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Alternative proteins vs animal proteins: The influence of structure and processing on their gastro-small intestinal digestion

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ABSTRACT

Background: Digestibility, an indicator of protein bioavailability, is essentially a measure of the susceptibility of a protein towards proteolysis. Proteins with higher digestibility have been linked with better health outcomes. Animal proteins are generally considered to be of better nutritional value than plant proteins not only because they are a good source of essential amino acids but also due to their higher digestibility in the human gastro-intestinal tract. With the recent emergence of alternative food protein sources, which are now processed in a completely new way to design new foods or new versions of the conventional foods, it has become extremely important to understand their digestion characteristics.

Scope and approach: This review discusses the factors that affect protein digestibility, including protein source, structure, type of processing, and modification, with a particular focus on the effects of non-protein components present in food matrix.

Key findings and conclusions: To obtain the desired functionality, particularly for alternate proteins, numerous physical, chemical, and enzymatic methods for modification have been reported. These modifications may alter structural characteristics of proteins by inducing structural modifications such as protein unfolding, crosslinking, and aggregation. Depending upon the protein reactivity during processing, the susceptibility of proteins towards hydrolysis by digestive enzymes might change, affecting not only the overall protein digestibility but also the rates of release of polypeptides and amino acids. The faster rates of protein digestion have been linked with muscle anabolism, suggesting the need and importance of classifying the new, emerging and alternative protein sources according to their rates of digestion into rapidly (RDP), slowly digestible (SDP) and resistant (RP) proteins. More research needs to be focussed on converting, through processing, the undigestible or RP into RDP or SDP to achieve better health outcomes.

1. Introduction

Proteins are a vital part of our diet because of their significant effects on our health and wellbeing. The consumption and demand of protein has been on the rise from the past two decades both in developed and developing countries (Akharume, Aluko, & Adedeji, 2021; Nikbakht Nasrabadi, Sedaghat Doost, & Mezzenga, 2021). The typical protein foods consumed globally include meats, beans, and dairy products. Muscle proteins play an essential role in human diets since they have been consumed in many cultures for many millennia. Nowadays, plant proteins which are perceived as healthy and sustainable protein foods have attracted a lot of attention due to growing consumer awareness towards sustainability and a desire to eat healthier diet. The plant-based trend has developed out of small-scale production for a niche market to established food companies bringing plant-based alternatives to market at a much larger scale. The raw materials used to produce this new category of foods are mainly from legume sources, such as soy and pea although some grain and cereal sources are also employed. In addition, emerging protein sources, such as cultured meat, insect proteins, algal protein, and microbial protein (Table 1), have great potential for meeting future protein supply and demand, but only a few kinds of them are commercialized.

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Table 1
Major sources of traditional and alternative protein foods and their new applications.

Sources	Categories	Alternative proteins and applications	Reference(s)
	Dairy products	• e.g., calcium caseinate & whey protein; being explored for meat analogues.	Wang, Dekkers, Boom, and van der Goot (2019)
	Insects	• e.g., *Alphitobius diaperinus* protein concentrate; being explored for meat analogues. • e.g., *Tenebrio molitor* flour; being explored for snacks. • e.g., cricket powder; being explored for pasta. • e.g., *Acheta domesticus* flour; being explored for bread.	Jantzen da Silva Lucas, Menegon de Oliveira, da Rocha, and Prentice (2020)
Plant	Legumes and beans	• e.g., hemp protein; being explored for meat analogues, plant-based milk, and bread.	–
	Cereals	• e.g., gluten, being explored for meat analogues. • e.g., oats, being explored for plant-based milk.	–
	Nuts and seeds	• e.g., peanuts; being explored for edible coatings • e.g., almonds, being explored for plant-based milks	Kazemian-Bazkiaee et al. (2020)
	Tuber	• e.g., potato protein; being explored for meat analogues	Kumar et al. (2017)
	Algae	• e.g., *spirulina*; being explored for meat analogues	Palanisamy, Töpfl, Berger, and Hertel (2019)
	Leaf protein	• e.g., grass protein (RuBisCO) being explored for food applications.	Kaur et al. (2021)
Fungal	Microbial proteins	• e.g., fermented fungus being explored for meat analogues	
	Mushroom	• e.g., *Colocvbeindica (dudhchatta)* mushrooms; being explore into analogue meat nuggets to increase meaty flavour.	Kumar et al. (2017)

According to the guidelines of the recommended dietary allowance for protein, it is suggested that a healthy adult with minimal physical activity should intake 0.8 g protein per kg body weight per day to meet their nutritional needs (Bhat, Morton, Bekhit, Kumar, & Bhat, 2021). It is notable that healthy adults over 65 are recommended to intake 1–1.2 g protein per kg body weight per day (Bauer et al., 2013). In addition to protein intake, they are also suggested to intake 25–30 g high quality protein, containing about 2.5–2.8 g of leucine in each meal to maintain muscle synthesis and quality of life (van den Helder et al., 2021).

The rate of protein digestion and the absorption of dietary amino acids are believed to have an impact on postprandial protein deposition (Dangin et al., 2003). The proteins that are digested at a faster rate have an enhanced postprandial protein gain especially in the elderly (Dangin et al., 2003). Throughout digestion, food breaks down into components with varying molecular weights. The products generated during protein digestion largely depend on the structural properties of food, including protein solubility and accessibility of digestive enzymes, protein source and processing history of foods. Unlike fat, which is almost digested completely in ileum, protein manages to enter the colon without complete digestion (Dallas et al., 2017). High protein digestibility reduces the amount of undigested proteinaceous substances entering the colon. The amount of undigested proteinaceous substances (both dietary and endogenous origin) reaching the human large intestine, based on a regular western diet has been reported to be approximately 12 g per day (Blachier et al., 2019). The undigested proteins are metabolised by colonic microflora either via proteolysis or a fermentation process, generating nitrogenous metabolites such as ammonia, amines, N-nitroso, phenolic, cresolic and indolic compounds. These products may contribute to long term detrimental effects on colonic heath, such as colorectal cancer and inflammatory bowel disease, depending on the balance between the rate of toxic metabolite generation, detoxification, and excretion from the large intestine (Peled & Livney, 2021).

According to the Food and Agriculture Organisation of the United Nations (FAO), true ileal digestibility is a measure of the difference between the amount of amino acids ingested and the amount of amino acids recovered from the ileum digesta, corrected with the basal and specific endogenous amino acid losses (Lee et al., 2016). Several studies have found that the true ileal digestibility of dietary plant protein in humans is usually lower than animal proteins. The *in vitro* protein digestibility values reported in the literature for different alternative protein sources are shown in Table 2. Nevertheless, the protein digestibility from both animal and plant sources could be altered by processing type and conditions (Kaur et al., 2016; Sá, Moreno, & Carciofi, 2020; van Lieshout, Lambers, Bragt, & Hettinga, 2020). As an example, pulsed electric fields (PEF) has been observed to disrupt the myofibrillar structure of beef, facilitating the accessibility of digestive enzymes that

Table 2
In vitro protein digestibility values for different alternative protein sources reported in the literature.

