

Effect of processing technologies on the digestibility of egg proteins

Zuhaib F. Bhat¹  | James D. Morton² | Alaa El-Din A. Bekhit³  | Sunil Kumar¹ | Hina F. Bhat⁴ 

¹ Division of Livestock Products Technology, SKUAST of Jammu, Jammu, Jammu and Kashmir, India

² Department of Wine Food and Molecular Biosciences, Lincoln University, Christchurch, New Zealand

³ Department of Food Sciences, University of Otago, Dunedin, New Zealand

⁴ Division of Biotechnology, SKUAST of Kashmir, Srinagar, Jammu and Kashmir, India

Correspondence

Hina F. Bhat, Division of Biotechnology, SKUAST of Kashmir, Srinagar, Jammu and Kashmir, India.

Email: bhat.hina@rediffmail.com

Abstract

Egg and egg products are a rich source of highly bioavailable animal proteins. Several processing technologies can affect the structural and functional properties of these proteins differently and can influence their fate inside the gastrointestinal tract. The present review examines some of the processing technologies for improving egg protein digestibility and discusses how different processing conditions affect the digestibility of egg proteins under gastrointestinal digestion environments. To provide up-to-date information, most of the studies included in this review have been published in the last 5 years on different aspects of egg protein digestibility. Digestibility of egg proteins can be improved by employing some processing technologies that are able to improve the susceptibility of egg proteins to gastrointestinal proteases. Processing technologies, such as pulsed electric field, high-pressure, and ultrasound, can induce conformational and microstructural changes that lead to unfolding of the polypeptides and expose active sites for further interactions. These changes can enhance the accessibility of digestive proteases to cleavage sites. Some of these technologies may inactivate some egg proteins that are enzyme inhibitors, such as trypsin inhibitors. The underlying mechanisms of how different technologies mediate the egg protein digestibility have been discussed in detail. The proteolysis patterns and digestibility of the processed egg proteins are not always predictable and depends on the processing conditions. Empirical input is required to tailor the optimization of processing conditions for favorable effects on protein digestibility.

KEYWORDS

egg proteins, gastrointestinal digestion, high-pressure processing, microwave, pasteurization, pickling, pulsed electric field, ultrasound

1 | INTRODUCTION

Laden with high-quality proteins and significant levels of vitamins, antioxidants, and other healthful compounds, eggs are considered as nature's functional food in its efficient ovoid container (Bhat, Kumar, et al., 2013). A large

hen egg generally meets 3%–4% of the energy requirements of an adult human being and provides 11% (6.5 g) of protein requirements and 12%–31% of the dietary reference intake for various essential amino acids (McNamara, 2013). A typical whole raw egg, egg white, and egg yolk (along with vitelline membrane) contain 12.50, 10.90, and 15.90 g

protein per 100 g, respectively (Réhault-Godbert et al., 2019). Egg white is composed of more than 150 different kinds of proteins and is considered as an ideal source of protein (Pathania et al., 2019). It contains all the essential amino acids in the proportion required for optimal human nutrition (Bhat, Kumar, & H.F. Bhat, 2015). Ovalbumin (54%), ovotransferrin (14%), ovomucoid (11%), lysozyme (3.5%), ovomucin (3.5%), and globulins (8%) are the major proteins present in the egg white, whereas low-density lipoprotein (68%), high-density lipoprotein (16%), livetins and other soluble proteins (10%), and phosvitin (4%) are the major yolk proteins (McNamara, 2013; Réhault-Godbert et al., 2019). In addition to its valuable nutritional contents, egg components are associated with various biological activities, such as anticancer, antimicrobial, immunomodulatory, antihypertensive, anti-inflammatory, and antiviral activities, protease inhibitory, and vitamin-binding properties (Bhat, et al., 2017; Bhat, Kumar, & H.F. Bhat, 2015). Regular consumption of at least 1 egg per day has been demonstrated to promote good health in several studies (Dimarco et al., 2017; Park et al., 2018; Shin et al., 2017). However, egg proteins could induce allergic reactions, especially among children and infants (Wei-Liang Tan et al., 2017). The six egg proteins that are commonly associated with allergies are ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), lysozyme (Gal d 4), α -livetins (Gal d 5), and the glycoprotein YGP42 (Gal d 6) (Dona & Suphioglu, 2020). Among these, digestion- and heat-labile proteins (e.g., Gal d 2) commonly cause milder or local reactions, whereas digestion- and heat-stable proteins (e.g., Gal d 1) are more likely to cause a severe allergic reaction (Caubet & Wang, 2011).

In addition to their direct consumption, egg whites and/or egg yolks are also ingredients in numerous products, such as cakes, biscuits, meringues, souffles, mayonnaise, and custards, due to their excellent culinary and functional properties such as foaming, emulsification, and gelation (Ma et al., 2020). Egg proteins are also used in some nutraceutical and pharmaceutical products (Christian et al., 2018). Denaturation of egg proteins at specific temperatures and their coagulation and formation of a stable matrix are mainly responsible for the functional properties of egg proteins (Pathania et al., 2019). Eggs are therefore processed in different ways before consumption and during preparation of different products. This processing affects their functional properties, microbial quality, shelf-life, and also have direct consequences on protein digestibility (Gharbi & Labbafi, 2018). Processing conditions can affect the allergenicity of egg proteins by affecting the linear and/or conformational epitopes of allergens and can be used to produce hypoallergenic egg products. Novel thermal and nonthermal processing technologies have shown promising results in reducing the allergic reac-

tions of egg proteins (Zhu et al., 2018a). Combined heat treatment and high-pressure processing (HPP) can partly inactivate antinutrients like avidin (Singh, & Ramaswamy, 2014). The effects of different methods of processing on egg white proteins and their allergenicity have been reviewed by Gharbi & Labbafi (2018) and Zhu et al. (2018a), respectively.

2 | IN VITRO SYSTEMS FOR FOOD DIGESTION

Although in vivo studies on humans and animals are the ideal models for studying the effect of processing on food digestion, there are often ethical and financial limitations (Bhat et al., 2021b, 2021c). Therefore, in vitro digestion models, both static and dynamic, have been proposed and widely used during last couple of decades to mimic the physiological conditions of upper human gastrointestinal tract. Static in vitro models have been commonly used for food, animal feed, and pharmaceutical purposes and are very useful in predicting outcomes of gastrointestinal digestion (Brodkorb et al., 2019; Ji et al., 2021). These models use a constant pH for each simulated digestive phase and a constant ratio of enzyme to substrates and electrolytes. A huge range of static and dynamic models (single static systems to multicompartmental) that vary in complexity, parameters and type, and number of steps included in the digestion sequence are available. Based on the purpose of the study, these models can simulate one or more of the oral, gastric, and small intestinal phases, and some include large intestinal fermentation phase. These models also vary in the duration, composition of the digestive solutions used in different phases (type, concentration, and activity of enzymes, the buffers and salts used), the surface-active components, the biological polymers, the fluid flows, and the mechanical stresses (direction and magnitude of stresses) applied (Lucas-González et al., 2018). This variation has made it difficult to compare the results of different research groups and published studies. Therefore, an international network of multidisciplinary experts (INFOGEST26) from more than 35 countries was established to harmonize the digestive conditions and to come up with an accepted set of digestion parameters for a static in vitro digestion simulation suitable for foods. The key parameters and conditions were defined and a general standardized static digestion method for foods (known as the INFOGEST method) was published based on relevant conditions in relation to available in vivo physiological data (Minekus et al., 2014). The INFOGEST method was recently amended and an improved digestion method, known as INFOGEST 2.0, was published to overcome some challenges such as the inclusion of the

oral phase and the use of gastric lipase (Brodkorb et al., 2019).

Although static *in vitro* digestion models are simple, low cost, and easy to perform, they cannot completely recreate the complexity of the digestive tract and are not suitable for simulating digestion kinetics. Dynamic *in vitro* models that allow flow of the food, pH regulation and real time injection of digestive enzymes in different compartments of the model more accurately mimic the digestive process and are more promising than static models (Brodkorb et al., 2019). These models are either multi- or mono-compartmental and simulate one or more phases of digestion and the performance of most of these systems has been compared to *in vivo* data obtained on humans or animals (Dupont et al., 2018; Havenaar et al., 2016; Maathuis et al., 2017). However, these dynamic models are comparatively complex, expensive to install and maintain and are therefore available to a minority of food researchers. The *in vitro* gastrointestinal digestion models and their applications in carbohydrate and protein rich foods have been reviewed (Brodkorb et al., 2019; Dupont et al., 2018; Griffin et al., 2014; Guerra et al., 2012; Ji et al., 2021; Lucas-González et al., 2018).

3 | FOOD PROCESSING AND ITS IMPACT ON FUNCTIONALITY, STRUCTURE, AND DIGESTIBILITY OF EGG PROTEINS

Food processing involves turning the raw material into consumable products (Bhat et al., 2021a) and varies from the simple processes of grinding, cooking, or packaging of foods (Bhat et al., 2018a; Dua et al., 2015a, 2015b, 2015c, 2016; Kalem, Bhat, Kumar, & Jayawardena, 2018; Kalem, Bhat, Kumar, Noor, et al., 2018; Kalem, Bhat, Kumar, Wang, et al., 2017, 2018; Noor, Bhat, Kumar, & Mudiyanse-lage, 2018; Noor, Bhat, Kumar, & Kalem, 2018; Noor et al., 2017; Sharma, Bhat, Kumar, Kumar, Bekhit, et al., 2021; Sharma, Bhat, Kumar, Kumar, Bhatti, et al., 2021; Singh, Kumar, Kumar, et al. 2014; Singh, Kumar, Bhat, et al., 2014; Mahajan et al., 2015a, 2015b, 2016a, 2016b) to the complex production of cultured cell-based products (Bhat, Bhat, et al., 2020; Bhat et al., 2014; Bhat, Morton, S. L. Mason, A. E. A. Bekhit, & Bhat, 2019; Bhat & Bhat, 2011a, 2011b; Pathak, Bukhari, et al., 2008; Pathak, Bhat, et al., 2008). Food processing fulfils some industrial and marketing needs such as improving storage quality or shelf-life (Ahmed et al., 2014; Bhat, Kumar, P. Kumar, et al., 2015, 2013; Bukhari et al., 2014-11; Jamwal et al., 2015; Kaur et al., 2015; Kumar, Bhat, et al., 2012; Kumar, Kumar, et al., 2012; Singh, Kumar, Bhat, Kumar, & Kumar, 2015; Zargar et al., 2017, 2016), production of functional food products

(Bhat, Kumar, & H.F. Bhat, 2015; Bhat, Kumar, & H. Bhat, 2015; Bhat & Pathak, 2009; Bhat & Pathak, 2012; Bhat, Pathak, Bukhari, & Ahmed, 2010; Bhat, Pathak, Bukhari, & Ahmed, Bhat, 2010; Bhat, Morton, S. Mason, A. E. B. Bekhit, & Bhat, 2019, 2011; Kumar et al., 2015, 2016; Kumar, Bhat, et al., 2013; Kumar, Kumar, et al., 2013; Kumar et al., 2011, 2014; Pathak et al., 2009; Bhat, Morton, Zhang, et al., 2020; Singh, Kumar, Bhat, & Kumar, 2015) and inducing special effects using novel technologies such as pulsed electric field (PEF; Bhat et al., 2018b; Bhat, Morton, Mason, Mungure, et al., 2018; Bhat et al., 2019a, 2019b; Singh et al., 2012). Before being ready in a final consumable form, food proteins therefore undergo several manufacturing processes, both thermal and nonthermal. All these processes have the potential to affect the quality and bioavailability of the proteins present in foods including eggs.

Egg proteins undergo several modifications and transformations during processing and cooking steps, which can affect their functional and nutritional properties (Rodrigues et al., 2020; Zhu et al., 2018a, 2018b). The main objectives of these processing steps are to stabilize egg and egg products, to inactivate microbes and enzymes, and to stabilize/improve nutritional, nutraceutical, and sensorial attributes (Aryee et al., 2018; Vanga et al., 2017). The effects of processing on the quality of food products including egg and egg products have received much attention in the last couple of decades and has led to the development of new technologies or improved processing strategies (Jia et al., 2019). Several nonthermal processing technologies, such as PEF, HPP, ultrasound, or thermal processing, such as microwave and pasteurization, gained attention due to their potential to improve functionality and quality of egg proteins (Farjami et al., 2020; Rodrigues et al., 2020; Zhu et al., 2018a). These processing technologies mediate the egg functionality and quality through structural transformations and by inducing modifications in the microstructure or structural properties of egg proteins, which can affect their digestion behavior within the gastrointestinal tract and influence their digestibility.

Processing results in varying degrees of protein unfolding and formation of aggregates/gelation, both of which can potentially affect the functional properties and digestibility of egg proteins (Hong et al., 2021). Determining the relationship between underlying mechanisms and processing parameters is necessary to control these processes and optimize functionality and protein digestibility. The microstructure and characteristics of egg white aggregates can be modified using several processing methods (Fang, & Nakagawa, 2021). Although thermal treatment is the original and most common method of processing that induces aggregation/gel formation in eggs, other novel and emerging methods such as HPP, ultrasound, and PEF are gaining importance (Farjami et al., 2020; Gharbi & Labbafi,

2018). The outcome of different methods of processing also depends on other processing conditions such as pH, ionic strength, quantity and type of salt, protein concentration, additives, heat/alkali treatment, and intensity of the treatment (Gharbi & Labbafi, 2018; Hong et al., 2021). Collectively, these various process options can also alter the allergenicity of the egg proteins (Claude et al., 2017).

