Food Science of Animal Products

Review Article

Eggstraordinary health: exploring avian egg proteins and peptides in boosting immunity and health maintenance

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Abstract: Amidst the outbreak of the Corona Virus Disease 2019 (COVID-19), there has been a pivotal shift in nutritional focus towards adopting healthier lifestyles to enhance immune function. The successive resurgence of COVID-19 serves as a stark reminder that prioritizing the enhancement of our own immune systems is of paramount importance. Eggs are nutritious, productive, cost-effective, and affordable for consumption. Likewise, eggs are exclusive among the numerous ways consumers can obtain high-quality animal protein. Egg proteins and their peptide derivatives are abundant and biologically active and have significant therapeutic effects on chronic diseases induced by a variety of factors, including metabolic syndrome, making eggs an important raw material for the study of proteins and peptide-active substances. This paper reviewed methods for the isolation and purification of avian egg proteins and their derived peptides; the effects of the proteins and peptides in enhancing the body's immunity, lowering blood pressure and expression levels of inflammatory factors; as well as the potential of the proteins and peptides in reducing the risk of chronic diseases such as osteoporosis and cardiovascular disease. This review was written with the expectation that it would provide a comprehensive understanding of the potential value of poultry egg proteins for maintaining health.

Keywords: egg protein; peptides; biological activity; healthy; immunity

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1 Introduction

The multiple rebounds of Corona Virus Disease 2019 (COVID-19) had dramatically impacted the lifestyle and health of the population. Individuals who have recovered from COVID-19 are more inclined to increase their intake of fruits, vegetables, and high-quality protein to promote wellness, as well as place more emphasis on immune enhancement[1]. Shortages and rising prices of food have altered the shopping habits of the population. Affordable and wholesome foods are the preference of most people, while consumption of meat and processed foods has declined. The habits and dietary structure of people have been gradually modified as the demand for eliminating sub-health and preventing the occurrence of diseases has increased[2]. The industries that focus on slowing down aging, improving immunity, and maintaining the health of life, are expected to continue to exhibit a significant rise. However, enhancing human immunity through medication might lead to different degrees of toxic side effects. Since each individual has a different constitution, some patients might have an allergic reaction to immunity-enhancing drugs, with symptoms such as skin itching and rashes, while severe allergic reactions would result in shock or even life-threatening conditions[3]. Furthermore, after entering the body, the medication might irritate the mucous membrane of the gastrointestinal tract after entering the body, resulting in symptoms such as abdominal pain, nausea, and vomiting[4]. In addition to the aforementioned side effects, medications aimed at enhancing the body's immunity may also entail other adverse effects, including weakness, dizziness, and headaches, among others. Increasingly, academics have been concerned about bioactive ingredients in the diet which have the potential to prevent chronic diseases or improve human health, thereby eliminating or reducing drug consumption[5]. Moreover, food-derived bioactive ingredients have been shown to be healthier than drugs and to have smaller adverse effects[6]. Up until now, there are a multitude of food-derived compounds and ingredients with different bioactivities obtained from animal and plant sources[7].

It is widely accepted that protein serves as the fundamental building block of all living organisms. Eggs are the least energy-intensive and most environmentally friendly of all protein-rich foods[8]. In view of the serious global climate change and the deteriorating environment, the egg has become the prime choice for protein supplementation[9]. In comparison to pork and beef, eggs are not only more productive and accessible, but also nutritionally valuable and extremely cost-effective (Figure 1A). Human beings can obtain eight essential amino acids and other nutrients from eggs[10]. Immunity could be boosted to some extent both by eating eggs directly and by taking egg proteins. However, if a high protein supplement with no allergic reactions were required,

