



Review

Impact of food processing on the structural and allergenic properties of egg white



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ARTICLE INFO

Keywords:

Egg white proteins
Avidin
Thermal processing
Non-thermal processing
Novel processing
Egg allergy

ABSTRACT

Background: Egg is one of the most nutritious foods that is easily available and it has become a favorite source of major nutrients like lipids and proteins around the world. However, eggs can trigger severe allergic reactions, especially in infants and children. The reactions are mostly IgE-mediated with a range of symptoms related to nose and throat and further can lead to life-threatening anaphylaxis. A total of ten allergens have been recognized to date and researchers are actively working on understanding their structure-function relationship which could help reduce the allergy incidences. Major egg allergens are abundant in egg white which include ovalbumin (OA), ovomucoid (OVM), ovotransferrin (OVT) and lysozyme (Lys). In addition to allergens, avidin present in egg white is also extensively studied due to its anti-nutritional properties. Avidin is known to form a complex with biotin which makes it unavailable for absorption.

Scope and approach: This review focuses on the effects of thermal and non-thermal processing methods on the structure and functional properties of various egg proteins including a wide range of allergens and anti-nutrients.

Key findings and conclusions: Novel processing techniques including various non-thermal techniques show promising results in reducing the allergic reactions of egg. Anti-nutrients like avidin can be partly inactivated under combined high-pressure processing and heat treatment.

1. Introduction

Eggs are abundant in nutrients which are beneficial to human health. The nutritional composition not only includes proteins and lipids but also contains various vitamins like thiamin, riboflavin, vitamin A, B, D, E and minerals like Ca, P, K, Na, Mg, Fe, Zn. However, beside cow milk, eggs are the other most important allergy-causing food item in children (Gray et al., 2015; Savage, Matsui, Skripak, & Wood, 2007). Exposure to egg in sensitive children can result in hives, rashes which are the symptoms of anaphylaxis. Most of egg allergens are presented in egg white, including ovomucoid (Gal d 1), ovalbumin (Gal d 2), conalbumin (Gal d 3) and lysozyme (Gal d 4) (Poulsen et al., 2001). The best way to manage egg allergy is complete abstinence from any food products containing eggs. However, eggs are incorporated in many food applications and complete avoidance is impossible (Mine & Yang, 2008).

Egg proteins are important in several functional properties, eg, emulsification, foaming, and flavor. The coagulative properties which lead to solidification upon heating and aeration play a key role in baking. Eggs also possess emulsification properties which are important in salad dressings and sauces that are widely consumed around the

world as part of various cuisines. They also have binding properties and can greatly improve texture and acceptability of products like health bars and baked products (Stadelman, 1999). An egg consists of 8–11% shell, 27–32% yolk and 56–61% albumen (Poulsen et al., 2001). Albumen comprises 88.5% water, 10.5% protein, 0.5% carbohydrate (Stevens, 1991). The protein composition of albumen is summarized in Table 1.

Recent research has also shown that eggs contain significant quantities of carotenoids like lutein and zeaxanthin that could play an important role both in well-being and in prevention of various diseases. The egg yolk contains high antioxidants which could provide further health benefits. They are also considered to be an important source of anti-bacterial and bioactive compounds which find their application in various industries including food, pharma and biotechnology (Lesniewski & Stangierski, 2018). Egg is also a vital source of choline nutrient (680 mg/100 g) which plays a major role in memory development and brain health (Applegate, 2000; Handelman, Nightingale, Lichtenstein, Schaefer, & Blumberg, 1999; Mellott, Williams, Meck, & Blusztajn, 2004). For example, research conducted on rat pups showed that the group treated with choline developed abilities in spatial memory three days sooner compared to control group. The choline was

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Acronyms

ELISA	Enzyme-linked immunosorbent assay
IgG	Immunoglobulin G
IgE	Immunoglobulin E
CD	Circular dichroism spectroscopy
UV	Ultraviolet absorption spectroscopy

SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ci-ELISA	Competitive indirect enzyme-linked immunosorbent assays
HABA	2-Hydroxyazobenzen-4'-Carboxylic Acid
MTS	Manothermosonication
PBS	Phosphate-buffered saline

applied to rat dams in their embryonic days of 12–17 (Mellott et al., 2004). Despite their health and nutritional profile, eggs contain few compounds that could cause adverse reactions in human body especially in form of allergic reactions. In this review, we outline the components of the egg proteins and the effects of various processing methods on their structure-functional relationship which could be of great interest to researchers and the industry.

2. Major egg white proteins

2.1. Ovalbumin

Ovalbumin is a phospho-glycoprotein composed of 385 amino acids with molecular weight of 45 kDa (Abeyrathne, Lee, & Ahn, 2014). Further, the ovalbumin molecular sequence contains a total of four thiol groups and one disulphide bond formed between the cystine residues 87–133. This molecule is generally linked with a carbohydrate chain at the amide nitrogen belonging to asparagine 298 residue (Stein, Leslie, Finch, & Carrell, 1991). The heavy glycosylation leads to the allergenicity of ovalbumin (Aalberse, 2000). Ovalbumin is an acidic molecule with an isoelectric point (pI) of 4.5. Half of the residues present in the molecule are hydrophobic in nature and one-third of them are charged amino acid residues (Huopalahti, Anton, López-Fandiño, & Schade, 2007). Although ovalbumin has certain attributes that make it heat stable, it can be denatured or aggregated by proteolysis due to exposure of the air-water interfaces during thermal processing (Huopalahti et al., 2007; Stevens, 1991). According to its structure, ovalbumin shows certain homology with serpin family proteins, but it does not exhibit any protease inhibitory activity (Huntington & Stein, 2001). Furthermore, ovalbumin plays a major role in immunological and nutritional studies. Ovalbumin acts as a standard protein in several experiments including protein assay, structural properties, animal testing on inhalant and dietary allergies (Abeyrathne et al., 2014; Mine & Yang, 2008).