The mechanism behind the thermal gelation of egg white proteins (e.g., raw to boiled eggs), a model of heat gelation for globular proteins, has three main steps, namely, native protein, denatured protein, soluble aggregated protein—gel/coagulation (Xue, Tu et al., 2020). The heat-induced denaturation causes unfolding of native proteins that disrupts the secondary and tertiary structures and exposes their inner hydrophobic regions. These denatured proteins interact with each other and form high molecular weight aggregates that in turn result in the formation of a three-dimensional gel matrix due to further interactions (Anton et al., 2009). The aggregation and unfolding of proteins are influenced by several factors that determine the kind and number of the interactions (predominantly hydrophobic and electrostatic) with final consequences on gel microstructure and digestibility of the proteins. Thermal processing of egg white exposes the four free sulfhydryl groups buried in the core of ovalbumin protein, the main egg white protein (54%) and the only one with free sulfhydryl groups, and results in the formation of disulfide bonds that can change the functional properties of the egg white proteins (Pathania et al., 2019). Though disulfide bonds are not required for gel formation, it makes the gel stronger (van der Plancken et al., 2007) and affect the protein digestibility. Gelation of yolk proteins by heat treatment is predominantly governed by unfolding of proteins that exposes functional groups that attract through hydrophobic interactions, forming a gel (Anton et al., 2009). The degree of protein unfolding is one of the main factors that affects the extend of digestion of egg white proteins by changing the available number of cleavage sites and their access to enzymes (Gharbi & Labbafi, 2018; van der Plancken et al., 2003). The shape and type of aggregates, which can be controlled by processing conditions such as pH, ionic strength, and protein concentration, affects the degree of protein unfolding during thermal and other processing. Four different types of aggregates (flexible strands, semiflexible fibrils, fractal, and spherical particles) can be formed depending on these processing conditions (Nicolai & Durand, 2013). Aggregates of different sizes and morphologies (spherical, spherical-agglomerated, linear, and linear-branched) can be produced by changing the combinations of ionic strength and pH in ovalbumin solutions (Claude et al., 2017; Nyemb, Guerin-Dubiard, et al., 2014). Although linear ovalbumin aggregates can be formed under condi-

tions of high electrostatic repulsion (low ionic strength and pH 9), the spherical-agglomerated morphologies are favored when there is minimal electrostatic repulsion (high ionic strength and pH 5; Claude et al., 2017; Nyemb, Guerin-Dubiard, et al., 2014). All types of ovalbumin aggregates in thermally processed samples are more susceptible to digestion compared to unheated samples (van der Plancken et al., 2003). The degree of unfolding before aggregation is the main factor in the digestibility of ovalbumin with lowest unfolding recorded in the spherical-agglomerated aggregates and highest in linear aggregates (Nyemb, Guerin-Dubiard, et al., 2014; van der Plancken et al., 2003).

Like thermal processing, nonthermal technologies, such as HPP, PEF, and ultrasound, affect the aggregation and gelation process of egg proteins and can have a direct effect on their digestibility. HPP can induce denaturation and aggregation of egg proteins and depends on processing conditions such as pressure, time, pH, temperature, and protein concentration. Although no aggregate formation happens at pressures below 100 MPa, it has been reported to break the ovomucin-lysozyme complex by disrupting the electrostatic interactions (Brand et al., 2014). This complex is responsible for maintaining the thickness of albumen and its destruction results in a reduction of viscosity and optical density (Brand et al., 2014; Panozzo et al., 2014). HPP treatment of 150 MPa resulted in the formation of aggregates stabilized by noncovalent interactions (not by S-S bonds), whereas the aggregation of egg white proteins above 150 MPa decreased the immunoreactivity (Panozzo et al., 2014; Singh, Sharma, & Ramaswamy, 2015). Although small soluble aggregates were formed at HPP of 550 MPa (20 min) at a protein concentration of 9.6 mg/ml by covalent interactions (S-S bonds), a translucent gel was formed at concentration of 96 mg/ml (15 min) by S-S bond crosslinking (Singh & Ramaswamy, 2015; van der Plancken et al., 2005). More soluble and smaller aggregates were formed under the HPP of 100–400 MPa compared to the aggregates formed under the thermal processing (70°C–85°C) due to lower surface hydrophobicity during HPP (van der Plancken et al., 2005; Zhu et al., 2014).

The effect of PEF on the aggregation of egg white proteins depends on the field intensity and the protein mixture. Although no aggregates were formed at 20 kV/cm (for 800 μ s) in ovalbumin solution, soluble aggregates were formed at 25 kV/cm after partial unfolding, exposure of SH groups and S-S bond formation between the protein molecules (Wu et al., 2015; Zhao & Yang, 2012). Presence of lysozyme in the solution resulted in the formation of insoluble aggregates causing an increase in turbidity and decreasing the protein solubility (Wu et al., 2014, 2015, 2016). PEF can affect the net charge on protein molecules and their electrostatic interactions, thereby aiding the

formation of aggregates between oppositely charged protein molecules such as positively charged lysozyme and negatively charged ovalbumin or ovotransferrin. PEF can also induce unfolding of protein molecules and expose the internal aggregation prone regions, such as in ovalbumin or ovotransferrin, making them available for non-covalent interactions, including hydrophobic interactions with lysozyme (Wu et al., 2014, 2015, 2016). PEF processing of lysozyme at 35 kV/cm caused unfolding and transition from α -helix to β -sheet along with an increased exposure of tryptophan residues (Yogesh, 2016; Zhao & Yang, 2008). Both covalent and noncovalent interactions have been reported after PEF treatment of egg proteins causing the formation of soluble or insoluble aggregates (Wu et al., 2016, 2015). All these effects of PEF processing on the aggregation and gel formation of egg protein have consequences on protein digestibility. Table 1 presents the effect of different processing technologies on digestibility of egg proteins. Figure 1a and b summarizes the mechanisms of how these different processing technologies affect egg protein digestibility during gastrointestinal digestion.

3.1 | Pasteurization/thermal processing

Eggs have a history of being the most important vehicle for *Salmonella* infection (Gantois et al., 2009), therefore an appropriate pasteurization process is crucial for ensuring the safety of egg products (Wei, Lau, et al., 2020). *Salmonella* contaminated eggs are responsible for 79,000 foodborne illnesses and 30 deaths annually in the United States alone (FDA, 2018). This becomes particularly important as processed egg products have become increasingly popular in recent decades and make up 40% of the total egg consumption (American Egg Board, 2015; Wei, Lau et al., 2020). The requirements for egg pasteurization vary according to the legislation of different countries (Da Silva et al., 2017). Although liquid whole eggs must be heated to no less than 60°C at least for 3.5 min in the United States, the recommendations in the United Kingdom for pasteurization are 64°C for 2.5 min (Cunningham, 1995), whereas France regulates the microbial results and there is no statutory thermal treatment. To achieve this, whole egg pasteurization may vary from 65°C to 68°C for 2–5 min to ensure 5–6 log reductions of vegetative microorganisms, especially *Listeria monocytogenes* and *Salmonella enteritidis* (Baron et al., 2010). The sensitivity of egg proteins to thermal treatments often limits the use of pasteurization temperatures in the egg industry and this prescribed range of time–temperature, namely, 60°C–68°C for 2–10 min has been reported to modify almost all egg proteins (Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al., 2017; Lechevalier, Guérin-

Dubiard, Anton, Beaumal, Briand, Gillard, Le Gouar, et al., 2017). Table 2 presents the effect of thermal processing and pasteurization on digestibility of egg proteins.

Given that thermal treatments are known to affect the structural and functional properties of egg proteins (Yang & Geveke, 2020) and the fact that the liquid egg products that undergo thermal pasteurization represent about 70% of total egg products (Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al., 2017), it is imperative to elucidate the impact of pasteurization on the digestibility of egg proteins. Liu et al. (2018) studied the effect of thermal treatments, including pasteurization, on the protein susceptibility of ovomucin-depleted egg white during in vitro gastrointestinal digestion simulation. The ovomucin-depleted egg white liquid was isothermally heated at 60°C or 80°C for 10 min and were compared with the unheated control samples. The peptide production of the samples was evaluated during the gastrointestinal digestion to assess the changes in the protein susceptibility to enzymatic hydrolysis. No significant effect of thermal pasteurization was observed on the susceptibility of ovomucin-depleted egg white and the ovalbumin dimers formed were equally resistant to peptic digestion as ovalbumin. A significant increase was observed in the susceptibility of the samples heated at 80°C and the protein ovalbumin and the protein aggregates formed during thermal processing were completely hydrolyzed during first 30 min of peptic digestion. The peptide production of the samples heated at 80°C after 30 min of the peptic digestion was similar or even higher than the control samples and the samples heated at 60°C after completion of peptic digestion. Similar results were observed during intestinal digestion and the peptide production of the samples heated at 80°C after 30 min of the intestinal digestion was similar or even higher than the control and the samples heated at 60°C after completion of intestinal digestion.

The protein denaturation/unfolding that was observed in the pasteurized samples heated at 60°C, while no increase was recorded in the protein susceptibility to gastrointestinal proteases, was explained by the fact that protein denaturation/aggregation at 60°C mainly involves ovotransferrin rather than lysozyme or ovalbumin (Liu et al., 2017). The denaturation of ovalbumin induced by thermal processing is believed to account for a significant increase in the overall protein susceptibility to enzymatic hydrolysis (van der Plancken et al., 2004). Thermal treatments as high as 65°C–75°C for up to 30 min have been observed to have little impact on the susceptibility of ovalbumin to gastrointestinal proteases (Jiménez-Saiz, Belloque et al., 2011; van der Plancken et al., 2003). Heating samples at 80°C resulted in severe protein denaturation and unfolding that involved most of the egg white proteins (Liu et al., 2017). Several studies have reported that

TABLE 1 Processing technologies that have an impact on the digestibility of egg proteins

Processing technologies/other factors	Effect on protein digestibility	Model studied	References	In vitro/in vivo study	Methods studied to assess the digestibility
Pasteurization/thermal 60°C for 10 min processing	No effect	Ovomucin-depleted egg white proteins	Liu et al. (2017)	In vitro	Enzymatic hydrolysis by OPA (o-phthaldialdehyde) assay, SDS-PAGE, peptide analysis by Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/TOF-MS)
80°C for 10 min	↑				
60°C for 10 min	No effect	Ovomucin-depleted egg white proteins	Liu et al. (2018)	In vitro	Enzymatic hydrolysis by OPA assay, SDS-PAGE, peptide analysis
80°C for 10 min	↑				
80°C for 6 h	↑	Ovalbumin	Nyemb, Guerin-Dubiard, et al. (2014)	In vitro	Peptide analysis, disappearance of intact ovalbumin
100°C for 10 min	↑	Egg white proteins	Lassé et al. (2015)	In vitro	OPA assay, SDS-PAGE, amino acid residues modification, peptide analysis using LC-MS/MS
65°C for 30 min	No effect	Ovalbumin and ovomucoid	Jiménez-Saiz et al. (2011)	In vitro	SDS-PAGE, RP-HPLC analysis
90°C for 15 min	↑				
100–120°C for 10 min	↑ (up to 105°C)	Spray-dried egg albumin and whole egg liquid	Schmidt et al. (2007)	In vitro	Protein digestibility (%), crude protein of digests
70°C for up to 14 days	No effect				
50°C–85°C	↑	Egg white proteins	Van der Plancken et al. (2004)	In vitro	Degree of hydrolysis by pH-stat method

(Continues)

TABLE 1 (Continued)

Processing technologies/other factors	Effect on protein digestibility	Model studied	References	In vitro/in vivo study	Methods studied to assess the digestibility
56°C for 32 min, 100°C for 5 min, 65°C for 30 min	↑	Egg white proteins	Wang et al. (2018)	In vitro	Nitrogen release (%), SDS-PAGE, peptide analysis
60°C (4, 7, and 10 min), 62°C (4 min), 64°C (4 min), 66°C (10 min)	↑	Whole egg proteins	Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al. (2017)	In vitro	Degree of hydrolysis, SDS-PAGE
62°C (10 min), 64°C (7 and 10 min), 66°C (4 and 7 min)	↓				
100°C up to 3 h	↑ (up to 1.5 h)	Egg proteins	Xue et al. (2020)	In vitro	In vitro protein digestibility (%) (protein in supernatant/total protein content × 100)
Microwave	↑	Egg white proteins	Zhu et al. (2018b)	In vitro	In vitro protein digestibility (%)
			Evenepoel et al. (2013)	In vivo	Ileal effluents and breath samples were collected for 15N- and 13C-content, respectively
Pickling	↑	Duck egg white proteins	Guo et al. (2019), Deng et al. (2020), Zhao, Cao, et al. (2020)	In vitro	Digestibility (%) by a formula (protein in supernatant/protein in samples × 100)
High-pressure processing	↑	Egg white proteins, ovalbumin	Evenepoel et al. (2013)	In vivo	Ileal effluents and breath samples were collected for 15N- and 13C-content, respectively.

(Continues)

TABLE 1 (Continued)

Processing technologies/other factors	Effect on protein digestibility	Model studied	References	In vitro/in vivo study	Methods studied to assess the digestibility
		Whole egg proteins	Hoppe et al. (2013)	In vitro	Peptide analysis of the digests (LC-MS/MS), SDS PAGE, 2D-electrophoresis
		Egg white proteins	Van der Plancken et al. (2004)	In vitro	Degree of hydrolysis by pH-stat method
Pulsed electric field	↑	Ovomucin-depleted egg white proteins	Liu et al. (2017)	In vitro	Enzymatic hydrolysis by OPA (o-phthalaldehyde) assay, SDS-PAGE, peptide analysis by MALDI-TOF/TOF-MS
			Liu et al. (2018)	In vitro	OPA (o-phthalaldehyde) assay, SDS-PAGE, peptide analysis by LC-MS
Ultrasound processing	↑	Egg white proteins	Zhu et al. (2018b)	In vitro	In vitro protein digestibility (%) by a formula (digestible protein/initial protein × 100)
			Jovanović et al. (2016)	In vitro	Degree of hydrolysis by pH-stat method
			Stefanovic et al. (2018)	In vitro	Degree of hydrolysis by pH-stat method
Tea polyphenols	↑	Ovalbumin and lysozyme (peptic digestion at pH 1.2)	Shen et al. (2014)	In vitro	SDS-PAGE
	↓	Ovalbumin and lysozyme (pancreatin digestion at pH 7.5)			
Presence of egg yolk	↑	Egg white proteins	Martos et al. (2013)	In vitro	SDS-PAGE
Glycation during Maillard reaction using D-ribose	↓	Egg white proteins	Yang et al. (2021)	In vitro	Soluble protein, peptide production

↑, Increased/higher protein digestibility.

