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Review

Ovalbumin: A potential functional protein

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Abstract Although ovalbumin makes up 54% of the total egg white proteins, individual protein usage is rare. The primary applications of ovalbumin in the food industry relate to other proteins, such as whole egg whites. Ovalbumin has remarkable functional properties, such as those of gelation, foaming, and emulsification, which are crucial in the processing of food, however, its application as a standalone functional protein is severely constrained due to separation issues. In recent years, new methodologies for the large-scale separation of ovalbumin have emerged. Meantime, ovalbumin was identified as a good source to produce bioactive peptides with a variety of functional properties, including antibacterial, antioxidant, and angiotensin-converting-enzyme inhibitory actions, according to research. Newly discovered bioactive peptides from ovalbumin can be used in the food sector in addition to their well-known functional properties to create health-promoting products. Benefits extend beyond the food business to numerous other sectors, such as the pharmaceutical and cosmetic industries. Consequently, a gap between the existing and prospective future uses is found. The main goals of this study were to determine some possible factors for the long-term neglect of the major protein and to determine the growing potential for applications of ovalbumin and peptides.

Keywords ovalbumin, enzymatic hydrolysis, separation techniques, functional properties

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1. Introduction

Poultry eggs are considered one of the naturally preserved nutritional components, which are rich in proteins and lipids. The hen egg, similar to other poultry eggs, has a compartmentalized structure and is composed of three main components: albumen (63%), yolk (27.5%), and eggshell (9.5%) (Kovacs-Nolan et al., 2005). An eggshell is a porous structure that is composed of an organic matrix (cuticle, shell matrix, mammillary core, and shell membranes) and an inorganic portion (mammillary knob layer, palisade layer, and surface crystal layer). Three inorganic layers are composed of calcite crystals. The thickness of the shell is recorded as 0.2 to 0.4 mm (Belitz et al., 2009; Roberts, 2004). Functions of the shell in eggs include gas exchange and controlling water loss (Solomon, 2010). Egg yolk is a complex mixture of microparticles held in suspension (Mine, 2022). The yolk is covered with a membrane called vitelline membrane. According to sources, half of the yolk (50%) is water. Rest is occupied by proteins (15-17%), lipids (31-35%), and carbohydrates (1%) (Abeyrathne et al., 2013a; Stadelman and Cortterill, 1995). Yolk is composed of triglycerol (66%), phospholipids (28%), cholesterol (5%), and other lipids in minor quantities. Lipids associated with proteins are called lipoproteins (Jolivet et al., 2006; Mine, 2022). A major water-soluble protein named livetin occupies around 9.3% of egg yolk proteins (Meram and Wu, 2017). Egg yolk is considered a supplement for carotenoids (around 200-300 μg) such

as lutein and zeaxanthin (Sanlier and Üstün, 2021). Egg yolk has multifunctional properties and is abundantly used in the food industry. Besides, yolk protein hydrolysates are reported to show functional properties such as antioxidant activity (Cho et al., 2014; Sakanaka et al., 2006).

Egg white is recorded as a system built from many globular proteins in an aqueous solution (Alleoni, 2006). Four layers - chalaziferous layer, a thin layer (outer thin and inner thin layers), a thick layer, and chalazae cord altogether form the egg white (Guha et al., 2019). The egg white is mainly composed of water (88%) and protein (11%) (Belchior and Freire, 2021). The protein component of egg white is categorized as major and minor proteins. Major proteins include ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.5%). Minor egg white proteins include ovoinhibitor (1.5%), ovoglycoprotein (1%), ovoflavoprotein (0.8%), avidin (0.05%), cystatin (0.05%), and ovomacroglobulin (0.05%) (Abeyrathne et al., 2013a; Kovacs-Nolan et al., 2005). However, with the identification of structural and functional diversification among the components, the utilization of eggs has diversified (Guha et al., 2019).

Applications of poultry eggs extend to broad areas. Eggs are utilized in the food industry mainly due to their protein composition (Cho et al., 2014). Eggs have been recognized for their higher digestibility and for supplying a significant amount of the daily requirement of nutrients (Belitz et al., 2009). Egg proteins are known as ‘sample proteins’ because of their higher biological value and ability to convert (about 94%) to body proteins (Sanlier and Ustun, 2021). Besides, studies have proven that egg white proteins are absorbed more quickly than whey proteins (Matsuoka et al., 2019). Applications of egg white proteins in producing health-promoting products have increased and resulted in the enhancement of the value of traditional food (Miguel et al., 2005). Even in the beverage industry, egg white is used on many occasions. According to a previous study, egg whites with milk produced a protein beverage with many advantages, like increasing the protein content and reducing microbial spoilage (Lotfian et al., 2019). Processed egg based high protein drink was also developed where egg white was utilized (Silva and Abeyrathne et al., 2016). The addition of egg albumen was considered in the production of carbonated beverages also (Hemanth et al., 2020). As an egg component, albumen was recognized for nutritional and functional properties (such as foaming, emulsification, and heat setting), which enhanced utilization in the food industry (Miguel et al., 2005; Omana et al., 2010). Besides utilizing egg white, the applications of egg white hydrolysates were also identified in the food industry and were utilized to develop new products (Garcés-rimón et al., 2016).