2.2. Ovomucoid

Ovomucoid is a heat-stable glycoprotein containing 186 residues and a molecular weight of 28 kDa and pI of 4.1 (Julià et al., 2007; Winiarska-Mieczan & Kwiecień, 2007). The structure of ovomucoid

consists of three domains: domain I and domain II (defined as α -type) and domain III (β -type). It contains both sulfated oligosaccharides and sialyloligosaccharides and a total of nine disulfide bonds (Huopalahti et al., 2007). Further, intra-domain disulfide bonds exist in each domain that contributes to its stability (Stevens, 1991). Domain III is different from domain I and domain II because it has shorter disulfide bonds between the first and second cysteine residues. The disulfide bonds not only contribute to the higher heat stability, but also are responsible for increasing difficulty in digestion and hence it is classified as a protease inhibitor which is also observed in other molecular like soy inhibitors (Vagadia, Vanga, & Raghavan, 2017; Vagadia, Vanga, Singh, & Raghavan, 2016; Winiarska-Mieczan & Kwiecień, 2007). Hen egg ovomucoid can only bind to one molecule of protease every time, which is different from ovomucoid in duck and turkey egg (Huopalahti et al., 2007). This is due to the flexible enzyme-inhibitor contact position resulting in varying inhibitory activity of ovomucoid present in different species. This phenomenon is beneficial to prevent a wide range of bacterial proteinases (Stevens, 1991).

Researchers also found that ovomucoid is the most allergic protein in egg (Dhanapala, Withanage-Dona, Tang, Doran, & Suphioglu, 2017). The allergic activity of ovomucoid remains stable under thermal treatment or enzymatic hydrolysis (Julià et al., 2007). However, this unique property can be used as a tool for detection of egg in a food products even after thermal processing.

2.3. Ovotransferrin/conalbumin

Conalbumin, also known as ovotransferrin, is a monomeric glycoprotein and one of the most allergic proteins found not only in egg white but also in the yolk. Its molecular weight is found to be 76 kDa, with a total of 686 amino acids and 15 disulfide bonds (Abeyrathne et al., 2014). Since each molecule of ovotransferrin can bind to two Fe^{3+} ions, they play a key role in iron transportation from the hen oviduct to the developing embryo (Huopalahti et al., 2007; Stevens, 1991). This property contributes to its applications in iron-fortified products like iron-fortified beverages and iron supplements. It has two different forms: apo-ovotransferrin (iron free) and holo-ovotransferrin (iron bound). After binding to iron, the stability of ovotransferrin was enhanced due to its increased resistance to physical and chemical stresses. Further, researchers also found that ovotransferrin has antimicrobial, antiviral and antioxidant properties (Abeyrathne et al., 2014). The iron-transferrin complex can effectively prevent both gram-positive or gram-negative bacteria from growing in different foods. Moreover, apart from Fe ion, bicarbonate ions can also enhance the antimicrobial activity (Huopalahti et al., 2007). At the same time, bacteriostatic ability due to the bicarbonate ion can be reversed by adding other Fe^{3+} . Other studies proposed different mechanisms on the origin of antimicrobial activity of ovotransferrin. They suggested ovotransferrin might have the ability to bind with bacterial cell membrane which in turn would affect the functional property (Huopalahti et al., 2007). The two opinions are both reasonable and can contribute to the strong antimicrobial activity of ovotransferrin as observed in various food products.

Table 1

Composition of egg white proteins (Desert et al., 2001; Forsythe & Foster, 1950; Mine, 1995; Stevens, 1991).

Protein	Amount in egg white (%)
Ovalbumin	54–66
Ovotransferrin	12–13
Ovomucoid	9.5–11
Lysozyme	2.3–4.5
Ovomucin	1.5–3.5
Ovoinhibitor	0.1–1.5
Ovoglobulin G2	1.0–6
Ovoglobulin G3	1.0–6
Riboflavin binding protein	1.0
Avidin	0.05–0.5
Ovostatin	0.5

2.4. Ovomucin

Ovomucin is a sulfated glycoprotein which is responsible for the thick gel texture of egg whites (Abeyrathne, Lee, Jo, Suh, & Ahn, 2016). It is considered as one of minor allergens present in egg (Bush & Hefle, 1996; Walsh et al., 1988). The molecular weight of ovomucin is 1.8–8.3 $\times 10^3$ kDa (Abeyrathne et al., 2014; Baumgartner & Schubert-Ullrich, 2010). Subunits in the structure of ovomucin can be divided into two forms based on their composition and properties: α - and β -ovomucin (Huopalahti et al., 2007). Furthermore, albumen contains both soluble and insoluble ovomucin. However, the primary difference is that the soluble ovomucin exists in both thick and thin albumen, but insoluble ovomucin occurs only in thick egg white portion (Huopalahti et al., 2007). Research showed that the physical stability and foaming properties of egg white are highly dependent on ovomucin and degradation of ovomucin can lead to changes in its functional properties (Stevens, 1991). Ovomucin was also reported to have good anti-tumor and antiviral activities (Huopalahti et al., 2007). The biologically active glycoproteins from ovomucin make its application interesting for food and nutraceutical studies.

