

Egg proteins: fractionation, bioactive peptides and allergenicity

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Abstract

Eggs are an important source of macro and micronutrients within the diet, comprised of proteins, lipids, vitamins, and minerals. They are constituted by a shell, the white (containing 110 g kg⁻¹ proteins: ovalbumin, ovotransferrin, ovomucoid, lysozyme and ovomucin), and the yolk (containing 150–170 g kg⁻¹ proteins: lipovitellins, phosvitin, livetins, and low-density lipoproteins). Owing to their nutritional value and biological characteristics, both the egg white and yolk proteins are extensively fractionated using different techniques (e.g., liquid chromatography, ultrafiltration, electrophoresis, and chemical precipitation), in which liquid chromatography is the most commonly used technique to obtain individual proteins with high protein recovery and purity to develop novel food products. However, concerns over allergenic responses induced by certain egg proteins (e.g., ovomucoid, ovalbumin, ovotransferrin, lysozyme, α -livetins, and lipoprotein YGP42) limit their widespread use. As such, processing technologies (e.g., thermal processing, enzymatic hydrolysis, and high-pressure treatment) are investigated to reduce the allergenicity by conformational changes. In addition, biological activities (e.g., antioxidant, antimicrobial, antihypertensive, and anticancer activities) associated with egg peptides have received more attention, in which enzyme hydrolysis is demonstrated as a promising way to break polypeptides sequences and produce bioactive peptides to provide nutritional and therapeutic benefits for human health.

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INTRODUCTION

Eggs are one of most prevalent foods over the world, regardless of religion and ethnic group. Over the last decade, there has been significant growth in the use of egg fractions in different food applications (e.g., cakes, pies, noodles, powdered soup, desserts, confectionary, and ice creams). Approximately 30% of total consumption of eggs is in the form of processed products.¹ Eggs contain three main components: the shell (90–120 g kg⁻¹), the white (600 g kg⁻¹), and the yolk (300–330 g kg⁻¹). Whole egg consists of water (750 g kg⁻¹), proteins (120 g kg⁻¹, mainly distributed between egg white and yolk), lipids (120 g kg⁻¹, mainly concentrated in the egg yolk), and carbohydrates, vitamins, and minerals (10 g kg⁻¹).²

Egg yolk is an effective food ingredient, as it has both nutritional (e.g., containing vitamins, minerals, essential fatty acids, and phospholipids) and organoleptic properties.³ It is composed of water (50%), protein (15–17%), lipids (31–35%), and carbohydrates (1%). The protein component includes lipovitellins (36%), livetins (38%), phosvitin (8%), and low-density lipoproteins (LDLs, 17%).⁴ From the solubility point of view, egg yolk is a complex system constituted by non-soluble granules (with particle size ranging from 0.3×10^{-6} to 2×10^{-6} m) and a clear yellow plasma, which can be easily separated by centrifugation. Granules account for 22% of egg yolk and contribute half of egg yolk proteins with 7% of egg yolk lipids, whereas the plasma accounts for 78% of egg yolk and attribute the rest of egg yolk proteins with 90% of egg yolk lipids.³

Because of high bioavailability, essential amino acid contents, and great functionality (e.g., gelling, forming, and emulsifying properties), egg white is used as a meaningful food ingredient

during processing.⁵ It is predominantly composed of water (88%) and protein (11%), with trace amounts of carbohydrates, ash, and lipids (1%). The egg white proteins mainly include ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.5%) with minor amounts of avidin (0.05%), cystatin (0.05%), ovomacroglobulin (0.5%), ovoflavoprotein (0.8%), ovoglycoprotein (1%), and ovoidinhibitor (1.5%).² Egg white proteins also exhibit various biological activities. For example, lysozyme has great antimicrobial activity and is used as a food preservative to avoid pathogenic bacteria attachment during the production of meat products.⁶ Ovotransferrin is used as a metal transporter, antimicrobial, and anticancer agent. Both ovomucin and ovomucoid present tumor suppression ability and can be used as anticancer agents.⁷ In this review, three important aspects related to egg proteins will be addressed, including: (i) the numerous fractionation approaches to extract individual egg proteins; (ii) bioactive properties of peptides produced by enzymatic hydrolysis of egg proteins; and (iii) the potential reduction of allergenicity of egg proteins using various processing methods.

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FRACTIONATION OF EGG PROTEINS

Egg fractions separated by chemical and physical techniques (Fig. 1) for nutritional and industrial purposes have been abundantly researched based on their physicochemical properties (e.g., molecular weight, affinity, surface charges, and acidity) in order to expand the application areas and increase the value of eggs.

Separation of egg white proteins

Ovalbumin (540 g kg^{-1}), ovotransferrin (120 g kg^{-1}), ovomucoid (110 g kg^{-1}), ovomucin (35 g kg^{-1}), and lysozyme (35 g kg^{-1}) are the major egg white proteins that are most commonly studied, whereas avidin (0.5 g kg^{-1}), cystatin (0.5 g kg^{-1}), ovomacroglobulin (5 g kg^{-1}), ovoflavoprotein (8 g kg^{-1}), ovoglycoprotein (10 g kg^{-1}), and ovoinhibitor (15 g kg^{-1}) are minor proteins presented in the egg white that are least investigated.² Ovalbumin is a major egg white protein with 45 000 Da molecular weight and 386 amino acids. It has an N-terminal amino acid of acetylated glycine and a C-terminal amino acid of proline. It is also known as a glycoprotein with a carbohydrate group attached to the N-terminus.⁸ Ovotransferrin is considered as a monomeric glycoprotein with 76 000 Da molecular weight and 686 amino acids.³ It has a unique metal-binding ability, in which one molecule of ovotransferrin can easily attach with two iron molecules at $\text{pH} > 7.0$ to further release into the human body at $\text{pH} < 4.5$.⁹ Ovotransferrin is presented as two forms with different physicochemical properties: an apo-form (also known as the iron-free form, colorless) and a holo-form (also known as the iron-bound form, salmon pink color). The holo-form ovotransferrin is more resistant to physical and chemical changes than the apo-form.⁷ Lysozyme is a strong basic egg white protein with 14 400 Da and a single polypeptide chain containing 129 amino acids, and it has a tendency to bind with negatively charged proteins (e.g., ovomucin). It has an N-terminal amino acid of lysine and a C-terminal amino acid of leucine. Four disulfide bridges on lysozyme result in high thermal stability.^{10,11} Ovomucin is another major egg white protein with soluble (8300 Da) and insoluble (220 000–270 000 Da) components.¹² Accounting for 330 g kg^{-1} of carbohydrates (e.g., galactose and galactosamine) attached on the protein structure, it is a glycoprotein that is distributed to the gel-like structure of egg white.^{13,14} Ovomucin is composed of two subunits: α -ovomucin, which is a homogeneous subunit consisting of acidic amino acids (e.g., aspartic acid and glutamic acid) with less carbohydrate groups, and β -ovomucin, which is a heterogeneous subunit mainly consisting of serine and threonine with more carbohydrates attached.^{12,15} Ovomuroid is a glycosylated egg white protein with 28 000 Da molecular weight.¹⁶ It is well known as a trypsin inhibitor with three disulfide bonds presenting on its three-dimensional structure, and even peptides derived from ovomucoid still retain the trypsin inhibitory activity and immunoglobulin E (IgE) binding ability. Therefore, ovomucoid is responsible for egg allergy.^{16,17}

Owing to the unique nature and multifunctional properties, egg white proteins have been widely used in the food industry; the isolation and purification of each of the components using different methods (Table 1) appeared to be a promising development. Liquid chromatography, including gel permeation, ion exchange, and immobilized ligand affinity chromatography, has played a prominent role in the fractionation of egg white proteins, in which ion-exchange chromatography is the most commonly used for its high production capability and the ease with which it can be scaled up for industrial application. Ion-exchange chromatography allows separation of molecules according to the charge, and the

charge distribution of the molecules can be modified by adjusting the pH and ionic strength in the mobile phase. Depending on the proteins of interest (e.g., sensitivity of protein to pH changes), both cation and anion-exchange chromatography have been used for egg protein analysis, in which anion-exchange chromatography is more commonly applied in egg white protein separation. Vachier and co-workers¹⁸ fractionated lysozyme, ovotransferrin, and ovalbumin by using anion-exchange chromatography with a Q-Sepharose fast-flow column, and achieved relatively high protein recovery ($>62\%$) and purity ($>75\%$). Based on the different molecular size and hydrodynamic volume in the solvent, Awadé *et al.*²² used gel-permeation chromatography to achieve a simultaneous isolation of ovomucin and lysozyme from native egg white that was diluted in a 0.05 mol L^{-1} tris(hydroxymethyl) aminomethane hydrochloride buffer ($\text{pH} 9$) containing 0.4 mol L^{-1} sodium chloride (NaCl), and obtained high purity of ovomucin (80%) and lysozyme ($>99\%$). In comparison with ion-exchange chromatography, gel-permeation chromatography is less applicable at a process level, because it requires the removal of lipids from egg protein sources before loading on the column, which is not necessary on the ion-exchange column.²⁸ However, there are no conformational changes on the protein structure, which still maintains the initial activity after separation by the gel-permeation chromatography.²² Immobilized-ligand-affinity chromatography is able to selectively extract single proteins from samples based on the formation of a complex between the protein of interest and a ligand covalently bound to a resin. Al-Mashikhi and Nakai²⁰ applied a single step to extract ovotransferrin from egg white proteins by using immobilized-ligand-affinity chromatography with a Cu-Sepharose 6B column to achieve extremely high recovery yield (95%) and protein purity ($>94\%$). However, protein denaturation could be possible, because drastic elution conditions might be necessary to break the strong interaction between the proteins and the columns. Immobilized-ligand-affinity chromatography is also relative expensive to limit the transfer to a process scale.²⁰

