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Effects of thermal, non-thermal and emulsification processes on the gastrointestinal digestibility of egg white proteins

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ABSTRACT

Background: Egg white proteins (EWPs) are an excellent source of essential amino acids for human nutrition. Also, egg white is commonly used in food processing because of its numerous technological functionalities. Processing can influence the EWPs digestibility and thereby amino acid bioavailability. A better understanding of processing effects on EWPs digestibility enables improving the nutritional value of EWP products.

Scope and approach: This review elucidates the impact of diverse processing methods on EWPs at molecular level (individual molecules), supramolecular level (aggregated state), and macroscopic level (gelled state).

Key findings and conclusions: Heat, high pressure, ultrasound, pulsed electric fields, and adsorption at interface can unfold native EWPs, causing exposure of hydrolysis sites and improvement of protein digestibility at molecular level. However, the Maillard glycation of EWPs may restrict the access of digestive enzymes to the proteins and reduce their digestibility. The Maillard reaction can also lower the IgE-binding capacity of ovalbumin, which could potentially reduce allergenicity. At supramolecular level, protein-protein interactions between the unfolded EWPs lead to the formation of aggregates with different morphologies, depending on the pH and ionic strength. The accessibility of digestive enzymes to the cleavage sites of heat-induced spherical aggregates is lower compared to the linear counterparts. However, gels formed from the linear aggregates show high resistance to digestion owing to the dense network of these gels. A combination of processes can increase the impact of digestibility. For instance, quick production of specific bioactive peptides can be achieved by applying enzymatic treatment to EWPs under high pressure.

1. Introduction

Egg is a highly nutritious food. It contains numerous essential micronutrients such as fat-soluble vitamins and minerals like zinc and selenium (Zhu, Vanga, Wang, & Raghavan, 2018). Egg proteins are generally considered an invaluable protein resource that provides all essential amino acids for human nutritional requirements (Wang, Chi, Cheng, & Zhao, 2018). Furthermore, egg proteins are known to be highly digestible: the true ileal digestibility of cooked whole egg proteins has been found to be 90.9% in humans (Evenepoel et al., 1998). Egg white protein (EWP) especially encompasses a variety of branched amino acids such as leucine (89 mg/g), isoleucine (56 mg/g) and valine (73 mg/g), which contribute to protein synthesis in skeletal muscle tissue (Hida et al., 2012). Deficiency in branched-chain amino acids can lead to liver cirrhosis (Dasarathy & Hatzoglou, 2018), urea cycle disorders (Molema et al., 2019), chronic renal insufficiency (Holeček, 2018), impaired mRNA translation and growth, and muscle wasting (Marchesini, Marzocchi, Noia, & Bianchi, 2005). The deficiency can also adversely influence synthesis of neurotransmitters, brain function and may consequently lead to Alzheimer's disease (Siddik, Moghaddam, Hegde, & Shin, 2019). Furthermore, EWPs are rich in sulfur-containing amino acids, which may increase HDL (high-density lipoprotein)-cholesterol levels (Oda, 2006).

Bioavailability of amino acids depends on protein digestibility (Böttger et al., 2019). Indeed, proteins in food must be accessible to digestive enzymes of the stomach and small intestine so that their amino

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acids can be released from the food matrix and absorbed by the intestinal epithelial cells for transportation into body cells (Horne, 2017; Singh & Gallier, 2014). Moreover, changes in digestibility may influence allergenicity of some proteins (Verhoeckx et al., 2019). Digestibility of EWPs is influenced by processing-induced changes to proteins at molecular level (individual molecules), supramolecular level (aggregated state), and macroscopic level (gelled state) (Fig. 1) (Golding, 2019).

Hen egg white contains a set of globular proteins mainly including ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), as well the enzyme lysozyme (3.4%), and fibrillar ovomucin (3.5%) (Wu, Majumder, & Gibbons, 2010). The native conformation of globular proteins might renders them resistant to enzymatic attack by burring potential cleavage sites inside the structure (Lorieau et al., 2018; Monti & Pucci, 2007). As a result, susceptibility of the proteins towards digestion is greatly affected by the alterations in their secondary and tertiary structures (Rahaman Vasiljevic, & Ramchandran, 2016). On the other hand, aggregates of various shapes (linear, branched and spherical) and sizes can be formed during processing of globular proteins, depending on solvent conditions such as pH and ionic strength, protein concentration, and temperature (Nyemb et al., 2016). It has been shown that the size and shape of the aggregated structures can influence enzyme accessibility to protein cleavage sites (Luo, Boom, & Janssen, 2015). With regard to protein gels, the digestibility strongly depends on gel microstructure, size of gel particles, and pH (Okolie, Aryee, & Udenigwe, 2018; Somaratne et al., 2020b; Thevenot, Cauty, Legland, Dupont, & Floury, 2017).

Zhu et al. (2018) reviewed the effect of processing on allergenic and structural properties of EWPs. However, they did not summarize the effect of processing on digestion of EWPs. Also, recently, Golding (2019) reviewed the effects of food microstructure on the digestion of proteins, lipids and carbohydrates present in foods. In the current paper, attention is directed towards alterations occurring in molecular conformation and aggregation of EWPs during processing and their effects on protein digestibility. The discussion is then focused separately on investigating the digestibility of EWPs in gelled state.

2. Processing operations

2.1. Conventional heat treatment

Heat treatment in the presence of water is by far the most commonly employed technique in food processing, with the aim of providing microbial safety, increasing shelf life and consumer acceptance (Gharbi, Labbafi, & Madadlou, 2017; Okolie et al., 2018). At molecular level, proteins undergo a varying degree of transition from the native state to less-folded or denatured state during heat treatment depending on time and temperature of heating, the intrinsic characteristics of proteins, and environmental factors (e.g. pH, and ionic strength) (Bloom et al., 2014; Nasabi, Labbafi, Mousavi, & Madadlou, 2017). Generally speaking, when a food protein solution is heated at temperatures between 50 °C and 60 °C, the protein tertiary structure starts to change (Zhu et al., 2018). By moderate heat treatments (60–90 °C, 1 h or less), proteins undergo alterations in secondary structure and become extensively denatured. This step is accompanied by an increase in surface hydrophobicity, and exposure of sulfhydryl groups previously hidden in the protein core (Damodaran, 2017; Van der Plancken, Van Loey, & Hendrickx, 2006). Such heat-induced changes in secondary and 3D structures of proteins lead to the exposure of cleavage sites and, as a consequence, protein digestibility is improved (Dupont & Nau, 2019, pp. 129–143).

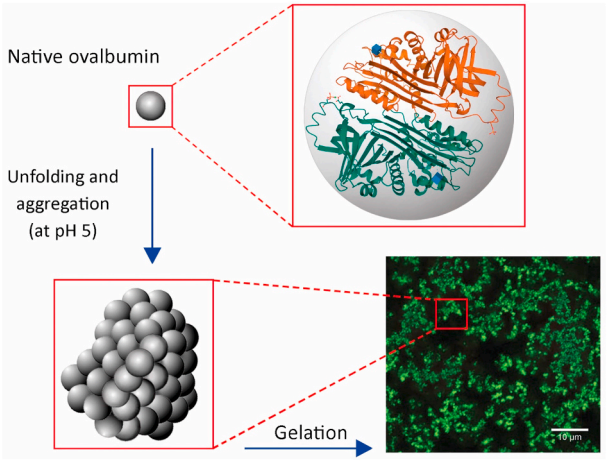

Fig. 1. Schematic representation of EWP changes at molecular level (individual molecules), supramolecular level (aggregated state), and macroscopic level (gelled state) (Somaratne et al., 2020a; Stein, Leslie, Finch, & Carrell, 1991).