2.5. Lysozyme

Lysozyme poses hydrolytic muramidase activity. It widely exists in nature, found in tissues of some animal or human organisms, secretions, and body fluids. It also occurs in bacteria and in some plants (Huopalahti et al., 2007). It should be noted that the role of lysozyme in egg allergy has been in controversy due to the conflicting findings from different studies (Anet et al., 1985; Langeland, 1983; Miller & Campbell, 1950). Further studies are required to assess and understand its role in egg allergy. The molecular weight of lysozyme is 14.4 kDa, and it has 129 amino acids in a single polypeptide chain (Abeyrathne et al., 2014). Lysozyme is a single polypeptide chain comprising two domains connected with an α -helix and is cross-linked by four disulfide bridges (Young, Tilton, & Dewan, 1994). Two of the four disulfide bridges in its structure contribute to extremely high heat stability and the other two are responsible for its enzymatic activity (Abeyrathne et al., 2014; Huopalahti et al., 2007). Its activity is unaltered under high temperature (100°C) or in acidic solutions (pH 3.0–4.0) (Huopalahti et al., 2007). However, it can be easily and rapidly inactivated upon reacting with thiol substances. Further, lysozyme also forms aggregates under 80 °C and neutral pH effects their functional properties (Iwashita, Handa, & Shiraki, 2017).

Lysozyme can protect the host from invasion of foreign bacteria, especially gram-negative bacteria (Stevens, 1991). Thus, it is widely used in food, pharmaceutical, and drug industries. Addition of lysozyme can avoid spoilage of food by preventing the growth of pathogens, which in turn can extend their shelf life. Furthermore, lysozyme can serve as an antimicrobial when combined into packaging polymers which finds its application in food packaging industry (Appendini & Hotchkiss, 2002; Huopalahti et al., 2007). In pharmaceutical and medical industries, lysozyme has been used to prevent infections or to serve as a stimulant in immunological studies to help treatment of bronchopulmonary diseases (Huopalahti et al., 2007).

2.6. Avidin

Avidin is a water-soluble glycoprotein which binds with biotin (Green, 1963; Yossa, Sarker, Karanth, Ekker, & Vandenberg, 2011). It is present in the oviducts or oviductal secretory tissues like egg white of birds, reptile, lizards, and amphibians (Elo, Räsänen, & Tuohimaa, 1980; Strzelczyk, Bujacz, Plažuk, Zakrzewski, & Bujacz, 2013). Avidin can be found in hen egg and it can make up 0.05–0.5% of egg white proteins (Green, 1975). However, the maturity of laying hen influences the content of avidin in egg white (Durance, 1991). Some findings showed that when a hen begins laying eggs, avidin content increases

from the first to the 15th egg and then becomes constant (Bush & White, 1989; Wet & Hsu, 1970).

Each monomer contains 128 amino acids residues (Yossa et al., 2011). Theoretical molecular weight of 15 kDa with corresponding theoretical combined molecular weight of 57 kDa (Malaikaew, Svasti, Kumar, & Buranaprapuk, 2011; Rothmund, Thomas, & Rylatt, 2002). Avidin contains eight antiparallel successive beta-strands associated with its three-dimensional crystal structure (Spolaore, Damiano, Raboni, & Fontana, 2014). This structure is the reason for high stability of avidin. A carbohydrate side chain and a disulfide bridge are also identified in avidin's structure that contribute to its stability (Laitinen et al., 2002). Avidin is an alkaline (Laitinen et al., 2002). Glycosylation and high pI results in avidin accumulation in liver and kidneys which may trigger a tumor if avidin is not purged by the body (Hytönen et al., 2003; Yao, Zhang, Sakahara, & Nakamoto, 1999). Further, avidin can bind to a material based on the charge of the material because of its high pI. Avidin aggregates usually when mixed at ambient temperatures with anionic detergents, such as sodium dodecyl sulfate (SDS). The resulting aggregates fail to penetrate the stacking gel during polyacrylamide gel electrophoresis (PAGE). To avoid the formation of such aggregates, avidin can be acetylated and the pI will be reduced (Bayer, Ehrlich-Rogozinski, & Wilchek, 1996).

Avidin is known to bind with biotin, resulting in vitamin H or B₇ deficiency (Green, 1975). Biotin is one of the water-soluble vitamins (Yossa et al., 2011). Up to 4 molecules of biotin can bind with one avidin molecule. Further, it suggests that the affinities are characterized by the dissociation constant which is around 10^{-15} M between them; it is remarkably higher than other antigen-antibody complexes making this complex very stable (Strzelczyk et al., 2013; Yossa et al., 2011). Biotin plays a significant role in human nutrition because it affects metabolic processes such as fatty acid synthesis and amino acid catabolism (Maeda, Kawata, Inui, & Fukuda, 1996). Humans cannot synthesize biotin and therefore depend on the supply of biotin from exogenous sources. The experiments (Maeda et al., 1996) showed that biotin-deficient rats had remarkably higher plasma ammonia concentration than biotin-supplemented ones. Therefore, biotin deficiency is proved to be related to 'hyperammonemia'. But the mechanisms that triggers this condition is not clear and warrants further investigation.