In addition to liquid chromatography, addition of chemicals (e.g., ethanol, ammonium sulfate, and calcium chloride) with or without physical procedures (e.g., electrophoresis and ultrafiltration) is another important stream for separation of egg white proteins. For example, Omana and Wu²³ tried to use different concentrations of calcium chloride and potassium chloride in combination with isoelectric precipitation (based on the minimum solubility of the protein at a certain pH) to extract ovomucin, because salts can alter the ionic species and concentrations to affect electrostatic interactions among proteins and protein solvents, to finally determine the protein solubility. They found the highest purity (97.3%) of ovomucin can be produced using a two-step method, in which 0.05 mol L^{-1} calcium chloride was first used to precipitate egg white proteins followed by the addition of 0.5 mol L^{-1} calcium chloride extraction. However, the use of potassium chloride in the preparation of ovomucin resulted in high impurities with ovotransferrin and low sample handling capacity.²³ Ovotransferrin and ovomucoid were fractionated from egg white by using a high level of ethanol treatment and acidic salt precipitation based on their solubilities in those solutions.²⁷ A 430 g kg^{-1} ethanol solution was first used to treat egg white, followed by centrifugation. The supernatant was then precipitated using 610 g kg^{-1} ethanol to get ovotransferrin (the purity was $>88\%$). Or the addition of 25 g kg^{-1} ammonium sulfate and 25 g kg^{-1} citric acid combination was applied to obtain ovomucoid (the purity was $>89\%$). The production yields of ovotransferrin and ovomucoid were $>92\%$ and $>96\%$ respectively. It is also easy to scale up with a high production yield.²⁷

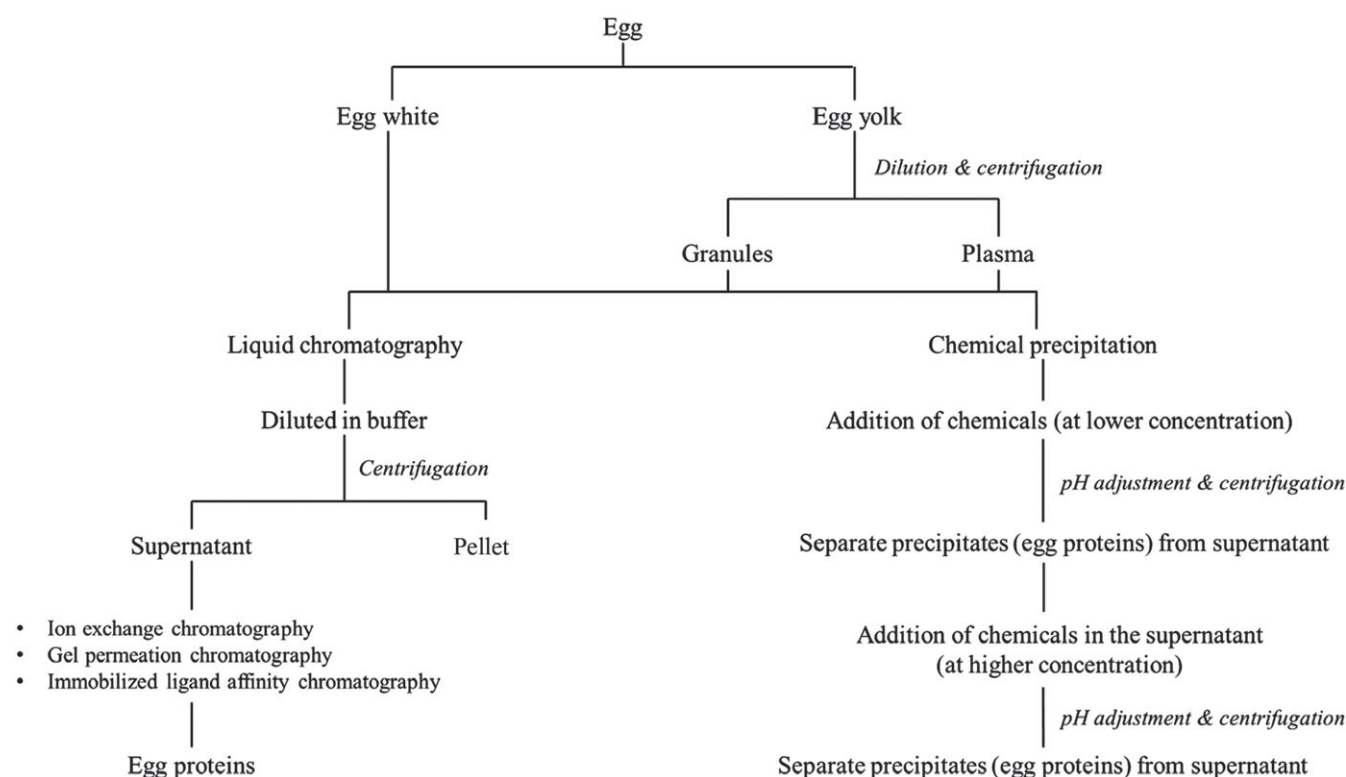


Figure 1. Schematic diagram to show the fractionation of egg proteins using liquid chromatography and chemical precipitation methods.

Table 1. Fractionation of major egg white proteins using different methods

Egg white proteins	Methods	Protein purity (%)	References
Ovalbumin	Isoelectric precipitation in the presence of 0.1 mol L ⁻¹ NaCl solution followed by centrifugation, in combination with ion-exchange chromatography	>82	12
	Anion-exchange chromatography	>75	18
	Ultrafiltration with 30 000 Da polyethersulfone in the Amicon stirred cell	75–99	19
Ovatransferrin	20–50% cold ethanol precipitation at room temperature	>80	9
	Anion-exchange chromatography	>75	18
	Immobilized-metal-affinity chromatography	>94	20
Lysozyme	Adding reductants (including ascorbic acid, cysteine, or cystine and β-mercaptoethanol)	–	21
	Ultrafiltration with 30 000 Da Biomax polyethersulfone and Ultracel Amicon YM regenerated cellulose membranes	>94	10
	Gel-permeation chromatography on a Superose 6 Prep Grade column	>99	22
Ovomucin	Calcium chloride in combination with isoelectric precipitation and gel filtration	>97	23
	Electrophoresis in combining with isoelectric precipitation	–	24
	Gel-permeation chromatography on a Superose 6 Prep Grade column	>80	22
Ovomucoid	Separated by 20% (v/v) ethanol at pH 5.0, followed by washing precipitates using 20% (v/v) ethanol	–	25
	Using sodium dodecyl sulfate polyacrylamide gel electrophoresis with linear gradient (40–200 g kg ⁻¹)	–	26
	Ethanol (430 g kg ⁻¹) treatment followed by ethanol (610 g kg ⁻¹) precipitation or acidic salt precipitation	>89	27

Although physical techniques (e.g., ultrafiltration and electrophoresis) are also used alone, liquid chromatography and chemical methods are the most common stream for the separation of egg white proteins, which provide high protein recovery and purity. Moreover, owing to the simple operation steps and rare interactions between column and proteins, liquid chromatography is easier to apply in industry.

Separation of egg yolk proteins

Egg yolk proteins are distributed in the granules, which are composed of lipovitelline (700 g kg⁻¹), phosvitine (160 g kg⁻¹), and LDLs (120 g kg⁻¹), and the plasma, which is composed of livetin (150 g kg⁻¹) and LDLs (850 g kg⁻¹).^{3,29} Granules and plasma can be easily fractionated based on two basic steps: dilution and centrifugation. Today, the method by McBee and Coterill³⁰ is still

the most popular, in which egg yolk was diluted in 0.17 mol L^{-1} NaCl solution, followed by centrifugation at $10\,000 \times g$ for 15 min to obtain the granules and the plasma. Recently, Strixner and Kulozik³¹ separated egg yolk by diluting into 0.15 mol L^{-1} NaCl solution at 1 : 2 ratio, followed by stirring the mixture for 1 h at 10°C before centrifugation at $10\,000 \times g$ for 45 min. This method obtained promising results for fractionating egg yolk on an industrial scale. Different methods to isolate egg yolk proteins are summarized in Table 2.

Lipovitellins (also known as high-density lipoprotein) are present in the egg yolk as liposoluble glycoproteins and consist of α -lipovitellin and β -lipovitellin, which precipitate at pH 7.5–7.8 and pH 6.5 and 7.0 respectively.⁴¹ It is believed they are able to prevent atherosclerosis by preventing the accumulation of cholesterol on the blood vessels. In work done by Luo *et al.*,³² after the separation of granules from egg yolk by NaCl washing and centrifugation, the granules were redissolved in NaCl solution, and lipovitellins were precipitated by adding ammonium sulfate followed by centrifugation at $11\,739 \times g$ for 30 min, but the production yield was only 21%. Based on the literature, lipovitellins cannot be prepared on a large scale, due to the lack of a promising method.

Phosvitin is a highly phosphorylated egg yolk protein with 100 g kg^{-1} phosphorus that is mono-esterified to the 123 serine residues from its 217 amino acids.⁴² It includes α -phosvitin, which is an aggregate of three or four subunits with 35 000–40 000 Da molecular weight, and β -phosvitin, which is an aggregate of four or five subunits with 45 000 Da molecular weight.⁴³ Owing to the excellent emulsifying properties, antioxidant capacity, antibacterial ability, and metal chelating property, phosvitin has great potential for use in food or functional food development. Ren and Wu³⁴ extracted phosvitin (for the development of functional foods with mineral-absorption-promoting ability) from egg yolk granules using 100 g kg^{-1} NaCl solution (at pH 7.25), followed by pH adjustment (from pH 8.0 to 3.5) to remove impurities. They found the purity was increased from 545 g kg^{-1} to 637 g kg^{-1} when the pH was decreased from 8.0 to 5.5, but the recovery reached a maximum (82.7%) at pH 8.0. The crude phosvitin (54.5%) was further purified using anion-exchange chromatography to achieve a purity of 97.1% with a recovery of 42.0%, and this method is industry applicable. Partially purified phosvitin was prepared by Ko *et al.*,³⁵ in which yolk granules containing phosvitin were first separated from yolk by pH adjustment (pH 4.0–8.0) and centrifugation. Subsequently, 85% ethanol was used to remove lipids and phospholipids to produce lipid-free granules that were homogenized with nine volumes of 10% ammonium sulfate or NaCl at pH 4.0 to generate phosvitin with the highest production yield. Finally, the salt was removed by ultrafiltration to obtain phosvitin (85% purity) with recovery rates of 72% (by ammonium sulfate) and 97% (by NaCl).