EWPs heated at 65 °C for 30 min showed the highest peptic hydrolysis compared to those heated at 56 °C for 32 min and 100 °C for 5 min. It was attributed to proper denaturation of EWPs at 65 °C, which exposed hidden hydrolysis sites; whereas heat treatment of EWPs at 100 °C led to (extensive) aggregation, which could hide some cleavage sites (Wang, Qiu, & Liu, 2018). Comparably, heat treatment of milk (at 62.5 °C for 30 min) resulted in aggregation of whey proteins on casein micelles surface and shielded β-casein inside the micelles, reducing the accessibility of pepsin to β-casein (De Oliveira et al., 2017).

The heat-induced conformational changes of ovalbumin destroy IgE and IgG-reactive epitopes, which contribute to the loss of ovalbumin allergenicity (Rahaman et al., 2016; Watanabe et al., 2014). In addition to modulating digestibility and allergenicity, heat treatment may also enhance the biological availability of the essential amino acids by inactivating antinutritional factors in some protein foods (Ustunol, 2015). For example, egg white has a considerable amount of protease inhibitors such as ovomucoid with anti-tryptic activity, ovostatin with inhibitory activity against several metalloproteases and serine proteases, and ovoinhibitor inhibiting trypsin, chymotrypsin, and some fungal proteases (Wu et al., 2010). The efficacy of all these inhibitors decreases when exposed to moderate thermal treatment (60–90 °C) in the presence of water (Damodaran, 2017). Decomposition and pyrolysis of some amino acids can occur as a result of more severe heat treatments (above 200 °C), leading to loss of the essential amino acids. Potential mutagens/carcinogens may be as well produced during such treatments (Ustunol, 2015).

At supramolecular scale, formation of aggregated structures with different sizes and morphologies during heat treatment can influence digestion of EWPs (Golding, 2019; Nyemb et al., 2016a). The extent and nature of aggregation depend on solvent conditions such as pH and ionic strength, protein concentration, and temperature (Kristo & Corredig, 2014; Nyemb, Guérin-Dubiard, et al., 2014). Depending on pH and ionic strength, aggregates of four different morphologies could be generated by heat treatment of ovalbumin solutions at protein concentrations below the critical gelation concentration (Nyemb, Guérin-Dubiard, et al., 2014, b). Linear aggregates (Fig. 2) were formed at pH 9 and low ionic strength (0.03 M NaCl), where intermolecular interactions were limited due to high net charge of the protein and the lack of charge-screening by salt ions. At intermediate net charges (pH 7) and low ionic strength (0.03 M NaCl), formation of linear-branched aggregates was favored. By increasing salt concentration (0.3 M NaCl) at pH 7, the structural and morphological properties of aggregates were modulated by the screening effect of salt ions, leading to formation of spherical aggregates. Under conditions where the protein net charge and electrostatic repulsions were minimized, i.e. at pH close to the isoelectric point of ovalbumin (pH 5) and at high ionic strength, spherical-agglomerated particles (Fig. 2) were generated (Dupont & Nau, 2019, pp. 129–143).

The aggregates of different morphologies exhibited different digestive behaviors during *in vitro* studies in terms of the extent of digestion,

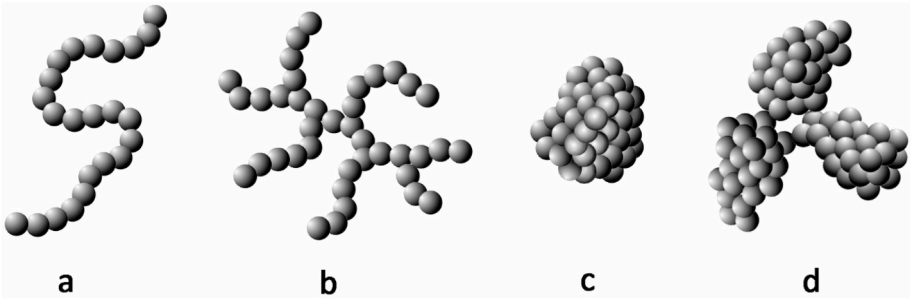

Fig. 2. Schematic representation of a) linear aggregate, b) linear-branched aggregate, c) spherical aggregate, d) spherical-agglomerated aggregate.

their susceptibility to each digestive enzyme, and the nature and relative concentration of generated peptides after digestion (Claude et al., 2016; Nyemb et al., 2014b). The extent of proteolysis was the greatest for linear aggregates that were almost entirely digested after only 10 min of *in vitro* simulated gastric digestion. The authors attributed this to the highest surface area to volume ratio of the linear aggregates, rendering greater exposure of cleavage sites in the aggregates. Another factor of considerable importance governing susceptibility to digestion of the aggregates could be the degree of unfolding of the proteins constituting aggregates (Liu, Oey, Bremer, Silcock, & Carne, 2018; Nyemb, Guérin-Dubiard, et al., 2014). As mentioned earlier, linear aggregates are formed under conditions where the net charge of protein molecules is very high. Such conditions favor the unfolding of proteins, especially at extreme alkaline pH values (Ustunol, 2014). The latter can be attributable to ionization of partially buried carboxyl, phenolic and sulfhydryl groups trying to migrate to the aqueous environment, which leads to unraveling of the polypeptide chain (Damodaran, 2017). Therefore, linear aggregates formed at pH 9 had most likely a greater degree of protein unfolding in addition to the high surface area to volume ratio, both of which contributing to the exposure of cleavage sites. In accordance, spherical-agglomerated assemblies of ovalbumin exhibited considerable resistance to *in vitro* digestion, as a result of the lowest degree of protein unfolding and the lowest surface area to volume ratio. It was reported that linear-branched and spherical aggregates showed intermediate digestive behaviors (Dupont & Nau, 2019, pp. 129–143; Nyemb, Guérin-Dubiard, et al., 2014).

Regarding the influence of aggregate morphology on susceptibility to each digestive enzyme, it was observed that *in vitro* tryptic cleavage occurred predominantly on spherical agglomerates as compared to the other morphologies (Nyemb et al., 2014b). Furthermore, the studies conducted by Nyemb et al. (2014a, b) showed that the nature and relative concentration of the released peptides could be tailored by modulating the aggregate morphology; the proteolysis regions were morphology dependent. For instance, the peptides released after digestion of the linear aggregates were mainly derived from the surface regions of parent (native) ovalbumin. Interestingly, even after extensive *in vitro* digestion, the peptide composition of the digestate considerably differed depending on the initial aggregate morphology (Nyemb et al., 2014b). Studies on the digestion of aggregated EWPs have applied *in vitro* digestion models, which gain mechanistic understanding of the digestion process of the aggregates. In order to ensure physiological relevance of the outcomes concerning the relationship between aggregate morphology and the release of peptides, future digestion studies using *in vivo* approaches on the various types of EWP aggregates are needed.

2.2. Dry heating

Dry heating process of EWPs refers to the storage of protein powder in a hot room under controlled temperature and humidity conditions (Mine, 1997). In egg processing industry, dry heating is usually performed with the aim of ensuring the microbiological safety, and/or improving the gelling, emulsifying, and foaming properties of egg white powders (Lechevalier et al., 2015; Van der Plancken, Van Loey, & Hendrickx, 2007).