Biotin plays an important role in the growth of microorganisms such as fungi and bacteria. Due to the stable complex compound between avidin and biotin, the growth of these microorganisms is efficiently inhibited (Elo et al., 1980; Green, 1990). However, the avidin-biotin complex has so many applications that it is, to some degree, advantageous to the biotechnology industry. Its applications in affinity chromatography, fluorescence-activated cell sorting, western blotting, ELISA and tumor targeting have been explored by scientific community (Strzelczyk et al., 2013). In addition, it can also be used for protein detection (Spolaore et al., 2014). However, the use of avidin-biotin complex applications is limited because its binding property with biotin is not specific (Huopalahti et al., 2007). Avidin has been used in anticancer treatment applications (Anton, Nau, & Nys, 2006). The research suggested that there are antibodies against avidin in human serum and hence humans can tolerate avidin to a certain extent (Bubb et al., 1993).

In 2007, a 2-year-old boy was found to be allergic to avidin (Enriquez-Matas et al., 2007). It is the first case reported regarding the egg allergy related to avidin. He was diagnosed to be hypersensitive to egg proteins. In addition, when he was 13-month-old, he got an acute and severe urticaria after contacting with raw egg incidentally. However, specific IgE to several major allergens in egg showed negative. Therefore, experts assumed the boy was allergic to avidin rather than the other proteins in eggs. However, there were no previous reports regarding avidin allergy, and the reported phenomenon itself should be further verified (Enriquez-Matas et al., 2007).

In recent years, avidin-like proteins were discovered from a wide range of sources. *Bradyrhizobium japonicum* is a symbiotic bacterium

which can fix nitrogen on the root nodules of soybean plants (Nordlund, Hytönen, Laitinen, & Kulomaa, 2005). When its gene encoding is examined, it was found that the protein is homologous with avidin. Rhizavidin from *Rhizohium etli* is also one of the avidin-like proteins (Helppolainen et al., 2007). A novel avidin-like biotin-binding protein was separated from *Bradyrhizobium japonicum* known as Bradavidin 2 (Helppolainen et al., 2008). Applications of this protein have been used in improving crop yields through inoculating legume seeds (Slaton, Roberts, & Ross, 2011). Avidin-like proteins were also identified in Tamogitake mushroom (Takakura et al., 2009), zebrafish (Taskinen et al., 2013) and shiitake mushroom (Takakura, Sofuku, Tsunashima, & Kuwata, 2016). These findings have renewed the interest of various researchers into the class of avidin-like proteins and their properties, biochemistry and applications in various industries.

3. Processing methods

A number of allergic reactions triggered by various foods have increased drastically in recent years. Therefore, many scientists attempted in technical food processing effects on allergies and further the structure-function relationship of proteins (Vanga & Raghavan, 2016; Vanga, Jain, & Raghavan, 2016). This section outlines the effects of thermal processing and non-thermal processing methods on various egg allergens and anti-nutritional compounds.

3.1. Thermal processing

3.1.1. Traditional heat treatment

Heat treatment is a traditional processing method aimed to increase microbial safety, eliminating toxins or improve texture and palatability of food. The structures of allergenic components tend to change after heat treatment, which explains why thermal processing can minimize allergenicity. The tertiary structures of proteins begin to change approximately at 50–60 °C according to different molecular weight, stability, and size followed by secondary structure changes as temperature increases beyond 65 °C (Vanga, Singh, & Raghavan, 2017).

Ovalbumin was treated at different temperatures (25–100 °C) and pHs (4.5, 7.0, 9.5) and their effects on structure and antigenicity were assessed by fluorescence spectroscopy, in silico prediction and enzyme-linked immunosorbent assay (ELISA) (Stănciuc, Banu, Turturică, & Aprodu, 2016). The antigenicity of ovalbumin reduced by 82% after thermal treatment at over 80 °C with pH 9.5 when compared to untreated control. This study further showed that pH played a vital role in denaturation as only 5.5% reduction was found when the same thermal treatment was performed at pH 7.0. The results proved the significance of temperatures and pH on structure and antigenic properties of a protein (Stănciuc et al., 2016). Claude et al. (2016) evaluated the effects of thermal processing on ovalbumin and its ability to cause an allergic reaction in both sensitization and elicitation stages using sera from patients' sensitive to ovalbumin and mouse model. They found that aggregation as a result of thermal treatments reduces the allergenicity.

Though there is an increased production of IgG, the IgE-binding is reduced both in human and mice samples.

In another study, ovomucoid was first isolated and dissolved in a basic PBS buffer. Then it was processed by heat from 85 to 98 °C in basic, neutral buffer and egg white. Ovomucoid in the basic buffer had more resistance to heat. Ovomucoid in egg white shared no similar denaturation patterns with the ovomucoid in the former two buffers (Julià et al., 2007).

Tong et al. (2012) studied changes in allergenicity and conformational structure of ovotransferrin after thermal processing. Circular dichroism spectroscopy (CD), ultraviolet absorption spectroscopy (UV) and fluorescence spectroscopy were used to analyze the alteration of conformational structure. ELISA was applied to examine changes in the allergenicity of ovotransferrin. The binding capacity of IgG and IgE to ovotransferrin was modified due to changes in conformational stabilization and structural alteration. They found that allergenicity can change in case of unfolding or changes to disulfide bonds in the structure.

In the early investigations, there was a belief that common cooking methods like boiling, frying could destroy avidin (Durance, 1991). However, their studies showed that some parts of egg white were not heated enough, resulting in residual avidin activity. Durance and Wong (1992) conducted an experiment to study the inactivation kinetics during thermal processing of avidin. Temperature ranged from 73 °C to 93 °C and the HABA-standard curve method was used to measure avidin activity. Inactivation kinetics was assumed to follow the first-order reaction kinetics. Their results showed that the inactivation of 90% avidin activity was reached at 121 °C for 20 min.