Ovalbumin is the major egg white protein that greatly impacts applications of egg white proteins (An et al., 2014; Stadelman and Cotterill, 1995). The use of ovalbumin mainly occurs with other egg white proteins. However, the protein itself has the potential to be used alone, with additional benefits of bioactive peptides. Production and identification of functional peptides have helped to add value to ovalbumin and extend its capabilities to be used in emerging food and non-food sectors. However, protein usage is at a lower level when compared with potential. Therefore, this review was focused on ovalbumin as an egg white protein, which is neglected compared to other egg proteins and to highlight future potentials in food and non-food sectors.

2. Ovalbumin

Ovalbumin is considered one of the first proteins to be isolated in pure form and is recognized as a globular, phosphorylated protein belonging to the serpin superfamily. Although protein owns a reactive center loop, it does not act as a protease inhibitor as other serpins (Huntington and Stein, 2001; Lv et al., 2015). The globular conformation of the protein was identified because of the hydrophobic core. Ovalbumin possesses a size of 45 kDa and a diameter of 5.5 nm (Li and Yan, 2017) and occupies the highest percentage (54%) of total egg white proteins (Stadelman and Cotterill, 1995). Therefore, ovalbumin is considered to have a great impact on applications of egg white (An et al., 2014). Ovalbumin percentage in egg whites differs from one species to another. The percentage of ovalbumin in chicken is 54%, in turkey 40%, and in ducks 40% (Weijers et al., 2002). Ovalbumin owns 386 amino acids, of which half of the amino acid sequence is considered hydrophobic (Huntington and Stein, 2001; Li and Yan, 2017). The amino terminus of the protein is considered acetylated, and the sequence possesses a ‘Cys-Val-Ser-Pro’ amino acid sequence (Nisbet et al., 1981). The protein contains four sulfhydryl groups and a single disulfide bond (Alleoni, 2006; Sheng et al., 2018). Ovalbumin is composed of three components A_1, A_2, and A_3, which contain two, one, and zero phosphate groups (Alleoni,

2006). Ovalbumin contains about 3.5% carbohydrate, a single carbohydrate moiety attached to Asn-292. As ovalbumin contains both hydrophobic and hydrophilic groups, the molecule is considered an amphiphilic molecule (Liu et al., 2021). The pI value and denaturation temperature of ovalbumin have been recorded as 4.5 and 84°C, respectively (Guha et al., 2019). Furthermore, it is converted to S-ovalbumin, a heat-stable form during storage. Factors that affect the formation of S-ovalbumin include pH and temperature, which are changed during storage. Therefore, S-ovalbumin is recognized for having the possibility to be used as a reference index in expressing the freshness of commercial eggs (Huang et al., 2012). Separating ovalbumin as an individual protein was considered by scientists for a longer period of time. Separating high-purity proteins is important in many circumstances, including studying bioactivity (Geng et al., 2012). Ovalbumin separation techniques are important in industrial applications and in identifying minor egg white proteins (Guérin-Dubiard et al., 2005). Ovalbumin must be separated successfully to isolate other minor proteins with high purity. Besides, some egg white proteins (ovalbumin, ovomucoid, ovotransferrin, and lysozyme) are considered food allergens and high-purified proteins are required for food allergy investigations of these components (Ma et al., 2020).

2.1. Separation techniques for ovalbumin

There are a large number of separation techniques that can be utilized in egg white protein separation. Egg white protein separation is considered to be mainly composed of two sectors as scale-up methods (iso-electric focusing, salting out, organic solvent precipitation, polyethylene glycol precipitation, and ion exchange chromatography) and laboratory-scale methods (electrophoresis, reverse micelles, affinity chromatography, and exclusion chromatography) (Ji et al., 2020). Separations of egg white proteins were carried out, focusing on the separation of individual proteins or multiple proteins. Similarly, different techniques have been utilized to separate ovalbumin (Table 1).

