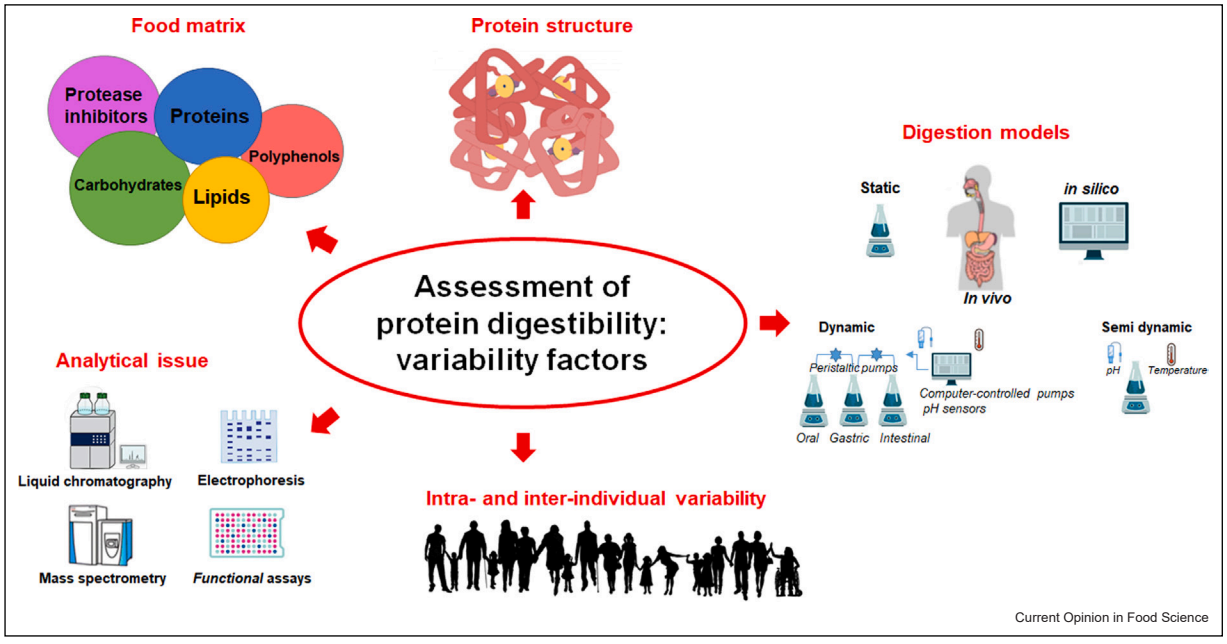
Food proteins experience 'harsh' conditions throughout the human gastrointestinal (GI) tract. Generally, dietary proteins are split into large- and medium-sized polypeptides by pepsin in acidic gastric conditions. Downstream, in the duodenum, a set of pancreatic proteases and peptidases with varying cleavage specificity cooperate to degrade the products of peptic digestion, which are further processed by a complex arsenal of intestinal brush border membrane exopeptidases before absorption [1]. Peptides that eventually cross the intestinal barrier and reach the bloodstream may undergo hydrolytic attack by a series of plasma peptidases [2].

Such a coordinated and finely tuned process of protein degradation has evolved to pursue a dual physiological purpose: 1) breakdown of proteins into simple derivatives that can be absorbed and used by the body; 2) reduction of the risk of abnormal reactions toward *non-self* potentially harmful sequences. Nevertheless, some food proteins or their large proteolytic products may relevantly survive GI degradation. The possible existence of a direct link between protein digestion stability and the capability of inducing immunoglobulin E (IgE)-mediated adverse reactions has been questioned and practically refused [3,4]. However, the way and the extent to which food proteins are degraded during digestion have several implications on human health regarding the absorption and bioavailability of the resulting amino acids, and the survival of possible bioactive or immunogenic polypeptides.

Is any specific structural trait that makes food proteins stable or sensitive to proteolytic degradation in the GI tract? Despite the extensive research on the topic, this question remains substantially unanswered due to uncertainty associated with several orders of variability factors (Figure 1): i) effects of the food matrix on protein structure and aggregation; ii) variety of *in vitro* and *in vivo* digestion models and variability of conditions for any given model; iii) intra- and interindividual variability in the case of *in vivo* digestion; iv) analytical issues related to the characterization of the 'digestomes' [5].

Based on selected recent investigations, we survey the structural features affecting the 'digestibility' of food proteins and the possible implications for human health. Furthermore, we emphasize those still unresolved aspects that are driving the upcoming research trends in this field. In nutrition, protein digestibility is usually a measure of the degree of net absorption of nutrients in the digestive tract. Herein, digestibility is intended in a

Figure 1



Variability factors challenge the assessment of food-protein digestibility. The combination of the multiple variability factors leads to uncertainness in determining the kinetics of food-protein degradation *in vivo*.