3.1.2. Microwave heating

Microwave has an electromagnetic frequency ranges of 300 MHz to 300 GHz (Vanga et al., 2017). Despite a wide range of applications in medical and telecommunication fields, microwave can also be used in food processing for applications such as pasteurization, drying, and defrosting (Richardson, 2001). Two frequencies of 915 MHz and 2.45 GHz can be used for industrial and food-related applications (Doona, 2010; Tewari & Juneja, 2008). Heat generation in food by microwave treatment results in numerous changes both in conformational structures and physical characteristics like flavor and texture (Vanga et al., 2017).

A molecular dynamics study reported that lysozyme was denatured in electromagnetic fields (English & Mooney, 2007). The nonequilibrium canonical ensemble (NNVT) simulations with the frequency in areas ranged from 50 to 500 GHz, and intensity of 0.1–0.5 V/Å were performed at 298 K. In their study, the secondary structure of lysozyme changed significantly, and the speed and extent of onset of denaturation were influenced largely by field intensity. Similar studies have also been performed on various other protein molecules including allergens like Ara h 6 in peanut (Ashutosh Singh, Vanga, Orsat, & Raghavan, 2017; Vanga, Singh, & Raghavan, 2015). Table 2 outlines the thermal processing effects on various egg proteins.

Table 2
Thermal processing methods of egg white proteins.

Protein	Processing method	Effects and outcome	Reference
Ovalbumin	Heat treatment at 25–100 °C for 20 min at pH 4.5, 7.0 or 9.0	Over 82% antigenicity was reduced and structure changed in 80 °C at pH 9.5	(Stănciuc et al., 2016)
Ovomucoid	Heat treatment (85–98 °C, pH 7.4, 9.1, 9.6)	Ovomucoid in the basic buffer showed more resistance than in neutral solutions	(Julià et al., 2007)
Ovotransferrin	Heat treatment	Changed IgG and IgE binding capacity and conformational structures	(Tong et al., 2012)
Avidin	Thermal processing (73–93 °C) with different time	Inactivation of 90% avidin activity (25 min at 121 °C)	(Durance & Wong, 1992)
Lysozyme	NNVT simulations with the frequency from 50 to 500 GHz and RMS intensity of 0.1–0.5 V/Å were performed at 298K for 0.5 ns	Significantly changed secondary structures	(English & Mooney, 2007)

3.2. Nonthermal processing

3.2.1. High-pressure processing

High-pressure processing was first employed to eliminate microorganisms for improving the shelf-life of food products (Hite, Weakley, & Giddings, 1914). It is also known as high hydrostatic pressure processing. Its regular working pressure varies from 100 to 800 MPa (Farkas & Hoover, 2000). It is found to be effective on conformational alteration of food components, resulting in structural and functional property changes of proteins including reduction in allergenicity (Rivalain, Roquain, & Demazeau, 2010). The conformational changes in proteins are due to the impact of pressure on regions stabilized by hydrophobic and electrostatic interactions which are pressure liable. Hydrogen bonds also play an important role in stabilizing protein structures, but are almost pressure insensitive (Ledward, 2000).

Ovalbumin was subjected to high pressure in the range of 0.1–600 MPa at 10 °C for 10 min to investigate the changes in its allergenicity and structure. A reduction in allergenicity was observed and the maximum inhibition rate was reported to be 89.7% at 500 MPa. A change in tertiary structures was detected by fluorometric measurement but no difference in secondary structures occurred in CD spectroscopy (Odani, Kanda, Hara, Matuno, & Suzuki, 2007). The effects of high pressure on enzymatic hydrolysis were studied in 2008 (López-Expósito et al., 2008). Ovalbumin was hydrolyzed by pepsin under 400 MPa at 37 °C for 10, 60, 120 min. The native structure broke down in minutes and produced lower allergenic hydrolysates. The result demonstrated high pressure could accelerate the proteolysis with reduced IgG and IgE binding. A recent study revealed that this novel food processing method can inactivate avidin (Singh & Ramaswamy, 2014). The processing impact on avidin was compared in high-pressure processing (HPP) with moderate temperatures and traditional thermal processing. Thermal processing was performed at different temperatures for pure avidin, avidin in albumen and avidin in the whole egg. The pure avidin was treated at 80, 90, 100 °C for 45 min while avidin in egg or egg white was treated at 30, 40, 50 °C for 10 min (Singh & Ramaswamy, 2014). Avidin was completely inactivated at 100 °C for 10 min. In terms of high-pressure processing the processing pressures of 500, 600, 700 MPa were applied combined with temperatures 30, 40, 50 °C to avidin in egg and albumen. The optimal condition for inactivation was 50 °C with a pressure of 700 MPa. The outcome showed that HPP is effective in inactivation of avidin. The investigation on the effects of different forms of the samples also showed avidin in the egg has a low resistance either to thermal or pressure processing compared to pure avidin.