LDL is a spherical molecule present in egg yolk plasma with a triglyceride core and cholesterol esters surrounded by a phospholipid and protein film. Generally, LDLs contain $830\text{--}890 \text{ g kg}^{-1}$ lipids, which are comprised of triglycerides (690 g kg^{-1}), phospholipids (260 g kg^{-1}), and cholesterol (50 g kg^{-1}), and $110\text{--}170 \text{ g kg}^{-1}$ proteins.⁴⁴ The low density (0.98 kg m^{-3}) makes LDLs relatively stable in aqueous solutions.⁴⁵ In the work done by Moussa *et al.*,³⁷ LDLs were extracted from egg yolk plasma by mixing with 400 g kg^{-1} ammonium sulfate for 1 h to precipitate livetins. Then, the supernatant was dialyzed for 6 h against distilled water to remove the salt to produce LDLs with 97% purity and 67% yield. Burley and Vadehra³⁸ used gel-permeation chromatography to

extract LDLs. In detail, egg yolk plasma was diluted using 2 mol L^{-1} NaCl solution; the mixture was then subjected to chromatography with an Ultragel Aca 34 column to isolate LDLs; finally, the LDLs were eluted using 1 mol L^{-1} NaCl solution.

Livetins are globular glycoproteins corresponding to chicken blood serum proteins and present in egg yolk plasma as α , β , and γ -forms, in which γ -livetins is primarily dominated by immunoglobulin Y (IgY) to be an ideal alternative to mammalian IgG antibodies.⁴⁶ Liquid chromatography is mostly commonly used to fractionate livetins from egg yolk plasma. Akita and Nakai³⁹ isolated livetins with 98% recovery from egg yolk plasma using gel-permeation chromatography with a Sephacryl S-200 column, and livetin was eluted using a 1.5 mol L^{-1} NaCl solution at pH 7.0. Burley and Vadehra³⁸ also isolated livetin in the order of γ -livetins, α -livetins, and β -livetins using gel-permeation chromatography with 4 mol L^{-1} NaCl elution.

Different fractionation methods have also been developed in order to extract egg yolk protein isolates and broaden the application of egg yolk, in which organic solvent washing was initially used,⁴⁷ which was also accompanied by phospholipid recovery. Owing to the higher affinity to phosphatidylcholine and phosphatidylethanolamines, ethanol is most commonly used to separate phospholipids and egg yolk proteins. For example, Juneja *et al.*⁴⁸ extracted phospholipids (with $800\text{--}850 \text{ g kg}^{-1}$ of phosphatidylcholine and $100\text{--}150 \text{ g kg}^{-1}$ of phosphatidylethanolamines) from fresh egg yolk using a combination of acetone and ethanol, followed by filter press separation. However, owing to the incompatibility with food application and possibility of organic solvent residues, heating methods were subsequently applied to isolate egg yolk proteins. Nevertheless, protein denaturation is the major problem to restrict its application in product development. Therefore, alternative and reasonable techniques to fractionate egg yolk proteins were investigated.

Precipitation by ammonium sulfate or sodium sulfate was used to extract protein isolates concentrated in IgY from egg yolk. But it also increased the salt concentration in the final products. In addition, several operational cycles (e.g., solubilization and crystallization) were also involved, which increased the production cost. In addition, the combination of water, ethanol, and hexane was also applied, in which water-soluble egg yolk proteins were first isolated through centrifugation; thereafter, non-soluble fractions of egg yolk were treated under a mixture of ethanol and hexane followed by ultrafiltration to produce non-soluble egg yolk proteins.⁴⁹ Since organic solvents can react with hydrophobic groups on the protein surface and further result in the denaturation of proteins, the fractionation of egg yolk proteins should be operated at low temperature (-5 to 0°C) to minimize protein denaturation.

Depending on the different molecular size, gel-permeation chromatography segregated proteins from defatted egg yolk fractions.⁵⁰ But it is not suitable for industrial-scale application, because of the fragile nature of gels used in gel-permeation chromatography and possible interaction between gels and proteins.⁵¹ Therefore, ion-exchange chromatography has been more widely used as a large-scale method to separate charged proteins from egg yolk. In the work by Castellani *et al.*,⁵² egg yolk proteins concentrated in phosvitin were extracted by two steps: salt precipitation by magnesium sulfate and ion-exchange chromatography without using organic solvents.

Compared with chromatographic methods, membrane filtration is a powerful tool to isolate proteins, owing to its low cost and

Table 2. Fractionation of major egg yolk proteins using different methods

Egg yolk proteins	Methods	Protein purity (%)	Reference
Lipovitellin	NaCl washing followed by ammonium sulfate precipitation	71.9	32
	Moving-boundary electrophoresis in combination with ultracentrifugation	–	33
Phosvitin	Using 100 g kg ⁻¹ NaCl followed by pH adjustment, anion-exchange chromatography	54.5–97.1	34
	Precipitation using ammonium sulfate or NaCl at pH 4.0, followed by ultrafiltration	~85	35
	Defatting by butanol and ether, followed by precipitation using 0.4 mol L ⁻¹ magnesium sulfate at pH 1.8	–	36
Low-density lipoprotein	Mixing with 400 g kg ⁻¹ ammonium sulfate for 1 h, followed by centrifugation to collect the supernatant, and using dialysis to remove salt	97	37
	Dilution with 2 mol L ⁻¹ NaCl, followed by gel-permeation chromatography	–	38
Livetin	Gel-permeation chromatography with a Sephacryl S-200 column	>99	39
	Anion-exchange chromatography with diethylaminoethyl–Sephacel column, followed by salting out with sodium sulfate	98	40

easy adaptation to industry. Surface properties of membranes, pH, and salt concentration are major factors in determining the success of protein fractionation.⁵³ Ultrafiltration is considered as a preliminary step to remove enzymes, whereas nanofiltration with negatively charged membranes successfully gets rid of salts from protein fractions.⁵¹ Generally, a serial membrane filtration process includes dilution, filtration, delipidation by hydrophobic filters, and diafiltration to desalt and concentrate proteins.⁵³ For instance, desalting of egg yolk proteins dominated by phosvitin was achieved using 10 000 and 30 000 Da molecular weight cut-off polyethersulfone membranes.⁵¹

BIOACTIVE PEPTIDES DERIVED FROM EGG PROTEIN HYDROLYSATES

Although it is well known that egg is a perfect biological item to provide various healthy materials, its consumption has been declining over the past few decades, due to its high cholesterol and fat content.⁷ Fractionation of targeted egg white/yolk proteins to develop functional food products is just one way to expand the utilization of egg. In addition to using whole egg and/or individual egg proteins to produce highly valued substances, there has been a tendency to consider the development of bioactive peptides. It is now well established that enzyme hydrolysis of individual egg proteins is an effective way to produce numerous bioactive peptides with potential and to demonstrate biological activities to provide specific benefits for human health (e.g., preventing chronic and infectious diseases, lowering blood pressure, and inhibiting proliferation of tumor cells).

Enzymatic hydrolysis of proteins

Enzymatic hydrolysis of proteins is defined as a process to decompose protein sequences into peptides with various molecular weights and/or individual amino acids with the participation of enzymes (e.g., pepsin, trypsin, chymotrypsin, bromelain, papain, and ficin).⁵⁴ It is highly specific and fully controlled by several factors: enzyme concentration, pH, temperature, ionic strength in the reaction medium, the degree of protein denaturation, and the rate of mass transfer in the enzyme–protein system. The hydrolysis of proteins results in the collapse of quaternary/ternary protein structure and the reduction of molecular weight to modify the functional and sensory properties.⁵⁵ Bioactive peptides are inactive

within the sequence of parent proteins, and they can be released during enzymatic hydrolysis to activate their biological activities that positively impact body functions and human health.⁵⁴ The specific activity (e.g., antioxidant, antihypertensive, antimicrobial, and anticancer activities) of a peptide is influenced by the type and location of amino acid residues on the protein structure (Table 3).⁷⁰

Antioxidant activity

Oxidation reactions not only induce adverse effects in food products (e.g., rancidity, flavor and color changes), but also trigger cardiovascular diseases.⁷⁰ Peptides derived from egg protein hydrolysates are considered as powerful antioxidants to prevent these diseases. The antioxidant activity of peptides is greatly dependent on the degree of hydrolysis, the type of enzyme used for hydrolysis, the position of amino acid residues on the peptides, and the type of amino acid.⁷⁰ For example, aromatic amino acids (e.g., tyrosine, tryptophan, and phenylalanine) exhibit strong free-radical-scavenging ability to donate hydrogen atoms to peroxide radicals and finally produce stable quinone-type compounds.⁷¹ In addition to scavenging free radicals, antioxidative peptides also are able to chelate metal ions, reduce hydroperoxides, and inhibit lipid peroxidation.⁷² Dávalos *et al.*⁵⁶ produced antioxidative peptides by enzymatic hydrolysis of ovalbumin with pepsin, and they found the antioxidant activity of Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu could be due to the presence of trypsin at the N-terminal end to scavenge free radicals, whereas methionine on Ser-Ala-Leu-Ala-Met is a sulfur-containing amino acid that readily reacts with oxidizing agents. Xu *et al.*⁵⁸ hydrolyzed phosvitin using trypsin to study the capability of inhibiting oxidation reaction in linoleic acid. They found that histidine, methionine, and tyrosine on the phosvitin peptides were responsible for the strong antioxidant activity rather than the content of phosphorylserine ligands.

