**ABSTRACT**

Egg white proteins (EWPs) are important components of many food products. To obtain optimal functionality, EWP aggregation needs to be controlled. Different treatments can lead to the formation of aggregates in diverse ways, depending on the parameters of the treatments. Recent articles on the effects of processing (heat treatment, alkali treatment, pulsed electric field, high pressure, ultraviolet irradiation, and high intensity ultrasound) on the aggregation of EWPs are reviewed. The relationships between the processing parameters and the aggregation mechanisms are discussed. The information may be helpful in controlling the aggregation mechanisms during the processing.

1. Introduction

Egg white (EW) is well-known for its excellent nutritional and functional attributes (foaming, gelation, and emulsifying) (Mine, 2002). Ovalbumin (OV) is the main protein in EW (54%), and its isoelectric point (pI) is 4.5. It is the only egg white protein (EWP) having free sulfhydryl (SH) groups. The four SH groups are buried in the protein core (Beveridge & Arnfield, 1979; Nisbet, Saundry, Moir, Forthgill, & Forthgill, 1981). During processing, the buried SH groups can become exposed. The exposed SH groups can form disulfide (SS) bonds through SH oxidation and sulfhydryl-disulfide exchange (SH-SS exchange). The SS bond is not essential for the formation of gel, but it can increase the gel strength (Totosaus, Montejano, Salazar, & Guerrero, 2002; Van der Plancken, Van Loey, & Hendrickx, 2003; Van der Plancken et al., 2005a). According to Van der Plancken et al. (2003), the extent of EWP digestion depends on the degree of protein unfolding. When compared with unheated OV, all types of OV aggregates (linear, linear-branched, spherical, and spherical-agglomerated) are more susceptible to digestion. The degree of OV unfolding before aggregation is a major factor in the OV digestibility, which is highest in the linear aggregate and lowest in the spherical-agglomerated aggregate (Nyemb et al., 2014). In addition, heat treatment can affect protein allergenicity. According to Claude et al. (2017), the allergenicity of OV aggregate depends on the aggregation process (conditions of pH and ionic strength). The OV allergenicity is lowest in the linear aggregate (form at pH 9 and low ionic strength), while it is highest in the spherical-agglomerated aggregate (form at pH 5 and high ionic strength). The most used processing method for EW is heat treatment, but the interest in emerging technologies (high pressure, high intensity ultrasound, ultraviolet, and pulsed electric field) is growing. Different processing methods can lead to the formation of aggregates in diverse ways, depending on pH, treatment intensity, type and quantity of salt, ionic strength, and protein concentration (Arzeni, Martinez et al., 2012; Doi, 1993; Ganesan & Benjakul, 2010; Gharbi, Labbafi, & Madadlou, 2017; Katekhong & Charoenrein, 2016; Mukhopadhyay, Tomasula, Luchansky, Porto-Fett, & Call, 2010; Nicolai & Durand, 2013; Nyemb et al., 2014; Totosaus et al., 2008).

Some main EW proteins, especially ovalbumin (OV), ovotransferrin (OT), and lysozyme (LY) were extensively studied. Apart from well-known proteins, some studies identified new proteins in the EW. A proteomic study (using 2-D electrophoresis and ESI LC-MS/MS) identified 16 proteins in hen EW, and two of them (VMO-1 and Tenp) were identified for the first time in 2006 (Guérin-Dubiard et al., 2006). In another proteomic study, the identification of 78 proteins from the EW (using FT-ICR LC-MS/MS and MS3) was reported, and among them, fifty four were recognized for the first time by Mann (2007).

Eggs are processed in different ways for microbial safety and shelf-life extension, which in turn can have consequences on protein digestibility. There has been a growing interest in the impact of processing on EWP digestion. The beneficial effects of heating, high pressure, and high intensity ultrasound on the EWP digestibility have been reported (Hoppe, Jung, Patnaik, & Zeece, 2013; Nyemb et al., 2014; Stefanović, Jovanović, Dojčinović et al., 2014; Stefanović, Jovanović, Grbavčić et al., 2014; Van der Plancken, Van Remoortere, Indrawati Van Loey, & Hendrickx, 2003; Van der Plancken et al., 2005a). According to Van der Plancken et al. (2003), the extent of EWP digestion depends on the degree of protein unfolding. When compared with unheated OV, all types of OV aggregates (linear, linear-branched, spherical, and spherical-agglomerated) are more susceptible to digestion. The degree of OV unfolding before aggregation is a major factor in the OV digestibility, which is highest in the linear aggregate and lowest in the spherical-agglomerated aggregate (Nyemb et al., 2014). In addition, heat treatment can affect protein allergenicity. According to Claude et al. (2017), the allergenicity of OV aggregate depends on the aggregation process (conditions of pH and ionic strength). The OV allergenicity is lowest in the linear aggregate (form at pH 9 and low ionic strength), while it is highest in the spherical-agglomerated aggregate (form at pH 5 and high ionic strength). The most used processing method for EW is heat treatment, but the interest in emerging technologies (high pressure, high intensity ultrasound, ultraviolet, and pulsed electric field) is growing. Different processing methods can lead to the formation of aggregates in diverse ways, depending on pH, treatment intensity, type and quantity of salt, ionic strength, and protein concentration (Arzeni, Martinez et al., 2012; Doi, 1993; Ganesan & Benjakul, 2010; Gharbi, Labbafi, & Madadlou, 2017; Katekhong & Charoenrein, 2016; Mukhopadhyay, Tomasula, Luchansky, Porto-Fett, & Call, 2010; Nicolai & Durand, 2013; Nyemb et al., 2014; Totosaus et al., 2008).
et al., 2002; Van der Plancken et al., 2005a, 2005b, 2006, 2007).