It has been shown that protein powders are extremely stable to thermal denaturation due to the fact that molecular chain mobility in proteins is restricted in dry state (Damodaran, 2006). To elucidate this, it is necessary to point out the role of water. Water can act as an effective plasticizer for polypeptide chains. In water, swelling of proteins occurs as a result of inclusion of water molecules into the surface cavities of proteins. The swelling leads to an increase in chain mobility and flexibility of protein molecules. Consequently, access of water to salt bridges and peptide hydrogen bonds of such a flexible protein structure during thermal treatment is greater than that in the dry state (Damodaran, 2017). Therefore, it is not surprising that dry heating only induces mild conformational changes in the molecular structure of proteins in comparison with heat and high-pressure treatments in solution (Van der Plancken et al., 2007). In agreement, at higher moisture contents of the powder samples, the degree of conformational changes is more pronounced (Van der Plancken et al., 2007).

The extent of conformational alterations and aggregation of proteins during dry heating process depends on several factors including temperature and time of the process, moisture content, and pH (Mine, 1996). Whereas slightly to moderately dry heated EWP powders (several days at 60 °C and 70 °C, 1 or 2 days at 80 °C, and 1 day at 90 °C) were observed to be more prone to *in vitro* digestion, intense dry-heating of EWP powders (over 2 days at 80 °C or 90 °C) slowed down the digestion (Lechevalier et al., 2015). Since intense dry heat treatments were shown to promote protein aggregation, causing the formation of large aggregates, it was assumed that such aggregated structures prevent the digestive enzyme from accessing cleavage sites (Lechevalier et al., 2015). Moreover, dry heating of EWP resulted in modification of protein antigenicity that depended on the protein considered. Whereas lysozyme antigenicity decreased when dry heating temperature increased, a surprising opposite effect was observed for ovotransferrin antigenicity, and no significant effect was observed for ovomucoid. Despite these results were obtained using monoclonal antibodies, which by definition interact with specific epitopes, an overall agreement with literature was thus revealed (Lechevalier et al., 2017).

2.3. Non-enzymatic browning

The Maillard glycation or non-enzymatic browning is a complex set of reactions initiated by an interaction between free amino groups of proteins and carbonyl groups of reducing sugars (Damodaran, 2017). In the food industry, glycation of edible proteins is mainly performed for improving their functional properties, such as emulsifying and foaming, solubility and gelling capacity, which make the glycation a promising technique for protein modification (Karbasi & Madadlou, 2018; Liu, Ru, & Ding, 2012; Nooshkam & Madadlou, 2016; Oliver et al., 2006). This reaction may also occur naturally and slowly during storage of food ingredients or more rapidly during regular meal preparation such as frying, baking, and cooking (Huber & BeMiller, 2017). While heating

itself is associated with an improvement in protein digestibility due to protein unfolding, and enhanced susceptibility to enzymatic proteolysis, cross-linking between amino acid and sugar during the Maillard reaction can cause a loss in bioavailability of the amino acids and decrease the digestibility of proteins (Golding, 2019; Teodorowicz, Van Neerven, & Savelkoul, 2017). The involvement of lysine in the Maillard reaction results in a major loss in the bioavailability of this essential amino acid (Damodaran, 2017). The oxidation of several other amino acids such as arginine, methionine, tryptophan, and histidine can as well occur as a consequence of the formation of reactive unsaturated carbonyls and free radicals during the Maillard reaction. Cross-linking of proteins by dicarbonyl compounds, which are intermediate products in the Maillard reaction, together with structural rearrangements and aggregation can have detrimental effects on protein solubility and digestibility (Damodaran, 2017). Moreover, the steric hindrance exerted by the sugar molecules attached to protein chains might restrict the access of digestive enzymes to the proteins and lower the reactivity of the enzymes (Corzo-Martínez, Soria, Belloque, Villamiel, & Moreno, 2010). It can also affect the ability of enzymes to hydrolyze particular sequences on a protein chain, which results in the formation of different polypeptide fragments (Golding, 2019).

For ovalbumin-glucose mixture, prolonged incubation time (96 h, 50 °C and 0.65 water activity) led to formation of high molecular weight aggregates. In fact, glucose could promote ovalbumin aggregation because of the rapid degradation of Amadori compounds into Maillard reaction products that show high reactivity toward other molecules. The ovalbumin aggregates formed during glycation could resist the gastric digestion. Unlike the impaired ovalbumin *in vitro* digestion by glycation, the digestion of ovomucoid, which is a heat-resistant glycoprotein, was not affected by this reaction (Jimenez-Saiz, Belloque, Molina, & López-Fandino, 2011).

Interestingly, several studies also reported an improvement in various proteins' solubility and *in vitro* digestion by the Maillard reaction (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Hiller & Lorenzen, 2010; Tanabe & Saeki, 2001; Wang & Ismail, 2012). The discrepancies observed most likely stem from differences in structural and physicochemical characteristics of the protein that is studied, as well as from the variety of experimental conditions used for the Maillard reaction (Huang et al., 2013; Teodorowicz et al., 2017). These reaction variables that control the degree of glycation include temperature and time of heating, pH, water content, type of sugar, and ionic strength of the medium (Huber & BeMiller, 2017). However, it is important to distinguish between different modifications when assessing the effect of glycation on digestion. For instance, Nooshkam and Madadlou (2016) observed that the Maillard conjugation enhanced gastric digestion of whey proteins, but this enhancement was probably more related to thermal denaturation of whey proteins. Generally, it seems that thermal denaturation could improve the digestibility of egg proteins during Maillard conjugation, while aggregation and the formation of the Maillard reaction products can reduce digestibility.

Moreover, some Maillard reaction products (MRPs) might resist to the action of gastric and intestinal digestive enzymes and reach the large intestine where they will be degraded by the human colonic microbiota. These products may affect *in vivo* the growth of colonic bacteria and modulate their composition (Ames, 2009; Seiquer, Rubio, Peinado, Delgado-Andrade, & Navarro, 2014). While some of the MRPs such as reductones are antioxidant due to their reducing power and metal chelation ability (Damodaran, 2017), others are detrimental to health. Further modification of Amadori compounds by dehydration, oxidation, rearrangement or other reactions causes the formation of a group of heterogeneous compounds such as pyrraline, imidazolone, pentosidine and carboxymethyllysine (CML), also known as advanced glycation end products (AGEs) (Davis, Prasad, Vijayagopal, Juma, & Imrhan, 2016; Liu, Teodorowicz, Wichers, van Boekel, & Hettinga, 2016). AGEs have been found to represent a pathogenic factor in aging and in chronic inflammatory diseases. There is also evidence that AGEs play a role in food allergy (Teodorowicz et al., 2017).

Potential allergenicity of the EWPs could also be influenced by glycation. The effectiveness of glycation in lowering antibody binding was reported to be dependent on the intensity of treatment and the intrinsic resistance of allergens to denaturation and digestive enzymes (Benedé, López-Expósito, Molina, & López-Fandiño, 2015). Enzyme-linked immunosorbent assay (ELISA) with human sera indicated that the ovalbumin glycated for 96 h with glucose had a lower IgE-binding capacity than native ovalbumin, attributed to the irreversible changes in the structure of protein causing the loss of conformational epitopes. Concerning ovomucoid, heating in the absence of sugar reduced its IgE-binding capacity, but glycation for 96 h increased its IgE-binding activity. It might be due either to the formation of new epitopes or to the fact that glucose favored recognition by IgE antibodies (Jiménez-Saiz, Belloque, Molina, & López-Fandino, 2011).

2.4. Emulsification

Emulsification is used in the food industry for the production of a wide range of products such as beverages, desserts, sauces, dressings, and dairy products (McClements, 2015). EWPs can be exploited as emulsifiers to stabilize edible emulsions due to their amphoteric nature and film-forming abilities (Chang et al., 2016).