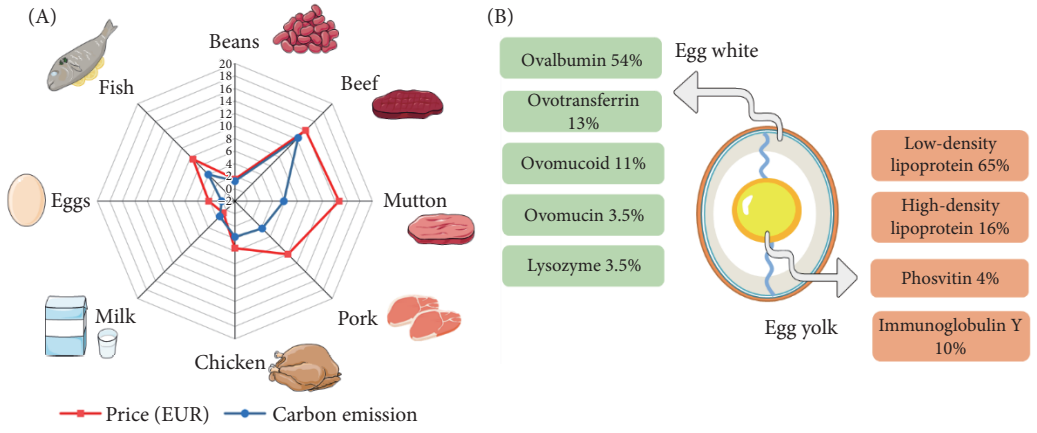

Figure 1 (A) Prices and carbon emissions of major food items providing protein; (B) Principal proteins in eggs and the predominant share.

specific egg protein or its derived peptides might be more suitable for enhancing the body's immunity and protecting the wellness of the organism[11]. Meanwhile, with powerful immunomodulatory effects, the functionally active proteins in eggs and their hydrolyzed peptides could effectively improve the resistance of the body to disease. As the demand for "nutrition, function and health" emerges, it is indispensable to look into the health-modulating effects of the proteins and their peptides in eggs. This work reviewed the health-modulating effects of functional proteins and peptides in eggs *in vivo* and *in vitro*.

2 Composition and distribution of poultry egg proteins

Eggs are mainly composed of egg white (55%), egg yolk (33%), egg shell (12%) and eggshell membrane[12]. The abundant proteins in eggs not only provide the material basis for all essential activities during the development of the avian embryo, but also for the numerous biological functional activities. According to the abundance of proteins, the proteins in eggs could be categorized into major proteins (more than 83% of the total protein) and minor proteins (less than 17% of the total protein). As shown in Table 1, the major proteins in egg whites are ovalbumin (OVA), ovotransferrin (OVT), ovomucoid (OVMD), ovomucin (OVMN), and lysozyme (LYZ), while minor proteins include G2 globulin, G3 globulin, ovomacroglobulin, ovalbumin inhibitor, riboflavin-binding protein, cystatin, and antibiotin protein[13–14]. The proteins in the egg yolk account for 32% of its dry weight[15] and are mainly low-density lipoprotein (LDL), high-density lipoprotein (HDL), phosvitin (PV), and yolk immunoglobulin Y (IgY) (Figure 1B). The eggshell membrane is a fibrous protein film located between the egg shell and the egg white that consists of a bi-layer structure of the outer shell membrane and the inner shell membrane, which serves as a physical barrier against pathogens invading the egg. 90% of the shell membrane is composed of protein and the rest contains about 2% ash and 2% glucose[16]. However, it is insufficient to thoroughly understand the abundant protein composition of eggs based on the existing protein classification criteria.

The structural properties and biological activities of the main proteins in eggs were shown in Table 1. In addition to the major proteins, a few unclassified proteins and peptides have been identified in eggs. The proteomics is the science that takes proteome as the research object and studies the structure, function, interaction, and its changing law of protein composition. In food science, proteomics is often used in quality control, nutritional analysis and functional identification[17]. Through proteomics technology, the content and composition of various proteins in food could be analyzed; the biologically active components could be identified; and the nutritional value of food could be evaluated[18]. Commonly employed methods for proteomics analysis encompass bidirectional electrophoresis, isobaric tags for relative and absolute quantification (iTRAQ), tandem mass tagging (TMT), and matrix-assisted laser desorption/ionization (MALDI) and so on[19]. Sun et al.[20] separated the major proteins in egg whites by reversed-phase chromatography, followed by TMT labeling of the proteins, and identified 148 proteins in egg whites by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Arena et al.[19] used membrane filtration followed by acetone/ethanol precipitation to isolate and purify peptides from egg white proteins. By means of MALDI-TOF-TOF-MS and LC-MS/MS, 506 peptides were identified from egg whites and some protein fragments were identified that might be associated with anti-hypertensive, antioxidant, anti-inflammatory, anticancer, anti-thrombotic, immunomodulatory and influencing cell adhesion activities. Similarly, 628 polypeptides were identified in poultry egg yolk after hydrolysis by chymotrypsin and pepsin. A total of 198 peptides were revealed to have anti-hypertensive, antibacterial, anti-cancer, anti-viral, calcium binding, and anti-inflammatory activities based on bioinformatics analysis of these 628 peptides and comparison with databases[21]. Ahmed et al.[22] characterized 427 proteins in eggshell membranes and identified a selection of proteins that promote wound repair and exert anti-inflammatory as well as antioxidant effects. In the proteomic study of egg proteins, an in-depth understanding of the structure and function of the proteome not only contributes to the clarification of the nutritional level of eggs, but also provides important guidance for the development of eggs and their products. It will also result in the innovative design and improvement of functional foods, promoting the food industry toward processing healthier and more nutritious foods.