Antihypertensive activity

Currently, hypertension plays a key role among the most increased lifestyle diseases. Peptides prominently present in egg albumin exhibit strong ability to inhibit the activity of angiotensinogen-I-converting enzyme (ACE, a peptidase working on angiotensin I to result in the hydrolysis of a terminal histidyl leucine dipeptide and the formation of angiotensin II), which can cleave a dipeptide from the angiotensin I (a decapeptide)

Table 3. Examples of bioactive peptides derived from egg proteins

Biological activity	Egg proteins	Enzymatic hydrolysis conditions	Peptides	Reference
Antioxidant activity	Ovalbumin	Pepsin, pH 7.4, 37 °C, 3 h	Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, Ser-Ala-Leu-Ala-Met	56
	Egg white proteins	Alcalase, pH 10.66, 50 °C	Asp-His-Thr-Lys-Glu, Phe-Phe-Glu-Phe-His, Met-Pro-Asp-Ala-His-Leu	57
	Phosvitin	Trypsin, pH 8, 37 °C, 24 h	His-Met-Tyr	58
	Egg yolk proteins	Pepsin, pH 3.5, 37 °C, 2 h	Tyr-Ile-Asn-Gln-Met-Pro-Gln-Lys-Ser-Arg-Glu	59
Antihypertensive activity	Ovalbumin	Pepsin (pH 2.0), trypsin (pH 7.8), and chymotrypsin (pH 8.0), 37 °C, 24 h	Arg-Ala-Asp-His-Pro-Phe-Leu, Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu	60
	Ovalbumin	Thermolysin (pH 7.5), pepsin (pH 2.0), trypsin (pH 7.5), and chymotrypsin (pH 7.5), 37 °C, 2 h	Leu-Trp, Ile-Lys-Trp	61
	Ovotransferrin	Thermolysin, pH 8.0, 55 °C, 3 h; pepsin, pH 2.0, 37 °C, 3 h	Ile-Arg-Try, Leu-Lys-Pro, and Ile-Gln-Try	62
	Egg white proteins	Alcalase, pH 10.0, 50 °C, 180 min	Thr-Asn-Gly-Ile-Ile-Arg	63
Antimicrobial activity	Ovalbumin	Trypsin and chymotrypsin, pH 7.8, 37 °C, 6 h	Ser-Ala-Leu-Ala-Met, Ser-Ala-Leu-Ala-Met-Val-Tyr, Tyr-Pro-Ile-Leu-Pro-Glu-Tyr-Leu-Gln, Glu-Leu-Ile-Asn-Ser-Trp, Asn-Val-Leu-Gln-Pro-Ser-Ser, Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu-Pro-Glu-Tyr-Leu, Gly-Ile-Ile-Arg-Asn, Thr-Ser-Ser-Asn-Val-Met-Glu-Glu-Arg	64
	Lysozyme	Pepsin, pH 1.0, 37 °C, 1 h, followed by trypsin, pH 8.0, 37 °C, overnight	Ile-Val-Ser-Asp-Gly- en (Asp-Gly-Met-Asn-Ala-Trp, His-Gly-Leu-Asp-Asn-Tyr-Arg	65
	Ovalbumin	Flavourzyme, pH 7.0, 50 °C, 1 h; pepsin, pH 2.0, 37 °C, 2 h; trypsin, pH 7.5, 37 °C, 3 h; neutrase, pH 7.0, 50 °C, 4 h; papain, pH 6.5, 50 °C, 5 h	Arg-Val-Ala-Ser-Met-Ala-Ser-Glu-Lys-Met-Lys-Ile	66
	Lysozyme	Alcalase, pH 7.5, 50 °C, 3 h	Asx-Ser-Gly-Arg-Ala-Leu	67
Anticancer activity	Ovomucin	Pronase (from <i>Streptomyces griseus</i>), pH 8.4, 37 °C, 24 h	Glycopeptides with 120 000 Da and 220 000 Da	68
	Phosvitin	Trypsin, pH 8.0, 37 °C, overnight	Phosphor-oligopeptides with phosphorus (0–19 wt%)	69

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Asx, aspartic acid or asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

to further convert to angiotensin II (a blood pressure booster). Therefore, the peptides can be considered as natural antihypertensive pharmacological compounds for bradykinin stimulation to inactivate the enzyme and to lower blood pressure.⁷³ For example, ovalbumin was transformed into octapeptides under the influence of pepsin, trypsin, and chymotrypsin, in which pepsin hydrolysis produced most active hydrolysates. Nine subfractions with a molecular weight less than 3000 Da were collected to show ACE inhibitory properties, in which Arg-Ala-Asp-His-Pro-Phe-Leu and Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu showed 500 g kg⁻¹ inhibitory concentration (IC₅₀) of 6.2 × 10⁻⁶ mol L⁻¹ and 4.7 × 10⁻⁶ mol L⁻¹ respectively.⁶⁰ Majumder and Wu⁶² hydrolyzed ovotransferrin using thermolysin and/or pepsin and tried to generate potent peptides of Ile-Arg-Try, Leu-Lys-Pro, and Ile-Gln-Try. However, two pentapeptides (Ile-Arg-Try-Cys-Thr and Ile-Gln-Try-Cys-Ala) and one quatrapeptide (Leu-Lys-Pro-Ile) containing the sequence of expected peptides were identified in the thermolysin–pepsin hydrolysate. They found pepsin hydrolysis

produced ovotransferrin hydrolysates with the weakest ACE inhibitory ability (IC₅₀: 320 µg mL⁻¹), followed by the hydrolysates produced by thermolysin treatment, whereas the hydrolysates generated by two-step hydrolysis (with thermolysin and pepsin) had the best ACE-inhibitory activity (IC₅₀: 198 µg mL⁻¹).

Antimicrobial activity

Theoretically, peptides derived from egg protein hydrolysates perform antimicrobial activities, especially those containing tryptophan and arginine. Owing to its amphiphilic property, tryptophan has high affinity to cell membranes to promote peptides folding and being beneficial to transport the peptides across the cytoplasmic membrane.⁷⁴ The presence of arginine can provide positive charges to form a hydrogen bond with the anionic molecules on the cytoplasmic membrane.⁷⁵ Therefore, peptides with tryptophan and arginine can accumulate on the cytoplasmic membrane to form channels, which cause destabilization and lead to cell death.⁵⁴ Ovalbumin was proteolytically digested by trypsin

and by chymotrypsin to produce five and three antimicrobial peptides respectively. The peptides produced by trypsin hydrolysis only exhibited strong bactericidal activity against *Bacillus subtilis*, whereas the peptides generated by chymotrypsin hydrolysis demonstrated antibacterial activity against both Gram-positive (e.g., *B. subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*) and Gram-negative (e.g., *Bordetella bronchiseptica*, *Escherichia coli*, and *Klebsiella pneumonia*) bacteria, as well as fungi (e.g., *Candida albicans*).⁶⁴ Mine *et al.*⁶⁵ applied two-stage hydrolysis with pepsin and trypsin on egg white lysozyme to produce two novel antimicrobial peptides, in which the peptide with the sequence of Ile-Val-Ser-Asp-Gly-Asp-Gly-Met-Asn-Ala-Trp inhibited Gram-negative bacteria (e.g., *E. coli* K-12), whereas His-Gly-Leu-Asp-Asn-Tyr-Arg peptide possessed antimicrobial activity against Gram-positive bacteria (e.g., *S. aureus* 23-394).

Anticancer activity

Since the last decade, epidemiological studies have showed the ingestion of egg protein hydrolysates can inhibit the proliferation of tumor cells.^{76,77} For instance, ovomucin is a highly viscous glycoprotein that consists of an α -subunit (~220 000 Da) and a β -subunit (~400 000 Da). It was hydrolyzed using pronase enzymes from *Streptomyces griseus* to release highly glycosylated oligopeptides with molecular weight of 220 000 Da (from the β -subunit), 120 000 Da (from the β -subunit), and 70 000 Da (from the α -subunit) to effectively inhibit tumor growth, in which the sialic acid residues on the 120 000 Da fragment were not essential for the anticancer activity.⁶⁸ In addition, Katayama *et al.*⁶⁹ prepared phospho-oligopeptides by tryptic digestion of phosvitin and found these oligopeptides increased enzyme activity for glutathione biosynthesis, which plays a key role in inducing cell death.

ALLERGENICITY FROM EGG PROTEINS

In addition to segregating the valuable individual egg proteins and generating bioactive peptides (as nutraceutical and pharmaceutical agents) by enzymatic hydrolysis, reducing allergenicity of egg proteins is another effective way to stimulate the consumption of eggs, which is discouraged by medical communities because of potential risks of allergy. Allergenicity induced by egg white proteins (e.g., ovomucoid, ovalbumin, lysozyme, and ovotransferrin) and egg yolk proteins (e.g., α -livetin and lipoprotein YGP42) cannot be neglected because of serious and immediate allergic reactions. In general, thermal processing, enzymatic hydrolysis, and high-pressure treatment are the most commonly studied techniques to induce conformational changes and to further decrease the allergenicity of individual egg proteins.