2.2. Functional properties of ovalbumin

Studies have shown that ovalbumin is a protein with exceptional thermal and functional properties and identified that functional properties are sensitive to environmental changes (Liu et al., 2021). For example, at an acidic pH, the

Table 1. Advantages and disadvantages of identified separating techniques used for ovalbumin

Description	Advantages	Disadvantages	References
Electrophoretic separations			
1. Native PAGE			Desert et al. (2001), Geng et al. (2012), Miguel et al. (2005)
Stacking gel - 4% acrylamide, separating gel - 7.5% acrylamide, pH 8.8	- Proteins did not undergo denaturation - Reflected the coexistence of A_1, A_2, and A_3 isoforms of ovalbumin	- Did not possess the ability to calculate the pI values of the molecules	
2. 2D electrophoresis			
pH gradient: 4-7 Subjected to SDS-PAGE (12%)	- Enabled the visualization of ovalbumin not corresponding to any known egg white molecule - Considering both the pI and molecular weight of the protein	- Limited resolution - Denaturation of polypeptides occurred when subjected to SDS-PAGE	
3. SDS-PAGE			
Stacking gel - 4%, separating gel - 12% acrylamide	- Abled to localize ovalbumin considering molecular weight	- Denaturation of protein	
4. Iso-electric focusing			
7.5% acrylamide gel pH gradient: 3-7 Running conditions - 1 h at 100 V, 1 h at 250 V, and 30 min at 500 V	- Visualization of ovalbumin did not correspond to any known egg white molecule - Identification of the availability of isoforms of ovalbumin - Can calculate pI values of isoforms	- Not suitable for egg white protein separation because iso-electric points (pI values) of most proteins are closer values	

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Description	Advantages	Disadvantages	References
Chromatographic techniques			
1. RP-HPLC			
C4 supelcosil LC-304 column, at 214 nm	- Better separation when compared to gel permeation chromatography	- Approximately 30% retention of ovalbumin on column	Awade and Efstathiou (1999)
2. Gel permeation			
With superose 12 HR 10/30 column, at 280 nm with FPLC system		- Not recommended (Ovalbumin was eluted together with ovotransferrin, ovomucoid, avidin, and ovoglobulin)	Awade and Efstathiou (1999)
3. Anion exchange chromatography			
i) Dimethylaminoethyl (DEAE) - cellulose (Whatman DE92 anion exchange cellulose)	- Better to use than Whatman DE52 because resin allows the application of a higher flow rate	- Adsorption of ovalbumin was observed - Lower capacity and protein recovery when compared with Whatman DE52	Awade (1996)
ii) Mono Q HR 5/5 using HPLC system (0.02 M Tris-HCl, pH 9, increasing NaCl concentration, detecting at 280 nm)	- Higher resolution compared with gel permeation and RP-HPLC and higher recovery than RP-HPLC (30% retention in RP-HPLC) - Low time consumption	- Sample preparation was essential for removing ovomucin - The complex separation procedure	Abeyrathne et al. (2013b), Awade and Efstathiou (1999)
iii) Q-Sepharose fast flow Mucin-free egg white is loaded to a cation exchanger (S Ceramic Hyper DF) to separate, and the resulting elute used in anion exchange chromatography (0.14 M NaCl isocratic elution, detected at 280 nm)	- Higher purity (91%) and yield (80%)	- Sample preparation was essential because mucin-free egg white was loaded, use of water in precipitation of ovomucin (Cause huge loss of proteins such as ovalbumin and lysozyme in the process)	Guerin-Dubiard et al. (2005), Omana et al. (2010)
iv) Q-Sepharose fast flow (Based on frontal chromatography)	- Simple - Low time consumption - High purity (94% in 0.14 NaCl and 83% in 0.5 M NaCl isocratic elusion) - Non-altered by-product	- Laboratory scale method - Sample preparation was required - Not following the two-step precipitation method for ovomucin. So, contamination of ovalbumin and lysozyme can occur during precipitation of ovomucin	Croguennec et al. (2000), Omana et al. (2010)
v) Q-Sepharose fast flow FPLC system (0.05 M Tris-HCl, pH 9, 0.3 NaCl)	- Non-denaturing method - Low time consumption	- Low protein recovery (54%) and purity (70%) - Laboratory scale - Sample preparation was essential	Belchior and Freire (2021), Tankrathok et al. (2009)
vi) Q Sepharose fast flow with FPLC (Co-extraction of egg white proteins) Two-step precipitation method for ovomucin precipitation and cation exchange chromatography (SP Sepharose FF) for separation of lysozyme, ovotransferrin, ovoinhibitor mainly	- High purity (estimated purity from 100 mM supernatant was 91.2%, estimated purities for three ovalbumin fractions resulted from 500 mM supernatants were 82%, 100%, and 100%) - High yield (yield of ovalbumin from 100 mM supernatant-40.70% from total egg white protein)	- High salt concentration in 500 mM supernatant demands dialysis prior to ion-exchange chromatography - Recovery of ovalbumin from 500 mM supernatant was practiced after cation exchange chromatography and resulted in low yields (0.40%, 0.38%, 1.62% from total egg white protein)	Omana et al. (2010)
4. Cation exchange chromatography			
CM-Sepharose FF chromatography	- Single protocol, purity was higher than commercial ovalbumin, low cost	- Egg white pretreatment was needed (removal of ovomucin and globulins)	Ma et al. (2020)