↓, Decreased/lower protein digestibility

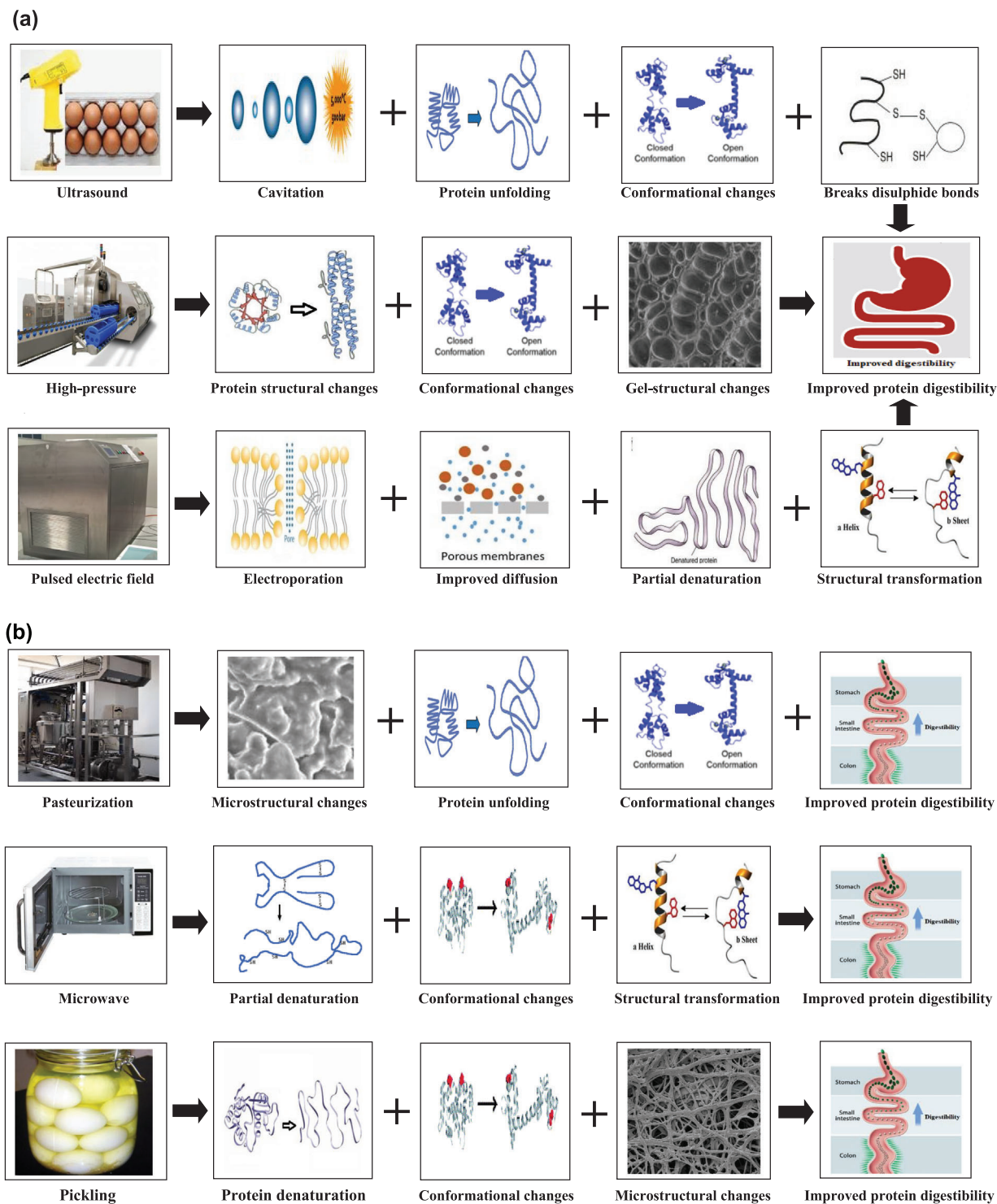


FIGURE 1 (a) Mechanisms of processing technologies for improving the digestibility of egg proteins, (b) mechanisms of processing technologies for improving the digestibility of egg proteins

heating of egg white proteins or ovalbumin at or above 80°C significantly increased the overall protein susceptibility to digestive proteases such as pepsin, pancreatin, trypsin, and chymotrypsin (Hoppe et al., 2013; Jiménez-Saiz et al., 2011; Lassé et al., 2015; Martos et al., 2011; Mine et al., 1990; Nyemb, Guerin-Dubiard, et al., 2014; Odani

et al., 1997; Takagi et al., 2003; van der Plancken et al., 2004, 2003; Yoshino et al., 2004).

The study by Liu et al. (2018) also evaluated the proteolysis of three major proteins present in the protein profile of ovomucin-depleted egg white, namely, ovalbumin, lysozyme, and ovotransferrin and analyzed their

peptide digests. Although ovotransferrin was completely hydrolyzed within 10 min and lysozyme was substantially hydrolyzed within 30 min of peptic digestion, ovalbumin remained largely intact even after the completion of the peptic digestion of the control samples. The ovalbumin was progressively hydrolyzed during intestinal digestion. Similar results have also been reported by Yoshino et al. (2004), who observed that lysozyme and ovotransferrin were rapidly hydrolyzed within 30 min of peptic digestion, whereas ovalbumin was only slightly degraded and remained intact for more than 2 hr of digestion. Contrary to these results, studies have reported that native lysozyme and ovalbumin were resistant to digestive proteases and have exhibited higher peptic resistance than ovotransferrin (Fu et al., 2002; Moreno, 2007). The proteolytic patterns of ovalbumin, lysozyme, and ovotransferrin of the samples heated at 60°C did not show any considerable change and the protein aggregates formed during thermal processing showed resistance to hydrolysis during peptic digestion for at least 1 h, however, these aggregates completely disappeared during intestinal digestion. For the samples heated at 80°C, the proteolytic patterns revealed that ovalbumin, lysozyme, and ovotransferrin were completely hydrolyzed within 10–30 min of peptic digestion and the protein aggregates of various molecular weight distributions formed during the thermal treatment were completely hydrolyzed within 10 min of peptic digestion.

Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al. (2017) studied the effect of pasteurization on in vitro protein digestibility of whole egg proteins. The whole egg liquid was homogenized at 5 MPa that was followed by pasteurization using an indirect tubular processing system at a temperature of 60°C, 62°C, 64°C, or 66°C for 4, 7, or 10 min. The raw homogenized whole egg liquid was used as a control. The samples were subjected to in vitro gastrointestinal digestion simulation and were compared after completion of gastric and intestinal phases. The samples pasteurized for 4 min at 60°C, 62°C, or 64°C and 7 min at 60°C showed significantly higher or equal degree of hydrolysis compared to control samples after completion of the gastric phase of digestion. All the higher time-temperature combinations showed significantly lower degree of hydrolysis than the control samples. After the completion of intestinal phase of digestion, samples pasteurized at 60°C (4, 7 and 10 min), 62°C (4 min), 64°C (4 min), and 66°C (10 min) showed significantly higher degree of hydrolysis than the control samples. The samples that were pasteurized at 62°C (10 min), 64°C (7 and 10 min), and 66°C (4 and 7 min) showed significantly lower degree of hydrolysis compared to control samples after completion of gastrointestinal digestion. These results were supported by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) anal-

ysis that also revealed higher hydrolysis in control and the low-intensity pasteurized samples.

The authors (Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al., 2017) concluded that low-intensity pasteurization (60°C [up to 10 min], 62°C, and 64°C [4 min]) favors higher protein digestibility in egg proteins during in vitro gastrointestinal digestion and should be recommended to improve protein digestion. A low-intensity pasteurization treatment (60°C, up to 10 min) was recommended to improve interfacial properties of egg proteins without any compromise on protein digestibility. However, for safety reasons, the authors suggested that the pasteurization at 62°C (up to 10 min) and at 64°C (up to 10 min) can also be performed without excessive damage to protein digestibility and functional properties. To prevent excessive damage to protein digestibility and other functional properties of egg proteins, it was suggested that the pasteurization at 66°C should be avoided. This loss in protein digestibility during high-intensity pasteurization was attributed to the protein aggregation induced by these intense thermal treatments, thereby reducing the degree of hydrolysis by masking the enzyme cleavage sites (Nyemb, Guerin-Dubiard, et al., 2014). The SDS-PAGE analysis indicated that the pasteurization below 66°C minimized the formation of protein aggregates. Some high molecular weight bands appeared in the samples heated above 66°C at the expense of some lower molecular components, suggesting the formation of protein aggregates.

Liu et al. (2017) studied the effect of conventional thermal pasteurization on the protein digestibility of ovomucin-depleted egg white. The ovomucin-depleted egg white liquid was heated to the pasteurization temperature (60°C for 10 min) within the glass test tubes in a thermostatic water bath and was compared with the untreated control samples and the samples heated at 80°C for 10 min. The samples were subjected to in vitro gastric digestion using pepsin. The protein digestibility of the digested samples was assessed by OPA (o-phthaldialdehyde) assay, SDS-PAGE, and peptide analysis by MALDI-TOF/TOF-MS. No effect of pasteurization was recorded on the protein digestibility of ovomucin-depleted egg white compared to untreated control, whereas a significant increase was observed in the digestibility of the samples heated at 80°C compared to both control and pasteurized samples. The susceptibility and degradation patterns of ovalbumin, ovotransferrin and lysozyme were also studied. Although no effect of pasteurization was recorded on the susceptibility of these egg white proteins to the hydrolysis by pepsin, a significant increase was observed in the susceptibility of ovalbumin and lysozyme in the samples heated at 80°C and pH also showed some effect on the degradation patterns. These results were

in agreement with the findings of previous studies that observed no significant impact of shell egg pasteurization (55°C for 3 h) on the *in vitro* digestibility of egg white (Hank et al., 2001) or recorded a minor increase in the digestibility of egg white solution (2% v/v) heated from 50°C to 60°C during protein digestion by chymotrypsin (Mine et al., 1990). Liu et al. (2017) stated that these low-temperature thermal treatments were not sufficient to induce major structural changes to substantially affect the enzymatic hydrolysis of the egg white proteins. On the contrary, intense thermal treatments (>80°C) induced protein structural modifications with a substantial impact on the enzymatic hydrolysis by exposing the active sites on the proteins that were otherwise inaccessible to digestive proteases. Results of SDS-PAGE and solution turbidity revealed that thermal treatments at 80°C induced different degrees of protein aggregation depending on the pH of the solution. Previous studies have reported formation of gels containing protein aggregates of different morphologies and organizations in the egg white proteins heated at 80°C (pH 2–9 at different ionic strengths; Nyemb et al., 2016). The differences in the gel structure can affect the diffusion of enzymes and access to hydrolytic sites resulting in varying degrees of peptic digestion. These results were in agreement with previous studies who also observed a significant increase in the digestibility of egg proteins subjected to such an intense thermal treatment (Lassé et al., 2015; Nyemb, Guerin-Dubiard, et al., 2014). Nyemb, Guerin-Dubiard, et al. (2014) reported that the extent of *in vitro* digestion and nature of peptides produced from ovalbumin heated at 80°C (pH 2–9 at different ionic strengths) was modulated by the morphology of protein aggregates. The extent of hydrolysis was measured by the appearance of soluble peptides and disappearance of intact ovalbumin and was lower for spherical aggregates compared to the linear aggregates. This difference was attributed to the differences in the degree of unfolding of the proteins during aggregate preparation or the surface area to volume ratio of the aggregates. The relative amounts of soluble peptides recorded was 27%, 43%, 61%, 77%, and 100% for nonaggregated ovalbumin and spherical-agglomerated, spherical, linear-branched, and linear ovalbumin aggregates, respectively. Peptide analysis by LC-MS/MS revealed that heat-induced aggregation rendered a number of peptide bonds susceptible to enzymatic digestion that were otherwise inaccessible to enzymes in native form. Furthermore, specific peptide bonds were hydrolyzed in aggregates of different morphologies, suggesting a link between structure of protein food matrix and its enzymatic hydrolysis during digestion. Such qualitative and quantitative differences can have significant nutritional consequences.

Lassé et al. (2015) studied the effect of the Maillard reaction, thermal treatment, and pH on digestibility of egg white proteins. The egg white samples of two salt concentrations (0 mM, 200 mM) were adjusted for pH (2, 5, 7, 9, and 12) and heated at 20°C or 100°C for 10 min. Methylglyoxal or glucose was mixed with samples (100 mM) to determine the effects of Maillard reaction on digestibility and amino acid modifications. The samples were subjected to *in vitro* gastrointestinal digestibility using pepsin and pancreatin and the degree of hydrolysis was measured by OPA method and SDS-PAGE. The digests were also evaluated for the modification of amino acid residues and peptide analysis using LC-MS/MS. The results of both SDS-PAGE and OPA assay were consistent with each other and a two- to threefold higher degree of hydrolysis was observed for samples heated at 100°C compared to 20°C during the entire period of digestion. A minimal effect of pH and salt was observed on the digestibility. The proteomic analysis revealed that increased digestibility came at the cost of a higher degree of amino acid residue-level modifications with dehydration and deamidation reactions being the predominant modifications, increasing with thermal exposure time. The results revealed that the presence of methylglyoxal increased the degree of modification by up to 38% of available arginine residues, establishing an important correlation between increased levels of Maillard reaction products and a decreased nutritional value of egg white proteins.

Jiménez-Saiz et al. (2011) studied the effect of pasteurization on the digestibility of ovalbumin and ovomucoid during *in vitro* gastrointestinal digestion. The pasteurization process was simulated by heating aqueous solutions (5 mg/ml, pH 7.0) of ovalbumin and ovomucoid in a water bath at 65°C for 30 min that were compared with the samples heated to a common cooking temperature (90°C for 15 min) and unheated samples (control). The study also evaluated the effect of Maillard reaction (protein glycation) on the digestibility and the binding properties of IgG (rabbit) and IgE (human) to ovomucoid and ovalbumin to assess the impact on their allergenicity. Although no difference was found in the susceptibility of the ovalbumin samples heated to the pasteurization temperature compared to the native ovalbumin, the samples heated at 90°C for 15 min showed an increased susceptibility to proteolysis during digestion. The Maillard reaction, however, impaired the digestibility of ovalbumin. Neither heat treatment nor Maillard reaction had any effect on digestibility of ovomucoid. Heat treatment significantly reduced the binding of IgG and IgE to both the proteins. A reduction of approximately 40% and 60% of the original values was observed in the binding of IgG and IgE to ovalbumin when samples were heated at 90°C. Although Maillard reaction

reduced the binding of IgE to ovalbumin, it increased the binding of IgE to ovomucoid, suggesting its resistance to denaturation and digestive enzymes. Heating ovalbumin samples at 100°C for 5 min has been reported to accelerate the proteolysis of ovalbumin (Takagi et al., 2003). The results also indicated the formation of protein aggregates, stabilized mainly by disulfide bonds, in the thermally treated samples particularly at 90°C that exceeded the denaturation temperature of ovalbumin of 78.3°C (de Groot & de Jongh, 2003). No effect of thermal treatments was observed on the in vitro digestibility of the ovomucoid. Several studies have reported the resistant nature of ovomucoid to thermal treatments in solution (Kosti et al., 2013; Shin et al., 2013).