Egg allergy

Hen's egg is one of 'the big eight' food sources (e.g., cow's milk, fish, peanuts, tree nuts, crustaceans, wheat, and soybeans) to induce food allergy over the world, especially for infants and children.⁷⁸ Food allergy is defined as the adverse health effects that are induced by the exposure of dietary allergens from foods resulting in immunological reactions on the sensitized subjects. The most common immunological reaction to implicate food allergy is the elaboration of IgE antibodies.⁷⁹ Therefore, egg allergy can be considered as an IgE antibody-mediated allergy produced by adverse immunological reaction of egg proteins resulting in atopic dermatitis and eosinophilic esophagitis.⁸⁰ The allergenicity of egg

proteins mostly depends on their resistance to heat and enzymatic digestion, which reflect on the ability to stimulate an immune response.⁸¹ Egg white proteins have a higher allergenic potential than egg yolk proteins. There are four major allergens from egg white: ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4); and there are two minor allergens from egg yolk: α -livetin (Gal d 5) and lipoprotein YGP42 (Gal d 6).⁸²

Ovomucoid is the most crucial and major allergen in egg white proteins. It consists of three structurally independent domains (I, II, and III), and each is internally cross-linked by disulfide bonds, which contribute to preventing heat denaturation and proteolytic digestion.⁸³ Ovomucoid also belongs to the serine protease inhibitor Kazal family, which against several proteolytic digestions by trypsin and elastase.⁸⁴ In comparison with domains I and II, domain III is the most allergenic reactive, with both IgE and IgG-binding epitopes, so it is considered as the major determinant for egg allergy.⁸³ Because of the presence of both conformational and linear epitopes, ovomucoid still displays allergenic reactivity when heat treatment (e.g., 100 °C for 30 min) or chemical treatment using denaturing detergents (e.g., urea) is applied to irreversibly disrupt protein conformation.^{85,86} Ovalbumin is a weaker allergen than ovomucoid. The disulfide bonds link six cysteines on the ovalbumin sequence between Cys74 and Cys121.⁸² It is a member of the clade B serpins family that is a subgroup of serine protease inhibitor family. The protein in this family typically has nine α -helices, three strands of β -sheets, and an exposed and mobile reactive center loop that is responsible for protease inhibitory activity.⁸⁷ Ovotransferrin is a minor egg white allergen and is comprised of 686 amino acids with 15 disulfide bonds. It belongs to the transferrin protein family, which has ferric-ion binding ability. The family includes two groups: soluble glycoproteins and membrane melanotransferrins, in which ovotransferrin is a soluble glycoprotein with a high degree of homology to inhibit iron metabolism.⁸⁸ Lysozyme is a weak egg white allergen with lower IgE reactivity. Four disulfide bonds make the protein structure highly compacted into five to seven α -helices and three-stranded anti-parallel β -sheets, so lysozyme presents good stability to heat denaturation.⁸⁹ It was demonstrated that lysozyme can tolerate heat treatment (at 100 °C) for up to 10 min at neutral pH.⁹⁰

α -Livetin is the first allergen identified in egg yolk proteins with 17 disulfide bridges, in which one free —SH is responsible for dimerization. The exposure of α -livetin results in both egg allergy symptoms and bird-egg syndrome that is induced by sensitization to α -livetin to cause respiratory difficulties.^{82,91} Lipoprotein YGP42 is the second egg yolk allergen, and only 18% of patients have IgE reactivity to YGP42 protein.⁹² Although it is a heat-stable allergen, it is digestible by pepsin in the gastrointestinal tract, so it only acts as a minor allergen.⁸²

Effect of processing on the allergenicity

Food processing in various ways is widely applied to improve physicochemical, functional, and nutritional properties of proteins. It may also alter the allergenic potential by impacting IgG-binding (antigenic) and IgE-binding (allergenic) properties of proteins. There are three factors to determine the allergenicity of proteins: heat stability, resistance to digestive enzymes, and IgE-binding epitopes on the protein molecules.⁹³ Theoretically, processing can potentially affect allergenic properties from two aspects: (i) processing can break the integrity of epitopes on the protein structure that are recognized by IgG and IgE antibodies to elicit allergic reactions, and this is most commonly investigated; (ii) processing

can reduce the ability of proteins to induce allergic sensitization, which is defined as the specific immunological priming through the intake of proteins.⁹⁴ Most commonly studied processing techniques on egg proteins include thermal processing, enzymatic hydrolysis, and high-pressure treatment (Table 4).

Generally, thermal processing induces secondary and tertiary structure changes on proteins to directly influence the allergenicity by destroying, masking, or exposing conformational epitopes.¹⁰⁸ Additionally, owing to reactions between allergenic proteins and other components (e.g., sugar, oxidized lipids, and polyphenols), allergenicity of protein can be chemically modified. For example, IgE binding ability of ovomucoid was even increased when purified ovomucoid was heated at 50 °C for 96 h with the presence of glucose, because a Maillard reaction between ovomucoid and glucose occurred to modify lysine and arginine residues through glycation, which might alter the affinity/accessibility of allergens for IgE antibodies; however, the digestibility of ovomucoid was not affected.⁹⁵ Watanabe *et al.*⁹⁶ used a murine model to assess ovalbumin-induced allergy, in which transgenic mice expressing T-cell receptors specific to ovalbumin were used for IgE production. Ovalbumin was treated in three different ways: 80 °C for 15 min, 100 °C for 5 min, and 121 °C for 40 min under 215 750 Pa pressure. They found the mice fed with ovalbumin heated at 100 °C for 5 min showed reduced T-cell responses and an IgE level that indicated the allergenicity of ovalbumin was decreased. Therefore, thermal processing can either decrease allergenicity of egg proteins or raise the formation of new allergenic compounds by a Maillard reaction between proteins and reducing sugar.

In comparison with thermal processing, enzymatic hydrolysis is a more effective way to modify the allergenicity of proteins, because both conformational and linear/sequential epitopes can respectively be collapsed and cleaved during hydrolysis, and digestibility can be easily improved.¹⁰⁹ In the study by López-Expósito *et al.*¹⁰¹, ovalbumin was hydrolyzed with pepsin under high-pressure (0.4×10^9 Pa) conditions in order to break the protein sequences and remove allergenic epitopes. They found that although proteolysis did not completely abolish the IgE reactivity, and some hydrolysates (e.g., Leu₁₂₄-Phe₁₃₄, Ile₁₇₈-Ala₁₈₇, and Leu₂₄₂-Leu₂₅₂) still carried IgE-binding epitopes, some hydrolyzed peptides (e.g., Phe₃₅₈-Phe₃₆₆) only contained one IgE-binding site, which improved the immune system's tolerance to ovalbumin. Moreover, Matsumoto *et al.*¹⁰² investigated enzymatic hydrolysis of ovalbumin using various proteases and found that alcalase (alkaline protease) from *Bacillus licheniformis* was most effective to completely degrade ovalbumin, by which the allergenic activity of the hydrolysates was eliminated almost completely. However, Jiménez-Saiz *et al.*¹⁰³ studied the immunological behavior of digested lysozyme under simulated gastric and gastroduodenal fluids with IgE-binding ability and basophil degranulation tests. They found the lysozyme hydrolysates still maintained IgE-binding and basophil activation capacities, because of incomplete gastric degradation on lysozyme and subsequent duodenal precipitation. The enzymatic digestion even promoted the release of immunoreactive peptides with IgE-binding epitopes of fragment 57–83 and fragment 108–122 that are linked by disulfide bonds. In the work done by Akita *et al.*,¹⁰⁵ the allergenicity of egg yolk IgY was studied using passive cutaneous anaphylaxis (PCA) test and enzyme-linked immunosorbent assay. It was observed that the hydrolysate fragments produced by pepsin digestion exhibited lower IgE antibody response, which was associated with higher suppressor T-lymphocytes or lower helper T-lymphocytes, or both.

Porta *et al.*⁹⁹ applied transglutaminase to modify the structure of ovomucoid to decrease its trypsin inhibitory activity. It was reported that Arg89-Ala90 dipeptide is a major trypsin-binding site on the ovomucoid residue 65–130 that is responsible for trypsin inhibitory activity,¹¹⁰ but transglutaminase-modified ovomucoid exposed Gln115 to be presented as an acyl donor for transglutaminase to form a covalent monodansylcadaverine conjugate, which decreased the IgE-binding opportunities and trypsin inhibitory activity of ovomucoid to lower the allergenicity. In the study by Abeyrathne *et al.*,¹¹¹ ovomucoid was hydrolyzed using various enzyme combinations: (i) pepsin, (ii) alcalase, (iii) alcalase + trypsin, and (iv) alcalase + papain. They found ovomucoid was completely broken down in to amino acid monomers and di and tripeptides under pepsin treatment to have the lowest possibility for the allergenicity and highest ACE inhibitory activity, whereas alcalase + trypsin-hydrolyzed ovomucoid had the highest antioxidant activity. Ovomucoid was also hydrolyzed using pepsin-coupled Sepharose 4B into three major fractions with molecular weights of 25 000, 18 000 and 13 000 Da. A PCA test showed that no positive PCA reaction happened against any fractions, which indicated that the antibody responses of hydrolyzed ovomucoid were remarkably weaker than the intact ovomucoid.¹⁰⁰ In addition, Mine *et al.*⁹⁸ also tried to reduce the allergenicity of egg white proteins, especially on ovomucoid domain III, by enzymatic hydrolysis with protease and glutathione-Sepharose 4B. Originally, two IgE binding sites (e.g., Lys29-Ser44 and Thr49-Cys56) were identified on ovomucoid domain III, whereas the enzymatic hydrolysis disrupted the α -helix structure of ovomucoid at the location of glycine 32 and phenylalanine 37, so the IgE epitopes were broken to decrease the allergenicity.

High-pressure treatment is considered as an effective processing technique to unfold the protein structure and change the position of epitopes on the protein sequence to decrease the allergenicity. Under the high pressure, the formation of a bridge between two IgE antibodies on the protein is rejected, and the production of basophils activation mediators is also suppressed.^{112,113} Ovomucoid was treated under high pressure (0.1×10^6 – 0.6×10^9 Pa) to measure the β -hexosaminidase released from KU812F human pre-basophilic cells to further determine the allergenicity of ovomucoid. It was found that the release of β -hexosaminidase was mostly inhibited when ovomucoid was treated under 0.4×10^9 – 0.5×10^9 Pa pressure. The unfolding of the tertiary structure of ovomucoid to expose hydrophobic cores at the surface of the molecule under high pressure also contributed to a reduction in allergenicity.¹⁰⁶ Moreover, Acero-Lopez *et al.*¹⁰⁷ investigated the effect of high-pressure treatment on ovotransferrin. They found that the number of sulfhydryl groups decreased when ovotransferrin was treated with pressure higher than 0.2×10^9 Pa at pH 8 that potentially predicts the reduction of allergenic activity from the protein, followed by the complete protein denaturation at pressures of 0.6×10^9 and 0.7×10^9 Pa with conformational structure changes from the α -helices, β -sheets, and β -turns. Overall, the modification of allergenicity and the degree of epitope changes really depend on the processing method used, exposure time, and the presence of other ingredients (e.g., sugar). Processing not only destroys epitopes on the protein sequence to decrease IgE-binding ability, but it is also able to create new epitopes to increase IgE-binding potential. Therefore, it is important to evaluate the processing effect on the inherent allergenicity of egg proteins.