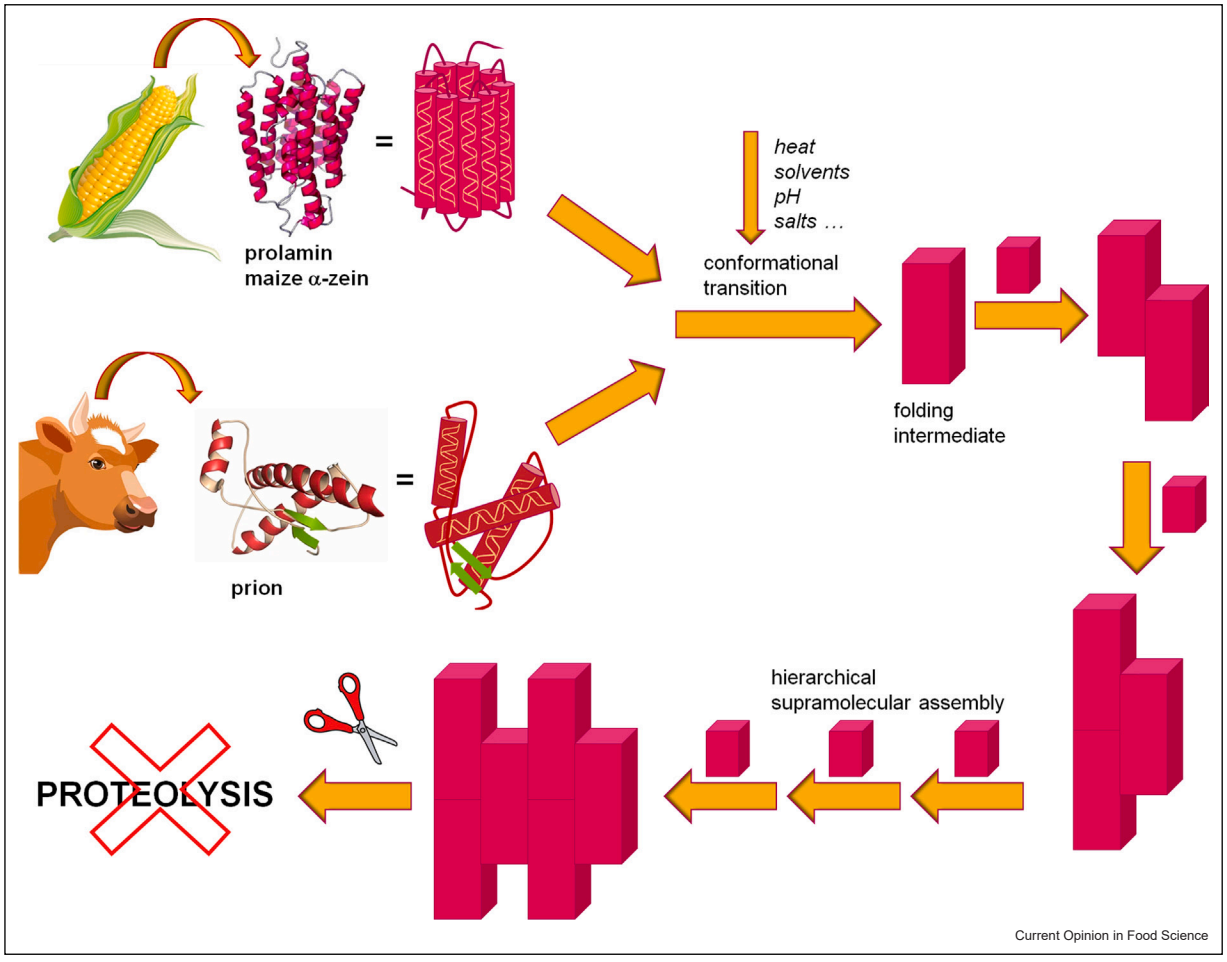
broader meaning, indicating the degree of hydrolysis that food proteins undergo during digestion, which measures the overall number of peptide bonds cleaved at any stage of the GI degradation process.

Structural traits affecting digestion stability of food proteins

Protein stability depends on all the levels of hierarchical organization, that is, primary, secondary, ternary, quaternary structures, and supramolecular assembly. The structural levels of a protein are interrelated, as the secondary structure depends on the primary one and the resulting three-dimensional (3D) organization is, in turn, affected by intrinsic factors, such as intrachain disulfide bonds, hydrophobicity, hydrogen bonding, post-translational modifications, as well as by the interaction with the environment (e.g. solvent, ions, chaotropic agents, pH, and temperature). By determining protein folding and accessibility of proteases to cleavage sites, these factors affect the inherent resistance of food proteins against process-induced denaturation and GI hydrolysis and, therefore, food-protein digestibility.

Maize (*Zea mays L.*) storage proteins, that is, zein, are generally considered resistant to GI digestion. Zeins have an elongated axial ratio, with a 3D organization different from compact globular proteins. In hydroalcoholic solution α-zeins, which are the most abundant and hydrophobic ones of the family, have coiled-coil tendencies that result in complex aggregates with a central section of nine helical segments constituted by nonpolar residue side chains connected by glutamine-rich loops or turns, forming a hardly accessible hydrophobic surface inside a triple superhelix [6]. Secondary structure of α-zeins is solvent-dependent and their amphiphilic character is the main driving force for their self-assembly properties that ultimately produce protein structures functionally analogous to a block copolymer. In the GI tract's water milieu, the overall organization of zein might be even more compact. Thus, zeins represent a paradigmatic example of food proteins with characteristic primary structure, determining the formation of secondary and tertiary structural elements with a subsequent supramolecular assembly that hinders digestibility (Figure 2). In this context, specific cysteine residues are critical for contributing to the formation of large protein aggregates [6]. The factors that control the supramolecular assembly of food proteins are still underexplored, even though they influence the sensory and nutritional quality of foods and could be modulated through processing [7]. Some seed storage proteins, such as the allergenic soybean 7S β-conglycinin subunits, natively assemble into amyloid aggregates that exhibit resistance to digestion [8]. However, even small conformational changes can drastically modify protein digestibility. For example, the so-called S-ovalbumin (S-Ova), a conformer thermodynamically more stable than native ovalbumin (Ova) generated by slight spontaneous conformational transitions, denatures at higher temperatures and is more proteolytically resistant than Ova,