Schmidt et al. (2007) studied the effect of different thermal treatments, including pasteurization, on in vitro protein digestibility of egg albumin and whole egg proteins. The raw egg albumin and whole egg liquid samples were freeze-dried and the developed powder was thermally processed in glass covered Petri dishes in a programmable laboratory autoclave at 100°C, 105°C, 110°C, 115°C, and 120°C for 10 min. The spray-dried egg albumin and whole egg liquid samples were also given same thermal treatments and were subjected to in vitro gastrointestinal digestion using pepsin and pancreatin. For pasteurization process (a regulatory requirement for *Salmonella* decontamination), spray-dried egg albumin and whole egg samples were packaged in a sealed polyethylene-lined corrugated boxes and stored in a controlled environment (hot room) at 70°C for 1, 2, 3, 5, and 14 days. The raw and spray-dried samples that were not thermally processed were also subjected to digestion. The digestibility of raw egg albumin increased significantly (39%–53%) following spray-drying process (mild heat treatment) and a significant increasing trend was observed in the protein digestibility with increasing processing temperature till 105°C (>80%). No significant change was observed in the protein digestibility thereafter till 120°C. No significant effect of spray-drying process or thermal treatments was observed on the digestibility of whole egg protein samples. The pasteurization process had a minimal effect and did not increase the protein digestibility of egg albumin and whole egg protein samples significantly in vitro. The authors stated that relatively low temperature and moisture maintained in the hot room during the pasteurization process were less effective compared with autoclave in affecting the digestibility. The effect of thermal processing was attributed to disrupting effect of heat on the structure of the proteins and inactivation of protease inhibitors, allowing greater hydrolysis by digestive enzymes.

An increase in the susceptibility to enzymatic hydrolysis of egg white proteins after heat treatment has been reported in other studies. Van der Plancken et al. (2003)

studied the effect of a thermal treatment (50°C–92°C) on the enzymatic hydrolysis of albumin and ovalbumin by trypsin and α -chymotrypsin (37°C and pH 8.0). A time-dependent change in the susceptibility of both the egg white proteins to enzymatic hydrolysis was recorded and a significant increase was observed in the degree of hydrolysis of the proteins after 10 min of enzymatic digestion. This heat-induced change in susceptibility of egg white proteins was described by a first-order fractional conversion model and the final degree of unfolding of the proteins was observed to be temperature dependent. In a similar study, van der Plancken et al. (2004) studied the effect of thermal processing (50°C–85°C) on the enzymatic hydrolysis of egg white solutions (10% v/v, pH 7.6) by a mixture of trypsin and α -chymotrypsin (37°C and pH 8.0) at atmospheric pressure. A time-dependent change in the susceptibility of the egg white proteins to enzymatic hydrolysis was recorded and a significant increase was observed in the degree of hydrolysis of the proteins after 10 min of enzymatic digestion that was described by a first-order fractional conversion model.

Wang et al. (2018) studied the effect of pasteurization and hard-boiling process on the digestibility of egg white proteins. To simulate the shell egg pasteurization and hard-boiling process, the egg white proteins were heated at 56°C and 100°C in a water bath for 32 and 5 min, respectively. Another set of samples were heated at 65°C in a water bath for 30 min to simulate the traditional Japanese way of cooking eggs in a hot spring (popularly known as Onsen-tamago). Control samples were not thermally processed and were stored at 4°C. All samples were freeze-dried and subjected to in vitro gastrointestinal digestion simulation using pepsin and pancreatin. A significant impact of pasteurization and boiling was observed during pepsin digestion and the in vitro digestibility of the egg white protein samples showed a significant increasing trend with increasing temperature with highest values observed for the samples heated at 65°C. The protein digestibility of the boiled samples was significantly lower than the samples heated at 65°C and comparable with pasteurized samples. The authors suggested that the thermal treatment at 100°C might have induced protein aggregation and reduced the available recognition sites for peptic hydrolysis. Proceeding to trypsin digestion, the protein digestibility of the thermally treated egg white protein samples showed a significant increasing trend with increasing temperature with significantly highest values observed for the boiled samples. This increase in the digestibility of the boiled egg white protein samples was attributed to the inactivation of trypsin inhibitors in the egg white during high-temperature cooking. The ovomucoid, a competitive trypsin inhibitor, present in egg white inactivates at a temperature of 90°C–120°C (van

der Plancken et al., 2004). The other egg white proteins have been reported to reduce the thermal stability of ovomucoid under high-temperature cooking conditions (van der Plancken et al., 2004). These findings were further supported by the results of SDS-PAGE as most of the bands were less than 35 kDa for the samples heated at 56°C and 65°C during peptic digestion, whereas the higher molecular weight bands for the samples heated at 100°C indicate a lower digestibility. However, after pepsin + pancreatin digestion, most of the bands were less than 25 kDa for the samples heated at 100°C, indicating that the digestibility increased after pancreatin digestion. Similar results have been presented by other studies (Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al., 2017; Lechevalier, Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Le Gouar, et al., 2017; Netting et al., 2015). These effects of the thermal treatments were supported by the results of peptide analyses (MALDI-TOF-MS and nano liquid chromatography-electrospray ionization-tandem mass spectrometry (Nano-LC-ESI-MS/MS)). In comparison to the samples treated at 56°C and control, a small proportion of high molecular weight peptides were found in the digested samples treated at 65°C and 100°C, possibly due to the hydrolysis of the egg white proteins into smaller peptides for these thermal treatments. Thus, the results of this study conclude that high-temperature thermal processing favors higher digestibility in egg white proteins during in vitro gastrointestinal digestion.

Xue, Tu, et al. (2020) studied the effect of braising on in vitro digestibility of egg white proteins. The fresh eggs were heated in boiling water for 10 min, shells were removed, water and marinades were added and the mixtures were allowed to boil for 3 h. The boiled eggs were used as control samples. The egg white samples were taken after every 30 min and were subjected to in vitro gastrointestinal digestion. The protein digestibility of the braised egg white samples was significantly ($p < .05$) higher than the control samples and showed a significant increasing trend with increasing cooking time up to 1.5 h. The protein digestibility of all the samples showed a decreasing trend after 1.5 h and the digestibility of the braised samples was significantly ($p < .05$) lower than the control samples after 2 h till 3 h of cooking time. The increase in the digestibility of the braised samples till 1.5 h of cooking was due to the destruction of egg white gel and formation of holes in the microstructure (Xue, Tu, et al., 2020).

The decrease in digestibility of the samples with further braising time was attributed to the formation of protein aggregates, the reactions of proteins with sugars or lipids, isomerization and desulfurization of amino acids, and formation of indigestible chelates, all of which have been reported to decrease the protein digestibility of egg white proteins (Hu et al., 2015; Lechevalier, Guérin-

Dubiard, Anton, Beaumal, Briand, Gillard, Le Gouar, et al., 2017). Correlation analysis revealed a negative relationship (-0.847 , $p < .05$) between ionic bonds and protein digestibility, whereas a positive correlation (0.834 , $p < .05$) was established between disulfide bonds and digestibility. These results were explained as the proteases found it difficult to disrupt ionic bonds (Xue, Tu et al., 2020). Further, it was speculated that the formation of β -turns was aided by formation of ionic bonds.

Thus, most of the studies suggest that thermal treatments tend to improve the digestibility of the egg proteins and in general the higher processing temperatures induce greater positive impact on the protein digestibility due to the favorable effects on the gel microstructure and protein aggregation that facilitate the diffusion of enzymes and their accessibility to the hydrolytic sites. Future studies should cover more intense treatments and for longer duration and correlate the effects on digestibility with the protein structural changes and gel microstructure. The studies should be designed with a particular emphasis on the sample protein concentrations and use of enzyme: protein ratios as close possible to the in vivo conditions to mimic the actual gastrointestinal conditions.

3.2 | Other processing factors

In addition to thermal processing, several other factors such as method of cooking, presence of other ingredients, such as polyphenols, during digestion and structural properties of the protein gels can influence the digestibility of the egg proteins. Nolasco et al. (2020) studied the effect of hen breed and cooking methods on the digestibility of the egg proteins assessed by degree of hydrolysis and peptide content (% w/w) during in vitro digestion. The eggs from White Leghorn (WL) and Rhode Island Red (RIR) were cooked in boiling water for 10 mins or the homogenized whole egg liquids were pan fried at 191°C for 90 s on each side and were subjected to simulated gastrointestinal digestion. Although a higher digestibility was expected for boiled eggs cooked at lower temperature compared to the fried samples, no significant effect of cooking methods or hen breed was observed on the digestibility of the egg proteins. However, the overall mean of all the boiled treatments (17.0% and 18.80% for WL and RIR, respectively) showed higher digestibility compared to the overall mean of all fried treatments (12.90% and 10.60% for WL and RIR, respectively) during gastric phase of digestion. Table 3 presents the effect of microwave, pickling and other processing factors on digestibility of egg proteins.

Martos et al. (2013) studied the effect of presence of egg yolk on the hydrolysis of egg white proteins during in vitro gastrointestinal digestion and observed a slight

TABLE 2 Effect of thermal processing and pasteurization on the digestibility of egg proteins

Authors	Aspects studied	Processing parameters	Main findings
Lechevalier, , Guérin-Dubiard, Anton, Beaumal, Briand, Gillard, Gouar, et al. (2017)	Studied the effect of pasteurization on in vitro protein digestibility of whole egg proteins	The whole egg liquid was homogenized at 5 MPa and was pasteurized at 60°C, 62°C, 64°C, or 66°C for 4, 7 or 10 min	The samples pasteurized for 4 min at 60°C, 62°C, or 64°C and 7 min at 60°C showed significantly higher or equal degree of hydrolysis compared to control samples after gastric digestion. After the intestinal digestion, samples pasteurized at 60°C (4, 7, and 10 min), 62°C (4 min), 64°C (4 min), and 66°C (10 min) showed significantly higher degree of hydrolysis compared to control samples. All the other combinations showed significantly lower degree of hydrolysis
Wang et al. (2018)	Studied the effect of pasteurization and hard-boiling process on the in vitro digestibility of egg white proteins	The egg white proteins were heated at 56°C and 100°C in a water bath for 32 and 5 min, respectively. The other samples were heated at 65°C for 30 min	The digestibility showed a significant increasing trend with increasing temperature up to 65°C compared to control samples. The protein digestibility of the boiled samples was significantly lower than the samples heated at 65°C and comparable with pasteurized samples
Liu et al. (2017)	Studied the effect of conventional thermal pasteurization on the protein digestibility of ovomucin-depleted egg white	The ovomucin-depleted egg white liquid was heated to 60°C for 10 min and was compared with the untreated control samples and the samples heated at 80°C for 10 min	No effect of pasteurization was recorded on the protein digestibility of ovomucin-depleted egg white compared to control samples. A significant increase was observed in the digestibility of the samples heated at 80°C compared to both control and pasteurized samples
Jiménez-Saiz et al. (2011)	Studied the effect of pasteurization on the digestibility of ovalbumin and ovomucoid during in vitro gastrointestinal digestion	The aqueous solutions (5 mg/ml, pH 7.0) of ovalbumin and ovomucoid were heated in a water bath at 65°C for 30 min and were compared with the samples heated to a common cooking temperature (90°C for 15 min) and unheated samples	No difference was recorded in the susceptibility of the pasteurized ovalbumin samples compared to the native ovalbumin. The samples heated at 90°C for 15 min showed an increased susceptibility to the enzymatic hydrolysis that resulted in an increased proteolysis during digestion
Schmidt et al. (2007)	Studied the effect of different thermal treatments, including pasteurization, on in vitro protein digestibility of egg albumin and whole egg proteins	Freeze-dried egg albumen and whole egg powders were thermally processed in glass covered Petri dishes in an autoclave at 100°C, 105°C, 110°C, 115°C, and 120°C for 10 min. For pasteurization process, spray-dried egg albumen and whole egg samples were packaged in a sealed polyethylene-lined corrugated boxes and stored in a controlled environment (hot room) at 70°C for 1, 2, 3, 5, and 14 days	A significant increasing trend was observed in the protein digestibility of egg albumin with increasing processing temperature until 105°C. No significant effect of spray-drying process or thermal treatments was observed on the digestibility of whole egg protein samples. The pasteurization process had a minimal effect and did not increase the protein digestibility of egg albumen and whole egg protein samples significantly in vitro.