It is often observed that protein adsorption at the oil-water interface increases its susceptibility to digestion, since protein conformation rearranges at the interface, resulting in the exposure of susceptible domains to hydrolytic enzymes (Li Zhai, Day, Aguilar, & Wooster, 2013). Besides, the non-adsorbed proteins which remain in the aqueous phase of an emulsion might undergo further peptic digestion (pepsinolysis) compared to counterpart proteins in a monophasic solution. The higher peptic digestibility of the non-adsorbed proteins to the oil-water interface has been ascribed to the homogenization-induced conformational alterations of proteins or the exchanges between adsorbed and non-adsorbed proteins during digestion (Nik, Wright, & Corredig, 2010; Sarkar, Goh, Singh, & Singh, 2009; Singh, & Ye, 2013).

In a study on digestibility and allergenicity of emulsified hen egg, Jiménez-Saiz, Ruiz-Henestrosa, López-Fandiño, and Molina (2012) compared *in vitro* digestion and human IgE-binding capacity of egg proteins in emulsion and in solution. It was found that because of the slightly higher digestibility of egg white allergens, IgE-binding of the gastroduodenal digestate of the emulsion was lower when compared to the solution. Nonetheless, the pepsinolysis extents of EWPs in emulsion and in solution were not remarkably different. The preferential adsorption of egg yolk lipoproteins (which were present together with EWPs) to the oil-water interface prevented significant conformational changes of EWPs upon emulsification (Benedé et al., 2015; Drakos & Kiosseoglou, 2008). Similarly, in an oil-in-water emulsion stabilized by β-lactoglobulin, some adsorbed β-lactoglobulin molecules remained inaccessible to pepsin since they were not completely unfolded. In fact, these adsorbed β-lactoglobulin molecules retained their native structure at the interface and remained resistant to pepsin digestion (Mackie & Macierzanka, 2010; Sarkar et al., 2009).

The digestion behavior of proteins in an oil-in-water emulsion can be affected by interactions with physiological surfactants, e.g. phosphatidylcholine (PC) and bile salts (BS). The introduction of vesicular PCs into simulated gastric fluid may lead to adhesion of PC vesicles to the adsorbed protein layer by weak electrostatic interactions and subsequent infiltration of PC through the protein layer. The oil droplets are then gradually covered by PC, associated with the removal of proteins from the oil-water interface. The competitive displacement of proteins from the interface causes protein digestion to take place mainly in the aqueous phase of emulsion, which could lower the difference between the digestibility extents (rates) of protein in solution and in emulsion (Jiménez-Saiz et al., 2012; Macierzanka, Sancho, Mills, Rigby, & Mackie, 2009). Other gastrointestinal enzymes, such as gastric and pancreatic lipases may play a significant role in the modification of

emulsion structure via hydrolysis of triglyceride oil phase into mono- and diglycerides as well as surface-active free fatty acids. As a result, the physicochemical properties of emulsions being digested and hence the digestion behavior of adsorbed proteins might change (Macierzanka et al., 2009; Singh & Ye, 2013). Likewise, the peptides produced as a consequence of gastrointestinal proteolysis can affect the physicochemical properties of emulsions. Fang, Bao, Ni, Choijilsuren, and Liang (2019) indicated that *in vitro* proteolysis of whey protein isolate-stabilized emulsions led to the exposure of more electrically charged residues, which stabilized the oil droplets by electrostatic repulsion. Moreover, the initial interfaces were displaced by some amphiphilic peptides, which protected the emulsion against complete coalescence (Fang et al., 2019). It is worth noting that emulsion coalescence and destabilization in the stomach can affect the gastric emptying rate (De Oliveira et al., 2016, 2017). Therefore, destabilization of emulsions stabilized by EWPs in the harsh condition of human gastric environment and its effect on the gastric emptying rate can be an interesting area for further research.

2.5. High pressure processing

High pressure processing as a well-established and innovative technology is employed in food industry to inactivate microorganisms and reduce the loss of nutrients, contributing to the development of novel food products (San Martin, Barbosa-Cánovas, & Swanson, 2002). This processing method is usually applied, either in the form of a static process in a vessel under pressures of 300–700 MPa, known as high hydrostatic pressure (HHP), or a dynamic process based on forcing a pressurized fluid (up to≈320 MPa) to pass through a small orifice, called high pressure homogenization (Bevilacqua, Campaniello, & Sinigaglia, 2010; Martínez-Monteagudo & Balasubramaniam, 2016; Patrignani, Siroli, Serrazanetti, & Lanciotti, 2018).

High pressure causes structural changes in proteins under various sets of processing conditions (pressure level, holding time, and temperature) and environmental factors (pH, ionic strength, protein type and concentration). Depending on the treatment and environmental variables chosen, high pressure can alter the quaternary, tertiary, and secondary structures of proteins (Hoppe, 2010; Yang & Powers, 2016). Irreversible denaturation with different degrees of structural modifications occurs at pressures greater than 300 MPa (Yang & Powers, 2016). These changes are associated with partial unfolding and the exposure of buried sulfhydryl and hydrophobic groups and cleavage sites of protein molecules (Van der Plancken et al., 2007). Unlike severe heat treatments, HHP processing (up to 1500 MPa) has no effect on covalent bonds from the primary structure of food proteins (Hoppe, 2010; Martínez-Monteagudo & Balasubramaniam, 2016). It is due to the fact that hydrostatic pressure principally changes the distance between molecules. This directly influences distance-dependent interactions including van der Waals interactions, hydrogen-bonding, electrostatic and hydrophobic interactions (Martínez-Monteagudo & Balasubramaniam, 2016). For ovalbumin solution (15% w/w), it was reported that the amount of β-turns became doubled under HHP treatments at 400–600 MPa, but the contents of β-sheet and α-helix slightly decreased. The alterations caused ovalbumin partial unfolding and greater molecular flexibility. In fact, β-turn structure has low hydrogen bonds, which leads to an increase in the flexibility of protein molecules (Ngarize, Herman, Adams, & Howell, 2004; Sheng, Wang, Huang, Xu, & Ma, 2016).

In contrast, the cleavage of the covalent bonds in protein structure can be possible by high pressure homogenization provided that protein molecules have long-chain fibrillar structure instead of globular. The extent of cleavage was reported to be dependent on the level of applied energy, considering the binding energy of covalent bonds (330–400 kJ mol^{-1} for peptide bonds and 213 kJ mol^{-1} for disulfide bonds) (Brand & Kulozik, 2016). It was shown that high pressure homogenization of ovomucin, which is a fibrillar protein forming a network through disulfide bridges, could cleave its covalent peptide bonds and modify disulfide bridges of the network (under pressures up to 100 MPa). At the intermediate state of this process, reduction of fibril size and destruction of ovomucin network occurred due to the breakage of peptide bonds in ovomucin molecule and the cleavage of network SS bridges into SH, respectively (Brand & Kulozik, 2016; Brand, Silberbauer, & Kulozik, 2016). An increase in energy input level resulted in greater amounts of small fibrils. Finally, a network of very small fibrils could be partially re-formed by building new SS bridges owing to the high reactivity of SH groups at pH values above 7 (Fig. 3) (Brand & Kulozik, 2016). Surface hydrophobicity, solubility, and digestibility of ovomucin can be improved by increasing its dissociation degree (Brand et al, 2014, 2016; Omana, Wang, & Wu, 2010). The pressure-induced changes at molecular scale, including increased molecular flexibility and greater exposure of potential cleavage sites, can lead to higher susceptibility to enzymatic proteolysis (Van der Plancken et al., 2005a, 2007).