To obtain optimal functionality, the aggregation of EWP needs to be controlled. Furthermore, the aggregates formed during the treatment can cause problem by sticking to tubes. Thus, a clear understanding of the aggregation mechanism is needed. Here, recent researches on the effects of processing (heat treatment, alkali treatment, pulsed electric field, high pressure, ultraviolet irradiation, and high intensity ultrasound) on the aggregation of EWPs are reviewed. The relationships between the processing parameters and the aggregation mechanisms are discussed. The information may be helpful in controlling the aggregation mechanisms during the processing.

2. Heat treatment

Heat treatment is often needed for microbial safety, yet severe heat treatment can have detrimental effects on the functional properties of EWP. Heat-denatured globular proteins can form different types of aggregates, depending on the balance between attractive and repulsive forces (Bryant & McClements, 1998). According to Totosaus et al. (2002), the heat-induced aggregation of globular proteins is influenced by extrinsic and intrinsic factors. The extrinsic factors include protein concentration, pH, ionic strength, type of ion, temperature, and duration. The intrinsic factors contain hydrophobicity, electrostatic interactions, molecular weight, amino acid composition, SS bonds, and SH-SS exchange. The molecular structures of globular protein gels (random and linear aggregates) were discussed in a review by Doi (1993), and their relationships with pH, ionic strength, the degree of unfolding, and heating method were also investigated. The size and type of the aggregates can be controlled by pH, type and quantity of salt, ionic strength, and protein concentration. Depending on these conditions, four types of aggregates (spherical particles, flexible strands, semi-flexible fibrils, and fractal) can be produced (Nicolai & Durand, 2013). The importance of combining pH and ionic strength in OV aggregation and gelation has been demonstrated (Claude et al., 2017; Nyemb et al., 2014). Depending on the combinations of pH and ionic strength in OV solutions, aggregates with different sizes and morphologies (linear, linear-branched, spherical, and spherical-agglomerated) can be formed. Under the condition of high electrostatic repulsion, the linear OV aggregate can be formed (pH 9 and low ionic strength). Nevertheless, the formation of spherical-agglomerated OV aggregate is favored under the condition of minimal electrostatic repulsion (pH 5 and high ionic strength) (Claude et al., 2014). Electrostatic repulsion is one of the main factors in solutions containing one protein type. Since all proteins have similar isoelectric point (pI), the electrostatic repulsive forces compete with hydrophobic interactions, which may inhibit the aggregate formation. In contrast, mixed proteins having different pI attract each other by electrostatic attractions as well as hydrophobic interactions, making the proteins prone to aggregation. When compared with the solutions of one protein type, in the mixed protein solution, each protein coexisting in the same condition forms aggregate at lower temperature (Hong, Iwashita, Handa, & Shiraki, 2017; Iwashita, Handa, & Shiraki, 2017; Wu, Zhao, Yang, & Yan, 2015), Matsudomi, Takasaki, and Kobayashi (1991) reported that after heating at 65°C for 30 min, no aggregate was formed in LY solution alone (0.15% w/w, pH 7–8, and no salt), and the solution remained transparent. However, when OT-LY mixture (0.06% w/w at ratio of 3:4: 1 OT: LY, and no salt) was heated at 65°C for 30 min, OT formed insoluble aggregate with LY. This insoluble aggregate was formed through electrostatic attraction between positively charged LY and negatively charged OT and hydrophobic interactions; furthermore, this electrostatic attraction between LY and OT became greater as pH increased from 7 to 9, and the turbidity increased significantly (at 65°C for 30 min, 0.06% w/w at 3:4:1 OT: LY, and no salt) (Matsudomi, 1991; Matsudomi et al., 1991). Hong et al. (2017) observed that the aggregation of LY in EWP solution (pH 7, 1 mg/ml, no salt and no additive) began at 65°C, which was far lower than the aggregation temperature of LY alone. They stressed that LY in hen EW was prone to the formation of aggregate as compared to LY alone. Since LY has positive charge at neutral pH, it has tendency to bind with other