Figure 2



Native food proteins can be partially converted into misfolded protein intermediates and aggregate into β-sheet-rich oligomers. These oligomers can stick together into protofibrils and then self-assemble into compact macroaggregates, with limited or no access by digestive proteases. Zein and prion are exemplary cases of protein supramolecular hierarchical self-assembly leading to hardly digestible or indigestible aggregates.

especially toward pepsin. Upon denaturation, Ova and S-Ova share comparably increased *in vitro* digestibility and similar immunological properties [9].

Environmental parameters, such as solution pH, are primary thermodynamic variables determining the structure, function, and dynamics of proteins by settling the charge of the polypeptides. Native β-lactoglobulin (β-Lg) is resistant to pepsin because of exceptional structural stability at gastric pH. Many potential cleavage sites for pepsin are buried inside the hydrophobic core of the native protein and are not readily accessible. Unlike β-Lg, α-lactalbumin (α-La) is pepsin-sensitive [10], despite a more compact 3D structure consisting of 123 amino acid residues stabilized by four disulfide bridges. In the gastric environment, also due to the loss of affinity for a Ca^{2+} ion at acidic pH, α-La undergoes conformational transitions populating the so-called 'molten globule state', which are relatively stable folding conformations, intermediate between native or denatured states. In these conformations, proteins maintain several native-like secondary structure elements but have a loose tertiary structure that involves the exposure of the hydrophobic core to the solvent and proteases. The substantial insensitivity of native α-La (holo form) to trypsin at nearly neutral pH [11] confirms the pH dependence of protein conformations and related digestibility. The pH-dependent structure of food allergens directly influences the kinetics of the proteolytic digestion after endosomal uptake by antigen-presenting cells. The subsequent exposure of the major histocompatibility complex (MHC) class-II molecules, which can vary as a function of pH, determines the T-cell polarization and the immune response [12].

Nonprotein food compounds affect GI protein degradation. Starch and fibers synergistically reduce protein digestibility by hindering the accessibility to

proteases and reducing hydration [13]. Food lipids alter protein structure and digestibility as well. β-Lg belongs to the lipocalin family, characterized by a hydrophobic β-barrel with a deep calyx that can allocate lipid molecules nonspecifically. The protonation of Glu_{89} at pH < 6.0 makes the calyx inaccessible [14] so that lipids do not directly influence the gastric digestion of β-Lg, while endogenous bile acids enhance the hydrolysis of β-Lg by pancreatic proteases [15]. Nonspecific lipid transfer proteins (nsLTPs), which also possess a lipid-binding pocket, are highly stable against thermal denaturation and GI digestion [16,17]. Like β-Lg, the resistance of nsLTPs to gastric proteolysis is a consequence of its compact globular structure at low pH [18]. A conserved tightly coiled core of eight cysteines featuring Cys–Cys and Cys–X–Cys motifs (where X represents any other residue) engaged in four intrachain disulfide bonds limits the mobility of the polypeptide backbone of nsLTPs. Other members of the prolamin superfamily share this signature motif (e.g. 2S albumins, cereal alpha-amylase/trypsin inhibitors), which also resembles that of cysteine-rich pathogenesis-related proteins (e.g. class-I chitinases, thaumatin-like proteins), described as food allergens as well [18]. Lipid binding to nsLTPs provokes the displacement of a flexible domain containing a pepsin-sensitive Tyr, thereby enhancing the overall susceptibility of nsLTPs to gastric hydrolysis [19]. The role of lipid ligands and related binding affinity is complex, given that, as for other lipid-binding proteins, the complexation could induce structural compaction or sterically hinder access to proteases, also increasing the sensitizing and eliciting food allergenic potential [20].

The impact of post-translational modifications, such as phosphorylation and glycosylation, on the stability of food proteins is still controversial and could vary for specific proteins [21].

A prosthetic atom or group can modify protein conformation and digestibility. Parvalbumins are small (9–11 kDa) calcium-binding proteins found in fast-contracting muscles and are major fish allergens exhibiting high resistance against GI digestion. A recombinant carp parvalbumin mutant lacking the calcium-binding aspartic acid residues was hydrolyzed within 10 min by pancreatic proteases. In contrast, the native counterpart with similar secondary structure elements was stable for over 45 min [22]. Like for α-La, the binding with Ca^{2+} ions stabilizes the holoprotein form compared with the calcium-free apo polypeptide, determining a more compact folding. A modified digestion sensitivity alters the proteolytic peptide patterns, immunological properties, and capability of parvalbumins to induce oral tolerance [22].