(Continues)

TABLE 2 (Continued)

Authors	Aspects studied	Processing parameters	Main findings
Van der Plancken et al. (2003)	Studied the effect of a thermal treatment on the enzymatic hydrolysis of albumen and ovalbumin	The albumen and ovalbumin samples were heated at 50°C–92°C	A time-dependent change in the susceptibility of both the egg white proteins to enzymatic hydrolysis was recorded and a significant increase was observed in the degree of hydrolysis of the proteins
Van der Plancken et al. (2004)	Studied the effect of thermal processing on the enzymatic hydrolysis of egg white	The egg white solutions (10% v/v, pH 7.6) were thermally processed at 50°C–85°C	A time-dependent change in the susceptibility of the egg white proteins was observed and a significant increase was observed in the degree of hydrolysis of the proteins
Xue, Tu, et al. (2020)	Studied the effect of braising on in vitro digestibility of egg white proteins	The fresh eggs were heated in boiling water for 10 min, shells were removed, water and marinades were added and the mixtures were allowed to boil for 3 h.	The protein digestibility of the braised egg white samples was significantly ($p < .05$) higher than the control samples and showed a significant increasing trend with increasing cooking time up to 1.5 h. The protein digestibility of all the samples showed a decreasing trend after 1.5 h and the digestibility of the braised samples was significantly ($p < .05$) lower than the control samples after 2 h till 3 h of cooking time
Liu et al. (2018)	Studied the effect of thermal treatments, including pasteurization, on the protein susceptibility of ovomucin-depleted egg white during in vitro gastrointestinal digestion	The ovomucin-depleted egg white liquid was isothermally heated at 60 or 80°C for 10 min and were compared with the unheated control samples	No significant effect of thermal pasteurization was observed on the susceptibility of ovomucin-depleted egg white. A significant increase was observed in the susceptibility of the samples heated at 80°C and the protein ovalbumin was completely hydrolyzed during first 30 min of peptic digestion. The peptide production of the samples heated at 80°C after 30 min of the peptic digestion was similar or even higher than the control samples
Nyemb, Guerin-Dubiard, et al. (2014)	Studied the impact of heat-induced aggregation on in vitro digestion of ovalbumin	The ovalbumin was heated at 80°C (pH 2–9) at different ionic strengths (0.03, 0.3, and 0.8 M NaCl) for 6 h	The extent of hydrolysis was lower for spherical aggregates compared to the linear aggregates. The relative amounts of soluble peptides were 27%, 43%, 61%, 77%, and 100% for nonaggregated ovalbumin and spherical-agglomerated, spherical, linear-branched, and linear ovalbumin aggregates, respectively.
Lassé et al. (2015)	Studied the effect of thermal treatment, pH, and glycation on in vitro digestibility of egg white proteins	The egg white samples of two salt concentrations (0 mM, 200 mM) were adjusted for pH (2, 5, 7, 9, and 12) and heated at 20°C or 100°C for 10 min. The methylglyoxal or glucose was mixed with samples (100 mM) to determine the effects of glycation	A two- to threefold higher degree of hydrolysis was observed for samples heated at 100°C compared to 20°C during the entire period of digestion. A minimal effect of pH and salt was observed on the digestibility. Presence of methylglyoxal increased the degree of amino acid modifications by up to 38% of available arginine residues

TABLE 3 Effect of microwave, pickling, and other processing factors on the digestibility of egg proteins

Authors	Aspects studied	Processing parameters	Main findings
Other processing factors			
Nolasco et al. (2020)	Studied the effect of cooking methods on the digestibility of the egg proteins during in vitro digestion	The eggs from White Leghorn and Rhode Island Red were cooked in boiling water for 10 mins or the homogenized whole egg liquids were pan fried at 191°C for 90 s on each side	No significant effect of cooking methods or hen breed was observed on the digestibility of the egg proteins
Martos et al. (2013)	Studied the effect of presence of egg yolk on the hydrolysis of egg white proteins	The egg white proteins were hydrolyzed in the presence of egg yolk during in vitro gastrointestinal digestion	A slight increase was observed in the susceptibility and hydrolysis of egg white proteins during digestion
Shen et al. (2014)	Studied the effect of tea polyphenols on the in vitro gastrointestinal digestion of ovalbumin and lysozyme	The ratio of protein and tea polyphenols used in the assay were 1:0, 1:0.25, 1:0.5, 1:1, 1:2, 1:4, and 1:8 w/w	The presence of tea polyphenols increased the susceptibility of both the proteins to peptic digestion at pH 1.2, whereas the hydrolysis of the proteins was inhibited during pancreatin digestion at pH 7.5
Nyemb et al. (2016)	Studied the effect of structural properties on the in vitro protein digestion of egg white gels and its digestion products	The pasteurized egg white (pH 5.0) and egg white ultrafiltrates (8 kDa cutoff, pH 2.0, 7.0, and 9.0) were used to obtain a range of gel structures and properties	The particulate gel comprising spherical aggregates showed greater digestion compared to the filamentous gel comprising linear aggregates
Microwave processing			
Zhu et al. (2018b)	Studied the effect of microwave processing on the digestibility of egg white proteins during in vitro gastrointestinal digestion	The liquid egg white samples were heated by microwave (2.45 GHz, 1000 W) at 60°C, 70°C, and 80°C for 1, 3, and 5 min	An increasing trend was observed in the protein digestibility with increasing temperature with highest values recorded for the samples heated at 80°C. A decreasing trend was observed in the protein digestibility with increasing processing time and significantly lower values were observed for the samples processed for 5 min
Evenepoel et al. (2013)	Studied true ileal digestibility of microwave cooked egg protein compared to raw egg protein during an in vivo study	Five human subjects were studied after having 25 g of microwave cooked ¹³ C- and ¹⁵ N-labeled egg protein and after having same test meal in raw form	A significant increase was observed in the true ileal digestibility of microwave cooked egg protein (90.9%) compared to raw egg protein (51.30%)
Pickling			
Guo et al. (2019)	Studied the effect of pickling on the protein digestibility of duck egg white during in vitro gastrointestinal digestion	Fresh duck shelled eggs were soaked in pickling solution (4% NaOH, 3% NaCl and 0.3% CuSO ₄) at 25°C for 42 days	A significant declining trend was recorded in the protein digestibility with increasing pickling time. The egg white samples pickled for 14 days showed significantly higher protein digestibility, whereas the samples pickled for 42 days showed significantly lower digestibility throughout the digestion

(Continues)

TABLE 3 (Continued)

Authors	Aspects studied	Processing parameters	Main findings
Zhao, Cao, et al. (2020)	Studied the effect of different pickling solutions on the protein digestibility of duck egg whites during in vitro gastric digestion	Fresh duck shelled eggs were soaked in pickling solution containing 4.5% NaOH, 3% NaCl and 0.3% CuSO ₄ or a mixture of CuSO ₄ (0.15%) and ZnSO ₄ (0.15%) or PbO (0.3%) at 25°C for 40 days	Significant differences existed among the samples pickled in different solutions on 4th, 8th, and 16th day of pickling and the egg white gel samples with lowest viscosity/density showed highest protein digestibility. After 40 days of pickling, no significant differences existed among the samples
Deng et al. (2020)	Studied the effects of alternative ions on the gelation behavior and digestion characteristics of alkali-induced egg white gel	The KCl, CaCl ₂ , ZnCl ₂ , and FeCl ₃ at different concentrations were separately mixed with alkaline solution (4% NaOH) and duck egg white liquid to form the egg white suspensions containing metal ions at a final concentration of 0%, 0.0083%, 0.0167%, 0.0333%, 0.0667%, and 0.133% were incubated in a water bath at 25°C for 2 h to allow the complete formation of the gel	In general, presence of metal ions increased the digestibility of egg white gels compared to control samples (0% metals) and the digestibility of the gels increased with increasing concentration of metal ions

increase in the susceptibility and hydrolysis of egg white proteins during digestion. Shen et al. (2014) studied the effect of tea polyphenols on the in vitro gastrointestinal digestion of protein ovalbumin and lysozyme. A dose-response experiment was carried out and the ratio of protein and tea polyphenols used in the assay were 1:0, 1:0.25, 1:0.5, 1:1, 1:2, 1:4, and 1:8 w/w. The results showed that the presence of tea polyphenols increased the susceptibility of both the proteins to peptic digestion at pH 1.2, whereas the hydrolysis of the proteins was inhibited during pancreatin digestion at pH 7.5. The spectroscopic analysis revealed that a strong conformational change occurred in both the proteins at pH 1.2 due to noncovalent interactions between these proteins and the tea polyphenols resulting in an increase in random and β -sheet structures at the expense of α -helix, leaving these proteins with loose structures. The noncovalent interactions between these proteins and the tea polyphenols resulted in a second opposite structural change in both the proteins at pH 7.5. The authors attributed this conformational change and the second opposite structural change as the reasons behind the promoting and inhibiting effect of tea polyphenols on the digestion of the proteins.

Nyemb et al. (2016) studied the effect of structural properties on the in vitro protein digestion of egg white gels and its digestion products. The pasteurized egg white (pH 5.0)

and egg white ultrafiltrates (8 kDa cutoff, pH 2.0, 7.0, and 9.0) were used to obtain a range of gel structures and properties. The egg solutions were heated at 80°C for 1 h in a water bath. The particulate gel comprising spherical aggregates showed greater digestion compared to the filamentous gel comprising linear aggregates that was attributed to the differences in the enzyme diffusion due to the differences in the microstructural characteristics of the gels or differences in the digestive enzyme adsorption or catalytic activity because of the local differences in pH within the gel matrices. The LC-MS/MS analysis revealed that the aggregate morphology modulated the hydrolysis of ovotransferrin that resulted in the production of specific peptides depending on the gel structure.

The conformational changes induced in the protein solutions during thermal processing affect their secondary and tertiary structures and trigger unfolding of the proteins that results in the formation of aggregates that join through cross linking and form gels (Weijers et al., 2003). The aggregate morphology, such as spherical particles or linear strands, determine the structural properties of these gels, which in turn depends on heating time and temperature, ionic strength, pH, and protein concentration (Croguennec et al., 2002). Thus, protein gelation has potential to develop food structures that is known as one of the important parameters that can affect the digestion of

proteins within the gut (Fardet et al., 2013; Norton et al., 2015). The physicochemical properties of the aggregates and the gel structure have been reported to determine the susceptibility of proteins to enzymatic hydrolysis and the rate of digestion of gelled protein systems (Zhang & Vardhanabhuti, 2014). The aggregated ovalbumin not only showed a lower resistance to digestion compared to nonaggregated ovalbumin, the morphology of the aggregates also affected the extent of the digestion and the nature and the amount of the digestion products (Nyemb, Guerin-Dubiard, et al., 2014; Nyemb, Jardin, et al., 2014). Several studies have reported variations in the digestive behavior between filamentous and particulate gels formed from whey and milk proteins (Guo et al., 2014; Macierzanka et al., 2012).

Yang et al. (2021) studied the effect of degree of glycation on the *in vitro* digestibility of egg white proteins. The nonenzymatic reactions between the free reducing sugars and the amino groups of the food proteins results in glycation during Maillard reaction that induces several favorable properties and flavors in the foods (Wang, Li, et al., 2020). The D-ribose powder was used to induce the glycation and different levels of the powder (0, 20, 30, 40, 50, 60 mg) were added to 30 ml fresh egg white liquid samples that were heated in a water bath at 90°C for 40 min to simulate common cooking processes. The samples were subjected to *in vitro* gastrointestinal digestion simulation. Addition of D-ribose improved the gelling properties and significantly reduced the protein digestibility assessed in terms of soluble protein and peptide production. This decrease in the digestibility of the egg white proteins was attributed to the changes induced by glycation in the structure of the gel. The glycation resulted in a more compact and dense gel structure that increased the resistance to the diffusion of the digestive enzymes. By modifying the ratio of granular and fibrous aggregates, the degree of glycation can be used to regulate the gelling properties and the digestibility of the egg white gel and can have applications for the development of controlled release food matrices for pharmaceutical and food applications.

Although glycation seems to have a negative impact, further research is required to validate the positive impact of additives, such as polyphenols, on egg protein digestibility.

3.3 | Microwave

Microwave is a dielectric heating method that uses electromagnetic waves typically at frequencies of 915 or 2450 MHz (Rombouts et al., 2020; Zhang et al., 2013). It is a rapid heating method that results in a low-temperature gradient within the food (Arzeni et al., 2012; Zhang et al., 2013) under certain processing precautions to eliminate shadow-

ing (low heating spots at the overlapping junctions of the microwaves). Microwave ovens are used in the food industry for several applications, such as sterilization, pasteurization, and enzyme inactivation of foods, and are very common in household kitchens due to several desirable characteristics such as safe handling, high heating efficiency, ease of the operation, low energy consumption, and low maintenance (Lin et al., 2020; Zielinska et al., 2019). The ions and polar molecular contents are important factors for generating heat energy by molecular friction and collision with surrounding molecules because of rapidly vibrating electromagnetic field (Tao et al., 2020). Compared to conductive heating, microwave processing induces minimal effects on the food compounds and less damage to the nutritional and sensory quality (Han et al., 2021; Vadivambal & Jayas, 2007). Microwave processing has been reported to affect the structural and functional properties of food proteins including egg proteins (Han et al., 2021; Rombouts et al., 2020; Tao et al., 2020; Wang, Gu, et al., 2020) and can therefore have an impact on the digestibility of egg proteins during gastrointestinal digestion. A significant decrease in the protein digestibility of microwave treated food proteins has been reported. For example, Xiang et al. (2020) observed a significant decrease in *in vitro* protein digestibility of microwave treated gluten and Kamble et al. (2020) reported a significant decline in *in vitro* protein digestibility of microwave treated wheat protein (900 W for 2 min). Contrary to these studies, several other studies have reported a positive impact of microwave processing on *in vitro* protein digestibility of food proteins, such as Alajaji and El-Adawy (2006) in microwave treated chickpea proteins, Vagadia et al. (2018) in microwave treated soymilk (1000 W, 70°C–100°C for 2–8 min) and Zhu et al. (2018b) in microwave processed egg white protein (60°C–80°C for 1–5 min). The increase in the protein digestibility in these studies were attributed to the reduction in the activity of endogenous protease inhibitors by microwave-induced protein denaturation and the proceeding digestion (Vagadia et al., 2018). This suggestion might explain the lower digestibility in refined proteins from wheat where both the samples and controls will lack the confounding effects of protease inhibitors.

Zhu et al. (2018b) studied the effect of microwave processing on the digestibility of egg white proteins during *in vitro* gastrointestinal digestion. The liquid egg white protein samples were heated by microwave (2.45 GHz, 1000 W) at 60°C, 70°C, and 80°C for 1, 3, and 5 min. The samples were freeze-dried after the treatment and then subjected to *in vitro* digestion using pepsin and pancreatin. The *in vitro* protein digestibility (%) was measured using a formula (digested protein/initial protein × 100). A processing time of 1 min led to significantly higher protein digestibility for all microwave processed samples compared to control

samples (73.44%). An increasing digestion trend was observed with increasing temperature with highest digestibility values recorded for the samples heated at 80°C (89.26%). A decreasing trend was observed in the protein digestibility with increasing processing time and significantly lower values were observed for the samples processed for 5 min (78.94%, 71.15%, and 75.97% for samples processed at 60°C, 70°C, and 80°C, respectively). All the microwave processed egg white protein samples were more digestible than the controls with the exception of the samples treated at 70°C for 5 min (71.15%). The microwave processing at 60°C–80°C increased the digestibility of egg white proteins by up to 15.82% compared to the control samples.

The changes induced by the microwave processing on the secondary structure of the egg white proteins were also studied. Microwave processing caused a significant increase in the β -sheets and unordered structures contents of the microwave processed samples compared to control. This increase was concomitant with a decrease in α -helical content in the microwave processed samples. The secondary structures of the samples processed at 60°C for 1 min were comparable with control samples. One important finding reported in this study was that no changes were observed in the secondary structures of the egg white proteins treated at 80°C with increasing processing time. The authors (Zhu et al., 2018b) stated that the results indicate a nonthermal effect of microwave that induced and accelerated the denaturation and folding process of the egg white proteins. The nonthermal irradiation produced during microwave processing has been reported to destabilize the conformation of egg white lysozyme (Singh, Burada, et al., 2018) and affect the kinetics of β -lactoglobulin folding (Bohr & Bohr, 2000). The structural transformations recorded during the study indicate that microwave processing induced partial unfolding of egg white proteins making them more flexible and extended. Microwave processing might have disrupted the hydrogen bonds that led to the conformational changes resulting in the exposure of hydrophobic regions and transformed the secondary structures (Han, Wang, et al., 2015).