	Protein type	Processing method	*In vitro* protein digestibility (IVPD, %)	Reference(s)
Pulse	Cowpea	Cooked	87–98	Khattab, Arntfield, and Nyachoti (2009)
	Pea	Cooked	73–94	Khattab et al. (2009)
	Pea protein isolate	Uncooked	87.2	Schimbator, Culeţu, Susman, and Duţă (2020)
	Kidney bean	Cooked	64–87	Khattab et al. (2009)
	Chickpea	Raw and soaked	74.3	Han, Swanson, and Baik (2007)
	Lentil flour	Uncooked	75.90–77.05	Barbana and Boye (2013)
	Lentil protein concentrates	Uncooked	82.80–83.20	Barbana and Boye (2013)
	Hemp	Uncooked	78.5	Schimbator et al. (2020)
	Soybean	Raw and soaked	71.8	Han et al. (2007)
	Soy protein isolates	Uncooked	85.9	Schimbator et al. (2020)
Cereal	Wheat	Cooked	85.5	Mertz et al. (1984)
	Maize	Cooked	85.3	Mertz et al. (1984)
	Rice	Cooked	83.8	Mertz et al. (1984)
	Sorghum	Cooked	56.8–63.2	Mertz et al. (1984)
	Sorghum	Extruded	79	Mertz et al. (1984)
	Millet	Cooked	74.8–85.5	Mertz et al. (1984)
	Oat protein concentrate	Uncooked	77.5	Schimbator et al. (2020)
Others	Protein from Pleurotus mushrooms	Uncooked	68.2	Schimbator et al. (2020)
	Sea buckthorn protein	Uncooked	76.2	Schimbator et al. (2020)
	Insect protein		77–98	Ramos-Elorduy et al. (1997)
	Algal protein	Extracted	78.4–88.9	Tibbetts et al. (2016)

led to an improvement in its protein digestibility (Chian et al., 2019). In contrast, an excessive protein aggregation caused by unfolding of proteins during thermal processing of foods has been linked with a reduction in protein degradation by digestive enzymes (Bhat et al., 2021).

This review provides an updated viewpoint and discussion on the structure and processing-related aspects of alternate and animal proteins and how those influence the protein digestion kinetics and protein bioavailability.

2. Protein classification according to their digestibility

Food protein quality is one of the major determining factors for healthy diet, muscle maintenance and growth. In the past, the dietary protein quality was determined by the protein digestibility corrected amino acid score (PDCAAS) (FAO, 2013). The PDCAAS system is derived based on the ratio of the amount of the first limiting dietary indispensable amino acid to the amino acid requirement of the reference population group (pre-school children), corrected for protein digestibility based on the true fecal nitrogen digestibility using a growing rat or pig model (Rutherfurd, Fanning, Miller, & Moughan, 2014). However, the fecal nitrogen digestibility does not accurately reflect the protein digestibility of foods as there might be contribution and variation of endogenous amino acid losses at the end of ileum caused by antinutritional factors. In addition, protein sources with PDCAAS of more than 1 have been truncated to 1, which lowers the credit of the extra dietary indispensable amino acids and overestimate the quality of proteins with limiting amino acids. Thus, a new protein quality scoring system, the digestible indispensable amino acid score (DIAAS) scoring system, was introduced by FAO in 2013, where the protein quality is calculated based on the essential amino acids composition of food and the extent of digestion of these protein at the ileum terminal (FAO, 2013). The DIAAS reflects the percentage of the total daily requirement of the most limiting essential amino acid along with the extent of amino acid absorption in the small intestine. According to the Food and Agriculture Organisation (FAO) of the United Nation (FAO), protein could be classified based on their DIAAS: excellent quality protein (DIAAS $\geq$100) and high-quality protein (DIAAS 75–99). Food with a DIAAS below 75 cannot have any protein quality claim.

In addition to the amino acid composition and protein digestibility of a proteinaceous food, the rate of protein digestion and amino acid absorption has also been suggested to contribute to postprandial protein deposition in humans and muscle protein turnover. Foods with fast protein digestion rate result in a steep elevation in postprandial aminoacidemia, providing a rapid amino acids supply for protein anabolism (Dangin et al., 2003). This rapid amino acid supply is beneficial for the elderly by limiting protein loss, where it can combat sarcopenia. Therefore, there is a need to classify proteins, especially the alternative protein sources, according to their rates of digestion in the gastro-small intestinal track, into rapidly (RDP), slowly digestible (SDP) and resistant (RP) proteins.

Nevertheless, not all excellent and high quality proteins have similar rates of digestion and amino acid absorption. The rate of protein digestion and absorption are affected by the chemical and physical properties of the food proteins. For instance, milk proteins whey and casein have a high DIAAS of >100 but their rates of digestion and absorption differ (Dangin et al., 2003). Ingestion of whey protein induces a steep but short elevation of postprandial plasma amino acids while casein protein results in a prolonged plateau of moderate increment in plasma amino acids. Whey protein is acid soluble and leads to faster gastric emptying and digestion rate. On the other hand, casein protein coagulates in acidic stomach conditions, slowing down the gastric emptying which in turns reducing the digestion and absorption rate. Among casein proteins with different structures, cross-linked sodium caseinate has a faster digestion rate when compared to micellar casein and calcium caseinate in healthy young men, showing the impact of protein structure on its digestive characteristics (Trommelen et al., 2020). Egg protein, another protein with a high DIAAS, is resistant to peptic digestion in its raw form, but has an improved digestion rate after cooking due to heat denaturation induced modification of amino acid residues (Lassé et al., 2015). Beef, which is an excellent quality protein, has the highest *in vitro* digestion rate when cooked at a temperature between 70 and 75 °C when compared to raw meat and meat cooked at 100 °C and above. Heating at a lower temperature induced changes to meat structure by exposing the cleavage sites to digestive enzymes, improving the digestion rate of meat. However, increasing the cooking temperature or time promotes protein oxidation and aggregation which may reduce the access to the cleavage sites, slowing down the digestion rate.

In general, animal proteins have higher DIAAS when compared to plant-based proteins with some exceptions such as soy protein and potato protein (Table 3). Despite having a slightly lower DIAAS than milk protein, soy protein isolate was found to have a faster digestion rate than micellar casein but was digested slower than whey protein hydrolysate (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). He, Spelbrink, Witteman, and Giuseppin (2013) reported that the increment rate of postprandial plasma amino acid was fastest in the subjects fed with whey protein isolate followed by sodium caseinate and high molecular weight potato protein.

Only a few studies have compared the protein digestion rates of emerging alternative protein sources with animal protein sources. Vangsoe, Thogersen, Bertram, Heckmann, and Hansen (2018) compared the protein digestion rate of whey protein isolate, soy protein isolate and insect protein isolates. This study reported that the ingestion of whey protein isolate leads to the fastest elevation in the plasma essential amino acids, branched-chain amino acids and leucine concentration. Soy protein isolate had a faster rate of increment in postprandial amino acids level than insect protein isolates during 1 h post-ingestion, but the rate of increment was insignificant after 1 h post-ingestion.

3. Protein composition and structural characteristics

This section discusses the digestibility and bioavailability of plant, and muscle proteins as well as several emerging protein sources; and analyzes how their composition and structural characteristics influence on protein digestibility and bioavailability.

3.1. Plant proteins

Legumes, cereals, and seeds are important sources of plant protein for humans, particularly in the vegetarian diet. It has been widely reported that legumes contain albumins and globulins largely, whereas cereal protein is mainly composed of prolamins and glutelins and each of these protein fractions have different digestibility. Given the presence of trypsin inhibitors in albumin fractions, the native albumins were the most resistant to hydrolysis (Mariotti, 2017).

As discussed in previous section, plant proteins have lower digestibility when compared to animal proteins, such as meat, poultry, egg, and milk (Mariotti, 2017). This is principally attributed to the presence of the antinutritional factors (ANFs) in plant proteins. Their effects on nutrients and the mechanisms of reducing the digestibility of plant proteins have been summarised by Sá et al. (2020). Employing adequate processing steps, such as cooking, boiling etc., is a strategy to improve the digestibility of plant proteins by inactivating or reducing the presence of these ANFs (Sá et al., 2020).

Commercial plant-based protein ingredients come in three primary forms: flours (20–30% of protein content), concentrates (50–80%), and isolates (>90%) (McClements & Grossmann, 2021). Isolation procedures have a profound effect on protein digestibility. The higher digestibility of plant protein isolates may link to a drastic reduction in the levels of tannins and phytates (Sá et al., 2020). As shown in Table 4, Barbana and Boye (2013) has reported that IVPD of lentil protein concentrates (82–83%) is higher than that of the flours (75–77%). Similarly,

Table 3
Digestible indispensable amino acid scores (DIAAS) and limiting amino acids for different plant and animal sources.