3 Major separation and purification techniques for poultry egg proteins

3.1 Isolation and purification of major proteins from egg whites

Poultry egg proteins are diverse and complex in composition.

Table 1 The physicochemical structure and potential uses of protein in eggs.

Egg protein		Relative content (%, *m*/*m*)	Molecular weight (kDa)	pI	Number of amino acids	Structure	Features	Application	References
Egg white 55%	Ovalbumin (OVA)	54	44.5	4.5	385	Five different immunoglobulin E binding epitopes	Phosphoglycoprotein	Hypotensive, immune-enhancing agent, antioxidant, oral delivery wall material	[55]
	Ovotransferrin (OVT)	12–13	77.9	6	686	Two (N- and C-) structural domains interconnected by α-helix	Binding metal ions	Prevention of iron deficiency anemia, immunomodulator	[57]
	Ovomucoid (OVMD)	11.00	28	4.1	186	Three structural domains, cross-linked by disulfide bonds within the structural domain	Trypsin and serine protease inhibition	Trypsin inhibitors, allergens, antioxidants	[68]
	Ovomucin (OVMN)	3.50	α: 21 β: 5 500–8 300	4.5–5.0		Two substituents linked by disulfide bonds	Contains 60% carbohydrates, structural protein of egg whites	Antibacterial agent, antiviral agent, inflammation regulators	[69]
	Lysozyme (LYZ)	3.40	14.4	10.9	129	Two (N- and C-) structural domains separated by a helix-loop-helix motif	Capable of lysing bacterial cell walls	Antibacterial, antioxidant	[137]
	Ovoinhibitor	1.50	49	5.1	447	21 disulfide bonds and 7 Kazal-type domains	Inhibition of serine protease activity	Pharmaceutical or anti-pathogen agent	[138]
	Riboflavin binding protein	0.80	32	4	219	Amino-terminal pyroglutamic acid residue	Capable of binding riboflavin	Visual fatigue relief agent	[139]
	Ovomacroglobulin	0.50	650	4.5	780	Four subunits linked by disulfide bonds	Inhibition of enzyme activity	Wound healing promoters, protease inhibitors, anti-inflammatory agents	[140]
	Cystatin	0.05	12.7	5.1	512	Homotetrameric proteins	Capacity to inhibit cysteine protease activity	Cysteine protease inhibitor, antibacterial agent, osteoporosis relief agent	[139]
	Avidin	0.05	68.3	10.5	512	Tetrameric protein	Combining biotin	Antimicrobial agents, drug-delivery materials	[139]
Egg yolk 33%	Low-density lipoprotein (LDL)	65.00	3 500	7.2		17–60 nm oil-in-water emulsified nanoparticles	Provide emulsification properties	Drug delivery materials, hypolipidemic agents	[141]
	High-density lipoprotein (HDL)	16.00	400	4.5		7–16 nm, the protein portion consists of four subunits that fold to form a hydrophobic funnel-shaped cavity	Lipid transporter protein	Hypolipidemic agents, hypoglycemic agents, anti-atherosclerotic	[105]
	Phosvitin (PV)	4.00	33–45	5	217	Highly phosphorylated glycoproteins	The most phosphorylated protein in eggs	Bone density enhancement and antioxidants	[113]
	Immunoglobulin Y (IgY)	10.00	180	5.7–7.6		Composed of four subunits, including two heavy chains and two light chains	No cross-reactivity in humans	Anti-bacterial, anti-viral, increase the immunity of the body	[90]

Note: pI, Isoelectric point.