A similar microwave induced transition from β -sheets to α -helix structures has been reported in microwave processed (300 to 800 W for 12 s) chicken myofibrillar proteins (Wei, Yang, et al., 2020). The effect of microwave treatment on egg protein appears to be dependent on the moisture content. For example, in contrast to Zhu et al. (2018b) findings, Wang, Gu, et al. (2020) observed an increase in the levels of α -helices and unordered structures, whereas a decrease was recorded in the β -sheet content compared to unprocessed control samples during microwave processing (440 W, 6 min) of spray-dried egg white protein powders. The microwave processing induced

the transition from β -sheet structures to α -helix and random coil structures. The secondary structure profile of the microwave processed spray-dried samples was very similar to that of the native liquid egg white proteins, indicating that the microwave treatment reversed the conformational changes induced by the spray-drying process (Wang, Gu, et al., 2020). Information relating changes in egg protein structure and its dielectric properties are rare and this information may help explaining the different effects observed above.

In agreement with the findings of Zhu et al. (2018b), Evenepoel et al. (2013) observed a significant increase in the true ileal digestibility of microwave cooked egg protein (90.9%) compared to raw egg protein (51.30%) during an *in vivo* study. Five human subjects were studied after consuming 25 g of cooked ^{13}C - and ^{15}N -labeled egg protein and after having same test meal in raw form. After consumption of the test meal, ileal effluents and breath samples were collected at regular intervals and assessed for ^{15}N - and ^{13}C -content, respectively.

These studies suggest that microwave processing tend to improve the digestibility of the egg proteins both *in vivo* and *in vitro*. In general, the higher processing temperatures induce greater positive impact on the protein digestibility due to the favorable protein structural changes, however, caution has to be taken to avoid negative effects of prolonged processing time.

3.4 | Pickling

Generally produced by pickling of shelled eggs for 6 weeks using a strong alkali, such as NaOH, preserved eggs are popular traditional egg products in China and South-east Asian countries (Zhao, He, et al., 2020; Guo et al., 2019). The alkali solution gradually permeates through the eggshell and its membranes into the egg white and induce denaturation of the egg white proteins to form a three-dimensional protein network gel at room temperature. Most of the egg white proteins undergo degradation during pickling and result in the formation of peptides and free amino acids that contribute a unique flavor and color to these pickled eggs (Zhao et al., 2016). The pickled egg white gels have been reported to possess anti-inflammatory properties in Caco-2 cells and animal models due to the presence of these bioactive peptides (Zhang, Zhao, et al., 2019; Zhang et al., 2018; Zhao, Yao, et al., 2017). The quality of the egg white gel is determined by the diffusion and concentration of the alkali, while a low concentration does not induce sufficient denaturation to form a gel, higher concentration can destroy the formed gel (Zhao, Tu, et al., 2014). Therefore, some heavy metal compounds, such as copper sulfate, zinc sulfate, and lead oxide, are added to

the pickling solution to control the rate of diffusion and the concentration of the alkali in the egg white. As the pickling process proceeds, these added metal ions produce some insoluble compounds by reacting with H_2S produced during protein degradation and block the eggshell pores from within and regulate the concentration of alkali by preventing any further diffusion into the egg white (Tu et al., 2013).

In general, consumption of pickled and preserved eggs is considered safe (Fu et al., 2014; Ganesan, 2010). While studying the potential health risk of Chinese traditional egg products including preserved eggs, Fu et al. (2014) reported a target hazard quotient and hazard index of less than 1, indicating that intake of heavy metals from preserved eggs and other egg products did not present a significant hazard risk to humans. Although heavy metals can cause toxicity if taken in higher levels, trace elements such as copper, iron, manganese, and zinc are required for normal catabolic body processes and are nutritionally essential for a healthy life. However, some traditional processes use lead, a toxic and carcinogenic element, to yield desirable characteristics in the pickled eggs. These are increasingly being replaced by alternative cations, especially calcium and zinc, due to safety concerns and consumer awareness (Ganesan, 2010). The maximum permissible limit for lead is 0.1 mg/kg for eggs (EOSQC, 1993). The U.S. FDA and European Commission have not established regulatory limits for concentrations of lead in eggs. The U.S. FDA has recommended a maximum level of 0.1 $\mu\text{g/g}$ of lead in candy and children have been suggested to consume less than 6 $\mu\text{g/day}$ of lead (Bautista et al., 2014). The European Commission has recommended a maximum permissible level of 0.02, 0.1, and 0.3 mg/kg of lead for milk, meat, and fish, respectively (European Commission Regulation (EC) No. 1881/2006). The maximum permissible limit of copper, zinc, cadmium, and chromium in eggs are 10, 20, 0.05, and 0.002 mg/kg, respectively (Islam et al., 2014).

Guo et al. (2019) studied the effect of pickling on the protein digestibility of duck egg white during *in vitro* digestion. Fresh duck shelled eggs were soaked in pickling solution (4% NaOH, 3% NaCl, and 0.3% CuSO_4) at 25°C for 42 days. The samples were taken on day 14, 28, and 42 of the pickling and were subjected to *in vitro* gastrointestinal digestion simulation. The digestibility (%) was measured using a formula (protein in supernatant/protein in samples $\times 100$). A significant effect of pickling was found and the protein digestibility showed a significant declining trend with increasing pickling time. The egg white samples pickled for 14 days showed significantly higher protein digestibility, whereas the samples pickled for 42 days showed significantly lower digestibility throughout the gastrointestinal digestion. The Fourier transform infrared (FTIR) spectroscopic analysis revealed that the pickling

process resulted in a decrease of α -helical content and increased the content of β -sheets. A negative correlation was established between the content of β -sheets and the protein digestibility of the pickled egg white proteins. The LC-MS/MS analysis revealed a negative correlation between a number of unique identified peptides and protein digestibility at the end of both gastric and intestinal digestion, although the number of peptides produced increased with increasing pickling time. The secondary structure has been reported to affect the digestion of food proteins and a positive correlation with the content of β -turns and a negative correlation with β -sheets content has already been established (Sun et al., 2014).

The transmission electron microscopy (TEM) results showed the presence of two types of microstructures, namely, fibrillar that existed from day 7 to 21 of the pickling and particulate, that existed from day 28 to 42. A positive correlation was found between the protein digestibility and the pore size of pickled egg white gels. The pore size of the internal aggregation of the egg gels has been reported as an important factor that affects the protein digestibility by influencing the diffusion rate of protein enzymes (Guo et al., 2019; Nyemb et al., 2016). A decrease in the protein digestibility during the muscle protein digestion was attributed to the presence of a tight protein network structure (Kaur et al., 2014).

Zhao, Cao, et al. (2020) studied the effect of different pickling solutions on the protein digestibility of duck egg whites during *in vitro* gastric digestion. Fresh duck shelled eggs were soaked in pickling solution containing 4.5% NaOH, 3% NaCl, and 0.3% CuSO_4 or a mixture of CuSO_4 (0.15%) and ZnSO_4 (0.15%) or PbO (0.3%) at 25°C for 40 days. The samples were taken on day 4, 8, 16, 32, and 40 of the pickling and were subjected to *in vitro* gastric digestion simulation using pepsin. Significant differences existed among the samples pickled in different solutions on 4th, 8th, and 16th day of pickling and the egg white gel samples with lowest viscosity/density showed highest protein digestibility. However, after completion of 40 days of pickling, no significant differences existed among the samples. As the protein digestibility of the egg white gel showed a marginal increase with increasing pickling time, the authors stated that higher proteolysis of the egg white gel into smaller peptides and amino acids had occurred in the samples pickled for 40 days and the effect of the metal ions disappeared.

The mean values of the protein digestibility of the alkali-induced egg white gel reported in this study were much higher than the heat-induced egg white gel and that difference was explained on the basis of the mechanism of the formation of the gel. Although the heat-induced egg white gel forms a relatively disordered dense structure, the preserved egg white gel is an ordered loose pore like

structure (Gharbi & Labbafi, 2018). Although Zhao, He, et al. (2020) speculated a relationship between the gel hardness and protein digestibility of preserved egg whites, such speculations have been experimentally demonstrated in the whey gels and the soft whey protein emulsion gel (with 10 mM added NaCl) has been reported to digest rapidly compared to a hard gel (with 200 mM of NaCl; Guo et al., 2014). The results showed that the pickling solutions containing different metal salts can affect the molecular interactions and the density of the internal structure of preserved egg white gel and can therefore change the digestive properties of the gel.

Deng et al. (2020) studied the effects of alternative ions (K^+ , Zn^{2+} , Ca^{2+} , and Fe^{3+}) on the gelation behavior and digestion characteristics of alkali-induced egg white gel. The KCl, $CaCl_2$, $ZnCl_2$, and $FeCl_3$ salts at different concentrations were mixed separately with alkaline solution (4% NaOH) and duck egg white liquid to form the egg white suspensions containing metal ions at a final concentration of 0%, 0.0083%, 0.0167%, 0.0333%, 0.0667%, and 0.133% and were incubated in a water bath at 25°C for 2 h to allow the complete formation of the gel. The study was aimed to improve the pickling process and to replace the heavy metals in preserved eggs to safeguard the consumer health. The samples were subjected to *in vitro* gastric digestion using pepsin. In general, the presence of metal ions increased the digestibility of egg white gels compared to control samples (0% metals) and the digestibility of the gels increased with increasing concentration of metal ions. Metal ions, especially at higher concentrations, decreased the cross-linking of gel networks by affecting the aggregation of protein molecules and resulted in the formation of an uneven and a loose gel network with a weak gel strength (Ngarize et al., 2005). Such gels allow greater diffusion and mixing of enzymes and digestive juices and are more susceptible to enzymatic hydrolysis. On the contrary, the gels with a denser microstructure having tighter and regular spatial structure and greater hardness does not allow an easy and a free diffusion of digestive enzymes into the gel structure/particles, thereby reducing the protein digestibility. Interestingly, the relatively denser microstructure and greater hardness of the egg white gels containing 0.0167% zinc ions showed lowest digestibility compared to control and other zinc ions induced gels. Although Zn^{2+} induced cold whey protein gels have been reported to digest faster and to a greater extent compared to Ca^{2+} and Mn^{2+} induced gels (Mohammadian & Madadlou, 2016) and the authors stated that the compact microstructure of Zn^{2+} induced whey protein gels imbibed more water and were more fragile and susceptible to enzymatic hydrolysis. It appeared that the zinc ions work differently during the formation of gels from different protein sources and may be attributed to the differences in

the binding of the proteins to the zinc ions. Further, there are greater structural differences between alkali-induced egg white gels and cold whey protein gels and caution has to be taken before extrapolating these results. In general, the results of the study (Deng et al., 2020) revealed that low concentration of metal ions favored the hardness and a regular microstructure, whereas the higher concentrations resulted in a disordered structure that were more susceptible to the digestive enzymes.

3.5 | High-pressure processing

HPP is an emerging and environmentally friendly mild processing technology that follows Le Châtelier's or Pascal's principles to induce various effects in foods during processing (Li et al., 2021). Known by several other names, such as cold pasteurization, bridgmanization, or pascalization, HPP has demonstrated very promising potential in several applications in food industry such as cold food sterilization and pasteurization, meat tenderization, extraction of bioactive components, reduction of allergenicity, sodium reduction, and enzyme inactivation (Bhat, Morton, Mason, & Bekhit, 2020; Bhat et al., 2018b; Duffuler et al., 2020; Huang et al., 2020; Morton et al., 2017). The HPP-processed foods represent an increasing market share in several food segments including egg and egg products (Xia, Zheng, et al., 2020). HPP has been reported to induce several effects in food proteins such as alteration in the secondary structure of cumin seed proteins (Chen et al., 2019), disruption or unfolding of the mussel proteins (Zou, Zhao, et al., 2020) and improved digestibility of cod and meat proteins (Xue, Wang, et al., 2020; Zhang, Bi, et al., 2019). As HPP has been reported to affect the secondary structure, microstructure, and the particle size of egg proteins (Zhang, Li, et al., 2020), it is very likely that it will affect the digestibility of the egg proteins during gastrointestinal digestion by increasing the access of enzymes to cleavage sites. Table 4 presents the effect of high-pressure, PEF, and ultrasound processing on digestibility of egg proteins.