The structural alterations induced by high pressure can also promote protein-protein interactions, leading to aggregation (Ngarize et al., 2004; Yang & Powers, 2016). Applying hydrostatic pressure in the range of 400–700 MPa led to the formation of aggregates stabilized mainly by SS bonds in EWP solution (9.6 mg/ml). By increasing pressure (from 400 to 700 MPa), protein solubility decreased and turbidity of solution increased, which is an indication of promoted aggregation. However, susceptibility of pressure-treated EWPs to enzymatic hydrolysis is improved (Van der Plancken et al., 2005 a, b; 2007). Moreover, proteolysis of ovalbumin can be promoted through simultaneous high pressure and proteolytic enzymatic treatment. In a study by Quiros et al. (2007), the impact of high hydrostatic pressure on the release of bioactive peptides from ovalbumin was evaluated. It was observed that enzymatic treatment of ovalbumin with trypsin, chymotrypsin, and pepsin under high pressure, up to 400 MPa, resulted in an improvement in its hydrolysis and accelerated the release of bioactive peptides in comparison with pre-pressurization.

The pressure-induced structural alterations in EWPs could reduce their allergenicity by destroying allergenic epitopes of EWPs (López-Expósito et al., 2008). Besides, HHP processing is less likely to initiate harmful chemical reactions compared to heating. While heat-induced EWP gelation produced lysino-alanine (known to be a renal toxic factor), this compound was not formed in HHP-induced EWP gels (Hoppe, 2010).

High pressure processing can be additionally applied in combination with non-proteolytic enzymes such as transglutaminase (TG). The combinatory use of HHP and the enzyme TG treatment, either simultaneously or sequentially increases susceptibility of EWPs to TG-mediated crosslinking. The HHP causes EWPs unfolding, making most buried glutamine and lysine residues accessible to TG. This catalyzes the formation of covalent inter and intramolecular bonds by TG between glutamine and lysine residues (Ma, Lozano-Ojalvo, Chen, Lopez-Fandiño, & Molina, 2015). Actually, the simultaneous application of HHP and TG led to a relatively greater extent of crosslinking and consequently a higher amount of large aggregates. The TG-induced crosslinking counterbalanced the enhanced protein digestibility by HHP treatment. Furthermore, IgE-binding and immuno-stimulatory properties of the HHP-TG-treated ovalbumin, as assessed by ELISA were not significantly different from those of native ovalbumin (Ma et al., 2015).

Addition of NaCl or sucrose to ovalbumin solution (2 mg/mL) prior to HHP (600–800 MPa) could increase sensitivity to trypsin proteolysis. Under the HHP in the presence of sucrose or NaCl (10%), ovalbumin lost its compactness but remained in a soluble form. On the contrary, in the absence of NaCl or sucrose, most of the proteins lost their solubility and no significant increase in ovalbumin digestibility was observed (Iametti et al., 1998; Quirós et al., 2007).

An inverse relationship between the processing temperature during HHP and subsequent digestibility of EWPs has been reported. When HHP processing of EWPs was performed at 700 MPa and at 40 °C (below the denaturation temperature), a higher proteolysis resistance was

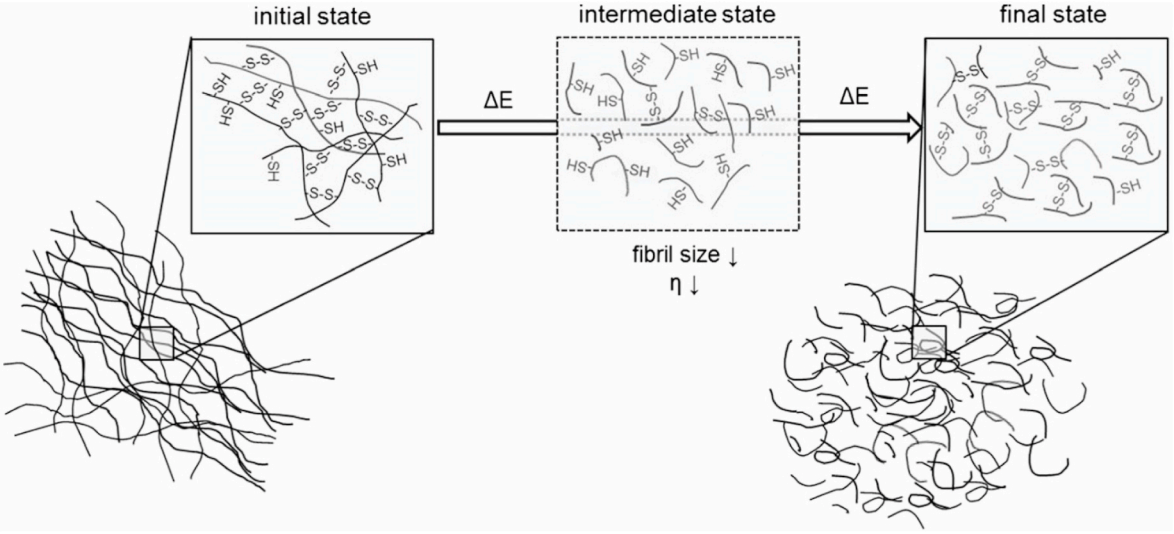

Fig. 3. Scheme of the changes to SH and SS in ovomucin due to mechanical treatments (ΔE energy input, fibril size↓ means fibril size decrease, η↓ means viscosity decrease). By ΔE, the SS bonds (initial state) were broken into free SH groups (intermediate state). Because of the small fibril size and low viscosity, the oxidation of free SH to SS and SH-SS exchanges were facilitated (final state) at high pH (Brand & Kulozik, 2016).

observed compared to HHP processing at lower temperatures (10 °C, and 25 °C). Similarly, for a lower pressure (600 MPa), lowering the treatment temperature (from 40 °C to 10 °C) increased the susceptibility of EWP solutions to enzymatic hydrolysis (Van der Plancken et al., 2004, 2005a).

2.6. High intensity ultrasound (HIU)

Ultrasound (US) is composed of a series of sound waves with frequencies beginning at 16 kHz, which is near the upper limit of the human hearing (Bermúdez-Aguirre, Mobbs, & Barbosa-Cánovas, 2011). The sound ranges employed can be classified into low and high intensity based on frequency range. Low intensity, which is called low power or low energy, possesses frequencies higher than 100 kHz at intensities below 1 W cm^{-2} (Güzey, 2002). This type of ultrasound is non-invasive and non-destructive. High intensity ultrasound (HIU) with frequencies between 16 and 100 kHz at intensities higher than 1 W cm^{-2}, also called high power or high energy, is disruptive and can have effects on the physical, chemical, or biochemical properties of foods (Wang & Guo, 2019). The effect of ultrasound is related mainly to cavitation. Once formed, cavities successively expand and compress, progressively enclosing gas and (water) vapor from the surrounding solution. The cavities finally burst due to the extreme internal pressure that increases temperature to roughly 5000 K. However, before the explosion, the water vapor is thermolyzed to free radicals, which recombine but might as well end to the surrounding solution upon cavity explosion. Accordingly, the influence of ultrasonication relies on shear forces and hot spots caused by cavitation, microcurrents generated around the oscillating cavities and free radicals released into the being-treated solution (Madadlou, Mousavi, Emam-Djomeh, Ehsani, & Sheehan, 2009).

HIU can be applied for modifying the structures of food proteins and altering their physical and functional properties, including rheological and emulsifying behaviors, surface hydrophobicity, solubility, and foaming capacity (Stefanović et al., 2014a). HIU-induced modifications are closely related to the type of proteins treated and several processing parameters such as intensity, frequency, temperature, presence of gases, pH of protein solution and solvent properties (vapor pressure, viscosity and surface tension of the solvent) (Stefanović et al., 2014a; Weiss, Gulseren, & Kjartansson, 2011).