negatively charged and denatured proteins, including OT and OV. In the study by Matsudomi (1991), no aggregate was formed in the LY solution (0.1% w/w, pH 7.6, and no salt), even at 90°C (2°C/min from 20 to 90°C); nevertheless, in OV-LY mixture (0.1% w/w at ratio of 1:0.1 mg/mg OV:LY, pH 7.6 and no salt), OV formed insoluble aggregate with LY at 70°C (2°C/min from 20 to 70°C). The aggregation between OV and LY was initiated by electrostatic attraction. Subsequently, partial OV unfolding caused the exposure of buried hydrophobic region and SH groups. This process led to hydrophobic interactions between OV and LY, followed by SH-SS exchange between the SS bonds of LY and the exposed SH groups of OV (Iwashita et al., 2017; Matsudomi, Yamamura, & Kobayashi, 1986). According to Iwashita et al. (2017), OV has an internal binding region, named S-peptide. The S-peptide of OV is suitable for hydrophobic interaction with native LY. Since the native LY has suitable region on its surface for hydrophobic interaction with the S-peptide, the exposure of S-peptide in OV causes the hydrophobic interaction between partially unfolded OV and native LY. This hydrophobic interaction may be strengthened by the electrostatic attraction between LY and OV (Iwashita et al., 2017). Heating at 70°C (for 4 min) led to the formation of soluble and insoluble aggregates in EWP solution (at pH 9.2, 10% w/w and no salt), and the main components of insoluble aggregates were OT (30.58%), LY (18.47%), and OV (14.20%) (Wu, Zhao, Yang, Yan, & Sun, 2016). At pH 5, after heating at 55°C for 10 min, insoluble aggregates with high OT and lower OV contents were formed in ovomucin-depleted EW solution (OdEW) (10% w/w, and no salt); however, as temperature increased from 55 to 60°C, the insoluble aggregate consisted mainly of OV and OT (Liu, Oey, Bremer, Carne, & Silcock, 2017a). It has been reported that at pH 5, denaturation temperature of OV in EW was 73°C (Németh et al., 2010). However, when OdEW (at pH 5, 10% w/w and no salt) was heated at 55°C for 10 min, OV contributed to the formation of aggregate, probably due to the proximity of pH 5 to OV pI. Heating at 55°C (10 min) caused the formation of insoluble aggregate at pH 5 (OdEW, 10% w/w, and no salt); nevertheless, at pH 9, no aggregate was formed under this condition (at 55°C for 10 min, OdEW, 10% w/w and no salt). Moreover, after heating at 60°C (10 min), OdEW (10% w/w and no salt) was more turbid at pH 5 as compared to pH 9 (Liu, Oey, Bremer, Silcock, & Carne, 2017b; Liu et al., 2017a). There were two main reasons for this difference in aggregation between pH 5 and pH 9. The first reason was the proximity of pH 5 to the overall pI (4.5–5 for most EWP). The second reason was due to differences in denaturation temperatures of OT and OV between the two pH values. As the pH of EW decreased from 8.9 to 5, the denaturation temperature of OT decreased from 63 to 57°C, and the OV denaturation temperature decreased from 77 to 73°C (Németh et al., 2010). At pH 5, the electrostatic attractions of LY with OT and OV were too weak to participate in aggregation (at 60°C for 10 min, OdEW, 10% w/w and no salt); thus, no LY was involved in the aggregation at pH 5, contrasting with aggregation at pH 9 (at 60°C for 10 min, OdEW, 10% w/w and no salt).

3. Alkali treatment

Alkali treatment is one of the oldest techniques in food preservation. Preserved eggs have many distinct characteristics, including unique flavors, dark green yolks, and brown transparent EW. Moreover, alkali treatment is effective in destroying aflatoxin in eggs of laying hens, fed diets containing aflatoxin (Chang, Tsai, & Li, 1999; Ma, Harwalkar, & Paquet, 1990; Zhao et al., 2016).

3.1. Alkali treatment of EW in liquid form

Alkaline solution caused the denaturation of EWPs, leading to the exposure of hydrophobic, SH and SS groups. These exposed groups
aggregated by SH oxidation, SH-SS exchange and, to a lesser extent, by hydrophobic interaction. This phenomenon was followed by the formation of gel (after 15 min). Afterwards, the strong alkaline solution destroyed the new-formed gel from liquid EW (after 7 h). Not only did the disruption of new-formed intermolecular SS and hydrophobic bonds occur, but also the disruption of native intramolecular SS bonds occurred. It is important to note that under alkaline condition, hydrogen bond cannot be formed (Chen et al., 2015; Ji et al., 2015).