3.2.2. Ultraviolet radiation

UV radiations with a wavelength of 100–400 nm are widely used in the food industry for pasteurization and post-harvest processing (Vanga et al., 2017). It has been used in reducing the growth of microorganism present in liquid egg white. A significant reduction of microorganism in the liquid egg white was observed, when the intensity and fluid depth increased to 0.153 cm and 1.314 mW/cm², respectively (Unluturk, Atilgan, Baysal, & Tari, 2008). Similar results were obtained in another study, the populations of microorganism (e.g. *E. coli* K-12) in liquid egg white were reduced by 0.896–1.403 log CFU at the same processing condition (Unluturk, Atilgan, Baysal, & Unluturk, 2010). This reduction may be due to the formation of thymine dimers after UV-C light treatment. These thymine dimers can prevent the microorganism from DNA transcription and replication, which in turn could result in the death of cell (Geveke, 2008).

Furthermore, ultraviolet radiation was applied to egg white and several physical and functional properties such as size, immunoreactivity, foaming properties were measured (Manzocco, Panozzo, & Nicoli, 2012). Samples of egg white were exposed to UV-C light (15 W) with an irradiance frequency of 35.4 W/m² for 5 and 30 min at 8 °C. For 5 and 30 min, the dose used was 10.6 and 63.7 kJ/m², respectively. Ultraviolet processing caused backbone cleavage and

aggregation of egg white but no alteration was made in immunoreactivity and gelling properties. However, foaming properties were enhanced (Manzocco et al., 2012). Additionally, a study found that the secondary structure and tertiary structure was disturbed after UV light treatment, which may be due to the reduction in disulfide bonds in lysozyme (Cys30-Cys115) based on the structural analysis (Wu, Sheng, Xie, & Wang, 2008). Thus, it indicates UV light can be used as a potential technique in changing the properties of egg white.

3.2.3. Ultrasound

Ultrasound is another novel non-thermal food processing method that employs sound waves with a wide frequency range (Feng, Barbosa-Cánovas, & Weiss, 2011). According to the frequency, three types of ultrasound are generally applied: power ultrasound (20–100 kHz), high frequency ultrasound (100 kHz–1 MHz), and diagnostic ultrasound (1–500 MHz) (Wu, Guo, Teh, & Hay, 2012). It can cause vibration of particles in the medium, resulting in occurrence of a cyclic succession of expansion and compression. The expansion and compression cycles change the surrounding pressure and cavitation bubbles may be generated. When these bubbles collapse, they will generate energy which leads to increase in temperatures and pressures (Wu et al., 2012). Studies reported that ultrasound can raise the temperature to 5000 K and pressures to 10–100 MPa (Weiss, Gulseren, & Kjartansson, 2011). These conditions in the solvent results in formation of free radicals like H⁺ because of sonochemical reactions (Valdramidis, Cullen, Tiwari, & O'Donnell, 2010). Thus, ultrasound can lead to both the conformational alterations and changes in physical properties of proteins like emulsification ability and solubility (Shriver & Yang, 2011; Soria & Villamiel, 2010).

Manas, Munoz, Sanz, and Condon (2006) investigated the inactivation kinetics of lysozyme using ultrasound under pressure. The inactivation reaction was conducted at different temperatures using phosphate buffer (pH 6.2) with heat (90–130 °C), ultrasound (117 µm, 20 kHz) and manothermosonication (MTS, 117 µm, 20 kHz, 200 kPa). MTS is a method in which a combination of heat, ultrasound, and pressure are applied. They concluded that ultrasound performed at room temperature with any pressure was unable to inactivate lysozyme. However, MTS at 70 °C for 3.5 min decreased lysozyme activity by 10-fold (Manas et al., 2006). Further, after introducing the step to pasteurize, MTS was able to inactivate 90% of lysozyme in 10 min.

3.2.4. Gamma irradiation

Gamma irradiation is a processing method which exposes food directly to an ionizing wave. Gamma irradiation was first developed to extend shelf life and to destroy microorganisms or food pathogens (Farkas, 2006). Radiation treatment at doses of 2–7 kGy can effectively eliminate potentially pathogenic non-spore-forming bacteria without negatively affecting the sensory and nutritional aspects of foods (József Farkas, 1998; Prakash, Guner, Caporaso, & Foley, 2000).

Kim et al. (2002) reported a novel allergenicity-reducing method which is the combination of gamma irradiation with heat treatment. Ovalbumin was tested under three different conditions: heat, irradiation after heat treatment, heat after irradiation. Heat treatment was conducted at various temperatures 37, 60, 70, 75, 80, 85, and 90 °C for 15 min. Gamma irradiation was operated at a dose of 10 kGy with the dose rate of 10 kGy/h. Denaturation of ovalbumin began at temperatures around 70 °C, and the most denaturation happened between 80 and 85 °C in a neutral solution. The study concluded that gamma irradiation, regardless of the temperature employed for heat treatment could not reduce the allergenicity of ovalbumin. Furthermore, a significant increase in the antigenicity of one of the samples processed by heat after irradiation was observed. However, the results observed by Lee, Seo, Kim, Lee, and Byun (2007) on the application of electron beam and gamma radiation were different from the previous study (Kim et al., 2002). Ovalbumin was irradiated by gamma or an electron beam under the same conditions (3, 5, 7, 10 kGy). The study showed that both

types of irradiation can reduce the antigenicity and IgE binding ability of ovalbumin. Contrary to Lee et al. (2007), other researchers observed no reduction in the allergic properties of ovalbumin under gamma radiation (Kim et al., 2002). Though the difference can be attributed to various reasons ranging from buffer concentrations, testing procedures and testing efficacy. Further studies are needed to understand changes in the structure of ovalbumin protein under radiation and its effect on the allergic properties of protein. These studies should concentrate on the difference in the secondary structure changes in ovalbumin under thermal and non-thermal processing conditions and their combination.