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Description	Advantages	Disadvantages	References
Salt Precipitation			
1. $(NH_4)_2SO_4$ or Na_2SO_4	- Separated ovalbumin successfully for the first time	- Product with high salt concentration - Irreversible unfolding of proteins can occur due to high salt concentration - Difficulties in the separation of lysozyme and ovalbumin	Datta et al. (2009), Hopkins (1900), Pereira et al. (2016)
2. Sequential separation of lysozyme, ovotransferrin, ovomucin, and ovalbumin [Amberlite FPC 3500 ion exchange resin iso-electric precipitation of ovomucin → 2.5% (w/v) citric acid and 5% (w/v) ammonium sulfate combination → 1.5% (w/v) citric acid and 2% (w/v) ammonium sulfate combination → ultrafiltration]	- Simple, non-toxic method, Better than ammonium sulfate and acetic acid combinations, which were practiced earlier (A lower percentage of ammonium sulfate has been utilized) - Effective separation of ovalbumin (purity was greater than 87%, yield greater than 97.7%) - The possibility to scale up can be used in the laboratory as well as on an industrial scale, provides cost benefits due to sequential separation of highly purified multiple proteins, within four days separation of four proteins can be accomplished	- Several stages of equilibrium/ purification of proteins correspond to a multiple-step process	Abeyrathne et al. (2013b), Abeyrathne et al. (2014a), Belchior and Freire, (2021), Pereira et al. (2016)
3. Successive extraction of egg white proteins (Modified from Abeyrathne et al., 2014)	- Higher purity (~95%) - Low cost - Simple	- Changes in the secondary structure of ovalbumin were identified	Ji et al. (2020)
Ultrafiltration			
1. Amicon stirred cell fitted with polyether sulfone (PES) membrane (Two-stage ultrafiltration scheme; 30 and 50 kD flat disks)	- High purity (98.7%)	- Preparation of the sample was required by steps such as centrifuging, dilution, and pH adjustments, can be highly affected by the operating and physicochemical conditions, difficult to use in the scaling-up process	Abeyrathne et al. (2013b), Datta et al. (2009)
Aqueous Biphasic Systems (ABS)			
1. High-speed counter-current chromatography; aqueous polymer two-phase system (polyethylene glycol and potassium phosphate)	- Proteins were purified from the crude solution of fresh egg white in one step, short elution time. denaturation and adsorption loss are lower compared to column chromatography, 95% purification recovery [16% (w/w) polyethylene glycol and 17% (w/w) potassium phosphate, pH 9.2]	- Require specialized equipment, environmental and economic problems have been identified in polyethylene glycol-salt (PEG-salt) systems. This is due to the large consumption of phase-forming chemicals and possesses difficulties in regeneration, waste disposal problems, scaling up is not practical	Abeyrathne et al. (2014a), Saravanan et al. (2008), Shibusawa et al. (1998), Shibusawa et al. (2001), Pereira et al. (2016)
2. The aqueous biphasic system coupled with high-speed counter-current chromatography [16% (wt) Polyethylene glycol/ PEG-1000 and 17% (wt) potassium phosphate, pH 9.2]	- Purified to 95% - Absence of solid support when compared to other chromatographic techniques	- Higher toxicity because it contains a phosphate-based system - High molecular weight polymer usage	Pereira et al. (2016), Zhi et al. (2005)
3. Single step purification [Polyethylene glycol/PEG-400 (25% (wt)), potassium citrate/citric acid aqueous biphasic system (25% (wt), 50% (wt) egg white aqueous solution (1:10v/v)), pH 7]	- A simple approach with a single step - Low time consumption and low cost - Sustainable method (recycling PEG phase during purification of ovalbumin by inducing precipitation at low temperature and centrifugation) - Better compared to other polymer salts ABS	- The recovery yield is 65%	Pereira et al. (2016)

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Description	Advantages	Disadvantages	References
	- Minimum loss of protein due to denaturation, minimized adsorptive loss, (absence of solid support when compared to other chromatographic techniques) - Do not require specialized equipment, can perform on an industrial scale - Biodegradable and less toxic (because ABS is citrate-based, not phosphate as in Zhi et al., 2005)		
4. Aqueous two-phase flotation (ATPF) (Polyethylene glycol 1000, ammonium sulfate)	- Low cost (Developed for separation of ovalbumin from byproduct formed in salted egg yolk production), simple, purity is approximately 92%, no difference in the ovalbumin structure or functional properties (Tested properties; oil binding capacity, foam capacity, emulsion ability, viscosity)	- Developed for the separation of ovalbumin from salted egg white	Jiang et al. (2019)
5. Simultaneous separation of egg white proteins by using ABS/TPP [30% (wt) PEG 2000, 13% (wt) $K_2H_2PO_4/KH_2PO_4$, pH 7.0]	- Recovery yield 82% - Can apply for crude suspensions directly - A single step to recover more than one protein	- Loss of ovalbumin due to use of high molecular weight PEG	Belchior and Freire (2021)
Polyethylene glycol precipitation combined with chromatography			
1. Co-purification using polyethylene glycol precipitation (PEG-8000) and anion exchange chromatography; Q Sepharose Fast Flow (20 mM Tris-HCl buffer, pH 8, 0.18 M NaCl)	- Simple - Desalination was not required prior to anion exchange chromatography - Has the potential to use at industrial level - Higher purity (three peaks with purity percentages 90.77%, 96.45%, 94.84%) and low cost since only one column is used	- Recovery percentage of ovalbumin is 88.64%	Geng et al. (2012)