3.2. Alkali treatment in preserved EW (pidan)

Preserved EW is prepared by soaking (30 days) shell egg in pickling solution containing NaOH, CuSO4 and NaCl. During pickling, the migration of alkali from the pickling solution to the EW occurs (Zhao et al., 2016).

The effect of alkali treatment on intact shell EW was different from its effect on liquid EW. Preserved EW gel exhibited higher hardness as compared to alkali-induced gel from liquid EW, and the preserved EW gel was stable during the treatment. As discussed earlier, during the alkali treatment, EWP became denatured and aggregated. In the preserved EW, the number of SS and non-covalent bonds increased with the extension of alkali treatment, contrasting with the alkali-induced gel from liquid EW. The increased covalent and non-covalent bonds could improve the hardness of preserved gel. In fact, the structure of preserved EW gel became stronger and more compact with the extension of pickling. During the pickling, water molecules migrated from the EW to the alkaline solution and yolk by osmosis process, which caused the reduction of free water in the preserved EW. As the free water decreased, the mobility and activity of OH− decreased; therefore, the detrimental effect of OH− on the covalent and non-covalent bonds diminished. Furthermore, the presence of cations (Cu2+ and Na+) in the pickling solution contributed to the reduction of electrostatic repulsion, through the shielding of protein negative charges. These cations maintained the preserved gel network together with the continuous dehydration. As the alkali processing continued, the stabilized gel became capable of keeping water. The retained water made it easy to form hydrogen bonds in the preserved EW. The ionic and SS bonds were the major forces of the preserved EW, and the hydrophobic and hydrogen bonds were secondary forces (Chen et al., 2015; Ganasen & Benjakul, 2011; Zhao, Tu, Xu, Li, & Du, 2014; Zhao et al., 2016).

3.3. Effect of glucose treatment after alkali treatment

After the alkali treatment, glucose soaking caused the destabilization of the gel, due to an enhanced Maillard reaction. It led to competition between glucose and cations for the making of bridge between EWPs, reducing the hardness and textural properties (Ganasen & Benjakul, 2014).

3.4. Difference between alkali treatments with KOH and NaOH

When compared with EW treated with NaOH, the gel of EW treated with KOH was harder and more stable, and it exhibited denser network. After the pickling, the moisture content of preserved EW from KOH was lower when compared with that from NaOH. The lower free water (gel from KOH) caused a larger reduction in the OH− mobility. This decreased mobility diminished the damaging effect of OH− on the covalent and non-covalent bonds. Thus, the preserved EW from KOH showed denser network with higher hardness as compared to that with NaOH (Zhang, Jiang, Chen, Ockerman, & Chen, 2015; Zhao et al., 2016).

3.5. Difference between preserved EW and hard-cooked EW

Microstructures of preserved EW and hard-cooked EW are shown in Fig. 1. Preserved EW exhibited loose structure with many regular voids, while hard-cooked EW showed denser network without the void. The aggregation mechanisms of the two gels were different, accounting for the different microstructures (Zhao et al., 2014). Under the strong alkaline condition, the protein net negative charge increased, which led to strong electrostatic repulsive forces between protein molecules. Though Cu2+ and Na+ were added to the pickling solution, they only formed low ionic strength in the EW (Zhao et al., 2016). At high pH and low ionic strength, the net charge of the EWP was high, and electrostatic repulsion between proteins was significant. Therefore, binding areas on denatured EWP molecules were limited, which favored the formation of linear aggregates (Kitabatake, Shimizu, & Doi, 1988).

As discussed by Chen et al. (2015), under the alkali treatment, denatured EWPs aligned with one another to form linear polymers. These linear polymers attached to one another, forming the mesh structure of the alkali-induced EW gel. According to Zhao et al. (2016), the migration of water contributed to the mesh structure of the preserved EW. During the pickling, the water molecules migrated from the EW to the pickling solution and yolk (by osmosis process). The water molecules left voids behind when they escaped from the EW, leading to the mesh structure of preserved EW gel (Zhao et al., 2016). The hard-cooked EW was harder than the preserved EW, due to the lower level of crosslinking under alkali processing. The preserved EW exhibited higher a* and b* values and lower L* value than the hard-cooked EW, and Maillard reaction under the alkaline condition contributed to the brown color of preserved EW (Zhao et al., 2014).

4. Pulsed electric field (PEF)

PEF processing is an emerging technology for pasteurization of liquid food, and it uses external electrical fields (moderate or high intensity) for a short time (Yogesh, 2016). In many references, PEF has been regarded as non-thermal processing; however, in many devices, temperature may increase during the treatment, and this increased temperature may produce a heating effect. In fact, PEF can be non-thermal treatment only in the absence of the heating effects.