Food materials	DIAAS (%)	Limiting amino acid
Cooked kidney bean [a]	88	Lysine
Cooked mung bean [a]	86	Leucine
Cooked chickpeas [a]	76	Lysine
Cooked peas [a]	68	Lysine
Cooked adzuki bean [a]	64	Leucine
Cooked broad beans [a]	60	Leucine
Corn [b]	36	Lysine
Cooked Rice [c]	59	Lysine
Wheat [b]	48	Lysine
Hemp [b]	54	Lysine
Cooked Oat [c]	54	Lysine
Soy [b]	91	Methionine + Cysteine
Potato [b]	100	N/A
Milk [b]	116	N/A
Egg [b]	101	N/A
Pork [b]	117	N/A
Chicken [b]	108	N/A
Beef [b]	112	N/A
Insect protein [d]	75	Lysine + Tryptophan

Han, Moughan, Li, and Pang (2020) [a]; McClements and Grossmann (2021) [b]; Loveday (2019) [c]
Huang et al. (2018) [d]
The rows have been colour-coded, with green as the best protein sources (DIAAS ≥100) followed by light green (DIAAS < 100 ≥ 85), yellow (DIAAS < 85 ≥ 70), pink (DIAAS < 70 ≥ 55) and orange (DIAAS < 55).

the refined soy protein isolates (85.9%) have higher protein digestibility than soybean (71.8%) (Schimbator, Culeţu, Susman, & Duţă, 2020).

The protein structure, especially the secondary structure, including α-helix, β-sheet, and β -turn also strongly associate with protein digestibility (Vanga, Wang, & Raghavan, 2020). Similarly, an increase in β-sheets and a loss in α-helices due to processing, such as ultrasonication has resulted in increased IVPD in the case of peanut (Ochoa-Rivas, Nava-Valdez, Serna-Saldívar, & Chuck-Hernández, 2017). This was possibly attributed to the local conditions within the structure, revealing new binding sites for the digestive enzymes to act on (Vanga et al., 2020).

The nutritional value of food protein is restricted by not only digestibility, but also the quantity of digestible indispensable amino acids as discussed in the previous section. Plants are commonly deficient in one or two essential amino acids and perceived as low protein nutritional value source (Schimbator et al., 2020). They have noted that lysine is the primary limiting amino acid in cereal proteins, whereas methionine and cysteine are in pulse proteins, see Table 2. The amino acid deficiency issue could be solved through protein complementation, such as the mixtures of cereals and legumes, plant, and animal proteins.

3.2. Muscle proteins

Muscle proteins are a valuable part of the human diet, as they have high digestibility and bioavailability compared to plant protein. Notably, muscle proteins contain all the dietary essential amino acids (Bhat et al., 2021). Muscle tissues have a complex hierarchical assembly of bundles of fibrous proteins embedded within connective tissue made from triple helices of collagen (McClements & Grossmann, 2021). Lean muscle is composed of about 17%–23% of the protein that can be classified into myofibrillar, sarcoplasmic and stromal based on their solubility at varying salt concentrations, accounting for 50 %–60%, 30% and 10 %–20%, respectively.

Data from IVPD have indicated a range of values of cooked beef/chicken//pork/fish, reaching around 92–95% (Queiroz Mendes, De Almeida Oliveira, Brunoro Costa, Vieira Pires, & Regina Passos, 2016). Moreover, the ovine myofibrillar protein and liver hydrolysates from lamb were found to be the most digestible, with a mean true ileal digestibility across all amino acids of 99%, while the value of bovine serum albumin was 93% (Cui et al., 2013).

3.3. Microbial and insect proteins

Microbial biotechnology has a long history of producing feeds and foods; and production of algae, yeasts, fungi, and plain bacterial cellular biomass is more efficient in land and water usage and less greenhouse gas emissions compared with pork and chicken and other plant-based meat alternatives (Colosimo, Warren, Finnigan, & Wilde, 2020). Mycoprotein is also being explored as a potential food protein source because it can give a meaty aroma, a savoury umami taste, and a meat-like texture (McClements & Grossmann, 2021). Some studies have reported that mycoprotein cell wells were highly resilient to digestion. It has been found that digestive enzymes promoted the most efficient release of protein from mycoprotein rather than physical extraction (Colosimo et al., 2020). However, the hyphal structure of mycoprotein was more susceptible to small intestinal proteases (Colosimo et al., 2020).

It has been reported that the IVPD of several types of brown seaweeds (e.g., *Ascophyllum nodosum, Saccharina latissimi*) and red seaweeds (e.g., *Palmaria palmata, Chondrus crispus*) was ranged between 78 and 88% (Tibbetts, Milley, & Lall, 2016). The soluble polysaccharides and oxidized polyphenols presenting at high levels in algae may inhibit protein digestion.

Insects are a potentially rich source of protein and lipids. The IVPD of insect proteins (*Honeybees, Liometopum ants, Sphenarium grasshoppers*) has been reported to be in the range of 77 %–91% (Ramos-Elorduy et al., 1997). Insect exoskeletons are mainly composed of chitin, a polymer of N-acetyl-d-glucosamine, which may inhibit protein digestion.

Conversely, some of edible insects are relatively soft at the larval stage with low chitin content, thus they can be easily digested by humans (Lee, Jo, Yong, Choi, & Jung, 2021). For example, the larvae of *P. brevitarsis* was found to have greater IVPD and bioavailability compared with beef. However, insect proteins are often lacking lysine and tryptophan (Huang et al., 2018). Currently, there is little knowledge about the bioavailability of insect-derived nutrients for humans.

Table 4
Protein modification methods and their effects on protein digestibility.

Modification type	Protein type	Effect on digestibility	Reference(s)
Physical			
High-Pressure Processing	Bovine *longissimus dorsi* muscle	• Pressure treatment induced the structural breakdown and protein denaturation resulted in increased susceptibility to pepsin action, thereby resulting in quicker digestion of the proteins	Kaur et al. (2016)
	Buckwheat	• Protein digestibility increased by 0.8% under high-pressure processing treatment	Deng, Padilla-Zakour, Zhao, and Tao (2015)
	Rabbit meat	• Different pressure treatments such as 100, 200 and 300 increased the protein digestion susceptibilities of different proteins like actin, myosin, and troponin T, respectively	Xue et al. (2020)
Ultrasound		• An improvement in the *in vitro* digestibility of myofibrillar proteins extracted from modified stored pork was observed after ultrasound treatment	Cheng, Ofori Donkor, et al. (2021)
Microwave	Pigeon Pea flour	• Microwave treatment increased *in vitro* protein digestibility	Sun et al. (2020)
	Peanut flour	• *In vitro* protein digestibility increased from 95.16 to 97.09% for microwave treated products.	Ochoa-Rivas et al. (2017)
	Soybean protein	• *In vitro* protein digestibility increased by 11% (conventional) and 7% (microwave treated) than the control	Vagadia et al. (2018)
Chemical			
Glycosylation	Rice protein	• Glycosylation reaction between rice protein and dextran increased *in vitro* protein digestibility	Cheng, Wei, et al. (2021)
	Milk protein (Caseinate)	• Cross-linked and glycosylated caseinate exhibited the highest *in vitro* protein digestibility, whereas control cross-linked caseinate (prepared under the same conditions but without oligochitosan addition) exhibited the lowest digestibility	Song and Zhao (2014)
Phosphorylation	Wheat gliadins	• Phosphorylated gliadins were more easily digested by digestive enzymes than raw gliadins	Xue et al. (2019)
Acetylation	Kidney bean protein isolate	• chemical acetylation treatment increased the *in vitro* trypsin digestibility	Yin et al. (2009)
Enzymatic			
	Hemp seed protein meal isolate	• Combined treatment of enzymes (carbohydrases and phytase) and ultrafiltration improved protein digestibility of hemp seed protein meal isolate due to pre-digestion reduction in polysaccharides and phytate	Malomo and Aluko (2015)
	Beef	• Overall digestibility of beef samples was improved through addition of actinidin	Zhu et al. (2018)

4. Processing and protein digestibility

Various processing technologies such as thermal, thermomechanical processing (TMP), pulsed electric field (PEF), microwave (MW), ultrasound (US), high-pressure processing (HPP), cold plasma technology (CPT) and the ultraviolet (UV) radiation have been employed to improve palatability, shelf life, functionality, nutraceutical, and organoleptic properties of food. Many new reports have illustrated the effects of different processing conditions on the digestion kinetics of different food proteins (plant, animal, insect, or fish), which are discussed in this section (Table 4). The mechanisms depicting the changes in protein structure induced by processing leading to changes in protein digestibility are presented in Figs. 1 and 2.