Ma et al. (2015) studied the effect of HPP-assisted crosslinking of ovalbumin and egg white by transglutaminase on their digestibility and allergenicity during *in vitro* gastrointestinal digestion simulation. The ovalbumin and egg white aqueous solutions (5 mg/ml) were prepared from their respective freeze-dried powders and were vacuum sealed and treated at 400 MPa with water as pressure-transmitting fluid. The pressure was applied for 30 min and the temperature was maintained at $40 \pm 2^\circ\text{C}$ during the treatment. The protein digestibility of the samples was assessed by SDS-PAGE and peptide analysis of the digests using reverse phase-high performance liquid chromatography (RP-HPLC). HPP treatment was found

TABLE 4 Effect of high-pressure, pulsed electric field, and ultrasound processing on the digestibility of egg proteins

Authors	Aspects studied	Processing parameters	Main findings
High-pressure processing			
Ma et al. (2015)	Studied the effect of HPP treatment on the protein digestibility of ovalbumin and egg white during in vitro gastrointestinal digestion simulation	The ovalbumin and egg white aqueous solutions (5 mg/ml) were treated at 400 MPa for 30 min	A significant increase was observed in the digestibility of both HPP treatment ovalbumin and egg white proteins during gastric as well as intestinal digestion
Hoppe et al. (2013)	Studied the effect of HPP treatment on in vitro digestion of whole egg white remained in liquid form, a minimal effect of HPP treatment was observed on the digestibility of ovalbumin, ovomucoid and lysozyme	The whole egg liquid was treated at 400, 600, and 800 MPa for 5 min	The highest increase was observed in the digestibility of the samples treated at 800 MPa followed by 600 MPa compared to untreated control samples
Van der Plancken et al. (2004)	Studied the effect of HPP as a pretreatment before thermal processing on the digestibility of egg white proteins	The egg white solutions (10% v/v, pH 7.6) were treated with HPP (100–700 MPa), which was followed by thermal processing at 10, 25°C, 40°C, and 60°C	A pressure-dependent increase was observed in the digestibility of the HPP treated samples
Pulsed electric field			
Liu et al. (2017)	Studied the combined effect of pH (4, 5, 7, and 9) and PEF on the in vitro protein digestibility of ovomucin-depleted egg white	The ovomucin-depleted egg white liquid was processed with PEF (1.4–1.8 kV/cm, 260–690 kJ/kg, 20 µs, and 300 Hz)	A significant ($p < .05$) increase was observed in the protein digestibility of the samples with pH 4 and processed with PEF at 690 kJ/kg. No significant effect was observed on the digestibility of other samples
Liu et al. (2018)	Studied the effect of the PEF processing on the protein susceptibility of ovomucin-depleted egg white during in vitro gastrointestinal digestion	The ovomucin-depleted egg white solutions (10% v/v) were processed with PEF (1.4–1.8 kV/cm, 259–695 kJ/kg, 20 µs, and 300 Hz)	The samples treated with PEF at highest energy input 695 kJ/kg (1.7 kV/cm) and at pH 4 showed significantly higher peptide production compared to control samples
Ultrasound processing			
Zhu et al. (2018b)	Studied the effect of ultrasound processing on the digestibility of egg white proteins during in vitro gastrointestinal digestion	The samples were processed with ultrasound at 400 W for 1, 4, 8, 12, and 16 min	A significant ($p < .05$) increase was observed in the digestibility of the samples processed for 16 min compared to control samples
Stefanovic et al. (2018)	Studied the effect of ultrasound treatment on in vitro hydrolysis of egg white proteins	A 10% (w/w) egg white aqueous solution was processed at a frequency of 20 kHz for 2, 5, 10, 15, and 20 min	A significant increase was observed in the degree of hydrolysis (%) for the samples processed with ultrasound up to 15 min compared to unprocessed control samples
Jovanović et al. (2016)	Studied the effect of ultrasound processing on the enzymatic hydrolysis of egg white proteins using an in vitro digestion model	The aqueous solution of egg white (5–20 mg protein/ml) was processed for 15 min using an ultrasound water bath (40 kHz, 600 and 1200 W, $25 \pm 1^\circ\text{C}$)	The ultrasound processing significantly ($p < .05$) improved the enzymatic hydrolysis of egg white proteins under different conditions compared to control samples

to improve the digestibility of both ovalbumin and egg white proteins during gastric as well as intestinal digestion. This increase in the digestibility was attributed to HPP mediated modifications in the structure of globular pro-

teins, which acquire a more flexible structure under HPP and then slowly refold at atmospheric pressure. This explained the higher digestibility of ovalbumin when it is subjected to HPP treatment in presence of

digestive enzymes compared to HPP treatment followed by enzymatic hydrolysis (López-Expósito et al., 2008; Quirós et al., 2007). The HPP-treatment did not reduce the allergenicity of the egg proteins. No difference was observed in the digestion (nitrogen analysis and SDS-PAGE) between transglutaminase cross-linked caseinate and native caseinate by Havenaar et al. (2013) using an in vitro gastrointestinal digestion model.

Hoppe et al. (2013) studied the effect of HPP treatment on in vitro digestion of whole egg white. The whole egg liquid was treated with high pressure at 400, 600, and 800 MPa (9°C) for 5 min and was compared with samples heated at various temperatures in the range of 65°C–95°C for 5 min in a water bath. All the samples were stored for up to 3 days at 4°C and were subjected to in vitro gastric digestion using pepsin. The digestion of the samples was assessed by SDS-PAGE, 2D-electrophoresis, and peptide analysis of the digests by LC-MS/MS. The HPP treatment at 800 MPa induced gel formation in the egg white solutions and formed a firm gel. Although no gel was formed at 400 MPa treatment and the solutions remained in liquid form, a minimal effect of HPP treatment was observed on the digestibility of ovalbumin, ovomucoid, and lysozyme. An increase in the digestion products in the range of 3–10 kDa was observed in 400 MPa treatment. The samples treated at 600 MPa resulted in the formation of a very soft gel and some aggregates, likely to be composed of ovalbumin and ovomucoid. Although a substantial increase was observed in the digestibility and the digestion products of the samples treated with 600 MPa compared to untreated control samples, the highest increase in digestibility was observed in the samples that were treated at 800 MPa. These results were also supported by the results of a LC-MS/MS analysis of low molecular weight fractions (<3.0 kDa) of pepsin digests that revealed numerous peptides for the samples treated at 800 MPa compared to others. HPP treatment in the range of 400–600 MPa has been reported to increase the digestibility of egg white proteins during in vitro gastrointestinal digestion simulation by several studies (Iametti et al., 1999; Miguel et al., 2007; van der Plancken et al., 2004).

Thermally processed samples also showed an increase in pepsin digestibility with highest increase observed for the samples that were treated at 95°C for 5 min. It is important to mention here that the samples treated with 800 MPa showed higher digestibility than the samples treated at 95°C for 5 min, particularly for ovalbumin and ovomucoid. This difference was attributed to the differences in the mechanisms for unfolding of the proteins by these two treatments. Differences in the denaturation between pressure and thermally treated ovalbumin has been reported to be linked to the increase in β -sheet and disulfide contents in thermally treated ovalbumin (Ngarize et al., 2005).

Therefore, compared to thermal processing, HPP presents a potential advantage by improving the protein digestibility without negatively affecting the quality of egg proteins.

Van der Plancken et al. (2004) studied the effect of HPP as a pretreatment before thermal processing on the digestibility of egg white proteins. Egg white solutions (10% v/v, pH 7.6) were treated with HPP (100–700 MPa), which was followed by thermal processing at 10°C, 25°C, 40°C, and 60°C. An increasing temperature range was used because the pressure induced protein denaturation depends on the temperature at which pressure is applied. The samples were subjected to in vitro intestinal digestion simulation using a mixture of trypsin and α -chymotrypsin at 37°C and pH 8.0. The degree of hydrolysis of the samples was measured by the pH-stat method. The HPP treated (20 min) samples showed a pressure-dependent increase in the digestibility of the egg white proteins that was accompanied by a loss of protein solubility. This increase in the digestibility was substantial for the samples that were treated with HPP above 500 MPa (20 min).

The results of above studies suggest that HPP tends to improve the digestibility of the egg proteins and in general, the higher pressures induce greater positive impact due to the favorable changes in protein structure and gel microstructure.

3.6 | Pulsed electric field

PEF is an emerging nonthermal technology that uses electric field pulses of shorter duration, ranging from milliseconds to nanoseconds, to induce several effects in the food matrices (Bhat et al., 2018b, 2018c, 2019d). Most of the PEF effects are the outcome of its potential to induce electroporation in cellular membranes or its capability to affect the structural and functional properties of food macromolecules including egg proteins (Bhat, 2019; Bhat et al., 2019d; Farjami et al., 2020; Rodrigues et al., 2020). When protein molecules with heterogeneous charge distributions are exposed to an electric field, it causes a gradual stretching or deformation of the protein molecule along the axis of the applied electric field (Freedman, Jürgensj et al., 2011, 2013). PEF induces unfolding of the proteins by destabilizing the tertiary and secondary structures of the proteins by disrupting the electrostatic interactions of individual polypeptide chains (Zhao, Tang, et al., 2014). PEF processing of whole egg white or isolated egg white proteins (e.g., lysozyme and ovalbumin; Perez & Pilosof, 2004; Zhao & Yang, 2008) has resulted in the exposure of buried SH and hydrophobic groups/regions and even induced the cleavage of S-S bonds (e.g., in lysozyme), consequently leading to protein aggregation (Liu et al., 2018; Wu, Zhao, Yang, et al., 2014; Zhao & Yang, 2012). Recently, PEF has

been reported to increase the digestibility of food proteins such as beef, venison, milk, and egg proteins (Bhat, Morton, Mason, Bekhit, et al., 2019; Bhat, Morton, Mason, Jayawardena, et al., 2019; Bhat, Morton, Mason, Jayawardena, et al., 2020; Liu et al., 2018, 2017). Although the structural changes induced by PEF processing have been widely studied in egg proteins, only a few studies have reported the impact of PEF on the digestibility of egg proteins and needs scientific attention. Studying the effect of PEF on the digestibility of egg proteins is important in the light of the fact that this technology is explored as an alternative for conventional pasteurization of liquid foods and has been successfully applied to pasteurize liquid whole eggs (Monfort et al., 2012; Rodrigues et al., 2020). Egg proteins appear to be less affected by PEF than thermal processing provided excessive ohmic heat is avoided, and both approaches produce similar levels of microbial inactivation (Marco-Moles et al., 2011; Monfort et al., 2012).

Liu et al. (2017) studied the combined effect of pH (4, 5, 7, and 9) and PEF on the *in vitro* protein digestibility of ovomucin-depleted egg white. Development of several beverages includes the adjustment of pH that is known to affect the unfolding and aggregation process of egg proteins during thermal or nonthermal processing (van der Plancken et al., 2006). Therefore, pH adjustment may affect the susceptibility of proteins to proteolysis by digestive enzymes. After the pH adjustment (4, 5, 7, and 9), ovomucin-depleted egg white liquid was processed with PEF (1.4–1.8 kV/cm, 260–690 kJ/kg, 20 μ s, and 300 Hz) and the samples were subjected to *in vitro* gastric digestion at pH 1.5 using pepsin. The protein digestibility of the digested samples was assessed by OPA assay, SDS-PAGE, and peptide analysis by MALDI-TOF/TOF-MS. A significant ($p < .05$) increase was observed in the protein digestibility of the samples processed with PEF at 690 kJ/kg at pH 4, however, no significant effect was observed on the digestibility of the samples at other pH (5, 7, and 9) and processed with PEF in the same range (260–690 kJ/kg). The susceptibility and degradation patterns of ovalbumin, ovotransferrin and lysozyme to hydrolysis by pepsin were also studied but no significant effect of PEF treatment was observed. Although ovotransferrin in samples with pH 5 and processed with PEF at 690 kJ/kg showed some noticeable aggregation, it did not reflect a perceptible effect on the proteolysis of ovotransferrin that is known to be highly susceptible to pepsin in its native form. PEF processing is known to modify the structure of the proteins through different mechanisms such as inducing the ionization of some chemical groups, breaking electrostatic interactions, exposure of hydrophobic amino acid residues (e.g., tryptophan) and cleavage of S-S and SH groups (Bhat et al., 2018b, 2019d; Giteru

et al., 2018; Liu et al., 2017). By inducing the unfolding of tertiary and secondary structures of the proteins, PEF has potential to increase the protein digestibility by modifying the protein intrinsic flexibility and increasing the availability of the recognition sites on the proteins (Bhat et al., 2019c; Bhat, Morton, Mason, Jayawardena, et al., 2019).

Liu et al. (2018) studied the effect of the PEF treatments on the proteolysis of ovomucin-depleted egg white during *in vitro* gastrointestinal digestion. The ovomucin-depleted egg white solutions (10% v/v, 10.3 mg protein/ml) were processed with PEF (1.4–1.8 kV/cm, 259–695 kJ/kg, 20 μ s, and 300 Hz) and the samples were subjected to *in vitro* gastrointestinal digestion. The peptide production, proteolytic pattern, and the final peptide profile were evaluated to assess the changes in the protein susceptibility to enzymatic hydrolysis. A significant effect of PEF processing and pH was observed on the protein susceptibility and the samples treated with PEF at highest energy input 695 kJ/kg (1.7 kV/cm) and at pH 4 showed significantly higher peptide production compared to control samples. Further, a lower rate of protein aggregation was observed under these PEF processing conditions (695 kJ/kg and pH 4). The proteolytic patterns of ovalbumin, lysozyme, and ovotransferrin for the samples processed with PEF at 259–695 kJ/kg and pH 4–9 were essentially the same as seen for the untreated control samples. However, the samples processed with PEF at 695 kJ/kg and pH 4 showed a faster and greater extent of digestion for ovalbumin, especially during the intestinal phase, as demonstrated by lower band intensity of intact ovalbumin and its ~40 kDa fragment, particularly during the intestinal digestion. Further, the protein aggregates formed in these samples during processing (695 kJ/kg and pH 4) were quickly hydrolyzed during digestion. Protein denaturation and degree of unfolding has been reported to increase enzymatic hydrolysis of egg proteins by exposing more hydrolytic sites on the polypeptide chains, whereas intense protein aggregation made egg proteins less digestible by reducing the availability of hydrolytic sites for the digestive enzymes (Gharbi & Labbafi, 2018). Investigations on the effects of PEF on digestibility of egg proteins have shown similar results to those studies investigating other nonthermal technologies, such as high-pressure and ultrasound, indicating the potential of these technologies to improve the digestibility of egg proteins through protein structural modifications without inducing significant protein aggregation or precipitation (Farjami et al., 2020; Gharbi & Labbafi, 2018; Rodrigues et al., 2020; Zhu et al., 2018b). The pH of the protein samples seems to have a role with lower pH favoring the digestibility and should be considered for future studies.

3.7 | Ultrasound

Ultrasound processing is a nonthermal technology that is widely used in the food industry especially for its applications for food proteins (Zhang, Wang, et al., 2020). Available as a traditional single-frequency ultrasound or as more efficient multifrequency sonication, this technology induces various effects through cavitation, dynamic agitation, shear stress, turbulence, and promotion of some chemical reactions (Xia, Pan, et al., 2020). Most of the ultrasound effects on proteins are mediated through cavitation, a process that involves the formation of small vapor bubbles inside a liquid medium triggered by a sudden decrease in pressure (Li et al., 2016). The bubbles collapse violently once the pressure recovers and releases the energy collected from the surrounding liquid, resulting in the formation of extreme local conditions such as pressure shocks of up to several 100 MPa (Zhang et al., 2015), microjets with velocities above 100 m/s (Jin et al., 2016) or hot spots with extreme temperatures of the order of several 1000 K (Jin et al., 2015). Ultrasound processing has been reported to affect the structure and functionality of food proteins such as rice protein, zein, egg white proteins, and gluten protein (Jin et al., 2016, 2015; Jun et al., 2020; Li et al., 2016; Zhang et al., 2015). Ultrasound processing has been reported to disrupt the disulfide bonds between protein molecules and increasing the thiol content and reducing the disulfide content, thereby affecting the protein interactions and the structure (Zhang, Wang, et al., 2020). All these findings suggest that ultrasound processing can positively affect the digestibility of food proteins including the egg proteins.