PEF could affect the aggregation of EWP, depending on the protein mixture and severity of the treatment. No aggregate was formed in the OV solution under PEF at 20 kV/cm (for 800 μs); however, PEF above 25 kV/cm resulted in partial OV unfolding, and the SH groups became exposed. This process was associated with the formation of soluble aggregates (in OV solution), through SH oxidation and SH-SS exchanges between OV molecules. The maintenance of balance between long-range repulsion and short-range attraction accounted for these small aggregates in the OV solution (Wu et al., 2015; Zhao & Yang, 2012). On the other hand, the presence of LY in the EWP solution led to only the formation of insoluble aggregates, and no soluble aggregate was formed. The formation of these insoluble aggregates caused the increase in turbidity and protein solubility loss (Wu, Zhao, Yang, & Chen, 2014; Wu et al., 2015, 2016). LY could form insoluble aggregate through a combination of two mechanisms under PEF (pH 7–9, 25–30 kV/cm, 600–800 μs, and 18–20 °C). The first mechanism was electrostatic attraction between positively charged LY and negatively charged OT and OV. PEF could accelerate protein movement, affected by the protein net charge. Therefore, PEF provided many opportunities for oppositely charged proteins to form aggregate. The second mechanism stemmed from the PEF-induced unfolding of OV and OT molecules. The partial unfolding of OV and OT caused the exposure of internal aggregation-prone regions. Therefore, the binding regions in OV and OT became available for non-covalent interactions with LY. In fact, the exposure of the aggregation-prone regions in OV and OT caused their hydrophobic interactions with LY. These hydrophobic interactions might be strengthened by the electrostatic attractions of LY with OV and OT (Wu et al., 2014, 2015, 2016). PEF processing at 35 kV/cm resulted in LY unfolding, accompanied by transition from α-helix to β-sheet and the exposure of more tryptophan residues (Yogesh, 2016; Zhao & Yang, 2008). At 25 kV/cm, LY interacted with OV and OT only through non-
covalent interactions (electrostatic and hydrophobic). In fact, no intermolecular SS bond between LY and other EWP was formed, as indicated in Fig. 2 at which intensities of LY bands were similar under non-reducing and reducing conditions. It might be hypothesized that SH-SS exchanges and hydrophobic interactions between OV and OT occurred under PEF, resulting in the formation of soluble aggregates in an OV-OT-LY mixture (25 kV/cm for 800 μs). Simultaneously the soluble aggregates, composed of OV and OT, interacted with LY through hydrophobic and electrostatic attractions, leading to the formation of insoluble aggregates. Although the interactions of LY with EWPs were non-covalent, intermolecular SS bond was the main binding force of the PEF-induced aggregate (25 kV/cm for 800 μs) (Wu et al., 2014, 2015, 2016).

The effect of PEF on the EWP aggregation depended on field intensity (kV/cm), energy input (W), and pH. PEF treatment at 1.7 kV/cm (W = 695 kJ/kg, and at below 50 °C) (Li et al., 2017a). As discussed earlier, LY formed insoluble aggregates with OV and OT at 25 kV/cm (pH 7–9, 18 °C, W = 2726.3 kJ/L). As pH decreased from 7 to 4 (25 kV/cm), the electrostatic attractions of LY with OV and OT became weaker, and the turbidity decreased significantly. However, when pH decreased to 2 (far away from LY pI), the electrostatic attractions of LY with OV and OT were completely averted. Thus, the formation of insoluble aggregate was prevented at pH 2, and the protein solution remained transparent (at 25 kV/cm). Since pH has significant effect on the protein charge, it can affect the electrostatic attractions between oppositely charged proteins under the PEF (Wu et al., 2015, 2016).

5. High pressure (HP)

High pressure (HP) processing has gained popularity as an alternative for conventional thermal treatment. It has advantages over thermal processing, including lower temperature and reduced extreme aggregation (Naderi, House, Pouliot, & Doyen, 2017; Singh & Ramaswamy, 2013).