surface hydrophobicity of ovalbumin is high. Therefore, greater emulsifying activity was identified at an acidic pH (Mine et al., 1991). Ovalbumin involves forming gels (transparent, semitransparent, or opaque gels) when subjected to heat over the denaturation temperature of 84°C (Alleoni, 2006). Ovalbumin is the main reason for egg whites to have foaming properties. It undergoes surface denaturation and interfacial coagulation that assist in the foaming of egg white (Croguennec et al., 2007). Moreover, ovalbumin can improve polysaccharides' antioxidant activity with the help of covalent bonds (Batiha et al., 2021). The functional properties of ovalbumin have been improved by many methods, including phosphorylation, glycation, interaction with tannic acid, electric pulse field, irradiation, and high-pressure microfluidization. Studies have shown that ultrasound pretreatment combined with glycation (treated with mannose) has caused enhancements in antioxidant activity, foaming capacity, and stability of ovalbumin (Yang et al., 2021). Similarly, ultrasound treatment and glycation with xylose can cause improvements in foaming properties and enhance solubility (Liu et al., 2021). Glycation with carboxymethyl cellulose caused increasing foaming ability (An et al., 2014). Improving functional properties can lead to increased protein applications. Moreover, enzymatic hydrolysis has also caused for increasing functionality of ovalbumin along with potential uses (Abeyrathne et al., 2014b).

2.3. Production of bioactive peptides from ovalbumin

Enzymes such as pepsin, alcalase, and trypsin are widely used for enzymatic hydrolysis (Bueno-Gavilá et al., 2021). Enzymatic hydrolysis has caused modifications in properties and enhanced food protein value (Garcés-rimón et al., 2016). Accordingly, egg white proteins are also hydrolyzed to

achieve enzymatic modifications. These modifications have been done to alter the functional characteristics of egg white proteins (Cho et al., 2014). The sequence of amino acids is involved in deciding the activity of a particular bioactive peptide (Eckert et al., 2013). Therefore, the activity of peptides formed is affected by the type of enzyme utilized (Chang et al., 2018; Patil et al., 2020). Enzymatic hydrolysis has considerably changed protein solubility, microstructure, and other functional properties (Bao et al., 2017).

Enzymatic hydrolysis of ovalbumin has been done in mainly two ways. The first method uses a single enzyme such as pepsin, trypsin, chymotrypsin, papain, alcalase (Dávalos et al., 2004; Miguel et al., 2004; Tang et al., 2013), and the second method uses combinations of enzymes (combinations of pepsin, trypsin, α-chymotrypsin, papain, and alcalase) (Abeyrathne et al., 2014b). However, factors like enzyme specificity, the concentration of the enzyme and substrate, pH, temperature, presence or absence of inhibitors, and ionic strength in the reaction medium affect the rate of hydrolysis (Eckert et al., 2013). Peptides derived from ovalbumin have many properties, like antioxidant, antimicrobial, metal chelating, and angiotensin converting enzyme (ACE)-inhibitory activities (Abeyrathne et al., 2014b).

The antioxidant activity of peptides helps to reduce oxidative stress, which is mainly due to imbalances in antioxidant status (Antolovich, 2002). Reactive oxygen species can damage macromolecules such as DNA, lipids, and proteins (Davalos et al., 2004). Oxidative stress can lead to several problems, such as cardiovascular diseases, cancers, and age-related diseases (Mishra et al., 2012). The antioxidant activity of peptides can be measured by considering different aspects such as radical scavenging activity, chelation of pro-oxidative transition metal ions, and inactivation of reactive oxygen species (ROS) (Bueno-Gavila et al., 2021). According to sources, peptides derived from ovalbumin have shown antioxidant activity. As per the record, the antioxidant activity and Fe^{2+} chelating activity of hydrolysates were significantly influenced by hydrolysis time and molecular weight (MW <3 kDa) (Bueno-Gavilá et al., 2021). Various levels of antioxidant activities were manifested in different studies. Peptides such as Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, and Ser-Ala-Leu-Ala-Met have shown very high antioxidant activities, while peptides such as Arg-Ala-Asp-His-Pro-Phe-Leu and Glu-Ser-Ile-Ile-Asn-Phe have shown low antioxidant activities (pepsin, 3 h) (Dávalos et al., 2004). During two enzyme treatments, it was identified that peptides derived from a combination of protease from *Bacillus licheniformis* (pH 6.5, 37°C for 3 h) followed by trypsin from bovine pancreas (pH 7.8, 37°C for 3 h) had shown lowest value to the antioxidant activity test done by using thiobarbituric acid/trichloroacetic acid solutions (TBA/TCA), strong metal chelating activities and angiotensin converting enzyme (ACE)-inhibitory activity. Other enzyme combinations, such as pepsin from porcine gastric mucosa (pH 2.5, 37°C for 3 h) followed by a protease from *Bacillus licheniformis* (pH 6.5, 37°C for 3 h), pepsin from porcine gastric mucosa (pH 2.5, 37°C for 3 h) and papain (pH 6.5, 37°C for 3 h) have also shown ACE-inhibitory, metal chelating and antioxidant properties (Abeyrathne et al., 2014b).