4.1. Thermal processing/heat treatment

Several types of thermal processes are used for food systems, such as high temperature-short time (HTST) pasteurization, ultra-high-temperature, sterilization, convectional cooking, boiling, poaching, baking, and frying, autoclaving, or sterilization, among others. Apart from the preservation effect, several studies have been conducted to utilise heat treatment as a tool to obtain peculiar functional properties (such as emulsification, gelation, etc.) in protein systems through controlled denaturation and aggregation (Akharume et al., 2021). However, the heat-induced structural modification of proteins could lead to cleavage sites becoming more or less accessible during protein digestibility. For example, the heating of dehulled proso millet flour (also known as common millet flour) with excess water leads to the formation of hydrophobic aggregates, leading to higher resistance towards gastric digestion, thus decreasing the overall protein digestibility compared to the unprocessed proso millet flour (Gulati et al., 2017). Similarly, Zhang et al. (2020) reported that heating water-soluble oyster protein at high temperatures (65–100 °C) resulted in digestive proteins becoming resistant to gastric proteases. In contrast, lower heat treatment (45, 55 °C) showed a higher digestibility. The cause of the impaired digestibility at higher temperatures was due to exposure of aromatic residues (tyrosine and phenylalanine), which reduced the affinity of pepsin during digestion (Zhang et al., 2020).

Inferior functionality (due to compact structure, high molecular weight), low digestibility, and the presence of antinutritional factors in plant proteins reduce their acceptance and food applications. Recent studies have reported improved protein functionality and digestibility for various new proteins such as soy and pea, when applying controlled heat-induced structural modifications (Nikbakht Nasrabadi et al., 2021). However, the denaturation patterns and proteins interactions are not always predictable as it depends on several factors such as protein source and processing conditions that could lead to different degrees of protein hydrolysis and peptide formation during digestion (Bhat et al., 2021; Kaur et al., 2016).

4.2. Thermomechanical processing

Extrusion has long been used to create healthier products while treating plant-based protein by-products to improve their consumer acceptability while reducing their antinutritional effects through enzyme inhibition. In recent years, the principle of thermomechanical treatment has been utilised in extrusion and shear-cell technology to produce a fibrous meat-like structure from plant proteins or a combination of animal and alternative proteins (Beniwal, Singh, Kaur, Hardacre, & Singh, 2021). This hybrid mixture of the structured plant proteins blends improved overall functional and sensorial properties and enhanced the protein quality score (Palanisamy, Franke, Berger, Heinz, & Töpfl, 2019). TMP includes the use of high temperature (95–160 °C) combined with shear that provides input energy to the protein system to undergo restructuring through the inter- and intramolecular aggregation of the amino acid chains and the formation of large molecular weight aggregates via cross-linking of protein molecules upon cooling. During TMP, the physicochemical and structural changes in concentrated protein systems accounted for unfolding the protein molecules, with a complete loss of tertiary structure and partial uncoiling of the secondary structure (Beniwal et al., 2021). Such extreme temperature and shear treatment has been reported to decrease antinutrients, trypsin, and chymotrypsin inhibitory activities and impart structural changes causing increased digestibility of various plant proteins (Palanisamy

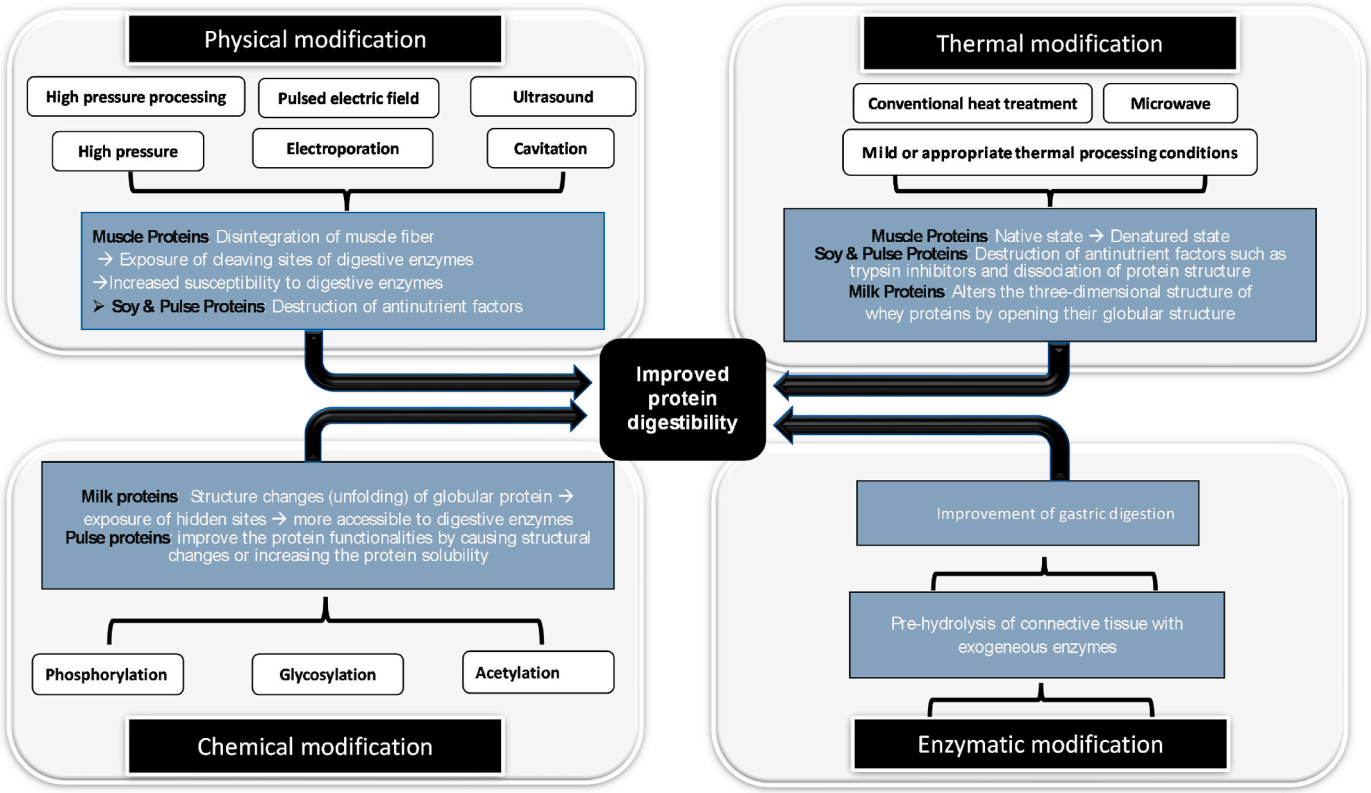

Fig. 1. Different methods of protein modification/processing and their influence on protein digestibility (Sources: Lee, Choi, et al., 2021; Zha, Rao, & Chen, 2021).