Zhu et al. (2018b) studied the effect of ultrasonic processing on the digestibility of egg white proteins during *in vitro* gastrointestinal digestion simulation. The liquid egg white protein samples were processed with ultrasound at a power output of 400 W for 1, 4, 8, 12, and 16 min and were freeze-dried after processing. The samples for digestion were generated from the freeze-dried egg white powder using phosphate buffer and the pH was adjusted to 7.9. The samples were subjected to *in vitro* digestion using pepsin (pH 1.5) and pancreatin (pH 7.8). The *in vitro* protein digestibility (%) was measured using a formula (digested protein/initial protein $\times$ 100). A significant ($p < .05$) increase (from 73.44 to 79.41%) was observed in the digestibility of egg white proteins processed with ultrasound for 16 min compared to control (unprocessed samples), equivalent to an 8% increase. A 5% increase ($p > .05$) was recorded in the protein digestibility of the samples processed for 12 min compared to unprocessed control samples. A loss in the activity of avidin, an antinutrient, was also recorded in all the processed samples and a significant decreasing trend was observed in the avidin activity

with increasing processing time. Avidin is known to bind with biotin and forms avidin-biotin complex, making it biologically unavailable. It is also an allergenic protein and is highly stable during normal cooking methods like boiling and proteolysis (pronase and trypsin; Durance & Wong, 1992; Green 1975). An 8% increase in protein digestibility might seem to be a small increase from an individual point of view, however, it can make a useful difference in reducing protein wastage over time and can be considered a small step toward sustainability and improved human health. An adult human being on the Western diet is considered to lose a significant amount of dietary protein that reaches the colon undigested each day (Roager & Licht, 2018). The fermentation of these nonhydrolyzed proteins by colonic flora is known to produce different mutagenic products (Bhat et al., 2021a). Further, the main food security threat facing humanity is that many people do not have access to nutritious and enough food (Hovhannisyan & Grigoryan, 2016).

The changes induced by the ultrasound processing on the secondary structure of the egg white proteins were also studied by Zhu et al. (2018b) using FTIR spectroscopy and circular dichroism spectroscopy. The results indicated that α -helix was the major secondary structure, accounting for about 40%–50% of the total secondary structures of the egg white proteins. A significant decrease was found in the α -helices content of all the samples treated with ultrasound compared to the control samples along with a concomitant increase of β -sheets contents. The turn structures remained almost constant regardless of processing time, accounting for about 28% of the total protein secondary structures. The authors stated that the ultrasound processing induced minimal effects on tertiary and secondary structures of egg white proteins and induced partial unfolding of the proteins, making the structure more flexible and extended. This study confirmed the earlier results suggesting that ultrasound processing can induce unfolding and refolding of the proteins causing a change in the secondary structure composition (Stanic-Vucinic, et al. 2012). The previous studies reported that ultrasound processing can induce irreversible changes in secondary structures of proteins leading to the formation of protein aggregates (Gülseren et al. 2007; Rombouts et al., 2020). For proteins with high α -helix content in their native structures, an increase in β -sheet content along with a concomitant decrease in α -helix content leads to the formation of aggregates (Stathopoulos et al., 2004).

In agreement with the results of Zhu et al. (2018b), Jun et al. (2020) reported similar changes in the secondary structure of the egg white proteins treated with high-power ultrasound (frequencies 20, 28, 35, 40, 50, 20/28, and 20/40 kHz, temperatures 25°C, 35°C, 45°C, and 55°C, durations 10, 20, 30, 40, 50, and 60 min). A significant

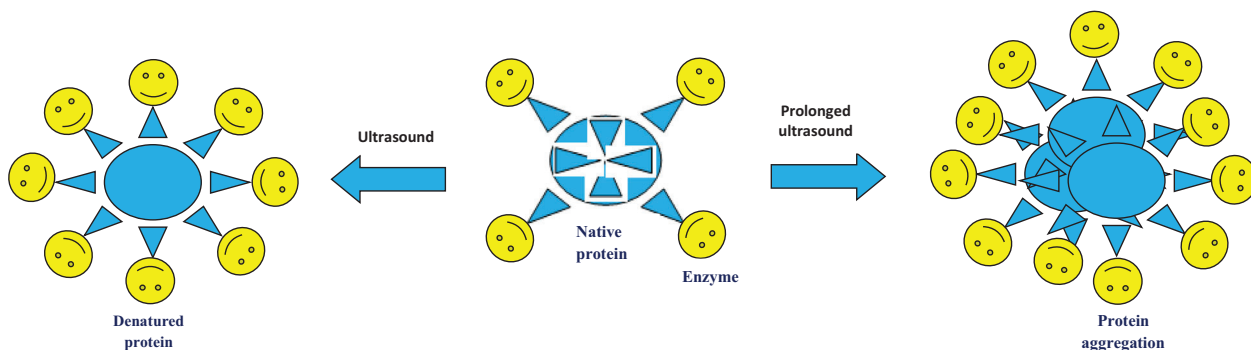


FIGURE 2 Mechanism of ultrasound processing for affecting the digestibility of egg proteins

decrease was observed in the α -helical content of the samples treated with single frequency ultrasound (40 kHz) compared to the control samples along with a concomitant increase of β -sheet and turn content. However, the continuous dual frequency of 20/40 kHz induced the unfolding of the β -sheet that resulted in an increase in the α -helix and random coil contents. The increase in the level of γ -random coil made the secondary structure less stable. No significant impact of ultrasound treatment was observed on the free sulfhydryl groups of the egg white proteins, however, the surface hydrophobicity increased significantly after the treatment. Although the ultrasound processing has been reported to increase the free sulfhydryl groups content by changing the conformation of the food proteins (Jin et al., 2015), the authors (Jun et al., 2020) stated that the oxidation of thiol groups by heat produced during the high-power ultrasound processing might have reduced the free thiol contents (S-S bond formation) or reversed the changes induced by the treatment.

Stefanovic et al. (2018) studied the effect of ultrasound treatment on in vitro hydrolysis of egg white proteins. A 10% (w/w) egg white aqueous solution was processed with ultrasound at a frequency of 20 kHz for 2, 5, 10, 15, and 20 min and the samples were subjected to in vitro enzymatic hydrolysis. A significant increase was observed in the degree of hydrolysis (%) for the samples processed with ultrasound up to 15 min compared to unprocessed control samples. However, the degree of hydrolysis for the samples processed for 20 min was significantly lower than the control samples, indicating a negative impact of the prolonged ultrasound processing on the hydrolysis of egg white proteins. A significant effect of ultrasound was observed on the total and reactive sulfhydryl groups and the processed samples showed an increase with increasing processing time up to 15 min. A significant decrease was observed in the total and reactive sulfhydryl groups for the samples processed for 20 min. The ultrasound processed samples also showed a significant increase in surface hydrophobicity and ζ potential with increasing processing time up to

15 min. A significant decrease was observed in the surface hydrophobicity and ζ potential for the samples processed for 20 min. These results indicate that the thiol groups present in the interior of the egg white protein molecules were exposed during the denaturation process induced by ultrasound processing up to 15 min, however, the prolonged processing induced S-S bond formation through oxidation of SH groups or through SH/S-S interchange reactions (see Figure 2). Oxidation of SH groups and formation of S-S bonds is known to decrease the digestibility of proteins (Hu et al., 2018). Initially established in sorghum protein (Hamaker et al., 1987), an inverse relationship between protein digestibility and disulfide content has been reported for different proteins (Bhat et al., 2021a; Zhao, He, et al., 2020).

FTIR spectra analysis revealed that ultrasound processing disrupted the interactions between the different parts of the protein molecules and the local sequences of amino acids, thereby inducing the unfolding of egg white proteins making them more exposed and susceptible to enzymatic hydrolysis. Based on the results of FTIR, surface hydrophobicity and SH groups content, the authors (Stefanovic et al., 2018) hypothesized that ultrasound processing facilitate the breakage of the hydrogen, disulfide and covalent/noncovalent bonds and thereby prevent the protein aggregation and facilitate enzymatic hydrolysis by increasing the availability of the efficient recognition sites. The cross-links between the protein molecules have been reported to be disrupted during the collapse of the cavitation bubbles and shear forces produced by shock waves and microstreaming (Li, Yang, et al., 2016; Zhang, Wang et al., 2020).

Jovanović et al. (2016) studied the effect of ultrasound processing on the enzymatic hydrolysis of egg white proteins using an in vitro digestion model. The enzymatic hydrolysis was carried out using different substrate and enzyme concentrations and at different temperatures to evaluate the kinetics of hydrolysis of egg white proteins. Aqueous solution of egg white (5–20 mg protein/ml) was

subjected to ultrasound processing for 15 min using an ultrasound water bath (40 kHz, 600 and 1200 W, $25 \pm 1^\circ\text{C}$) and a probe-type sonicator (20 ± 0.2 kHz) to induce a more efficient cavitation in the egg white protein solutions. Untreated samples that were stirred and heated to a temperature of 25°C were used as a control. The ultrasound processing was observed to improve the enzymatic hydrolysis of egg white proteins significantly ($p < .05$). The mean values of hydrolysis activation (E_a) energy for the samples treated with probe and bath ultrasound were 61.70% and 53.10% lower than control samples (86.70 kJ/mol), respectively, indicating that a lower energy was required for ultrasound treated samples to attain a faster reaction. The ultrasound processed samples showed significantly lower values for enzyme deactivation (E_d) energy, enthalpy (ΔH), and entropy (ΔS) of activation compared to control samples (61.6%, 63.6%, and 32.2% lower for the probe-type sonication, respectively). These significantly lower values for different thermodynamic parameters for ultrasound processed samples might be attributed to the cavitation effects that caused the disruption of hydrogen bonds, hydrophobic cores, and the aggregation of proteins (Zou, Ding, et al., 2016). All these results indicate a positive impact of ultrasound processing on the digestibility of egg white proteins.

The ultrasound processing also resulted in a significant increase in both total and exposed sulfhydryl groups and the probe-type sonicator was more effective in inducing these changes compared to the ultrasound water bath. These changes were attributed to the conformational changes induced by ultrasound processing that is known to induce partial or complete unfolding of protein polypeptides, depending on the treatment conditions, and thereby exposing the buried SH groups. Several studies have reported a significant increase in the sulfhydryl content along with the changes in the secondary structure of the ultrasound processed egg white proteins (Huang et al., 2008; Xiong et al., 2016). The increase in the SH groups was also accompanied with the decrease of both particle size and particle size distribution (Jovanović et al., 2016) and was attributed to ultrasound-assisted disruption of protein interactions responsible for the formation of protein aggregates. Ultrasound processing has been reported to break the protein aggregates into smaller particles (Jambrak et al., 2014) and to significantly decrease the particle size of native egg white proteins (Arzeni et al., 2015). A significant increase ($p < .05$) was also recorded in the ζ potential of the ultrasound processed egg white proteins and was attributed to the structural rearrangement of the proteins caused by ultrasonic cavitation (Jovanović et al., 2016). All these results point toward positive changes induced by the ultrasound processing in egg white proteins that increased their susceptibility to the enzymatic hydrolysis. However, excessively prolonged ultrasound processing can nullify

these positive effects on protein digestibility. This positive effect of ultrasound on the enzymatic hydrolysis of egg white and eggshell membrane proteins has been confirmed by several other studies (Jain & Alan, 2016; Stefanović et al., 2014). In addition to an increased susceptibility to enzymatic hydrolysis, these studies observed a significant increase in functional properties of ultrasound treated samples such as emulsion stability, foam stability, solubility, and antioxidant and antimicrobial activities, which can have health and industrial importance.

4 | CONCLUSIONS

Egg is an important source of high-quality animal proteins and undergoes several processing and cooking steps before being presented in its final consumable form. Most processing technologies, including PEF, high-pressure, ultrasound, microwave, pasteurization, and pickling, can modify the functional and nutritional properties and can also affect the digestibility of egg proteins. By affecting the egg microstructure and structural properties of egg proteins, these processing technologies result in conformational changes and disrupt the secondary and tertiary protein structure, thereby improving the digestibility of egg proteins by exposing the recognition sites and increasing the access of digestive enzymes to these hydrolytic sites. Although both thermal and nonthermal processing technologies were found to improve the digestibility of egg proteins, the nonthermal technologies, such as high-pressure, ultrasound, and PEF, improve the digestibility without negatively affecting the functional and nutritional properties of egg proteins. Thermal processing at lower temperatures ($<60^\circ\text{C}$) was generally found to be ineffective, however, higher temperatures (close to 100°C) mostly improved the digestibility of egg proteins due to the favorable changes in protein aggregation, gel microstructure, and protein structure. Maillard reaction products or glycation in general reduced the digestibility and nutritional value of egg white proteins. In addition to their favorable effects on protein digestion, both thermal and nonthermal processing technologies were found to reduce the allergenicity of egg proteins. Developing egg products with superior digestibility by employing these processing technologies is relatively nascent area and offers ample opportunities for the egg and poultry industry in the development of novel egg-based foods for future consumers. However, for all these technologies, attention should be paid to the effective processing conditions that avoid unfavorable protein and microstructural changes and subsequent reduction in digestibility. The future studies may focus on the processing induced protein structural and microstructural changes and correlate them with the

changes in the protein digestibility. As most of the dietary egg proteins are consumed in the form of cooked products, which present entirely different conditions and matrices, studies should focus on evaluating the impact of the processing technologies on the digestibility of cooked egg products.

AUTHOR CONTRIBUTIONS

Zuhaib F. Bhat: Conceptualization; data curation; formal analysis; methodology; visualization; writing—original draft; writing—review and editing. **James D. Morton:** Conceptualization; data curation; formal analysis; visualization; writing—review & editing. **Alaa El-Din A. Bekhit:** Conceptualization; data curation; formal analysis; visualization; writing—review & editing. **Sunil Kumar:** Conceptualization; data curation; formal analysis; writing—review & editing. **Hina F. Bhat:** Conceptualization; data curation; formal analysis; visualization; writing—review & editing.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ORCID

Zuhaib F. Bhat  <https://orcid.org/0000-0003-1563-0710>

Alaa El-Din A. Bekhit  <https://orcid.org/0000-0003-2569-8620>

Hina F. Bhat  <https://orcid.org/0000-0002-0031-251X>

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