5.1. Effect of high pressure (HP) on aggregation of EWP

Pressure treatment could induce the EWP denaturation and aggregation, depending on pressure range, protein concentration, time, pH, and temperature. No aggregate was formed under pressure below 100 MPa (native EW). However, it resulted in the destruction of fibrillar ovomucin–LY complex. In the native EW, ovomucin can interact with LY through electrostatic attractions, which are between the carboxylic groups of ovomucin sialic acids and the amino groups of LY lysine residues. The ovomucin–LY complex is the main reason for the gel-like structure of native EW. The HP could disrupt this complex by the breakage of electrostatic attraction between LY and ovomucin; therefore, the bound LY became released from the complex. The destruction of the complex caused the reduction in viscosity and optical density (Brand, Pichler, & Kulozik, 2014; Panozzo et al., 2014). HP treatment at 150 MPa caused the formation of aggregates stabilized by non-covalent interactions (not by SS bonds), despite the exposure of SH groups. At pressures above 150 MPa, at which the EWP aggregation occurred, the viscosity and optical density increased. This aggregation led to change in color from yellow to white, and it was associated with the decrease of immunoreactivity (Panozzo et al., 2014; Singh, Sharma,
& Ramaswamy, 2015). HP at 550 MPa (20 min) caused the formation of soluble aggregates at concentration of 9.6 mg/ml. These small aggregates were formed by SS bonds, arising from SH oxidation and SS-SH exchanges. However, at concentration of 96 mg/ml (native EW), HP treatment (at 550 MPa for 15 min) induced the formation of translucent gel, associated with SS bond crosslinking (Singh & Ramaswamy, 2015; Van der Plancken et al., 2005b).

The effect of HP on SH groups was dependent on temperature. When compared with higher temperature (40–60 °C, 500 MPa), the exposure of SH groups was faster at lower temperature (10 °C) for a given pressure (500 MPa). In fact, temperature had antagonistic effect on the HP-induced exposure of buried SH groups. However, under the HP, the exposed SH groups were more prone to oxidation at higher temperature. As compared to high temperatures at atmospheric pressure (70–85 °C), the SH groups were more sensitive to oxidation at elevated pressures and low temperature (500–700 MPa, 25 °C) (Van der Plancken et al., 2005b).

The aggregates formed under the HP (100–400 MPa) were smaller and more soluble than those formed under the heating (70–85 °C), due to the lower level of surface hydrophobicity under the HP (Van der Plancken et al., 2005b; Zhu, Zhou, Yu, Li, & He, 2014).

The effect of HP on EW in shell egg was different from its effect on liquid EW. HP treatment at 500 MPa (10 min) induced the exposure of hydrophobic and SH groups in shell egg; however, neither of these exposed groups contributed to the formation of aggregate. This behavior was attributed to reversible protein unfolding in the shell egg. After HP treatment, these exposed groups became buried within the proteins, contrasting strikingly with liquid EW in which the aggregation and gelation occurred at the same pressure (Lai et al., 2016; Singh & Ramaswamy, 2015; Singh et al., 2015).

5.2. Combined HP and transglutaminase (TG) treatment

Transglutaminase (TG) induces the formation of crosslinks, which may alter functional properties. This enzyme catalyzes the formation of intermolecular or intramolecular covalent bonds between glutamine and lysine residues. Applying TG either under HP or under heat treatment allows protein unfolding, making more of the glutamine and lysine residues. Applying TG either under HP or under heat treatment allows protein unfolding, making more of the glutamine and lysine residues. Applying TG either under HP or under heat treatment allows protein unfolding, making more of the glutamine and lysine residues. Applying TG either under HP or under heat treatment allows protein unfolding, making more of the glutamine and lysine residues. Applying TG either under HP or under heat treatment allows protein unfolding, making more of the glutamine and lysine residues.

The aggregates formed by TG after HP, contrasting with the simultaneous use of TG and HP. However, the LY partial unfolding was reversible after the HP. After the HP, the exposed glutamine and lysine residues became buried within the LY tertiary structure, and they became unavailable for the TG. Thus, no LY oligomer was formed by the use of TG after HP, contrasting with the simultaneous use of TG and HP (Schuh, Schwarzenbolz, & Henle, 2010).

6. Ultraviolet (UV) irradiation

UV processing can eliminate pathogens such as Salmonella, Escherichia coli and Listeria in the egg. However, it may produce free radicals, a serious concern with regards to consumer health and food quality (Abdanan Meh dizadeh, Minaei, Karimi Torshizi, & Mohajerani, 2015). UV irradiation can enhance the oxidation of fatty acids, although it may cause no change in the color or pH of the liquid eggs (De Souza & Fernandez, 2011).