Peptides derived from ovalbumin have also shown antibacterial activity (Pellegrini et al., 2004). As per sources, ovalbumin hydrolyzed with pepsin (pH 2.0, 37°C, 4 h) had shown the highest antimicrobial activity when compared with the hydrolysis of ovalbumin with trypsin (pH 7.5, 37°C), papain (pH 6.5, 50°C), alcalase (pH 9.0, 60°C), neutrase (pH 7.0, 50°C), and flavourzyme (pH 7.0, 50°C) at a different time (1, 2, 3, 4 and 5 h). Arg-Val-Ala-Ser-Met-Ala-Ser-Glu-Lys-Met-Lys-Ile was also identified as a peptide with antimicrobial activity (Tang et al., 2013). Enzymatic hydrolysis of ovalbumin by chymotrypsin and trypsin has produced a few peptides (five peptides from trypsin and three peptides from chymotrypsin) which contain antimicrobial activity (Pellegrini et al., 2004). Similarly, peptides produced by ovalbumin were identified as strongly active against *Bacillus subtilis* and lesser on *Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *Bordetella bronchiseptica* like species (Kovacs-Nolan et al., 2005).

Egg white protein hydrolysates were recognized as biologically active compounds with specific health benefits (Cho et al., 2014). Chicken eggs and other poultry species, such as ostrich, have also manifested ACE inhibition (28-57%) (Bueno-Gavilá et al., 2021). As per records, ACE-inhibitory activity was shown by the peptides derived from ovalbumin. An octapeptide (Phe-Arg-Ala-Asp-His-Pro-Phe-Leu) named ovokinin has been identified as a peptide (due to pepsin digestion) that reduced the systolic blood pressure of spontaneously hypertensive rats (SHR) (Fujita et al., 1995). However, unhydrolyzed egg white or ovalbumin (purity 99%) did not manifest ACE-inhibitory activity as peptides. Peptides such as Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, Phe-

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Arg-Ala-Asp-His-Pro-Phe-Leu, and Arg-Ala-Asp-His-Pro-Phe-Leu have shown higher ACE-inhibitory activity (Chang et al., 2018; Miguel et al., 2004). Hydrolysis of ovalbumin using two enzyme combinations also has manifested around 80% ACE-inhibitory activity (Abeyrathne et al., 2014b). A summary of the bioactivities of the peptides derived from ovalbumin is given in Table 2.

3. Current uses of ovalbumin

Ovalbumin is a protein mainly utilized along with egg white for its functional properties. In the food industry, this scenario is typical, and the protein is primarily used as a food protein (Lv et al., 2015). Protein utilization is seen in the bakery and sweet industries and even in baby food production

Table 2. Summary of the bioactivity of the peptides derived from ovalbumin

Bioactivity	Hydrolysis condition	Peptides formed from ovalbumin	References
1. Antioxidant activity	i) Pepsin pH 2.0, 37°C, 3 h	High activity Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu Ser-Ala-Leu-Ala-Met Intermediate activity Tyr-Gln-Ile-Gly-Leu Tyr-Arg-Gly-Gly-Leu-Glu-Pro-Ile-Asn-Phe Low activity Phe-Arg-Ala-Asp-His-Pro-Phe-Leu Arg-Ala-Asp-His-Pro-Phe-Leu	Davalos et al. (2004)
2. ACE-inhibitory/ Antihypertensive activity	i) Egg white with pepsin (pH 2.0, 37°C, 3 h) Ultrafiltration (3000-Da cut-off membrane)	Potent activity Arg-Ala-Asp-His-Pro-Phe-Leu Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu Phe-Arg-Ala-Asp-His-Pro-Phe-Leu (Ovokinin) Moderately active Tyr-Gln-Ile-Gly-Leu Phe-Ser-Leu Ile-Val-Phe	Miguel et al. (2004)
	ii) Pepsin	Glu-Arg-Lys-Ile-Lys-Val-Tyr-Leu (Most potent) Leu-Trp Phe-Phe-Gly-Arg-Cys-Val-Ser-Pro	Iroyukifujita et al. (2000)
	iii) Protease from *Cynara scolymus* (mature artichoke flower extract), pH 6.2, 36°C, 2 h, 4 h, 16 h, < 3 kDa	Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu	Bueno-Gavila et al. (2021)
3. Antimicrobial activity	i) Trypsin (pH 7.8, 37°C, 6 h) Chymotrypsin (pH 7.8, 37°C, 6 h)	Trypsin digestion; 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 Chymotrypsin digestion; 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	Pellegrini et al. (2004)
	ii) Flavourzyme (pH 7.0, 50°C) Pepsin (pH 2.0, 37°C) Trypsin (pH 7.5, 37°C) Neutrase (pH 7.0, 50°C) Papain (pH 6.5, 50°C) Alcalase (pH 9.0, 50°C)	Arg-Val-Ala-Ser-Met-Ala-Ser-Glu-Lys-Met-Lys-Ile	Chang et al. (2018), Tang et al. (2013)