Thermal processing has complex effects on protein digestibility. Heating causes conformational transitions with progressive loss of secondary and tertiary structure elements. As a rule of thumb, mild heating improves while severe thermal treatments reduce digestibility [23]. β-Lg undergoes an abrupt conformational shift and adopts a molten globule-like metastable monomeric state at approximately 65°C [24]. Partially unfolded β-Lg is more prone to pepsin hydrolysis, while interchain cross-linking triggered by heat-induced thiol/disulfide exchange reactions combined with the irreversible intermolecular aggregation *via* hydrophobic alignment of β-sheets can provoke the formation of gels and amyloid-like fibrils with limited sensitivity to proteases [25].

Prion-related diseases or transmissible spongiform encephalopathies represent the extreme cases of formation of highly heat- and hydrolysis-stable (food) protein macroaggregates. Amyloid fibril growth starts with the misfolding of partially structured folding intermediates of PrP proteins into β-sheet-rich oligomers, which stick together into protofibrils, and progresses through the supramolecular hierarchical autocatalytic assembly of amyloid fibrils (Figure 2) [26]. *In vitro*, the misfolding is activated by either heat or pH-/ionic strength, while *in vivo* the trigger remains to be ascertained, confirming the necessity to study the dynamics of protein supramolecular assembly. The ability of prion aggregates to endure exposure to digestive proteases and to cross the intestinal epithelial barrier underlies the oral transmission mechanisms of prion diseases [27].

Digestion stability of food peptides

Dietary bioactive peptides often are very short sequences (< 10 residues or shorter), and the determinants of food allergies are, in general, proteolytic products of food allergens, that is, peptides, and not the proteins themselves. Nevertheless, small-/medium-sized peptides resulting from protein hydrolysis escape the detection by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which is commonly used to monitor protein degradation. For this reason, we believe that an informative characterization of the food-protein 'digestomes' requires necessarily a combination of mass spectrometry-based peptidomics and proteomics [5].

The peptide patterns obtained from the digestion of a food product depend on the process technology. For example, digestion kinetics and peptide patterns resulting from *in vitro* or *in vivo* digestion differ among differently manufactured cheese types and from those of yogurt and raw milk [28,29]. Similarly, isolated gluten protein fractions and whole-wheat flour produce different peptides, regardless of the wheat genotype [30]. On the other hand, some specific peptide 'core' sequences appear intrinsically stable toward digestive degradation [31]. A recent meta-analysis has individuated some traits of the primary structure common to many

peptides that have been described as resistant to GI digestion [32]. These traits include lower hydrophobicity, higher positive net charge at intestinal pH, branched-chain aliphatic N-terminal residues, absence of C-terminal leukine, and higher histidine and proline content, especially at the C-terminal, compared with susceptible peptides. However, there is no straightforward correlation between physiochemical properties and peptide stability; thus, the current *in silico* approaches are inadequate to predict the behavior of peptides exposed to hydrolytic enzymes. For example, low-sized peptides generally may show better structural stability than longer ones. Nevertheless,low-molecular-weight angiotensin converting enzyme (ACE)-inhibitor fish-derived peptides were more promptly hydrolyzed by the pepsin–pancreatin system under simulated conditions of GI digestion than their high-molecular-weight precursors [33]. Similarly, several casein-derived small molecular weight fractions appear less stable than longer casein-derived peptides during simulated GI digestion [32]. The supposed antihypertensive Val–Pro–Pro and Ile–Pro–Pro lactotripeptides are among the most studied bioactive peptides with well-assessed digestion stability [34]. Anyway, size does not appear to be a determining factor *per se*, since the pea-derived ACE-inhibitor tripeptide Leu–Arg–Trp fails to lower blood pressure in spontaneously hypertensive rats due to chymotrypsin-mediated hydrolysis, unlike its egg-derived isomer Ile–Arg–Trp, which exhibits antihypertensive and multifunctional activities *in vivo* [35].

Several *in silico* attempts have been made to predict the potential blood stability of food-bioactive peptides [36]. A high frequency of negatively charged (Glu) and small-sized residues (Ala, Glu, Ile, and Leu) correlates with long half-life in mammalian blood. In contrast, aromatic (Tyr and Phe) and other near-neutral amino acids (Gly, His, and Ser) predict a short peptide half-life. A Trp-to-Val substitution drastically reduces the peptide stability to blood peptidases [36]. However, the predictive power of these approaches based on the analysis of primary structure alone is limited and experimental validation is required.

Gluten peptides are exceptionally resistant to processing by GI digestive enzymes because of the high content of proline and glutamine residues [37]. However, Pro (formally an imino acid and not an amino acid) increases disorder and flexibility in Pro-rich peptides through *cis*-/*trans*-isomerization [38], thus potentially reducing peptide stability. Pro-rich domains are not intrinsically resistant to enzymatic hydrolysis since many microbial or mold-derived endoproteases are able to cleave gluten peptides in pseudogastric conditions [37]. Intestinal mucosa does include prolyl- or dipeptidyl-peptidases, which can cleave Pro-containing peptides [32]. Therefore, peculiar conformational arrangements of proline-rich peptides and their relatively low water solubility might contribute to explaining why these enzymes are the rate-limiting ones in the digestive breakdown [39,40]. The macropeptides of gliadins and low-molecular-weight glutenin subunits promptly released by pepsin contain hepta- and dodecapeptide repeat proline-/glutamine-rich motifs. Circular dichroism and nuclear magnetic resonance (NMR) determinations have confirmed that gliadin macropeptides, including the α2-gliadin-derived 33-mer immunodominant peptide in celiac disease, adopt a polyproline-2 (PP2) conformation both in water and in hydroalcoholic solvents [38]. At high concentrations and depending on the temperature, the PP2 conformation can convert toward a β-parallel structure, inducing a self-assembly process into macro-aggregates that proteases could hardly access.