UV irradiation (at 35 W/m²) induced the oxidation of LY tryptophan groups in the native EW. The photoexcited tryptophan groups caused the breakage of intramolecular SS bonds in the LY molecules; therefore, new free SH groups were formed (Manzocco, Panazzo, & Nicoli, 2012; Wu, Sheng, Xie, & Wang, 2008). The new-formed SH groups in the LY could form intramolecular SS bonds, leading to the formation of aggregates during the irradiation. This intermolecular crosslinking increased the turbidity and apparent viscosity of the EW (Kuan et al., 2011; Manzocco & Nicoli, 2012; Manzocco, Panazzo, & Nicoli, 2013; Manzocco et al., 2012; Wu et al., 2008). In the native EW, different proteins were differently sensitive to the UV light. While LY was the most photosensitive protein in the native EW, UV was photostable in the native EW; thus, the intramolecular SS bond in the OV molecule remained unaffected by the UV light (35 W/m²), contrasting with LY molecule in which the tryptophan group oxidation and the SS bond disruption occurred. Nevertheless, UV treatment (20 J/l, native EW) induced the exposure of buried SH groups in the OV, and it might stimulate the formation of intramolecular SS bonds between EWPs. When compared with heat pasteurization (56 °C, native EW), UV treatment (at 24 W/m²) induced the lower level of OV unfolding in the native EW (Mendes de Souza, Briviba, Müller, Fernández, & Stahli, 2013; Mendes de Souza et al., 2015). Foams prepared from UV-treated EW exhibited higher stability as compared to those from untreated EW. This high foam stability was due to the increase in viscosity, brought about by the intermolecular crosslinking; moreover, the formation of elastic network at air-water interface could account for the improved foam stability (Kuan et al., 2011; Manzocco et al., 2012, 2013; Mendes de Souza et al., 2015).

The susceptibility of EWP molecules to the UV light was substantially dependent on protein concentration. When the protein concentration decreased, the protein photosensitivity increased. As discussed earlier, at the concentration of 96 mg/ml (native EW), the intramolecular SS bond in the OV was UV-resistant. However, when the concentration decreased to below 2.2 mg/ml, the intramolecular SS bond in the OV became prone to breakage (at 29 W/m²). This breakage caused the generation of new free SH group, which could lead to backbone fragmentation or aggregation. Nevertheless, at concentration of 2.2 mg/ml (and above), the intramolecular SS bond in the OV was UV-resistant. At this concentration, neither the photoexcitation of tryptophan groups nor the disruption of SS bonds occurred. This critical concentration was associated with macromolecular crowding effects, which improved the OV photostability (Manzocco & Nicoli, 2012; Manzocco et al., 2012).

7. High intensity ultrasound (HIU)

High intensity ultrasound (HIU) treatment has gained importance in the last decade. It contains high energy mechanical waves (20–100 kHz) inducing cyclic cavity generation and collapse (sonication bubbles). Therefore, it can bring about the protein denaturation and aggregation (Stefanović, Jovanović, Grbavić et al., 2014).

The effect of HIU on EWP was dependent on frequency, pH, and duration. HIU treatment at 20 kHz for 2–14 min enhanced protein solubility in EWP solution (10% w/w and pH 8), and it reduced protein particle size. As discussed earlier, in the native EW, part of the LY is bound to ovomucin network through electrostatic attraction. Acoustic cavitation could break the electrostatic attraction between positively
charged LY and negatively charged ovomucin (at pH 8). This phenomenon caused the release of LY from the ovomucin network, rendering LY in soluble form. The destruction of the fibrillar ovomucin network reduced the viscosity of EWP solution (Stefanović et al., 2017). HIU treatment at 20 kHz for 20 min caused the exposure of SH and hydrophobic groups. These exposed groups could form aggregates, depending on pH (Xiong et al., 2016). One of the determinant factors in protein aggregation under the HIU is pH. Since pH can affect the protein net charge and the reactivity of SH groups (Handa, Takahashi, Kuroda, & Froning, 1998), it may influence the size and structure of the aggregates under the HIU. At pH 9, both SS bonds and non-covalent bonds were the driving forces for the formation of aggregates (at 20 kHz, for 20 min, and 10 mg/ml), and these aggregates were small and soluble. When compared with pH 9 (at 20 kHz for 20 min, and 10 mg/ml), at pH 7, HIU-induced aggregates were larger and less soluble (at 20 kHz for 20 min, and 10 mg/ml). Furthermore, no SS bond contributed to the formation of the aggregate at pH 7, contrasting with the aggregates at pH 9 (Arzeni, Pérez, & Pilosof, 2012; Arzeni, Martínez et al., 2012; Stefanović, Jovanović, Dojčinović et al., 2014). At higher pH (9), SH groups are more reactive, leading to a higher likelihood of SS-SH exchanges. On the other hand, pH 7 is closer to pl of main EWPs than pH 9. At lower pH (7), electrostatic repulsive forces between EWPs are weaker, leading to larger aggregate and lower solubility (Van der Plancken et al., 2005a, 2005b, 2006). The HIU-induced aggregates could affect the foaming properties of EWP, depending on the size and structure of the aggregates. At pH 7 (20 kHz, 20 min, 10 mg/ml), the large aggregates had detrimental effect on foam volume. However, at pH 9, the small aggregates had no damaging impact on the foam volume (20 kHz, 20 min, 10 mg/ml) (Arzeni, Pérez et al., 2012; Stefanović, Jovanović, Dojčinović et al., 2014). HIU above 60 kHz (for 4 min) was associated with the cleavage of water molecules, leading to the generation of hydroxyl and hydrogen free radicals in OT solution (5%). These free radicals caused the breakdown of intramolecular SS bonds in the OT molecules, generating new SH groups. Nonetheless, HIU below 60 kHz (for 4 min) was not sufficient to disrupt these intramolecular SS bonds (Lei, Majumder, Shen, & Wu, 2011).