(Weijers et al., 2002). These applications in the food industry are mainly due to gelation and foaming properties (Geng et al., 2019). In addition, ovalbumin is widely used in research studies. Ovalbumin is utilized in diverse areas, such as biochemistry, immunology, and nutritional studies. As an example, ovalbumin is used as a standard protein in protein assays (Abeyrathne et al., 2014a; Geng et al., 2019), and different research has been carried out to identify methods such as phosphorylation, glycation, and enzymatic hydrolysis to increase the functional properties of ovalbumin (Abeyrathne et al., 2014b; Yang et al., 2021). However, the large-scale application of ovalbumin as an individual food protein was not identified within the current study. Yet, as more research is conducted, the greater the potential for using it as an individual protein.

4. Future potential of ovalbumin

Although ovalbumin was identified as an egg allergen (Yang et al., 2021), methods such as thermal processing and enzymatic hydrolysis have been identified as methods that can affect conformations and impact allergenicity (Chang et al., 2018). Therefore, along with modifications, ovalbumin has the potential to be introduced as a functional protein to the wider community. The potential usage of the protein spreads across a wide area and is not limited to the food industry.

Ovalbumin possesses tumor necrosis-releasing factors that can be applied in tumor suppression (Abeyrathne et al., 2013a), and studies have also been done to recognize its potential to be used as a drug carrier. As per sources, discoveries were made about ovalbumin's ability to form nanocomplexes and its potential in use as PUFA vehicle (Kratz, 2008; Sponton et al., 2015). Similarly, Yu et al. (2006) described ovalbumin usage to create nanogels (Chitosan-ovalbumin). Ovalbumin was discovered as a food-globular protein used in Retinol (RET) vehiculation strategies to introduce retinol into food matrices and potentially produce fortified foods (Visentini et al., 2017). Ovalbumin, therefore, has the potential to be used in the nutraceutical, pharmaceutical, and cosmeceutical industries, as demonstrated by the findings cited above.

Enhancing utilization of the proteins depends on improving functional characteristics (Lv and Chi, 2012). As an example, improving the foaming properties of ovalbumin can expand its utilization in different kinds of food products. Glycated ovalbumin may be useful in food products (frankfurters, creams) with emulsification as their functional property (Lv et al., 2015).

A significant increase in functionality (properties like antioxidant, antimicrobial, metal chelating, and ACE-inhibitory activities) of native ovalbumin occurred due to enzymatic hydrolysis (Abeyrathne et al., 2014b). Therefore, there is a potential for using these bioactive peptides in different industries to obtain both nutritional and functional benefits. Pragmatic usage of hydrolysates has been done in different studies related to the food industry and evaluated the utilization of egg white hydrolysates. The development of haute cuisines such as custard, cream, cheese, and junket-like products was done with egg white hydrolysates, which have the ability to provide new textures to the food industry (Garcés-rimón et al., 2016). The development of functional ice cream was carried out without including dairy solids (López-Martínez et al., 2021). Furthermore, egg white hydrolysates were identified for dairy-like technological properties (Garcés-rimón et al., 2016). In the meantime, milk allergy is considered one of the most common food allergies in children, with an estimated prevalence in developed countries (Flom and Sicherer, 2019). Therefore, there is a potential to utilize egg white hydrolysates as an alternative to dairy products so that the lactose intolerance community can benefit and consume foods that have the same technological properties as dairy products do. Being a major component in egg white, the peptides from ovalbumin do contribute to technological properties.

There has been a decline in egg consumption during the past few decades due to its high cholesterol and fat content (Chang et al., 2018). Hence, the utilization of the functional properties of peptides grabs the opportunity to be used to increase egg consumption. According to studies done with Spontaneously Hypertensive Rats (SHR), there is a potential of developing food products with antihypertensive activity by using egg white hydrolysates (Miguel et al., 2006). Furthermore, a previous study identified antioxidant peptides derived from goose egg white proteins, which might be useful as a food additive (Baratzadeh et al., 2013). As discussed in Table 2, ovalbumin is a rich source of bioactive peptides that can produce different products, such as nutraceuticals or food additives.