Heating or other food processing can modify several amino acids or induce the formation of unnatural compounds. Nonenzymatic glycation decreases protein digestibility, masking some amino acids, especially lysine, to protease recognition. Other chemical modifications may influence protein digestion, including oxidation, racemization, dephosphorylation, and covalent cross-linking. The overall impact of these modifications on protein and peptide bioaccessibility/bioavailability and their relationship with human health require urgent assessment [41,42], especially for formula-fed infants in early life, when severely processed milk or milk substitutes are the sole or prevalent source of food proteins [23,41].

Role of proteases and *in vivo* bioavailability

In addition to the intrinsic stability, the digestibility of food proteins depends on the efficiency of GI proteases/peptidases [43]. Under physiological conditions, GI proteases/peptidases cover broad ranges of enzymatic activities and cleavage specificities [32].

Human digestion efficiency and absorption can vary according to numerous factors, such as genetics, age, sex, assumption of drugs, dietary pattern, oral chewing, and physical and psychological status. As for the protein substrates, the chemical environment affects the activity of digestive enzymes. Furthermore, GI proteolytic action is modulated by endogenous protease inhibitors and exogenous food components (e.g. plant protease inhibitors and polyphenols) [44]. The complexity of food peptide patterns found *in vivo*, even evidencing unexpected cleavage specificities [45], suggests the possible contribution of additional factors to digestion, such as GI microorganisms. The *in vitro* models of food-protein digestion cannot account for the intra- and interindividual variability of digestion and absorption capacity, which should be investigated as key 'primary endpoints' in nutritional research [46].

For this reason, *in vivo* bioavailability of food-protein components should be considered the main goal for establishing the relationship between dietary proteins and human health. Few investigations have addressed the bioavailability of food-derived peptides due to many technical challenges [5,47]. However, some recent studies have demonstrated that dietary peptides are stable enough to be monitored in biological fluids. Peptides from peanut allergens have been detected in both human plasma and breast milk by immunochemical techniques [48]. More reliably, circulating milk-derived peptides have been characterized by high-resolution mass spectrometry [49]. It has been demonstrated that food-derived peptides can survive digestion and are then excreted into breast milk [50–52]. Immunological active gluten peptides have been identified in the urine of children who had eaten cereals [53]. Milk peptides have been identified in the stool of breastfed infants, demonstrating that some sequences can survive the entire process of GI degradation, including the proteolysis by colon microorganisms, and may even influence infant gut development [54]. These results have been obtained with small cohorts of individuals and need to be supported by studies on extended populations of healthy and diseased subjects. Under physiological conditions, dietary peptides occur in human biological fluids at very low abundance. The hypothesis that peptide concentration might be underestimated because part of them is converted into bioactive nonpeptide metabolites deserves further exploration [55].