Table 4. Effect of processing on the allergenicity of egg proteins

Processing	Egg proteins	Processing conditions	Detection method	Allergenic changes	Reference
Thermal processing	Ovomucoid	50 °C 96 h	Inhibition ELISA	Increasing IgE-binding ability	95
	Ovalbumin	80 °C for 15 min, 100 °C for 5 min, and 121 °C for 40 min under 215 750 Pa pressure	Heterozygous mice model with ELISA test in sera	Reducing T-cell responses and IgE levels	96
Enzymatic hydrolysis	Egg white proteins	Baking conditions	Clinical trials, including skin prick test and oral food challenge, and oral immunotherapy	Ovomucoid and ovalbumin-specific IgE decreased, and egg protein tolerance increased	97
	Ovomucoid	Protease and glutathione – Sepharose 4B, room temperature, 6 h	Western immunoblot	Disruption of α -helix structure, and break the structural integrity of ovomucoid domain III to decrease the allergenicity	98
		Transglutaminase, pH 7.5, 37 °C, 24 h; trypsin, pH 8.1, 37 °C, 10 min	ELISA	Modification of ovomucoid to decrease IgE-binding opportunities and trypsin inhibitory activity	99
		Pepsin coupled with Sepharose 4B, pH 9.0, 37 °C, 3 h	PCA	Break ovomucoid into three fractions with molecular weights of 25 000, 18 000, and 13 000 Da to decrease antibody responses	100
	Ovalbumin	Pepsin, pH 2.5, 37 °C, 10, 60, and 120 min	ELISA	Loss of conformational and linear/sequential epitope, and reduction of IgE-binding site	101
High-pressure treatment	Lysozyme	Alcalase, pH 8.3, 37 °C, 15 h	ELISA	Almost completely destroy ovalbumin to eliminate antigenicity of the hydrolysate	102
		Gastric digestion: pepsin, pH 1.5, 2.0, and 3.2, 37 °C, 15, 30, and 60 min	Inhibition ELISA (to test IgE-binding ability), MALDI-TOF/TOF (to test immunoreactive products), and basophil activation test	Released immunoreactive peptides with IgE-binding epitopes	103
	Egg white proteins	Duodenal digestion: lipase, colipase, trypsin, and α -chymotrypsin, pH 6.5, 37 °C, 30 min, 0.25 mol L ⁻¹ bile salt	Immunoblotting test, EAST inhibition	Loss of tertiary structure, increase the digestibility, and reduction of the IgE-binding potential	104
	IgY	Protamex and Flavourzyme (from Novozyme), 55 °C, 2 h	PCA and ELISA	IgE antibodies were not detectable in IgY hydrolysates, which had relatively lower antigenicity than the whole IgY molecule	105
High-pressure treatment	Ovomucoid	0.1 × 10 ⁶ –0.6 × 10 ⁹ Pa, 10 min, 10 °C	Cell mediator release assay (to measure β -hexosaminidase released from KU812F human pre-basophilic cells that sensitized with sera from egg allergic patients)	Reducing β -hexosaminidase release up to 89.7%, and unfolding of tertiary structure of ovomucoid to decrease the allergenicity	106
	Ovotransferrin	0.2 × 10 ⁹ , 0.4 × 10 ⁹ , 0.5 × 10 ⁹ , 0.6 × 10 ⁹ , and 0.7 × 10 ⁹ Pa at pH 3.0 and 8.0	Sulphydryl group determination, in-gel digestion	A decrease in total sulphydryl groups, improving digestibility	107
	Ovalbumin	0.4 × 10 ⁹ Pa	ELISA	Enhancing the digestibility and reducing allergenicity by removing epitopes	101

CONCLUSION

In conclusion, egg proteins are distributed into egg white (including ovalbumin, ovotransferrin, ovomucin, ovomucoid, and lysozyme) and egg yolk (including lipovitellins, livetins, phosvitin, and LDLs). Fractionation of individual egg proteins with high purity and protein recovery using various techniques (e.g., liquid chromatography, chemical precipitation, ultrafiltration, and electrophoresis) has been maturely investigated, but future research regarding the transformation of those laboratory techniques onto an industrial scale would be essential to efficiently expand the utilization of egg proteins. In addition, bioactive peptides with various bioactive properties (e.g., antioxidant, antihypertensive, antimicrobial, and anticancer activities) produced by enzymatic hydrolysis of egg proteins have aroused more attention for their potential pharmaceutical and nutraceutical applications. However, continuous research to legally prove therapeutic functions of existing peptides and explore more novel peptides would be meaningful to transfer them into real applications to satisfy nutritional requirements of human beings. The consumption of eggs is greatly limited by the protein (e.g., ovomucoid, ovalbumin, ovotransferrin, lysozyme, α -livetins, and lipoprotein YGP42) induced allergenicity, which could be diminished or solved by thermal processing, enzymatic hydrolysis, and high-pressure treatment through destroying allergenic epitopes and reducing IgE-binding ability. But appropriate processing conditions to avoid the generation of new allergenic epitopes would need to be assessed in the future.

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REFERENCES

- 1 Froning GW, Egg products industry and future perspectives, in *Egg Bioscience and Biotechnology*, ed. by Mine Y. John Wiley & Sons Inc., Hoboken, NJ, pp. 307–325 (2008).
- 2 Kovacs-Nolan JKN, Phillips NM and Mine Y, Advances in the value of eggs and egg components for human health. *J Agric Food Chem* **53**:8421–8431 (2005).
- 3 Anton M, Egg yolk: structures, functionalities and processes. *J Sci Food Agric* **93**:2871–2880 (2013).
- 4 Stadelman WJ and Cotterill OJ, *Egg Science and Technology*, 4th edn. Avi Publishing Company, Westport, CT (2001).
- 5 Powrie WD and Nakai S, Characterization of edible fluids of animal origin: eggs, in *Food Chemistry*, ed. by Fennema OR. Marcel Dekker, New York, NY, pp. 829–850 (1985).
- 6 Froning GW, New product innovations from eggs, in *New and Developing Sources of Food Proteins*, ed. by Hudson BJF. Chapman & Hall, London, pp. 71–94 (1994).
- 7 Abeyrathne EDNS, Lee HY and Ahn DU, Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents – a review. *Poult Sci* **92**:3292–3299 (2013).
- 8 Huntington JA and Stein PE, Structure and properties of ovalbumin. *J Chromatogr B* **756**:189–198 (2001).
- 9 Ko KY and Ahn DU, An economic and simple purification procedure for the large scale production of ovotransferrin from egg white. *Poult Sci* **87**:1441–1450 (2008).
- 10 Wan Y, Lu J and Cui Z, Separation of lysozyme from chicken egg white using ultrafiltration. *Sep Purif Technol* **48**:133–142 (2006).
- 11 Huopalahti R, Fandino RL, Anton M and Schade R, *Bioactive Egg Compounds*. Springer, New York, NY (2007).
- 12 Omana DA, Wang J and Wu J, Co-extraction of egg white proteins using ion-exchange chromatography from ovomucin-removed egg white. *J Chromatogr B Anal Technol Biomed Life Sci* **878**:1771–1776 (2010).
- 13 Hiidenhovi J, Aro HS and Kankare V, Separation of ovomucin subunits by gel filtration: enhanced resolution of subunits by using a dual-column system. *J Agric Food Chem* **47**:1004–1008 (1999).
- 14 Mine Y, *Egg Bioscience and Biotechnology*. John Wiley & Sons Inc., Hoboken, NJ (2008).
- 15 Robinson DS and Monsey JB, The composition and proposed subunit structure of egg-white β -ovomucin. *Biochem J* **147**:55–62 (1975).
- 16 Kovacs-Nolan JKN, Zhang JW, Hayakawa S and Mine Y, Immunochromatographic and structural analysis of pepsin-digested egg white ovomucoid. *J Agric Food Chem* **48**:6261–6266 (2000).
- 17 Oliveira FC, Coimbra JSR, Silva LHM, Rojas EEG and Silva MCH, Ovomucoid partitioning in aqueous two-phase system. *Biochem Eng J* **47**:55–60 (2009).
- 18 Vachier MC, Piot M and Awadé AC, Isolation of hen egg white lysozyme, ovotransferrin and ovalbumin using a quaternary ammonium bound to a highly cross-linked agarose matrix. *J Chromatogr B Biomed Sci Appl* **664**:201–210 (1995).
- 19 Datta D, Bhattacharjee S, Nath A, Das R, Bhattacharjee C and Datta S, Separation of ovalbumin from chicken egg white using two-stage ultrafiltration technique. *Sep Purif Technol* **66**:353–361 (2009).
- 20 Al-Mashikhi SA and Nakai N, Separation of ovotransferrin from egg white by immobilized metal affinity chromatography. *Agric Biol Chem* **51**:2881–2887 (1987).
- 21 Chang HM, Yang CC and Chang YC, Rapid separation of lysozyme from chicken egg white by reductants and thermal treatment. *J Agric Food Chem* **48**:161–164 (2000).
- 22 Awadé AC, Moreau S, Mollé D, Brulé G and Maubois JL, Two-step chromatographic procedure for the purification of hen egg white ovomucin, lysozyme, ovotransferrin and ovalbumin and characterization of purified proteins. *J Chromatogr A* **677**:279–288 (1994).
- 23 Omana DA and Wu J, Effect of different concentrations of calcium chloride and potassium chloride on egg white proteins during isoelectric precipitation of ovomucin. *Poult Sci* **88**:2224–2234 (2009).
- 24 Desert C, Guerin-Dubiard C, Nau F, Jan G, Val F and Mallard J, Comparison of different electrophoretic separations of hen egg white proteins. *J Agric Food Chem* **49**:4553–4561 (2001).
- 25 Tanabe S, Tesaki S and Watanabe M, Producing a low ovomucoid egg white preparation by precipitation with aqueous ethanol. *Biosci Biotechnol Biochem* **64**:2005–2007 (2000).
- 26 Yousif AN and Kan JW, Visualization of chicken ovomucoid in polyacrylamide gels. *Anal Biochem* **311**:93–97 (2002).
- 27 Abeyrathne EDNS, Lee HY and Ahn DU, Separation of ovotransferrin and ovomucoid from chicken egg white. *Poult Sci* **93**:1010–1017 (2014).
- 28 Awadé AC, On hen egg fractionation: applications of liquid chromatography to the isolation and the purification of hen egg white and egg yolk proteins. *Z Lebensm Unters Forsch* **202**:1–14 (1996).
- 29 Meulenaer BD and Huyghebaert A, Isolation and purification of chicken egg yolk immunoglobulins: a review. *Food Agric Immunol* **13**:275–288 (2001).
- 30 McBee LE and Cotterill OJ, Ion-exchange chromatography and electrophoresis of egg yolk proteins. *J Food Sci* **44**:656–660 (1979).
- 31 Starixner T and Kulozik U, Continuous centrifugal fractionation of egg yolk granules and plasma constituents influenced by process conditions and product characteristics. *J Food Eng* **117**:89–98 (2013).
- 32 Luo Y, Xiong R, Hou L, Li J and Chen J, Process for coproduction of crude immunoglobulin Y and high-density lipoproteins from hen egg yolk. *J Agric Food Chem* **58**:11420–11427 (2010).
- 33 Bernardi G and Cook WH, An electrophoretic and ultracentrifugal study on the proteins of the high density fraction of egg yolk. *Biochim Biophys Acta* **44**:86–96 (1960).
- 34 Ren J and Wu J, Preparation of high purity egg phosvitin using anion exchange chromatography. *Food Chem* **158**:186–191 (2014).
- 35 Ko KY, Nam KC, Jo C, Lee EJ and Ahn DU, A simple and efficient method for preparing partially purified phosvitin from egg yolk using ethanol and salts. *Poult Sci* **90**:1096–1104 (2011).
- 36 Mecham DK and Olcott HS, Phosvitin, the principal phosphoprotein of egg yolk. *J Am Chem Soc* **71**:3670–3679 (1949).
- 37 Moussa M, Martinet V, Trimeche A, Tainturier D and Anton M, Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen–thawed bull semen. *Theriogenology* **57**:1695–1706 (2002).
- 38 Burley RW and Vadehra DV, Chromatographic separation of the soluble proteins of hen's egg yolk: an analytical and preparation study. *Anal Biochem* **94**:53–59 (1979).