Gomaa and Boye (2015) evaluated the changes in the allergenic and structural properties of various egg proteins using a combination of gamma radiation and thermal processing. They reported that no significant changes in the allergenicity as well as the secondary structures were observed due to gamma radiation. However, they reported that changes in food matrix played a vital role in allergy detection.

The allergenicity of ovomucoid was also evaluated (Lee et al., 2002) after the processing with gamma irradiation (10 kGy) combined with heat treatment (100 °C, 15 min) in different pH solutions (pH 7.0, 9.0 and 10.0). The study indicated that the concentration of ovomucoid could be effectively reduced by the combination of gamma irradiation and thermal processing. Table 3 outlines the effects of various non-thermal processing methods on egg white proteins.

3.3. Chemical treatments

3.3.1. Enzymatic hydrolysis

Enzymatic hydrolysis is one of the primary and widely used methods to reduce allergenicity. Hydrolysis can alter not only the structure of proteins including the conformational or linear epitopes present in the molecule, but also can influence their functional characteristics (Vanga et al., 2017). However, the unspecific proteolysis nature of the enzyme on these proteins may instigate new challenges. New epitope centers or unexpected changes in the functional properties that can occur after enzymatic hydrolysis could risk food safety (Sathe, Teuber, & Roux, 2005; Vanga et al., 2017).

Yang et al. (2017) evaluated the effects of alcalase hydrolysis on allergenicity and antigenicity of albumen. The hydrolysis was conducted at different temperatures (30–80 °C with 10 °C intervals) and pH 6 to pH 12. The optimal pH and temperature for both free and immobilized alcalase was found to be 8.0 and 60 °C, respectively. The calibration curve method was used to calculate the degree of hydrolysis and hydrolysates were characterized by SDS-PAGE. After 3-h hydrolysis, the degree of hydrolysis of free alcalase (25%) is more than that of immobilized one (20%). Both were beneficial to reduce the IgG and IgE binding activity. However, immobilized alcalase can be cyclically used

as it is more stable compared to free alcalase.

Enzymatic hydrolysis method was also applied to enhance the functional properties of ovomucin (Abeyrathne et al., 2016). Pepsin, trypsin, papain, and alcalase were used for hydrolysis in this study. Each enzyme reacted with ovomucin at 37 °C for up to 24 h and ended with enzyme inactivation at 100 °C for 15 min. The optimal condition for inactivating ovomucin with an enzyme was 37 °C for 3 h. Findings revealed that pepsin, papain, and alcalase could hydrolyze ovomucin. The hydrolysates of ovomucin had an antioxidant activity and ACE-inhibitory activity (Abeyrathne et al., 2016). In another study, hydrolysis using trypsin and pronase showed no significant effect on avidin (Green, 1975).

3.3.2. Acid treatment

To date, many studies found that acidic conditions are helpful in improving food digestibility and in reducing the activity of allergic proteins. Armentia, Duenas-Laita, Pineda, Herrero, and Martín (2010) reported that addition of vinegar to some food proteins (e.g. chicken, lentils) improved their digestive function in the stomach, leading to reduction in the symptoms pertaining to food allergy. Specifically, it revealed that the patients showed a negative result through the test of double-blind placebo controlled challenge when they consumed 10 g of chicken with vinegar. However, the results were positive after taking the same quantity of chicken without vinegar. Similarly, this study reported that the IgE-binding activity was reduced when tested in vinegar treated lentils compared to the untreated ones. Addition of vinegar provides a more acidic condition for the stomach which will enable it to fully activate the digestive pepsin moieties. This might result in the protonation of acidic amino acid residues in allergens which can potentially reduce the risk of food allergy. A study found that acetic acid treatment of egg white is effective in reducing the allergenicity (Lee et al., 2017). Eggs were boiled for 10 min, and then were treated with acetic acid solutions (pH 2, 3 or 4) for 24 h. The results showed that pH 2.0 or pH 3.0 acetic acid treatment significantly reduced the allergenicity of ovalbumin. Reduction of allergenicity under acidic treatment can be primarily attributed to the protein cleavage and the polarity changes on the protein surface. These changes can lead to conformational change that will not trigger an allergic reaction in the patient. Further studies are required to evaluate the effect of acidic conditions on the protein structures and their functional properties including their allergenicity and digestibility.

3.3.3. Protein glycation

Protein glycation involves a reaction between monosaccharides and amino acids present in proteins which may result in structural deviations and functional property changes in proteins. A Schiff base is

Table 3
Nonthermal processing methods of egg white proteins.