Information regarding the digestive behavior of HIU-treated EWPs is currently scarce. Therefore, in this section, the non-digestive proteolysis of HIU-treated EWPs is discussed. Enzymatic hydrolysis of EWPs appeared to be improved by controlled ultrasound treatments under optimal conditions due to the structural changes in the protein molecules. The content of free sulfhydryl groups and surface hydrophobicity of EWPs during HIU treatment (0–480 W for 10 min) was reported to increase at first and then decline. This indicated that HIU-induced unfolding caused the inner hydrophobic and sulfhydryl groups exposure and disulfide bond breakdown at first. The increase in sulfhydryl groups might be caused by the degradation of ovomucin subunits by HIU (Sheng et al., 2018), which is again ascribed to the long-chain fibrillar conformation of ovomucin (Brand & Kulozik, 2016; Sheng et al., 2018). The decreased content of free sulfhydryl groups and surface hydrophobicity by increasing ultrasound power was attributed to protein aggregation or thiol oxidation by the hydrogen peroxide formed as a result of ultrasonic cavitation (Sheng et al., 2018). Lei, Majumder, Shen, and Wu (2011) observed that HIU above 60 kHz (for more than 4 min) resulted in structural alterations in ovotransferrin. The treatment caused generation of hydroxyl and hydrogen free radicals in ovotransferrin solution (5%). The structural changes associated with the production of free radicals led to the breakdown of intramolecular native SS bonds in the ovotransferrin molecules, generating new SH groups in protein. Despite the considerable increase in reactive sulfhydryl groups, there was no improvement in the overall degree of ovotransferrin hydrolysis by thermolysin (Lei et al., 2011). On the other hand, the breakage of SS bonds could alter the spatial configuration of ovotransferrin and favor the release of bioactive peptide sequences, which were encrypted in the ovotransferrin molecule. Short-time HIU treatment of EWPs (frequency of 40 kHz for 15 min) at a low temperature (25 °C) caused an increase in alcalase hydrolysis, which could be attributed to the enhanced accessibility to the cleavage sites of EWPs. A significant decrease in the hydrolysis rate of EWPs was observed when the calorimetric power, treatment time and temperature were increased (at 35 kHz and 55 °C from 15 to 60 min). Formation of protein aggregates which lowers the accessibility of the appropriated protease to the unfolded protein cleavage sites accounts for the reduced hydrolysis rate (Stefanović et al., 2014a).

Regarding the effect of substrate pH during HIU treatment, the highest hydrolysis rate of EWP solutions was reported at high pH values (in the pH range of 9–10). This could be due to limited aggregation and greater solubility and unfolding of proteins, as well as denaturation and inactivation of ovomucoid (Knežević-Jugović et al., 2012; Stefanović et al., 2014a). At higher pH levels, further away from the average pI of the major EWPs, the electrostatic repulsion between EWPs was stronger,

which led to smaller aggregates and higher EWP solubility. Thus, at higher pH values, the maximum proteolysis rate was observed for sonicated EWP. In addition, the exposure of EWPs to extreme pH conditions might lead to the significant unfolding of protein molecules, which increased the susceptibility to alcalase hydrolysis (Stefanović et al., 2014a). On the other hand, the SH-SS exchanges between EWPs containing cysteine residues may reduce the inhibitory activity of protease inhibitors such as ovomucoid. In fact, at alkaline pH, the reactive SH groups could interact with native-intramolecular SS bonds of ovomucoid. The SS-SH exchanges caused the formation of non-native SS bonds in ovomucoid, resulting in the irreversible denaturation of ovomucoid. The processes could inactivate ovomucoid inhibitory activity against alcalase, improving proteolysis (Knežević-Jugović et al., 2012).

2.7. Pulsed electric fields

Pulsed electric fields (PEF) is an emerging non-thermal technology for pasteurization of liquid foods by exposure to electric fields of high voltage (10–50 kV/cm) for durations ranging from micro-to milliseconds (Zhao, Yang, & Zhang, 2012). Protein molecules undergo alterations during PEF processing due to ionization of some chemical groups or rupturing of electrostatic interactions in their structure. These changes are associated with the exposure of previously buried hydrophobic regions or sulfhydryl groups, leading to the unfolding and subsequent aggregation of proteins (Guo & Shen, 2019; Wei et al., 2018; Zhao, Tang, Lu, Chen, & Li, 2014). The PEF-induced unfolding depends on various parameters such as energy input, field strength, and treatment time as well as electrical conductivity and pH of solution (Martın-Belloso & Soliva-Fortuny, 2011).

Comparing PEF treatment at an energy input of 690 kJ/kg with heat treatment at 80 °C, under different pH conditions (pH 4–9), indicated that the PEF processing at pH 4 increased the digestibility of ovomucin-depleted egg white at a similar level to that induced by heating at 80 °C for 10 min (regardless of the pH of the heat-treated solutions) (Liu, Oey, Bremer, Silcock, & Carne, 2017). Noteworthy is the extent of aggregation induced by these treatments. While heating at 80 °C caused a noticeable protein aggregation and turbidity, PEF treatment at pH 4 (690 kJ/kg) could maintain the high protein solubility and the solution clarity. These make the application of PEF suitable for protein-fortified drinks, where neither turbidity nor precipitation is desired (Liu, Oey, Bremer, Silcock, & Carne, 2017). By evaluating the effect of pH (4, 5, 7, and 9) and PEF on the *in vitro* peptic digestion of ovomucin-depleted egg white, it was observed that PEF at pH 5 and high energy input (690 kJ/kg) led to noticeable ovotransferrin aggregation and insolubilization. However, such aggregation did not significantly alter the pepsinolysis of ovotransferrin, which is highly susceptible to pepsin digestion in its native form. When the PEF-induced ovotranferrin aggregates were subjected to pepsinolysis, these insoluble aggregates were quickly hydrolyzed. In contrast, PEF under the same conditions induced no aggregation of ovalbumin and lysozyme. Furthermore, the resistance of ovalbumin and lysozyme to pepsinolysis remained unaffected by PEF (pH 5, 690 kJ/kg), attributed to the lack of sufficient structural modification (Liu, Oey, Bremer, Carne, & Silcock, 2017; b, 2018).

It was reported that at alkaline pHs and energy inputs above 2700 kJ/kg (field strength above 25 kV/cm), electric field could strengthen the electrostatic attractions between oppositely charged proteins, which enhances the aggregation by electrostatic and hydrophobic attractions. Under these conditions, lysozyme could form insoluble aggregates with ovalbumin by two major mechanisms: (i) the electrostatic attraction between the positively charged lysozyme (provided that pH is lower than 10) and negatively charged ovalbumin and (ii) the hydrophobic interactions between the partially unfolded ovalbumin and the lysozyme (Wu, Zhao, Yang, & Chen, 2014; Wu, Zhao, Yang, & Yan, 2015; Wu, Zhao, Yang, Yan, & Sun, 2016). Although not investigated, such protein aggregation might influence protein digestibility.

3. Digestibility of EWPs in gelled state

There are differences between the digestion of protein gels and protein solutions. These differences may be due to the fact that protein substrate is immobilized in a network in the gelled systems, and also that there is steric hindrance against the diffusive ingress of pepsin and the egress of peptides generated during digestion (Golding, 2019; Luo et al., 2015). This explains why protein gels are slowly digested in comparison to protein solutions (Luo et al., 2015). The most important factors that influence the digestibility of protein gels include the degree of structural alterations at molecular level, pH, and microstructure of gel as well as size of gel particles (Dupont & Nau, 2019, pp. 129–143; Golding, 2019).

3.1. Structural alterations at molecular level

The structural alterations in individual molecules, induced through various treatments such as heating and high pressure processing can also influence the digestibility of protein gels. The degree of these alterations depends on the type of treatment and its conditions, as explained earlier for different processing methods.