- 39 Akita EM and Nakai S, Immunoglobulins from egg yolk: isolation and purification. *J Food Sci* **57**:629–634 (1992).
- 40 Hatta H, Kim M and Yamamoto R, A novel isolation method for hen egg yolk antibody, "IgY". *Agric Biol Chem* **54**:2531–2535 (1990).
- 41 Sugano H, Studies on egg yolk proteins. III. Preparation and properties of two major lipoproteins of the egg yolk, alpha-lipovitellin and beta-lipovitellin. *J Biochem* **45**:393–401 (1958).
- 42 Byrne BM, Schip ADV, Vandeklundert JAM, Arnberg AC and Gruber M, Amino-acid-sequence of phosvitin derived from the nucleotide-sequence of part of the chicken vitellogenin gene. *Biochemistry* **23**:4275–4279 (1984).
- 43 Anton M, Castellani O and Guerin-Dubiard C, Phosvitin, in *Bioactive Egg Compounds*, ed. by Huopalahti R, López-Fandiño R, Anton M and Schade R. Springer, Berlin, pp. 17–24 (2007).
- 44 Cook WH and Martin WG, Egg lipoproteins, in *Structural and Functional Aspects of Lipoproteins in Living Systems*, ed. by Tria E and Scanu AM. Academic Press, New York, NY, pp. 579–615 (1969).
- 45 Powrie WD and Nakai S, The chemistry of eggs and egg products, in *Egg Science and Technology*, ed. by Stadelman WJ and Cotterill OJ. Avi Publishing Company, Westport, CT, pp. 97–139 (1986).
- 46 Laca A, Paredes B, Rendueles M and Diaz M, Egg yolk plasma: separation, characteristics and future prospects. *LWT – Food Sci Technol* **62**:7–10 (2015).
- 47 Sheumack DD and Burley RW, Separation of lipid-free egg yolk proteins by high-pressure liquid chromatography using solvents containing formic acid. *Anal Biochem* **174**:548–551 (1988).
- 48 Juneja LR, Sugino H, Fujiki M, Kim M and Yamamoto T, Preparation of pure phospholipids from egg yolk, in *Egg Uses Process Technology Development*. CAB International, Wallingford, pp. 139–149 (1994).
- 49 Ahn DU, Lee SH, Singam H, Lee EJ and Kin JC, Sequential separation of main components from chicken egg yolk. *Food Sci Biotechnol* **15**:189–195 (2006).
- 50 Tsutsui T and Obara T, Preparation and characterization of phosvitin from hens egg yolk granule. *Agric Biol Chem* **48**:1153–1160 (1984).
- 51 Chay Pak Ting BP, Mine Y, Juneja LR, Okubo T, Gauthier SF and Pouliot Y, Comparative composition and antioxidant activity of peptide fractions obtained by ultrafiltration of egg yolk protein enzymatic hydrolysates. *Membranes (Basel)* **1**:149–161 (2011).
- 52 Castellani O, Martinet V, David-Briand E, Guerin-Dubiard C and Anton M, Egg yolk phosvitin: preparation of metal-free purified protein by fast protein liquid chromatography using aqueous solvents. *J Chromatogr B Anal Technol Biomed Life Sci* **791**:273–284 (2003).
- 53 Kim H and Nakai S, Immunoglobulin separation from egg yolk: a serial filtration system. *J Food Sci* **61**:510–513 (1996).
- 54 Eckert E, Zambrowicz A, Pokora M, Polanowski A, Chrzanowska J, Szoltysik M *et al.*, Biologically active peptides derived from egg proteins. *World's Poult Sci J* **69**:375–386 (2013).
- 55 Vinnars E and Wilmore D, History of parenteral nutrition. *J Parenter Enteral Nutr* **27**:225–232 (2003).
- 56 Dávalos A, Miguel M, Bartolomé B and López-Fandiño R, Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. *J Food Prot* **67**:1939–1944 (2004).
- 57 Liu JB, Jin Y, Lin SY, Jones GS and Chen F, Purification and identification of novel antioxidant peptides from egg white protein and their antioxidant activities. *Food Chem* **175**:258–266 (2015).
- 58 Xu X, Katayama S and Mine Y, Antioxidant activity of tryptic digest of hen egg yolk phosvitin. *J Sci Food Agric* **87**:2604–2608 (2007).
- 59 Zambrowicz A, Pokora M, Setner B, Dąbrowska A, Szoltysik M, Babik K *et al.*, Multifunctional peptides derived from an egg yolk protein hydrolysate: isolation and characterization. *Amino Acids* **47**:369–380 (2015).
- 60 Miguel M, Recio I, Gómez-Ruiz JA, Ramos M and López-Fandiño R, Angiotensin I-converting enzyme inhibitory activity of peptides derived from egg white proteins by enzymatic hydrolysis. *J Food Prot* **67**:1914–1920 (2004).
- 61 Fujita H, Yokoyama K and Yoshikawa M, Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *Food Chem Toxicol* **65**:564–569 (2000).
- 62 Majumder K and Wu J, A new approach for identification of novel antihypertensive peptides from egg proteins by QSAR and bioinformatics. *Food Res Int* **43**:1371–1378 (2010).
- 63 Yu ZP, Liu BQ, Zhao WZ, Yin YG, Liu JB and Chen F, Primary and secondary structure of novel ACE-inhibitory peptides from egg white protein. *Food Chem* **133**:315–322 (2012).
- 64 Pellegrini A, Hulsmeier AJ, Hunziker P and Thomas U, Proteolytic fragments of ovalbumin display antimicrobial activity. *Biochim Biophys Acta Gen Subj* **1672**:76–85 (2004).
- 65 Mine Y, Ma F and Lauriau S, Antimicrobial peptides released by enzymatic hydrolysis of hen egg white lysozyme. *J Agric Food Chem* **52**:1088–1094 (2004).
- 66 Tang W, Zhang H, Wang L and Qian H, Antimicrobial peptide isolated from ovalbumin hydrolysate by immobilized liposome-binding extraction. *Eur Food Res Technol* **237**:591–600 (2013).
- 67 You SJ, Udenigwe CC, Aluko RE and Wu J, Multifunctional peptides from egg white lysozyme. *Food Res Int* **43**:848–855 (2010).
- 68 Watanabe K, Tsuge Y, Shimoyamada M, Ogama N and Ebina T, Antitumor effects of pronase-treated fragments, glycopeptides, from ovomucin in hen egg white in a double grafted tumor system. *J Agric Food Chem* **46**:3033–3038 (1998).
- 69 Katayama S, Xu X, Fan MZ and Mine Y, Antioxidative stress activity of oligophosphopeptides derived from hen egg yolk phosvitin in Caco-2 cells. *J Agric Food Chem* **54**:773–778 (2006).
- 70 Park PJ, Jung WK, Nam KS, Shahidi F and Kim SK, Purification and characterization of antioxidative peptides from protein hydrolysate of lectin-free egg yolk. *J Am Oil Chem Soc* **78**:651–656 (2001).
- 71 Kawashima K, Itoh H, Miyoshi M and Chibata I, Antioxidant properties of branched-chain amino acid derivatives. *Chem Pharm Bull* **27**:1912–1916 (1979).
- 72 Elias RJ, Kellerby SS and Decker EA, Antioxidant activity of proteins and peptides. *Crit Rev Food Sci Nutr* **48**:430–441 (2008).
- 73 Miguel M and Aleixandre A, Antihypertensive peptides derived from egg proteins. *J Nutr* **136**:1457–1460 (2006).
- 74 Chan DI, Prenner EJ and Vogel HJ, Tryptophan and arginine-rich antimicrobial peptides: structures and mechanisms of action. *Biochim Biophys Acta Biomembr* **1758**:1184–1202 (2006).
- 75 Powers JPS and Hancock REW, The relationship between peptide structure and antibacterial activity. *Peptides* **24**:1681–1691 (2003).
- 76 Azuma N, Suda H, Iwasaki H, Yamagata N, Saeki T and Kanamoto R, Antitumorigenic effects of several food proteins in a rat model with colon cancer and their reverse correlation with plasma bile acid concentration. *J Nutr Sci Vitaminol* **46**:91–96 (2000).
- 77 Belobrajdic DP, McIntosh GH and Owens JA, Whey proteins protect more than red meat against azoxymethane induced ACF in Wistar rats. *Cancer Lett* **198**:43–51 (2003).
- 78 Török K, Horváth V, Horváth A, Hajas L, Bugyi Z and Tömösközi S, Investigation of incurred single and multi-component model food matrices for determination of food proteins triggering allergy and coeliac disease. *Eur Food Res Technol* **239**:923–932 (2014).
- 79 Sampson HA, Update on food allergy. *J Allergy Clin Immunol* **113**:805–819 (2004).
- 80 Hill DJ, Hosking CS, de Benedictis FM, Oranje AP, Diepgen TL and Bauchau V, Confirmation of the association between high levels of immunoglobulin E food sensitization and eczema in infancy: an international study. *Clin Exp Allergy* **38**:161–168 (2008).
- 81 Astwook JD, Leach JN and Fuchs RL, Stability of food allergens to digestion *in vitro*. *Nature Biotechnol* **14**:1269–1273 (1996).
- 82 Watanabe H, Takushi S, Nakajima-Adachi H, Hachimura S, Vieths S and Toda M, Physiological, biochemical, and allergenic properties of egg allergens, in *Handbook of Eggs in Human Function*, ed. by Watson RR and DeMeester F. Wageningen Academic Publishers, Wageningen, pp. 549–564 (2015).
- 83 Maeno A, Matsuo H and Akasaka K, Tyrosine/tyrosinate fluorescence at 700 MPa: a pressure unfolding study of chicken ovomucoid at pH 12. *Biophys Chem* **183**:57–63 (2013).
- 84 Shah RB and Khan MA, Protection of salmon calcitonin breakdown with serine proteases by various ovomucoid species for oral drug delivery. *J Pharm Sci* **93**:392–406 (2004).
- 85 Hirose J, Kitabatake N, Kimura A and Narita H, Recognition of native and/or thermally induced denatured forms of the major food allergen, ovomucoid, by human IgE and mouse monoclonal IgG antibodies. *Biosci Biotechnol Biochem* **68**:2490–2497 (2004).
- 86 Mine Y and Zhang JW, Comparative studies on antigenicity and allergenicity of native and denatured egg white protein. *J Agric Food Chem* **50**:2679–2683 (2002).
- 87 Stein PE, Leslie AG, Finch JT and Carrell RW, Crystal structure of uncleaved ovalbumin at 1.95 Å resolution. *J Mol Biol* **221**:941–959 (1991).
- 88 Giansanti F, Leboffe L, Pitari G, Ippoliti R and Antonini G, Physiological roles of ovotransferrin. *Biochim Biophys Acta Gen Subj* **1820**:218–225 (2012).