Conclusions

The bioavailability and excretion peptide patterns emerging from the preliminary studies in *humans* reflect the extremely high variability and complexity of protein digestion. Many knowledge gaps still prevent us from offering a concrete perspective of preparing food products that meet specific nutritional and functional requirements [56]. Most of the current studies carried out with *in vitro* digestion models are focused on the detailed kinetics of food-protein degradations rather than on the fate of food-protein-derived products in our body. The structural and mechanistic factors that rule the organization of nutrients within raw and processed food matrices, their digestion, absorption, distribution, effects on target organs, and ultimate impact on human health should be addressed with opportunely designed investigations, which combine molecular assessments with *in vivo* clinical interventional trials and observational studies.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Picariello G, Ferranti P, Addeo F: **Use of brush border membrane vesicles to simulate the human intestinal digestion**. *Food Res Int* 2016, **7**:3402.
2. Cavaco M, Andreu D, Castanho MARB: **The challenge of peptide proteolytic stability studies: scarce data, difficult readability, and the need for harmonization**. *Angew Chem Int Ed Engl* 2021, **60**:1686.
3. Akkerdaas JH, Cianferoni A, Islamovic E, Kough J, Ladics GS, McClain S, Poulsen LK, Silvanovich A, Pereira Mouriès L, van Ree R: **Impact of food matrices on digestibility of allergens and poorly allergenic homologs**. *Front Allergy* 2022, **3**:909410.
4. Verhoeckx K, Bøgh KL, Dupont D, Egger L, Gadermaier G, Larré C, Mackie A, Menard O, Adel-Patient K, Picariello G, Portmann R, Smit J, Turner P, Untersmayr E, Epstein MM: **The relevance of a digestibility evaluation in the allergenicity risk assessment of novel proteins. Opinion of a joint initiative of COST action ImpARAS and COST action INFOGEST**. *Food Chem Toxicol* 2019, **129**:405-423.
5. Picariello G, Di, Stasio L, Nitride C, Mamone G, Ferranti P: **Food protein digestomics**. In *Comprehensive Foodomics*. Edited by Cifuentes A. Elsevier; 2020:748-761.
6. Zhang X, Dong C, Hu Y, Gao M, Luan G: **Zein as a structural protein in gluten-free systems: an overview**. *Food Sci Hum Wellness* 2021, **10**:270-277.
7. Boire A, Renard D, Bouchoux A, Pezennec S, Croguennec T, Lechevalier V, Le Floch-Fouéré C, Bouhallab S, Menut P: **Soft-matter approaches for controlling food protein interactions and assembly**. *Annu Rev Food Sci Technol* 2019, **10**:521.
8. Han K, Feng G, Li T, Deng Z, Zhang Z, Wang J, Yang X: **Digestion resistance of soybean 7S protein and its implications for reinforcing the gastric mucus barrier**. *J Agric Food Chem* 2022, **70**:8776.
9. Röder M, Wiacek C, Lankamp F, Kreyer J, Weber W, Ueberham E: **Improved sensitivity of allergen detection by immunoaffinity LC-MS/MS using ovalbumin as a case study**. *Foods* 2021, **10**:2932.
10. Dupont D, Tomé D: **Milk proteins: digestion and absorption in the gastrointestinal tract**. In *Milk Proteins*. Edited by Boland M, Singh H. Elsevier Inc; 2020:701-714.
11. Permyakov EA: **α-Lactalbumin, amazing calcium-binding protein**. *Biomolecules* 2020, **10**:1210.
12. • Hofer F, Fisher AL, Kamenik AS, Waibl F, Fernández-Quintero ML, Liedl KR: **pH-dependent structural diversity of profilin allergens determines thermal stability**. *Front Allergy* 2022, **3**:1007000.

Analyzing a class of structurally related allergens, this study emphasizes the need of considering the pH-dependent nature of the food protein stability and, in turn, of the proteolytic patterns, which affect the immunological properties.

13. Opazo-Navarrete M, Tagle Freire D, Boom RM, Janssen AEM: **The influence of starch and fibre on *in vitro* protein digestibility of dry fractionated quinoa seed (Riobamba variety)**. *Food Biophys* 2019, **14**:49-59.
14. Barbiroli A, Iametti S, Bonomi F: **Beta-lactoglobulin as a model food protein: how to promote, prevent, and exploit its unfolding processes**. *Molecules* 2022, **27**:1131.
15. Wang R, Wang Y, Edrington TC, Liu Z, Lee TC, Silvanovich A, Moon HS, Liu ZL, Li B: **Presence of small resistant peptides from new in vitro digestion assays detected by liquid chromatography tandem mass spectrometry: an implication of allergenicity prediction of novel proteins?** *PLoS One* 2020, **15**:e0233745.
16. Missaoui K, Gonzalez-Klein Z, Pazos-Castro D, Hernandez-Ramirez G, Garrido-Arandia M, Brini F, Diaz-Perales A, Tome-Amat

J: **Plant non-specific lipid transfer proteins: an overview**. *Plant Physiol Biochem* 2022, **171**:115.

17. Skypala IJ, Asero R, Barber D, Cecchi L, Diaz Perales A, Hoffmann-Sommergruber K, Pastorello EA, Swoboda I, Bartra J, Ebo DG, Faber MA, Fernández-Rivas M, Gomez F, Konstantinopoulos AP, Luengo O, van Ree R, Scala E, Till SJ: **Non-specific lipid-transfer proteins: allergen structure and function, cross-reactivity, sensitization, and epidemiology**. *Clin Transl Allergy* 2021, **11**:e12010.

18. Wangorsch A, Scheurer S, Blanca M, Blanca-Lopez N, Somoza ML, Martín-Pedraza L: **Allergenic properties and molecular characteristics of PR-1 proteins**. *Front Allergy* 2022, **3**:824717.

19. Abdullah SU, Alexeev Y, Johnson PE, Rigby NM, Mackie AR, Dhaliwal B, Mills E: **Ligand binding to an allergenic lipid transfer protein enhances conformational flexibility resulting in an increase in susceptibility to gastroduodenal proteolysis**. *Sci Rep* 2016, **6**:30279.

20. Chruszcz M, Chew FT, Hoffmann-Sommergruber K, Hurlburt BK, Mueller GA, Pomés A, Rouvinen J, Villalba M, Wöhrl BM, Breiteneder H: **Allergens and their associated small molecule ligands-their dual role in sensitization**. *Allergy* 2021, **76**:2367-2382.

21. Costa J, Villa C, Verhoeckx K, Cirkovic-Velickovic T, Schrama D, Roncada P, Rodrigues PM, Piras C, Martín-Pedraza L, Monaci L, Molina E, Mazzucchelli G, Mafra I, Lupi R, Lozano-Ojalvo D, Larré C, Klueber J, Gelencser E, Bueno-Diaz C, Diaz-Perales A, Benedé S, Bavaro SL, Kuehn A, Hoffmann-Sommergruber K, Holzhauser T: **Are physicochemical properties shaping the allergenic potency of animal allergens?** *Clin Rev Allergy Immunol* 2022, **62**:1-36.