Pepsin digestion of HHP-induced EWP gels produced at 800 MPa was higher than that of heat-induced EWP gels formed by heating at 95 °C. It could be attributed to the greater protein unfolding, degradation, solubility, and flexibility caused by HHP treatment (Hoppe, Jung, Patnaik, & Zeece, 2013; Van der Plancken et al., 2005b). Hoppe et al. (2013) observed a conversion of a very soft gel to a more solid gel when the pressure was raised from 600 to 800 MPa. This caused the greatest pepsin hydrolysis for ovalbumin and other EWPs. In fact, as pressure increased from 600 to 800 MPa, a reduction in α-helix content and increase in β-sheet took place. The changes led to an increase in digestion and health benefits of EWP gels, accompanied by the release of more bioactive peptides from EWPs (Hoppe 2010, 2013). In another study, heat-induced ovalbumin gels showed a greater increase in β-sheet structure as compared to HHP-induced ovalbumin gels. Moreover, the involvement of β-sheet structures was less intense in HHP-induced gelation (Ngarize et al., 2004). Similarly, Nyemb et al. (2016a) observed the increase of *in vitro* digestion of heat-induced EWP gels when the proportion of β-sheet structures increases.

3.2. Gel pH

An important factor in disintegrating protein gels is the performance of digestive enzymes. Gel pH can influence the activity of enzymes (Luo et al., 2015). Luo et al. (2015) investigated the digestibility of EWP gels at different pH values. They showed that increasing pH from 1.8 to 3.0 decelerated the disintegration of protein gel. This was due to the lower activity of pepsin at pH 3.0, since pepsin exhibits an optimum activity at pH of ~2 (Lamond et al., 2019; Luo et al., 2015).

It has been reported that at pH values higher than 6.5, the apparent size of pepsin increases, which may hinder its diffusion through the gel matrix manufactured at such pH values. The repulsive forces between the protein molecules and digestive enzymes in gel networks at pHs 9.0 and 2.0 are expected to be strong due to the highly charged proteins, with the exception of pepsin at pH 2.0 due to its very low pI. In contrast, for gels prepared at pHs close to the pI of proteins, the electrostatic repulsions should be minimal. This pH effect can also contribute to the degree of proteolysis (Campos & Sancho, 2003; Nyemb et al., 2016 a). However, electrostatic interactions between EWPs and pepsin due to specific pH conditions would be less influent than the differences of gel microstructure due to different pH gelation (Somaratne et al., 2020a).

3.3. Gel microstructure

Porosity and tortuosity of protein gel network, as two main microstructural parameters, significantly influence the digestion of protein gels (Lorieau et al., 2018). A slower rate of disintegration has been

reported for EWP gels with compact-dense microstructure, leading to a slower kinetics of nutrient release from the gels when compared to EWP gels with looser microstructure (Somaratne et al., 2020b). Higher protein concentration results in an increase in the tortuosity and a decrease in the pore size of protein gels, leading to a limited ability of enzyme to diffuse into the gel network (Golding, 2019; Luo et al., 2015). Preparation of egg white gels at 90 °C caused formation of a more compact microstructure than preparation at 120 °C and 140 °C. A much slower hydrolysis of the gels prepared at the lower temperature was explained by the decreased porosity of their microstructure. Previous studies on the gelation mechanism of EWPs thermal gels showed that aggregation in a mixture of all EWPs occurred at two distinct coagulation temperature ranges. At the first range, 61.5–62.5 °C, ovotransferrin and possibly some other protein(s) were partially aggregated. At the range of 71.0–73 °C, aggregation of EWPs was accelerated and the main body of the gel was formed (Campbell, Raikos, & Euston, 2003; Xue et al., 2020). Heating at elevated temperatures, especially those higher than 120 °C, could cause progressively lower protein solubility and weakened hydrogen bonding (Opazo-Navarrete, Altenburg, Boom, & Janssen, 2018). Compaction of gel network can also result from extensive syneresis of gel, which inhibits enzymes diffusion into the interior of the gel structure (Golding, 2019). The presence of thickening and gelling biopolymers can influence the microstructural attributes of protein gels. Composite EWPs/gellan gels were shown to have denser microstructures than the single-component EWP gels. The enrichment with gellan gum caused a decrease in *in vitro* degradation of the gels (Babaei, Khodaiyan, & Mohammadian, 2019). In composite EWP/gelatin gels, porosity of gel network significantly reduced with increasing gelatin content (0, 0.3, 0.4 and 0.5%). The extent of *in vitro* gastric degradation of mixed gels was lower than that of gelatin-free EWP gel (Babaei, Mohammadian, & Madadlou, 2019).

A noteworthy point is the role that the morphology of aggregates (ranging from strands to particulates) plays in determining the structural properties of protein gels. Four different types of egg white gels thus formed under various physicochemical conditions (Fig. 4).

3.3.1. *Fracturable gel (pH ~2/ionic strength 0.05 M)*

Under highly acidic conditions (pH 2) and low ionic strengths, the protein net charge and electrostatic repulsions are high, and proteins tend to denature rather than aggregate; thus, the intermolecular interactions are limited. In such conditions, formation of semi-flexible fibrils probably only by hydrophobic interactions is favored. These fibrillar aggregates, which are rich in strongly hydrogen bonded β-sheets, can make a fracturable filamentous gel with a very tight, dense and homogeneous structure (Fig. 4) (Farjami, Madadlou, & Labbafi, 2016; Nyemb et al., 2016 a, b; Weijers, Sagis, Veerman, Sperber, & van der Linden, 2002). For the fracturable EWP gels, the smallest pore size and lowest digestion extent were observed. Since fibrillar aggregates themselves provide the most accessibility for enzymes, the proteolysis resistance observed for these gels was mainly attributed to the low enzyme diffusion (Nyemb et al., 2016 a, b). A positive correlation has also been reported to exist between the resistance of fibrillary structures to trypsinolysis and the percentage of β-sheets secondary structure, meaning that the higher β-sheet percentage, the higher proteolysis resistance (Soto & Castano, 1996).

3.3.2. *Granular-spongy gel (pH ~ 5.0/ionic strength 1 M)*

At high ionic strength and pH about 5.0, i.e. close to the pI of most EWPs, the protein net charge and the electrostatic repulsions between proteins are minimized. Under these conditions, the predominance of attractive forces can favor the formation of granular-spongy gels composed of large spherical aggregates. Although, at supramolecular scale, the accessibility of cleavage sites in the large spherical aggregates is low (as discussed earlier), the granular-spongy gel made of these aggregates showed the highest degree of proteolysis compared to other gel microstructures (Nyemb et al., 2016 a, b). This is due to the fact that the microstructure of such gel is heterogeneous and contains large inter-connected pores, which can enhance enzyme diffusion into the gel network (Fig. 4) (Somaratne et al., 2020a).