- 89 Diamond R, Real-space refinement of the structure of hen egg-white lysozyme. *J Mol Biol* **82**:371–391 (1974).
- 90 Tomizawa H, Yamada H and Imoto T, The mechanism of irreversible inactivation of lysozyme at pH 4 and 100 °C. *Biochemistry* **33**:13032–13037 (1994).
- 91 Quirce S, Marañón F, Umpiérrez A, De las Heras M, Fernández-Caldas E and Sastre J, Chicken serum albumin (Gal d 5*) is a partially heat-labile inhalant and food allergen implicated in the bird-egg syndrome. *Allergy* **56**:754–762 (2001).
- 92 Jolivet P, Boulard C, Chardot T and Anton M, New insights into the structure of apolipoprotein B from low-density lipoproteins and identification of a novel YGP-like protein in hen egg yolk. *J Agric Food Chem* **56**:5871–5879 (2008).
- 93 Yoshino K, Sakai K, Mizuha Y, Shimizuike A and Yamamoto S, Peptic digestibility of raw and heat-coagulated hen's egg white proteins at acidic pH range. *Int J Food Sci Nutr* **55**:635–640 (2004).
- 94 Verhoeckx KCM, Vissers YM, Baumert JL, Faludi R, Feys M, Flanagan S et al., Food processing and allergenicity. *Food Chem Toxicol* **80**:223–240 (2015).
- 95 Jiménez-Saiz R, Belloque J, Molina E and López-Fandiño R, Human immunoglobulin E (IgE) binding to heated and glycated ovalbumin and ovomucoid before and after *in vitro* digestion. *J Agric Food Chem* **59**:10044–10051 (2011).
- 96 Watanabe H, Toda M, Sekido H, Wellner A, Fujii T, Henle T et al., Heat treatment of egg white controls allergic symptoms and induces oral tolerance to ovalbumin in a murine model of food allergy. *Mol Nutr Food Res* **58**:394–404 (2014).
- 97 Leonard SA, Sampson HA, Sicherer SH, Noone S, Moshier EL, Godbold J et al., Dietary baked egg accelerates resolution of egg allergy in children. *J Allergy Clin Immunol* **130**:473–480 (2012).
- 98 Mine Y, Sasaki E and Zhang JW, Reduction of antigenicity and allergenicity of genetically modified egg white allergen, ovomucoid third domain. *Biochem Biophys Res Commun* **302**:133–137 (2003).
- 99 Porta R, Giosafatto CVL, di Pierro P, Sorrentino A and Mariniello L, Transglutaminase-mediated modification of ovomucoid: effects on its trypsin inhibitory activity and antigenic properties. *Amino Acids* **44**:285–292 (2013).
- 100 Matsuda T, Tsuruta K, Nakabe Y and Nakamura R, Reduction of ovomucoid immunogenic activity on peptic fragmentation and heat denaturation. *Agric Biol Chem* **49**:2237–2241 (1985).
- 101 López-Expósito I, Chicón R, Belloque J, Recio I, Alonso E and López-Fandiño R, Changes in the ovalbumin proteolysis profile by high pressure and its effect on IgG and IgE binding. *J Agric Food Chem* **56**:11809–11816 (2008).
- 102 Matsumoto K, Yoshimaru T, Matsui T and Osajima Y, Enzymatic degradation of ovalbumin by various proteases. *J Fac Agric Kyushu Univ* **41**:239–245 (1997).
- 103 Jiménez-Saiz R, Benedé S, Miralles B, López-Expósito I, Molina E and López-Fandiño R, Immunological behavior of *in vitro* digested egg-white lysozyme. *Mol Nutr Food Res* **58**:614–624 (2014).
- 104 Hildebrandt S, Kratzin HD, Schaller R, Fritsche R, Steinhart H and Paschke A, *In vitro* determination of the allergenic potential of technologically altered hen's egg. *J Agric Food Chem* **56**:1727–1733 (2008).
- 105 Akita EM, Jang CB, Kitts DD and Nakai S, Evaluation of allergenicity of egg yolk immunoglobulin Y and other egg proteins by passive cutaneous anaphylaxis. *Food Agric Immunol* **11**:191–201 (1999).
- 106 Odani S, Kanda Y, Hara T, Matsuno M and Suzuki A, Effects of high hydrostatic pressure treatment on the allergenicity and structure of chicken egg white ovomucoid. *High Pressure Biosci Biotechnol* **1**:252–258 (2007).
- 107 Acero-Lopez A, Ullah A, Offengenden M, Jung S and Wu J, Effect of high pressure treatment on ovotransferrin. *Food Chem* **135**:2245–2252 (2012).
- 108 Rahaman T, Vasiljevic T and Ramchandran L, Effect of processing on conformational changes of food proteins related to allergenicity. *Trends Food Sci Technol* **49**:24–34 (2016).
- 109 Sabadin IS, Villas-Boas MB, de Lima Zollner R and Netto FM, Effect of combined treatment of hydrolysis and polymerization with transglutaminase on β -lactoglobulin antigenicity. *Eur Food Res Technol* **235**:801–809 (2012).
- 110 Kato Y and Matsuda T, Glycation of proteinous inhibitors: loss in trypsin inhibitory activity by the blocking of arginine and lysin residues at their reactive sites. *J Agric Food Chem* **45**:3826–3831 (1997).
- 111 Abeyrathne EDNS, Lee HY, Jo C, Suh JW and Ahn DU, Enzymatic hydrolysis of ovomucoid and the functional properties of its hydrolysates. *Poult Sci* **94**:2280–2287 (2015).
- 112 Han GD, Matsuno M, Ikeuchi Y and Suzuki A, Effects of heat and high-pressure treatment on antigenicity of beef extract. *Biosci Biotechnol Biochem* **66**:202–205 (2002).
- 113 Suzuki A, Hara T, Koyama H, Nogami N, Han GD, Odani S, et al., Effect of high pressure treatment on the allergenicity of bovine serum albumin evaluated by histamine release assay using sera from allergic patients and human basophilic KU812F cells, in *Proceedings of the 50th International Congress of Meat Science & Technology*. Helsinki, Finland, pp. 1163–1166 (2004). Available: http://icomst-proceedings.helsinki.fi/papers/2004_05_37.pdf [8,13 August 2004]