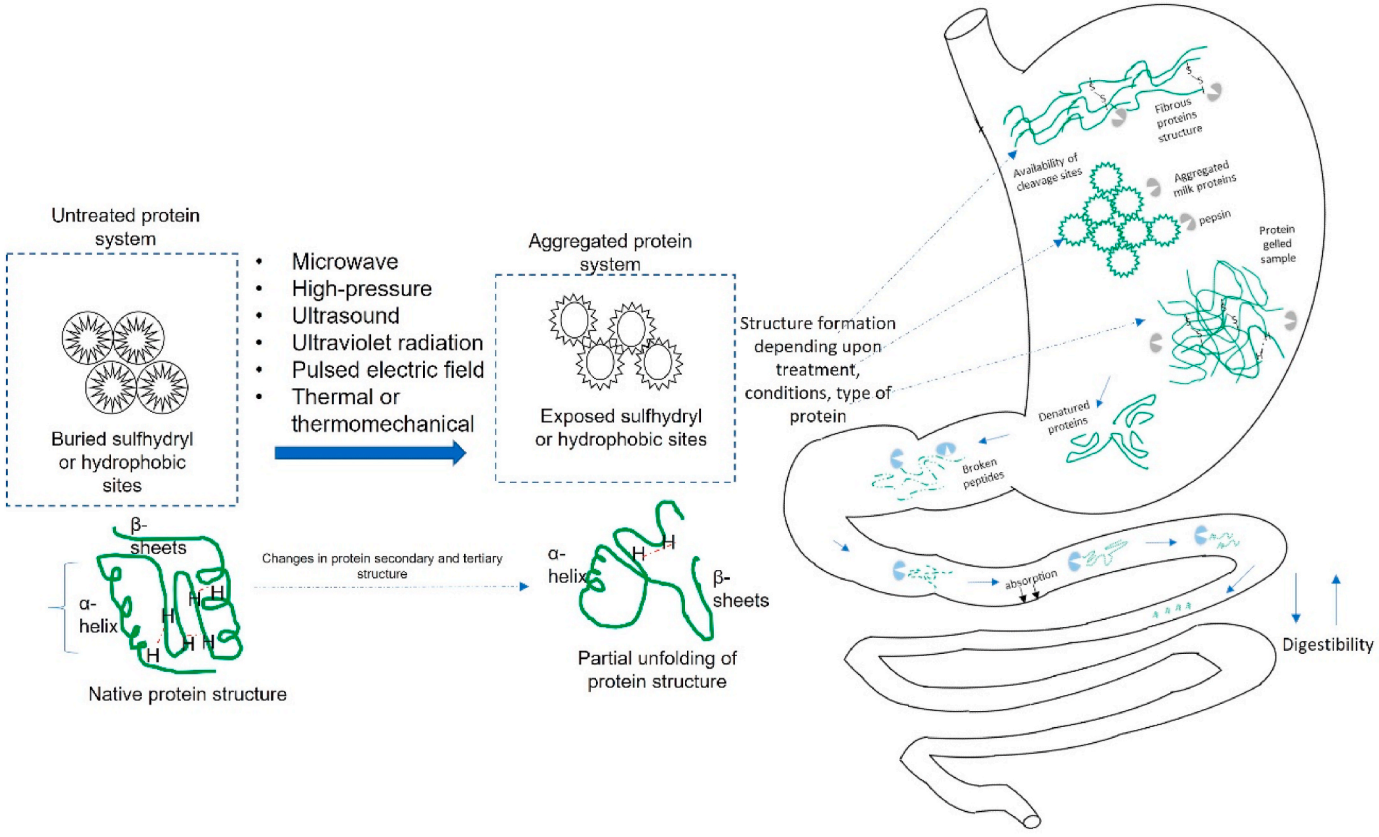

Fig. 2. Mechanisms depicting changes in protein structure induced by processing leading to changes in protein digestibility in the gastro-small intestinal tract.

et al., 2019). Depending upon the moisture in the feed, the extrude proteins could be classified as low-moisture (10%–30% moisture) or high-moisture (40%–70% moisture) structured proteins (Beniwal et al., 2021). It is important to note that the complex interplay of processing variables during TMP could affect protein degradation and the interaction of components that influences protein digestibility (Beniwal et al., 2021). However, degradation reactions may also occur during TMP due to the formation of covalent bonds by β-elimination in cysteine, leading to an increase in dehydroalanine (DHA) and lanthionine (LAN) or due to the Maillard reaction that could affect the nutritional properties of extruded products (Beniwal et al., 2021).

4.3. Ultrasound

High-intensity ultrasound (HIU) has gained interest due to its disruptive action on food's physical and chemical properties, while low-intensity ultrasound (LIU) provides information on the physicochemical properties of food materials. The mechanical nature of HIU induces protein aggregation and crosslinking through partial protein unfolding, exposing hydrophobic and sulphuryl sites, breaking the non-covalent bonds, and fragmentation of peptides in protein structure. This impacts its overall functionality but may or may not affect the digestibility of food proteins. The ultrasound treatment at 20 kHz, pulsed on-time 10 s, off-time 5 s, the amplitude of 60%, and duration of 10 min improved the protein surface activity and overall digestibility of buckwheat protein from 41.4% (control) to 58.2% (Jin, Okagu, Yagoub, & Udenigwe, 2021). Ultrasonication showed potential applications in the reduction of trypsin inhibitors' activity and improving the digestibility of soymilk through changing the secondary structures of proteins (Vanga et al., 2020). Cheng, Wei, Liu, Xu, and Chen (2021) reported that US treatment improved the viscoelastic properties of whey protein emulsion gels with the formation of a compact and fine network and improved their digestion during *in vitro* gastric and small-intestinal digestion. The digestion characteristics of animal proteins could also be enhanced by ultrasound treatment. Zhang, Zhang, Chen, and Zhou (2018) reported that ultrasound could improve the digestibility of tropomyosin due to changes in its secondary structure, and protein fragmentation through HIU induced free radicals. The high intensity ultrasonication (HIU) imparted structural changes in the abalone muscles and led to improved overall protein breakdown during *in vitro* digestion (Bagarinao, Kaur, & Boland, 2020). Ultrasonication of native β-lactoglobulin (β-Lg) led to a decrease in its allergenicity and improved antioxidant properties during digestion.

Several studies have reported that pre-treatment of proteins with ultrasound prior to enzymatic hydrolysis breakdown the proteins into smaller fragments (Bagarinao et al., 2020; Jin et al., 2021). The possible mechanism for this could be the generation of drastic physical forces and highly reactive free radicals due to the acoustic cavitation, which can attack the side chains and backbone of protein molecules, leading to alternations in their secondary and microstructures (Jin et al., 2021). Therefore, the structural changes induced by ultrasonication could improve the enzyme accessibility for protein hydrolysis during digestion.

4.4. High pressure processing

Depending on the HPP conditions (pressure, temperature, and time) and the protein system, HPP can induce changes in protein conformations such as denaturation, aggregation, or gelation (Kaur et al., 2016). Additionally, it has been reported that high-pressure processing treatment (100–300 MPa for 9 min) increased the IVPD of rabbit meat by exposing hidden peptide cleaving sites. Interestingly, different pressure treatments such as 100, 200 and 300 MPa increased the protein digestion susceptibilities of different proteins like actin, myosin, and troponin T, respectively (Xue et al., 2020). Similarly, after HPP treatment, 2-3-fold increased release of peptides were observed in cod meat than in control (Zhang, Bi, Wang, Cheng, & Chen, 2019). The high pressure processing of meat at 600 MPa for 10 min caused textural changes in meat structure similar to cooking of meat, however, led to better IVPD in terms of free amino N release (Kaur et al., 2016).