22. Freidl R, Gstöttner A, Baranyi U, Swoboda I, Stolz F, Focke-Tejkl M, Wekerle T, van Ree R, Valenta R, Linhart B: **Resistance of parvalbumin to gastrointestinal digestion is required for profound and long-lasting prophylactic oral tolerance**. *Allergy* 2020, **75**:326-335.

23. •• van Lieshout GAA, Lambers TT, Bragt MCE, Hettinga KA: **How processing may affect milk protein digestion and overall physiological outcomes: a systematic review**. *Crit Rev Food Sci Nutr* 2020, **60**:2422-2445.
This review surveys the impact of technological processes on milk protein modifications and the kinetics of degradation.

24. Broersen K: **Milk processing affects structure, bioavailability and immunogenicity of β-lactoglobulin**. *Foods* 2020, **9**:874.

25. Golding M: **Exploring and exploiting the role of food structure in digestion**. In *Interdisciplinary Approaches to Food Digestion*. Edited by Gouseti O, Bornhorst G, Bakalis S, Mackie A. Springer; 2019:81-128.

26. Shoup D, Priola SA: **Cell biology of prion strains *in vivo* and *in vitro***. *Cell Tissue Res* 2022,1-15.

27. Richt AJ, Haley N: **Transmissible spongiform encephalopathies**. In *Veterinary Microbiology*. Edited by McVey DS, Kennedy M, Chengappa MM, Wilkes R. John Wiley & Sons Ltd; 2022:728-741.

28. Nguyen H, Gathercole JL, Day L, Dalziel JE: **Differences in peptide generation following *in vitro* gastrointestinal digestion of yogurt and milk from cow, sheep and goat**. *Food Chem* 2020, **317**:126419.

29. Baptista DP, Gigante ML: **Bioactive peptides in ripened cheeses: release during technological processes and resistance to the gastrointestinal tract**. *J Sci Food Agric* 2021, **101**:4010-4017.

30. Boukid F, Prandi B, Faccini A, Sforza S: **A complete mass spectrometry (MS)-based peptidomic description of gluten peptides generated during *in vitro* gastrointestinal digestion of durum wheat: implication for celiac disease**. *J Am Soc Mass Spectrom* 2019, **30**:1481-1490.

31. Torcello-Gómez A, Dupont D, Jardin J, Briard-Bion V, Deglaire A, Risse K, Mechoulan E, Mackie A: **The pattern of peptides released from dairy and egg proteins is highly dependent on the simulated digestion scenario**. *Food Funct* 2020, **11**:5240.

32. •• Ahmed T, Sun X, Udenigwe CC: **Role of structural properties of bioactive peptides in their stability during simulated gastrointestinal digestion: a systematic review**. *Trends Food Sci Technol* 2022, **120**:265-273.
This review systematically summarizes the peptide structural properties associated with stability to GI digestion.

33. Wang B, Xie N, Li B: **Influence of peptide characteristics on their stability, intestinal transport, and in vitro bioavailability: a review**. *J Food Biochem* 2019, **43**:e12571.

34. Mirzapour-Kouhdasht A, Garcia-Vaquero M: **Cardioprotective peptides from milk processing and dairy products: from bioactivity to final products including commercialization and legislation**. *Foods* 2022, **11**:1270.

35. Fan H, Wu K, Wu J: **Pea-derived tripeptide LRW fails to reduce blood pressure in spontaneously hypertensive rats due to its low gastrointestinal stability and transepithelial permeability**. *Food Biosci* 2022, **49**:101964.

36. • Gülseren İ, Vahapoglu B: **The stability of food bioactive peptides in blood: an overview**. *Int J Pept Res Ther* 2022, **28**:2.
This study attempted to determine the half-life of known food bioactive peptides in blood using *in silico* approaches, based on structural factors such as primary structure, molecular weight, and hydrophobicity.

37. Freitas D, Gómez-Mascaraque LG, Brodkorb A: **Digestion of protein and toxic gluten peptides in wheat bread, pasta and cereal and the effect of a supplemental enzyme mix**. *Front Nutr* 2022, **9**:986272.

38. Alcantara J, Stix R, Huang K, Connor A, East R, Jaramillo-Martinez V, Stollar EJ, Ball KA: **An unbound proline-rich signaling peptide frequently samples *Cis* conformations in Gaussian accelerated molecular dynamics simulations**. *Front Mol Biosci* 2021, **8**:734169.

39. • Herrera MG, Dodero VI: **Gliadin proteolytical resistant peptides: the interplay between structure and self-assembly in gluten-related disorders**. *Biophys Rev* 2021, **13**:1147-1154.
This review collects studies about the structural traits determining the immunological properties of gliadin peptides involved in celiac disease and emphasizes the need of combining multiple methods to obtain an integrative understanding of complex biological issues.

40. Herrera MG, Nicoletti F, Gras M, Dörfler PW, Tonali N, Hannappel Y, Ennen I, Hütten A, Hellweg T, Lammers KM, Dodero VI: **Pepsin digest of gliadin forms spontaneously amyloid-like nanostructures influencing the expression of selected pro-inflammatory, chemoattractant, and apoptotic genes in Caco-2 cells: implications for gluten-related disorders**. *Mol Nutr Food Res* 2021, **65**:e2100200.