8. Conclusion

Different treatments led to the formation of aggregates in diverse ways, depending on the parameters of the treatments. At pH 5, after heating at 55 °C for 10 min, insoluble aggregates with high OT and lower OV contents were formed in the OdEW; nevertheless, at pH 9, no aggregate was formed under this condition (55 °C for 10 min, OdEW). When OT-LY mixture was heated at 65 °C for 30 min (pH 7–8, and no salt), OT formed insoluble aggregate with LY. This insoluble aggregate was formed through electrostatic attraction between positively charged LY and negatively charged OT and hydrophobic interactions. After heating at 70 °C, OV formed insoluble aggregate with LY in the OV-LY mixture (pH 7.6 and no salt). The aggregation between OV and LY was initiated by electrostatic attraction. Subsequently, partial OV unfolding caused the exposure of buried hydrophobic region and SH groups. This process led to hydrophobic interaction between OV and LY, followed by SH-SS exchange between OV and LY.

During the pickling (shell egg), the migration of water (from EW to yolk and alkaline solution) reduced OH− mobility. This reduced mobility diminished the damaging effect of OH− on the covalent and non-covalent bonds. Therefore, the number of SS and non-covalent bonds increased, leading to the increase in the hardness of preserved EW.

No aggregate was formed in the OV solution under PEF at 20 kV/cm (for 800 μs). However, PEF above 25 kV/cm caused the formation of soluble aggregates (in the OV solution) through SS bonds. The presence of LY in the EWP solution led to the formation of insoluble aggregates, and no soluble aggregate was formed. LY could form insoluble aggregate through the combination of two mechanisms under the PEF (pH 7–9, 25–30 kV/cm, 600–800 μs). The first mechanism was the electrostatic attraction between positively charged LY and negatively charged OT and OV. The second mechanism resulted from the unfolding of OV and OT molecules under the PEF. The partial unfolding of OV and OT caused the exposure of internal aggregation-prone regions in these molecules; therefore, the binding regions in OV and OT became available for hydrophobic interactions with LY. These hydrophobic interactions might be strengthened by the electrostatic attractions of LY with OV and OT. When pH decreased from 7 to 2 (25 kV/cm, 800 μs), the electrostatic attractions of LY with OV and OT were averted. No aggregate was formed under the pressure below 100 MPa, and the EWP solution remained transparent. Nevertheless, HP at 150 MPa induced the formation of aggregates stabilized by non-covalent interactions (not by SS bonds), resulting in the increase in optical density. HP at 550 MPa caused the formation of soluble aggregates at concentration of 9.6 mg/ml. These small aggregates were formed by SS bonds, arising from SH oxidation and SS-SH exchanges. However, at concentration of 96 mg/ml (native EW), HP treatment (at 550 MPa) induced the formation of translucent gel, associated with SS bond crosslinking. The combination of TG and HP (400 MPa) caused the formation of aggregates stabilized by intermolecular SS bonds.

UV irradiation (native EW, at 35 W/m²) caused the breaking of intramolecular SS bonds in the LY, leading to the generation of new SH groups. These new SH groups in the LY could form intermolecular SS bonds, leading to the formation of aggregates. This intermolecular crosslinking caused the increase in turbidity and apparent viscosity. UV treatment (20 J/l, native EW) induced the exposure of SH groups in the OV, stimulating the formation of intermolecular SS bonds. The intramolecular SS bond in the OV was resistant to the disruption at the concentration of 96 mg/ml (native EW). However, when protein concentration decreased to less than 2.2 mg/ml, the intramolecular SS bond in the OV became prone to breaking (at 29 W/m²). This breaking caused the generation of new free SH groups.

At pH 9, both SS bonds and non-covalent bonds were the driving forces for the formation of aggregates (at 20 kHz, for 20 min, and 10 mg/ml), and these aggregates were small and soluble. When compared with pH 9 (at 20 kHz for 20 min, and 10 mg/ml), at pH 7, HIU-induced aggregates were larger and less soluble (at 20 kHz for 20 min, and 10 mg/ml). Furthermore, no SS bond contributed to the formation of the aggregate at pH 7, contrasting with the aggregates at pH 9. Since pH could affect the protein net charge and the reactivity of SH groups, it influenced the size and structure of the aggregates (20 kHz, 20 min, and 10 mg/ml).

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