The pressure treatment of dairy protein at 200 MPa for 5 min exposed the buried peptide fragments and improved the protein digestibility (Hu et al., 2017). The pressure treatment of milk during homogenization has been reported to improve the protein hydrolysis by pepsin. The even distribution of fat globules in larger protein fractions formed a fragmented and crumbled coagulum with cracks, therefore, giving more accessibility to pepsin during the gastric digestion phase (Ye, Cui, Dalgleish, & Singh, 2017).

Similarly, in the case of pulse proteins, high pressure treatment has been reported to increase the IVPD of lentil and faba protein concentrates than the untreated controls by reducing the trypsin inhibitor activities (Hall & Moraru, 2021).

The ultra-high-pressure homogenization (UHPH) has been reported to preserve the lysine content due to milder thermal conditions on a plant-based beverage without affecting the protein digestibility but reducing the allergenic response (Munekata et al., 2020). In addition, UHPH has been reported to enhance bioactivity of plant or dairy protein-based beverages due to conformational modifications that proved to be less allergenic based on their lower gut immune response in comparison with heat-treated samples (Hu et al., 2017).

4.5. Microwave

Microwave is a form of electromagnetic radiation with a frequency range of 300 MHz to 300 GHz (Bhat et al., 2021). The microwave treatment is based on a mechanism of dipole movement in water molecules present inside the food material, under the influence of electromagnetic field due to high-frequency MW radiation. The protein undergoes partial unfolding of the secondary and tertiary structure with the breaking of non-covalent interactions such as disulphide linkages and hydrogen bonds that influences the digestion kinetics of protein digestibility (Bhat et al., 2021). However, the intensity and processing time significantly influences protein polymerization and thus influence its digestibility (Phongthai, Lim, & Rawdkuen, 2016). The microwave treatment required a shorter processing time to improve the digestibility and antinutrient inhibition in soymilk than conventional heating (Vagadia, Vanga, Singh, Gariepy, & Raghavan, 2018). Vanga et al. (2020) reported that 10-min microwave processing at 85 °C of soy milk successfully reduced the trypsin inhibitors while imparting changes in protein secondary structure that led to an improvement in soymilk digestibility. However, MW treatment of gluten protein for an extended period could lead to crosslinking and isopeptide bonds between specific amino acid groups that resist enzymatic digestion, thereby reducing the amount of total available amino acids after digestion (Xiang, Zou, Liu, & Ruan, 2020). The longer duration of the MW treatment on gluten flour reduced the amount of available lysine due to the establishment of crosslinks (due to presence of sugars or metals that lead to oxidation or Maillard reaction) that are also resistant towards gastric digestion (Xiang et al., 2020). In the case of shrimp protein, the microwave treatment reduced the protein digestibility due to changes associated with protein secondary structure with an increase in β-sheets and loss of turns (Luo, Taylor, Nebl, Ng, & Bennett, 2018). The microwave treatment has been employed in assisting the extraction of rice bran proteins with no negative impact on the digestibility of the obtained protein powders (Phongthai et al., 2016).

4.6. Pulsed electric fields

Pulsed Electric Fields (PEF) treatment is a technology that involves applying electric fields to semi-solid or liquid food products by placing them between two electrodes and subjecting them to very a short burst of strong pulses at high voltages (1–80 kV/cm), thus causing minimum

damage to the quality (Bhat et al., 2021; Munekata et al., 2020). The latest research suggests that PEF can modify protein conformation, change their unfolding mechanisms, and lead to aggregation and disruption of protein structure that impart improved/or impaired digestibility of proteins and immunological responses (Bhat et al., 2021). The possible mechanism could be the breakdown of electrostatic interactions and ionizations of numerous chemical groups, which can modify the secondary and tertiary structure of proteins, thereby losing α-helix and β-sheet (Bhat et al., 2021). Using optimum processing parameters, low-intensity PEF could be used in foods to improve their organoleptic properties and nutritional value (Chian et al., 2019). This treatment induces electroporation and increases membrane permeability, enhancing protease diffusion into the membrane-bound muscle mass and allowing greater digestion of myofibrils (Bhat et al., 2021). PEF treatment has shown a positive impact on the *in vitro* digestion kinetics, causing greater and faster digestion (*in vitro*) of meat proteins, irrespective of the meat type, species, or muscle type (Bhat et al., 2021). Chian et al. (2021) reported that a combination of cooking along with PEF treatment further enhances the vitro protein digestibility and free amino nitrogen released at the end of simulated gastro-small intestinal digestion compared with the cooked meat control. PEF-treated muscle fibres displayed more severe Z-disks and I-bands disruption that was linked with greater susceptibility to the action of digestive enzymes (Chian et al., 2019).

4.7. UV radiation, cold plasma, and supercritical carbon dioxide technology

UV light irradiation can effectively change protein functionality through an increase in polymerization of protein chains through breakage of intramolecular disulphide bonds and formation of new intermolecular disulphide or protein aggregates that may affect the protein digestibility and nutritional value (Gharbi & Labbafi, 2018). Hu et al. (2017) reported that ultraviolet light- C (UV–C) (250–270 nm) treatment for 5 min positively affected the amino acid residues and protein structure of α-casein (α-ca) fragments in bovine milk leading to an improvement in protein digestibility by 37–43% compared to the untreated sample. Prolonged UV-C treatment for 15 min resulted in decreased protein digestibility compared to UV-C for 5 min, suggesting a negative effect of the prolonged UV-C treatment on IVPD.

Cold plasma technology (CPT) is an emerging alternative to thermal sterilization of food products due to its direct application onto the food surface. The plasma used in this technology results from the ionization of a cocktail of reactive gases (mainly oxygen and nitrogen), which generate UV radiation under the influence of input energy provided through an energy source that could be the electrical, thermal, optical, or electromagnetic source. The cold plasma technology imparts high energy to the food system containing proteins that undergo denaturation reactions and change the protein conformation (Sruthi et al., 2022). A recent review by Akharume et al. (2021) showcased the controlled modification of various plant proteins such as zein, peanut protein, and pea flour protein under the influence of applied voltage and time combination under plasma technology to enhance functionality.

Cold plasma treatment provides advantages over conventional thermal treatments causing lesser loss of nutritional functionality due to the formation of Millard reactions products which are often linked to reduced digestibility score (Venkataratnam, Sarangapani, Cahill, & Ryan, 2019; Sadhu, Thirumdas, Deshmukh, & Annapure, 2017; Segat, Misra, Cullen, & Innocente, 2015). However, it may cause oxidative reduction of amino acids containing (-SH) group and carbonyls formation due to decomposition of amide bond with the reactive species from the plasma (Segat et al., 2015). Numerous works have showcased its promising result in reducing food allergens and mycotoxins associated with new protein sources. Sadhu et al. (2017) have reported improving the hydrophilicity of mung bean upon CPT while displaying an enhanced activity of amylases and proteases and reduction in antinutritional factors. Venkataratnam et al. (2019) reported the reduction of allergens Ara h 1 associated with peanut protein ingredients with the application of cold plasma at 80Kv treatment for different time duration. Circular dichroism (CD) studies revealed that CPT -induced changes in the secondary structure of Ara h1 with a decrease in α-helix structure led to reduced IgG binding capacity of the allergen. In conclusion, the application of CPT in food shows its potential usages of improving the functionality and digestibility of underexplored protein sources. Supercritical carbon dioxide technology has acquired some interest lately as a structural modification method to improve the functional characteristics of proteins. The minimal damage of proteins during supercritical carbon dioxide treatment results in unfolding of secondary and tertiary structures of proteins thereby allowing efficient and complete attack of proteolytic enzymes in the gut.