3.3.3. *Intermediate gel (pH ~7.0/ionic strength 0.05 M)*

At low ionic strength and pH about 7.0, close to the pI of ovotransferrin (about 6.5), the formation of random and spherical

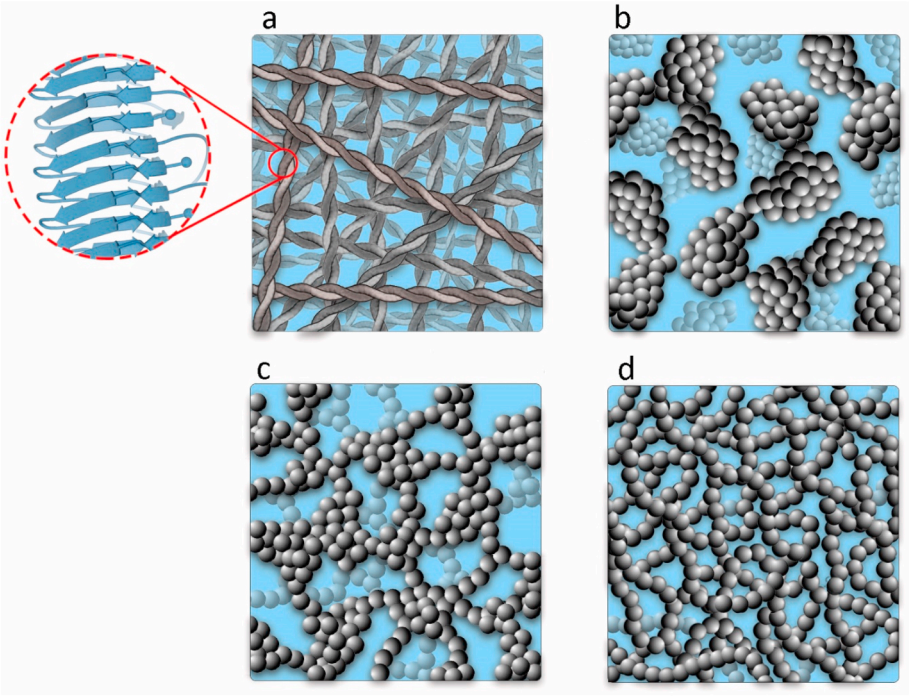

Fig. 4. Schematic representation of the microstructure of EWP gels produced under different processing conditions: (a) fracturable gel; (b) granular-spongy gel; (c) intermediate gel; (d) smooth-rigid gel.

aggregates of ovotransferrin predominates. On the other hand, pH 7 is far from the pI of ovalbumin (about 4.5), which induces the formation of linear-branched aggregates of ovalbumin. As a result, the EWP gel prepared at pH 7 consists of a mixture of different aggregated structures: spherical aggregates of ovotransferrin dispersed in a protein network of linear-branched aggregates of ovalbumin. The heterogeneity of the gel generated at pH 7 is associated with the formation of large pores in its microstructure. These large pores can facilitate enzyme diffusion into the gel, resulting in high digestion extent (Nyemb, Guérin-Dubiard, et al., 2014; Nyemb et al., 2016 a).

3.3.4. Smooth-rigid gel (pH ~9.0/ionic strength 0.05 M)

At low ionic strength and pH about 9, i.e. far from the pI of most EWPs, especially ovalbumin and ovotransferrin, the protein net charge and electrostatic repulsions strongly increase, and the required activation energy barrier to unfold proteins is reduced (Van der Plancken et al., 2006). Under these conditions, proteins tend to unfold rather than aggregate, leading to the longest required time for gelation to start. These conditions favor the formation of smooth-rigid EWP gels with homogeneous network. In such gels, fewer ovotransferrin spherical aggregates exist, but a greater amount of ovalbumin linear aggregates are present. The dominance of ovalbumin linear aggregates allows a dense network with smaller unconnected pores, as compared to the pH 7 intermediate gel (Fig. 4) (Nyemb et al., 2016 a, b; Somaratne et al., 2020a). The dense and tight network of the smooth-rigid EWP gel can hinder enzyme diffusion. This leads to a lower digestion extent for the gels despite comprising linear aggregates which per se are highly sensitive to proteolysis (Campos & Sancho, 2003; Nyemb et al., 2016 a). The influence of gel firmness on digestibility has been as well shown for whey protein gels. Guo, Bellissimo, and Rousseau (2017) reported that soft and homogenous whey protein gels made with low salt concentrations broke down more rapidly during *in vitro* intestinal digestion as compared with firmer gels (with higher salt concentration) having denser and more spatially heterogeneous protein matrix.

3.4. Size of gel particles

Reduction of gel particle size as a result of mechanical actions can increase the surface area available for the activity of digestive enzymes (Singh & Gallier, 2014). Diffusion processes of enzymes into the gel and the generated peptides outwards from the gel might also be facilitated by reducing the gel particle size. The type of gel network structure can influence the size of gel particles produced during mastication. Fine-stranded gels formed from flexible linear aggregates (at pHs above the pI) were demonstrated to break down into large and inhomogeneous particles with irregular shapes during mastication. In contrast, for particulate gels, it was reported that they break down rapidly into a homogeneous distribution of small particles (Foegeding, 2006).

4. *In vivo* digestion of EWPs

Most studies on the digestion of EWPs have used *in vitro* digestion models, which cannot accurately simulate the gastrointestinal digestion conditions in human due to the complexities of the digestive system, the complex nature of foods and meals, and the wide variability between individuals (Muttakin, Moxon, & Gouseti, 2019). Nau et al. (2019) considered for the first time *in vivo* investigation of EWP gel digestion in the gastric compartment of the animal model pigs. Such *in vivo* digestion has proven that the gastric emptying was slower for the smooth-rigid EWP gels than for the granular-spongy and intermediate EWP gels. The higher cohesiveness and elasticity of the smooth-rigid gels (compared with the two other EWP gels) caused greater resistance to breakdown and produced harder particles in the stomach, which led to the slower gastric emptying rate of the gels. Furthermore, the higher intra-gastric pH for the smooth-rigid gels could limit the proteolytic action of pepsin on these gels, contributing to the lower gastric emptying of these gels (Luo, Chen, Boom, & Janssen, 2018; Nau et al., 2019). Noteworthy, although processing has sometimes a limited impact on protein digestibility *in vivo*, *in vitro* studies have been able to demonstrate that processing can have a major impact on the kinetics of protein hydrolysis which can be critical for some specific populations. As an example, it is known that liquid and gelified dairy products have similar digestibility but that the liquids are more rapidly metabolized than gels (Barbé et al., 2013, 2014). This results in a faster release of dietary amino acids in the bloodstream. This is particularly important for elderly people who need a fast and high uptake of amino acid in order to restore muscle protein synthesis when suffering from sarcopenia.

5. Conclusion

Processing can induce alterations in the native structure of individual EWPs as well in their interactions with each other. Digestibility of EWPs, which most of them are resistant to digestion in their native form, increases under processing due to unfolding and the exposure of hydrolysis sites. Nevertheless, in certain conditions, the unfolded proteins can form spherical aggregates that are resistant to proteolysis. Interestingly, these aggregates can produce protein gels that show higher degree of proteolysis than the gels made of linear aggregates that are more susceptible to proteolysis. Although all processing methods can unfold EWPs, the unfolded proteins are rather intermediate states. The processing-induced unfolded proteins can either refold or aggregate. Thus, after processing, proteolytic enzymes are mainly faced with an aggregate or a non-aggregated native state.

Improvement in digestibility without influencing biologically important micronutrients and sensory quality of egg white is of great importance. While enhancing EWPs digestibility through heat treatments may be associated with negative effects on egg white nutritional quality and safety, alternative low-thermal (e.g., PEF) and non-thermal technologies (e.g., HHP and HIU) could enhance the health benefits of egg white without negatively affecting its nutritional aspects.

By employing specific processing strategies, it can be possible to modulate the digestive behavior of EWPs in terms of the extent of digestion and the nature and relative concentration of the peptides released. One way to enhance the efficacy of a processing technique is to combine it with other processing methods. Applying enzymatic treatment to EWPs under high pressure allows quick production of specific bioactive peptides. Given the importance of industrial-scale production of health-promoting food products, further works on the development of applicable processing techniques that will result in an increase in protein digestibility and release of highly bioactive peptides as well a reduction in food allergens are required.

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