Even though many studies show its potential, why has

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ovalbumin been overlooked by the food industry?

5. Possible reasons for ovalbumin being neglected as a protein

Ovalbumin is recognized as a major food allergen (Ma et al., 2020), which is a concern that can indeed limit the utilization of protein in the food industry. Other than that, during this study, several reasons have been identified for neglecting ovalbumin in the current scenario. The hydrophobicity of ovalbumin is one such identified reason. The protein contains an amino acid sequence of 386 amino acids, where half of the amino acids (such as valine, leucine, and tryptophan) are considered to be hydrophobic (Huntington and Stein, 2001; Li and Yan, 2017). Therefore, insolubility can become a problem during product development. Especially in developing beverages.

Denaturation can hurt industrial applications. The denaturation temperature of ovalbumin is recorded at 84℃ (Alleoni, 2006). Moreover, ovalbumin can readily denature when exposed to new surfaces (Sheng et al., 2018; Stadelman and Cotterill, 1995). Therefore, denaturation can occur due to agitation. According to sources, precipitation can be seen in beverages like whey protein beverages due to thermally denatured proteins (Goudarzi et al., 2014). Similar behavior can be shown by ovalbumin due to the possibility of denaturation. Precipitation can also occur when pH is close to the iso-electric point (Geng et al., 2019).

Another identified reason is that many ovalbumin experiments are still at the research level. Research experiments on separating techniques can be considered an example. Several techniques cause protein denaturation, while others cause low yields and purity levels (Table 1). Those can be the reasons for ovalbumin to be neglected, although it is considered a major residue in separating techniques. The presence of a few separating methods (successive extraction of egg white proteins and sequential separation of egg white proteins) for ovalbumin on an industrial scale can be a reason for lower utilization. The sequential separation of egg white proteins (lysozyme, ovotransferrin, ovomucin, and ovalbumin) by Abeyrathne et al. (2014a) can be considered a successful technique for separating ovalbumin. Chemical compositions of 2.5% (w/v) citric acid and 5% (w/v) ammonium sulfate, and 1.5% (w/v) citric acid and 2% (w/v) ammonium sulfate combinations have been used to yield ovalbumin (>97% yield) to separate ovalbumin. The protocol was identified as a simple and non-toxic method that can be used at the industrial level (Abeyrathne et al., 2013b). But purity was recorded as >85%. The purity level was increased in ‘successive extraction of egg white proteins’. This proves that research studies on ovalbumin are currently at continuous development, considering pros and cons.

Furthermore, neglect of ovalbumin can be due to protein behavior under different conditions. According to studies, limitations in functional performance can occur due to protein aggregation under some conditions, such as pH, ionic strength, and temperature conditions (Yang et al., 2021). Gel formation depends on many factors, which include protein concentration, pH, and ionic strength. Treatment with strong alkali can induce ovalbumin gel, but continuous treatment can cause irreversible damage to protein. The hydrophobic core is exposed when ovalbumin is treated with a strong alkali. A crystal gel formation was identified when treated with alkali (Zhao et al., 2016). Studies have also shown that the emulsification and interfacial stabilization of ovalbumin as a polymeric stabilizer depends on pH. The aggregate state can be seen at a lower pH than pI (4.5), and at an alkali pH ovalbumin unfolds (Xu et al., 2020). The presence of salts, the concentration of protein dispersion, and the oil-phase volume also affect ovalbumin’s emulsifying properties (Mine et al., 1991).

In its native state, ovalbumin shows less functional properties (such as not showing antioxidative activity) being an amino acid source (Abeyrathne et al., 2014b). Therefore, no additional benefit other than nutritional value was identified. This can be a cause for the limitation of applications.

New product development with ovalbumin or ovalbumin hydrolysates requires more experiments. The behavior of peptides in a food system, whether exhibiting functional properties within the food system or the behavior of peptides with other food components, has to be thoroughly understood. Although different products of egg white hydrolysates have been identified in the review, no records on the utilization of egg white hydrolysates in beverages have been identified. So, there is also a knowledge gap in some sectors. Ovalbumin is a huge residue that is separated during the separation of minor proteins and wasted without being used, but a low understanding can cause low usage in the industry.

6. Conclusions

In summary, ovalbumin is removed as the major residue when separating egg white proteins. The protein and its peptides were recognized for their enormous potential to be used in the cosmeceutical, pharmaceutical, and nutraceutical industries. However, using ovalbumin as a stand-alone protein on larger scales is minimal. Several potential causes that could have contributed to the protein's long-term neglect were discovered during the study and emphasized the necessity of further investigations to minimize the highlighted obstacles.

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Conflict of interests

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Author contributions

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