Other than as a structural modification method, the supercritical carbon dioxide treatment can be used in protein processing as a pretreatment for the removal of lipophilic substances or oils from the food matrices, precipitation of proteins and separation of the different peptides (Kang et al., 2017; Lima et al., 2019). Generally, proteins are denatured by the elevated temperatures during extraction of oils or proteins from the food matrixes. However, in case of supercritical carbon dioxide process, protein nature (activity and stability) depends on the protein type, pressure, and temperature used in the processing. Kang et al. (2017) reported that supercritical carbon dioxide treatment removed the protein from the soy flour without denaturation and improved the functional characteristics like solubility and water absorption capacity than hexane treated and control soya flour. However, in another study, structural analysis exhibited the partial denaturation of α-helix structure of alpha-lactalbumin protein, while β-lactoglobulin was in its native state at highest temperature and pressure (65 C and 24 MPa) (Lima et al., 2019). To date, there is no as such study focusing on the digestibility of supercritical carbon dioxide extracted/treated proteins and thus there is a need of more research in this area.

4.8. Enzymatic processing

During recent years, enzymatic modification of proteins to improve their functionalities and biological properties have attracted a lot of attention. Enzymatic modifications involve the controlled use of non-proteolytic and proteolytic enzymes to change the structural properties of proteins.

In case of meat proteins, stromal proteins have limited digestibility, so proteolysis or pre-degradation by exogeneous enzymes can increase their digestibility. Pre-treatment with the kiwifruit enzyme, actinidin led to breakdown of the muscle structure and improved the IVPD of beef brisket (Zhu, Kaur, Staincliffe, & Boland, 2018).

Hemp seed protein isolates obtained after a combined treatment of enzymes (carbohydrases and phytase) and ultrafiltration had higher IVPD than the traditional protein isolates and commercial protein concentrate (Malomo & Aluko, 2015). Pre-hydrolysis with enzymes is also an effective method to modify the immune reactivity of food proteins because it affects the resistance of food allergens to gastrointestinal digestion. Enzymatic hydrolysis results in rapid collapsing of conformational epitopes while linear epitopes are broken down, and their further existence depends on the type of enzyme used and the degree of hydrolysis. Other than the pre-hydrolysis, follow up treatment of proteins with enzymes after extraction can also increase their purity and *in vitro* digestibility. In comparison with alkali extracted rice protein, protein extracted by enzyme assisted microfluidization by using amylase and glucoamylase has been reported with higher protein purity and digestibility. This could be due to the difference in chemical composition of the extracted proteins, as alkali extracted protein contained complex of polypeptides including prolamin (indigestible protein) and globulin, while enzyme assisted microfluidization extracted protein was rich in glutelin (easy to digest protein) (Xia et al., 2012). Similarly, legume protein (chickpeas and peas) extracted by enzyme assisted-aqueous

extraction by using enzymes (alcalase, trypsin, papain, pepsin, and a combination of papain and alcalase) exhibited higher digestibility than the proteins extracted by direct aqueous extraction. The reason could be proteolytic activity of enzymes, which released more digestible free amino acids and protein fractions.

4.9. Chemical modification of proteins

4.9.1. Glycosylation

Glycosylation, also referred to as glycation, is a food grade reaction that can improve protein functionalities. Different methods including heating and molecular crowding can be used for chemical or nonenzymatic glycation. This reaction, also called an early step of Maillard reaction, takes place through covalent conjugation between a free carbonyl group of reducing sugars and amine group of protein/peptide/amino acid in the presence of water and controlled heat treatment. Structure changes (unfolding) of globular protein due to carbohydrate binding and heating, may make expose the hidden sites more accessible during glycation and may increase the protein digestibility. It has been reported that glycosylation reaction between rice protein and dextran leads to protein unfolding, thereby increasing their susceptibility to digestive enzymes (Cheng, Ofori Donkor, et al., 2021). Similarly, Song and Zhao (2014) documented that glycosylated and crosslinked caseinate exhibited higher IVPD in comparison with caseinate. In the case of whey proteins, based on the appropriate processing, a balanced steric hindrance and protein unfolding will determine either increase or decrease in its digestibility after glycation (van Lieshout et al., 2020). Glycation induced-direct and indirect blocking of lysine residues, and cross-linking may decrease the accessibility of cleavage sites for proteases and can decrease digestibility of proteins (van Lieshout et al., 2020). It has also been reported that increasing protein digestion may alleviate its allergenicity. Interestingly, glycation is one of the most promising and novel method to reduce the allergenicity of the β-lactoglobulin, which is a most predominant whey milk protein. It can reduce the immunoglobulin E binding sites of β-lactoglobulin by covalent bonds with reducing sugars. Several research studies have shown physical-assisted chemical modification like glycation exert better effect to reduce allergic potential of allergens, especially β-lactoglobulin than the glycation alone. The pre-treatment of proteins with the physical methods may help to improve the glycation by decreasing the immunoglobulin E binding sites of β-lactoglobulin and thus increasing the glycation sites. In this regard, various physical methods like ultrasound, pulsed electric field and high-pressure, micro fluidization with combined use of glycation has been already reported in the literature (Shao, Zhang, Zhu, Liu, & Tu, 2020). An improved IVPD of proteins was observed owing to the structural changes in both protein and starch due to MW treatment (Sun, Ohanenye, Ahmed, & Udenigwe, 2020).

4.9.2. Phosphorylation

Phosphorylation is another chemical modification technique that has been reported to affect the protein digestibility. The study of Xue et al. (2019) reported that phosphorylated gliadins were more easily digested by digestive enzymes than raw gliadins which partially resisted the hydrolysis by gastric juice. Phosphorylation through dry heating also enhanced the digestibility of Fag e 2, which is a major allergen present in buckwheat and is highly resistant to pepsin digestion (Ahmad, Athamneh, Suzuki, Nakamura, & Katayama, 2020).

4.9.3. Acetylation

Acetylation of proteins by using acetic and succinic anhydride at suitable anhydride -to-protein ratios has been reported to improve the protein functionalities by causing structural changes or increasing the protein solubility. The *in vitro* trypsin digestibility of kidney bean protein isolates was also increased with aceylation, depending on the level and anhydride type (Yin, Tang, Wen, & Yang, 2009).

5. Food ingredient interactions and their effects on protein digestion

The digestibility of proteins is also affected by the other components present in food (described in detail by Nikbakht Nasrabadi et al., 2021), as shown in Fig. 3. These are discussed briefly below.

5.1. Starch

The starch-protein complex formation or crosslinking between the proteins along with the presence of phenolic complexes has resulted in a reduction in protein digestibility (Wen, Li, Gu, Wang, & Wang, 2019). Wen et al. (2019) reported that IVPD was enhanced with heat treatment of gluten in the presence of starch compared to gluten alone. It was attributed to the loose spatial structure of the gluten-starch complex and therefore exposing more sites for enzymatic digestion. However, the protein digestibility decreased in the presence of starch when treated at temperatures lower than 60 °C. It was proposed that the increased hydration and reduced disulphide bonding during thermal processing resulted in changes in protein's secondary structure, which might have affected its digestibility (Wen et al., 2019). Following a similar theory, black beans were processed using a combination of decortication, germination, and extrusion (de la Rosa-Millán, Heredia-Olea, Perez-Carrillo, Guajardo-Flores, & Serna-Saldívar, 2019). The stronger molecular interaction between starch and protein, as shown by ATR-FTIR, was suggested to enhance the IVPD of black beans from 85.4% to 94.4% for the processed beans (de la Rosa-Millán et al., 2019).

5.2. Polysaccharides

Polysaccharides such as xanthan gum, carrageenan, and pectin have been shown to increase the viscosity of digesta, reduce protein hydrolysis rate, and slow gastric emptying rate when added to dairy and plant proteins (Niu, Xia, Jung, & Yu, 2019; Opazo-Navarrete, Freire, Boom, & Janssen, 2019), while fewer studies have explored their effects on animal protein digestibility (Lin et al., 2019).