Protein	Processing method	Effects and outcome	Reference
Avidin	High-pressure processing (500, 600, 700 MPa) with heat treatment: 80, 90, 100 °C in 45 min for pure avidin while 30, 40, 50 °C with 10 min for avidin in egg, compared with thermal processing (80, 90, 100 °C within different time for both forms of avidin)	Complete inactivation of avidin at 100 °C for 10 min or at 50 °C with a high-pressure treatment at 700 MPa.	(Singh & Ramaswamy, 2014)
Lysozyme	Ultrasound with heat treatment at different temperatures; Ultrasound (117 µm, 20 kHz) and manothermosonication (MTS, 117 µm, 20 kHz, 200 kPa)	Ultrasound performed at room temperature with any pressure was unable to inactivate lysozyme. MTS can inactivate 10-fold the activity within 3.5 min at 70 °C.	(Manas et al., 2006)
Egg white	Ultraviolet with an irradiance frequency of 35.4 W/m ² for 5 and 30 min at 8 °C. The dose for 5 and 30 min was 10.6 and 63.7 kJ/m ²	1. Backbone cleavage and aggregation of egg white 2. No effects on immunoreactivity and gelling properties 3. Enhanced foaming properties	(Manzocco et al., 2012)
Ovalbumin	Gamma irradiation (10 kGy) combines with heat treatment (37–90 °C)	Highest denaturation at 80–85 °C Reduced allergenicity	(Kim et al., 2002)
	Gamma and an electron beam irradiation at 3, 5, 7, 10 kGy High pressure (0.1–600 MPa) at 10 °C for 10 min	Reduced IgG and IgE binding ability and allergenicity Maximum inhibition rate reached 89.7% at 500 MPa but no changes were observed in tertiary and secondary structures	(Lee et al., 2007) (Odani et al., 2007)
Ovomucoid	Gamma irradiation (10 kGy) with heat treatment (100 °C, 15 min) at pH 7.0, 9.0 and 10.0)	Reduced allergenicity	(Lee et al., 2002)

Table 4
Chemical modification techniques and changes on functional properties of egg white proteins.

Protein	Processing method	Effects and outcome	Reference
Egg white	Acetic acid treatment	Partly decreased allergenicity in egg white but little effects on ovomucoid	(Lee et al., 2017)
Ovalbumin	N-acetylglucosaminidase	Reduced allergenicity and antigenicity	(Hwang et al., 2016; Park et al., 2017)
Ovomucoid	Glycation with moist-heat processing (a water bath at 60 °C for 30 min with different concentration of sodium carbonate-bicarbonate buffer at pH 9.6) Deglycosylated processing	1. Increased allergenicity 2. IgE binding capacity increases with the concentration of sodium carbonate-bicarbonate buffer increases Unaltered IgE binding capacity	(Ma et al., 2013)
Avidin	Enzymatic Hydrolysis (trypsin and pronase)	No effects	(Besler, Steinhart, & Paschke, 1997)
Albumen	Enzymatic hydrolysis (free and immobilized alcalase) Conducted at 30–80 °C with 10 intervals and pH 6–12 with 1 interval.	1. Optimal condition: 8.0 and 60 °C for 3 h. Degree of hydrolysis of free Alcalase was 25% while 20% of immobilized alcalase. 2. Reduced IgG and IgE binding	(Green, 1975) (Yang et al., 2017)
Ovomucin	Enzymatic hydrolysis at 37 °C with 24 h (Pepsin, trypsin, papain and alcalase)	1. The optimal inactivation condition was 37 °C for 3 h. 2. The hydrolysates of ovomucin displayed an antioxidant activity and ACE-inhibitory activity.	(Abeyrathne et al., 2016)

formed after the reaction of carbonyl group of glucose with the amine group of protein during the initial process (Van Buren & LeWinter 2011). Although this primary reaction is reversible, the carbonyl-protein adduct is eventually converted to a stable product, Amadori. Finally, it is possible to form an advanced glycation end product (AGE), a stable protein which is widely used in the food industry (Van Buren & LeWinter 2011). Thus, protein glycation has the potential to reduce food allergenicity as it involves chemical reactions that can lead to permanent changes in the protein structure. Ma, Gao, and Chen (2013) investigated the effects of glycation with the moist-heat processing of ovalbumin on the antigenicity, allergenicity, and conformations of ovalbumin. The experiments were conducted in different concentrations of sodium carbonate-bicarbonate buffer (final concentration of 0.2, 0.05 and 0.02 mol/L at pH 9.6). The reaction was performed in a water bath at 60 °C for 30 min followed by an immediate cooling in ice water. They concluded that glycation would increase the allergenicity as IgE binding capacity increased with an increasing concentration of sodium carbonate-bicarbonate buffer.

Many studies found that *N*-acetylglucosaminidase can hydrolyze N-terminal to reduce the allergenicity of an allergic protein. Hwang et al. (2016) reported the allergenicity of ovalbumin was reduced through a natural *N*-acetylglucosaminidase which was isolated from *ascidian viscer*. Park, Yoon, Kim, Han, and Choi (2017) also found that ovalbumin allergenicity was decreased by 1000-fold by *N*-acetylglucosaminidase treatment after measurement of OVA-specific IgE binding and histamine levels which were then analyzed by Ci-ELISA. Table 4 outlines chemical modifications techniques that can change functional properties of egg white proteins.

4. Conclusion

The understanding of the scientific community regarding the interdependency of structure-function relationships including their allergenicity is limited. Hence, considerable resources are being devoted on exploring the influence of innovative thermal and non-thermal processing techniques on the physicochemical properties of various egg proteins. Novel processing techniques including high pressure, microwave processing show promising results in reducing the allergic reactions without compromising the nutritional quality and safety of the products. Ultrasound and UV applications have greatly reduced the allergic activity in egg proteins like lysozyme, but at the same time improved other functional properties like foaming stability which might be desirable in few food products. The activity of anti-nutritional components like avidin was also reduced by high pressure processing and their combination with heat treatment. However, further research is required in understanding the conformational changes in proteins

due to various processing methods. Further, innovative techniques like molecular modeling, proteomics and genome associated studies must be undertaken to evaluate these allergens.

Conflicts of interest

The authors report no conflict of interest.

Acknowledgement

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada for their financial support.

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