41. Ye Y, Engholm-Keller K, Fang Y, Nielsen CF, Jordà A, Lund MN, Chatterton DEW: **UHT treatment and storage of liquid infant formula affects protein digestion and release of bioactive peptides**. *Food Funct* 2022, **13**:344-355.

42. Accardo F, Leni G, Tedeschi T, Prandi B, Sforza S: **Structural and chemical changes induced by temperature and pH hinder the digestibility of whey proteins**. *Food Chem* 2022, **387**:132884.

43. Fu Z, Akula S, Thorpe M, Hellman L: **Marked difference in efficiency of the digestive enzymes pepsin, trypsin, chymotrypsin, and pancreatic elastase to cleave tightly folded proteins**. *Biol Chem* 2021, **402**:861-867.

44. Kårlund A, Paukkonen I, Gómez-Gallego C, Kolehmainen M: **Intestinal exposure to food-derived protease inhibitors: digestion physiology- and gut health-related effects**. *Healthcare* 2021, **9**:1002.

45. Vivanco-Maroto SM, Santos-Hernández M, Sanchón J, Picariello G, Recio I, Miralles B: ***In vitro* digestion of milk proteins including intestinal brush border membrane peptidases. Transepithelial transport of resistant casein domains**. *Food Res Int* 2022, **157**:111238.

46. Walther B, Lett AM, Bordoni A, Tomás-Cobos L, Nieto JA, Dupont D, Danesi F, Shahar DR, Echaniz A, Re R, Fernandez AS, Deglaire A, Gille D, Schmid A, Vergères G: **GutSelf: interindividual variability in the processing of dietary compounds by the human gastrointestinal tract**. *Mol Nutr Food Res* 2019, **63**:e1900677.

47. •• Caira S, Picariello G, Renzone G, Arena S, Troise AD, De Pascale S, Ciaravolo V, Pinto G, Addeo F, Scaloni A: **Recent developments in peptidomics for the quali-quantitative analysis of food-derived peptides in human body fluids and tissues**. *Trends Food Sci Technol* 2022, **126**:41.
This review summarizes capabilities and challenges of peptidomics for characterizing food-derived peptides in biological fluids and tissues.

48. Pahlow Mose A, Mortz E, Stahl Skov P, Mortz CG, Eller E, Sprogøe U, Barington T, Bindslev-Jensen C: **The quest for ingested peanut protein in human serum**. *Allergy* 2020, **75**:1721-1729.

49. •• Caira S, Pinto G, Picariello G, Vitaglione P, De Pascale S, Scaloni A, Addeo F: **In vivo absorptomics: identification of bovine milk-derived peptides in human plasma after milk intake**. *Food Chem* 2022, **385**:132663.
In this article, hundreds of dietary peptides were purified from the plasma of human volunteers and characterized by mass spectrometry.

50. Picariello G, De Cicco M, Nocerino R, Paparo L, Mamone G, Addeo F, Berni, Canani R: **Excretion of dietary cow's milk derived peptides into breast milk**. *Front Nutr* 2019, **6**:25.

51. Zhu J, Garrigues L, Van den Toorn H, Stahl B, Heck AJR: **Discovery and quantification of nonhuman proteins in human milk**. *J Proteome Res* 2019, **18**:225.

52. Dekker PM, Boeren S, Wijga AH, Koppelman GH, Vervoort JJM, Hettinga KA: **Maternal allergy and the presence of nonhuman proteinaceous molecules in human milk**. *Nutrients* 2020, **12**:1169.

53. •• Palanski BA, Weng N, Zhang L, Hilmer AJ, Fall LA, Swaminathan K, Jabri B, Sousa C, Fernandez-Becker NQ, Khosla C, Elias JE: **An efficient urine peptidomics workflow identifies chemically defined dietary gluten peptides from patients with celiac disease**. *Nat Commun* 2022, **13**:888.
In this article, dietary gluten peptides have been purified from the urine of healthy and celiac disease individuals and, for the first time, characterized by mass spectrometry.

54. • Beverly RL, Huston RK, Markell AM, McCulley EA, Martin RL, Dallas DC: **Milk peptides survive *in vivo* gastrointestinal digestion and are excreted in the stool of infants**. *J Nutr* 2020, **150**:712.
This article demonstrates that some food-derived peptides can survive the degradation along the entire GI tract, also including the microbial fermentation in the colon.

55. • Sato K: **Metabolic fate and bioavailability of food-derived peptides: are normal peptides passed through the intestinal layer to exert biological effects via proposed mechanisms?** *J Agric Food Chem* 2022, **70**:1461-1466.
This article hypothesizes the metabolic transformation as an alternative mechanism through which dietary peptides exert systemic bioactive effects.

56. • Capuano E, Janssen A: **Food matrix and macronutrient digestion**. *Annu Rev Food Sci Technol* 2021, **12**:193-212.
This review surveys the structural and compositional aspects of foods that modulate macronutrient digestibility, the mechanistic basis to elucidate the effects of nutrition on human health and the possibility of designing foods with improved functionality.