A study involving insoluble and soluble dietary fibres from wheat bran evaluated their effects on *in vitro* protein digestion of Surimi (Pagrosomus major) (Lin et al., 2019). The IVPD decreased with an increase in the dosage of these dietary fibres. This effect could be due to any of the several mechanisms suggested in the literature, including the interaction of carboxyl and amino groups of proteins, and negatively charged groups on polysaccharides, high viscosity caused by polysaccharides leading to reduction in protein digestibility, or direct inhibition of digestive enzyme activities due to the presence of polysaccharides (Niu et al., 2019).

In the case of plant proteins, the presence of fibre and starch in the reconstituted quinoa protein concentrate/isolate significantly affected the *in vitro* gastric digestibility of quinoa protein (Opazo-Navarrete et al., 2019). The presence of starch alone reduced the rate and extent of protein hydrolysis of preheated samples at 120 °C due to the hindrance of pepsin by gelatinised starch.

5.3. Fat

Fat has been reported to hinder the protease binding sites, leading to a reduction in protein hydrolysis. The degree of protein hydrolysis of intact cotyledon cells from soybean has been reported to reduce from 37.17% to 29% when subjected to hydrolysis in the presence of lipase inhibitor (Zahir, Fogliano, & Capuano, 2018). Horstman et al. (2021) investigated postprandial aminoacidemia in older participants ($n = 10$) after consumption of dairy products differing in fat contents. They reported that the essential amino acid blood response was delayed by fat when comparing full-fat milks (UHT and pasteurised) with low-fat milks. On the other hand, Elliot, Cree, Sanford, Wolfe, and Tipton (2006) demonstrated that whole milk consumption by healthy adults

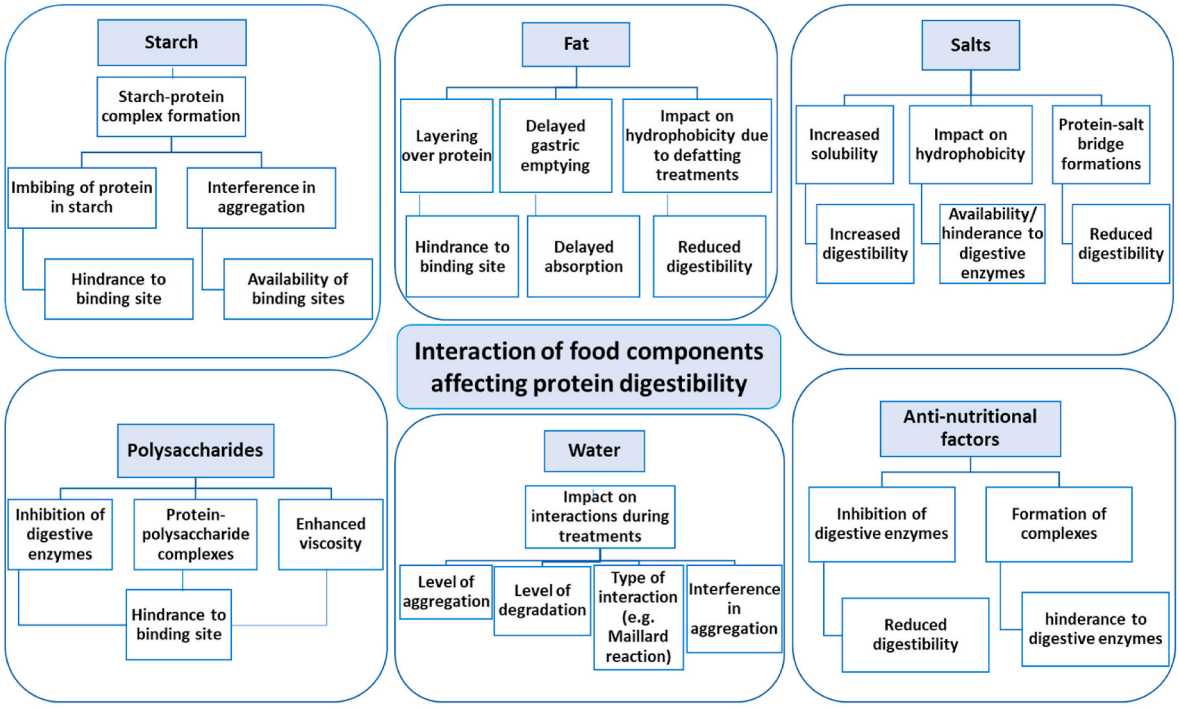

Fig. 3. The effects of different food components on protein digestibility.

after leg resistance exercise could increase the utilization of available amino acids for protein synthesis than fat-free milk.

Defatting significantly reduced beef protein digestibility in an *in vitro* digestion model (Lee, Jo, et al., 2021). This could be due to decreased sulfhydryl groups and increased surface hydrophobicity of beef protein. However, the protein digestibility of larvae samples (insect protein) was not impaired by defatting but increased slightly, which might be attributed to the structural changes in insect proteins through endogenous proteases (Lee, Jo, et al., 2021). Interestingly, greater early postprandial plasma leucine availability has been observed in young men after egg white consumption (34 ± 2%) as compared to whole eggs (25 ± 2%); however, greater stimulation of myofibrillar protein synthesis was observed in the case of whole eggs (van Vliet et al., 2017) indicating a positive impact of the presence of non-protein components such as lipids.

5.4. Salt

Salts have been reported in the literature to affect the bioaccessibility of proteins. Sodium salts are known to reduce antinutrients of legumes when added to the cooking water (Alpos, Leong, & Oey, 2021). The spatial conformation of proteins is affected by various salts as they change the surface charges of proteins. The digestibility of Surimi with NaCl was higher in the gastric phase; however, there was no significant difference after the small-intestinal phase when compared to the control (Liu et al., 2021). Salt (NaCl) destroyed the hydration layer on the protein's surface and hence provided more binding sites for digestion by pepsin (Liu et al., 2021). However, the *in vitro* digestibility of chicken sausage with high salt (1.8%) and phosphate (0.3%) concentration was found to be lower than the sample without salt or phosphate (Choi & Chin, 2021). Meat products with salt and phosphate could result in a dense and hard structure due to protein denaturation and aggregation during heating which could reduce the digestibility of proteins (Choi & Chin, 2021). Calcium salt, $CaCl_2$, was also reported to lower the protein hydrolysis of black beans when added to the cooking medium (Alpos et al., 2021).

6. Conclusions and future research directions

This review found a gap in the literature to understand how emerging protein sources respond to the new methods of processing currently being employed to process them into new food textures, in terms of their digestibility. A careful screening of alternative protein sources for their digestion rates to categorise them into rapidly digestible, slowly digestible or resistant proteins; and application of innovative ways of processing to SDP and RP to make desired changes in their structure that allow their conversion into rapidly and more completely digestible proteins will be an innovative approach. Therefore a fundamental understanding on the structural changes that occur in proteins during different types of processing will be helpful to adapt the optimum amount and type of processing required to create protein foods with improved digestibility. More research should also be directed towards prediction of protein digestibility from their structural and physicochemical characteristics. Advanced *in vitro* digestion models which are equipped with oral, gastric and small-intestinal simulations alongwith peristaltic movements are an economical way and will be particularly useful to screen a large number of samples. Developing rapidly digestible protein ingredients by physical modification of plant protein sources is an important area of research which can help in improving the nutritional properties of plant proteins. Similarly, food microstructure of naturally occurring whole foods with high protein digestibility needs to be studied to create model foods that provide higher protein digestibility. The microstructure of plant foods and how rigid cell walls, protease inhibitors, and tannins present in plant proteins limit their digestibility compared to animal proteins still needs to be fully understood.

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