Numerous egg white proteins have comparable physiochemical properties and molecular weights, which largely prevent the isolation and purification of specific functional proteins. In addition, the viscous nature of egg whites does not facilitate the chromatographic separation of individual proteins, making it difficult to achieve co-purification of multiple proteins and maintain the biological activity of functional proteins. Previously, salts such as ammonium sulfate, sodium chloride or potassium chloride were often used to separate OVA, OVT; however, the purity of the purified proteins was usually not adequate. The separation and purification of OVA, LYZ, and OVT were usually carried out by ion exchange chromatography[23], where OVT and LYZ could also be separated by affinity chromatography[24]. Although the chromatographic method could yield high-purity

proteins, it was unsuitable for large-scale industrial production due to its high price, slower speed, and lower yield. Currently, ultrafiltration has become an efficient method for the separation and purification of proteins from eggs. It has already been demonstrated that the purity of LYZ and OVA separated by ultrafiltration could reach more than 90%[25], but the operation conditions were more complicated. It has been reported that LYZ was separated by fpc3500 resin, followed by the isolation of OVMN from LYZ - free egg white. In recent years, electrodialysis of ultrafiltration film (EDUF) technology has gradually attracted the attention of scientists. Due to the ability to effectively separate natural active ingredients, EDUF was used to isolate and purify LYZ from model solutions OVA and LYZ. The total yield of LYZ obtained using the EDUF method was 32.2% with 100% purity after running at a voltage of 5 V for 240 min. However, EDFU has merely isolated lysozyme from the OVA model solution in previous work. The actual egg white proteins are more complex in composition; therefore, the purity of a single protein obtained using the EDFU method may not be 100%, which is somewhat limiting[26]. The precipitation of OVT was carried out by adding ammonium sulfate and citric acid to the egg-white-free LYZ solution. The separation of OVMD and OVA from the obtained supernatant was carried out with ethanol. Further purification of the isolated proteins was performed, yielding four proteins with purity higher than 90% except for OVMD, and the percentage yield was higher than 77%[27], which was a straightforward and efficient process that maintains the original spatial structure of the proteins. Currently, there are numerous innovative green and efficient separation methods for the major proteins in egg whites by cation exchange chromatography, which could ultimately result in yields of 60.0%, 52.1%, 29.6%, and 90.2% for OVMD, OVA, OVT and LYZ, respectively[28]. Therefore, the search for greener, more efficient and cost-effective isolation and purification methods has become a key direction to promote egg protein research to achieve greater breakthroughs in the field of food science.

3.2 Isolation and purification of major proteins from egg yolks

The major methods for the isolation and purification of PV from egg yolk include salt precipitation, salt leaching, membrane separation techniques, and the use of iron-based inorganic nanomaterials with affinity activity for the enrichment of PV[29]. Among them, the methods of salt precipitation and salt extraction were simpler, but the PV obtained is of lower purity. The enrichment of PV by membrane separation techniques and the use of affinity-active iron-based inorganic nanomaterials were relatively complex, but the purity of the enriched PV was relatively higher[30]. Extraction by salt precipitation, salt leaching, membrane separation techniques, and the use of inorganic nanomaterials were all effective means, while chromatography has been well established in this regard[31]. The majority of methods for purifying PV were based on chromatographic techniques (ion exchange chromatography, hydrophobic chromatography, gel chromatography). These methods vary in efficiency, but all of them could obtain a certain purity of PV[32]. It is necessary to avoid the contamination of protein hydrolyzing enzymes in the purification process of PV because the protein would be decomposed by the enzymes, which would lead to the decrease of purity. Meanwhile, the interaction between PV and metal ions *in vivo* needs to be further investigated.

The relative simplicity and low cost of the IgY preparation process make the isolation and purification of IgY more feasible on a production scale, offering the possibility of large-scale production. To isolate and purify IgY from egg yolk, the main methods include ammonium sulfate precipitation and polyethylene glycol precipitation. The advantages of the ammonium sulfate precipitation method are effective separation and more purified products, with yields up to more than 60% and purity up to more than 90%. In addition, the extraction and separation conditions of this method were relatively mild, which was conducive to maintaining the natural conformation and biological activity of IgY[25]. Furthermore, the process had a short cycle time, low distilled water consumption, and simple steps, making it suitable for large-scale and efficient production. The advantages of the polyethylene glycol precipitation method were that the equipment used was simple, easy to operate, and does not cause specific adsorption of proteins. However, the long precipitation time would lead to lower production efficiency. Moreover, the IgY extracted by polyethylene glycol precipitation was not pure enough and had a relatively low viability and concentration, which cannot meet the market demand[33]. Almeida et al.[34] employed label-free quantitative nano-LC-MS/MS to identify the proteins present in the water-soluble components of egg yolk. Subsequently, IgY was purified by centrifugal partition chromatography using polyethylene glycol and potassium phosphate buffer. By changing the ratio and concentration of polyethylene glycol to salt, the purity of purified IgY was improved, and the purity of the final product IgY could reach 50.6%. Notably, these methods had some limitations, such as the possibility of affecting the natural conformation and biological activity of IgY or requiring a large number of reagents and equipment. Recently, our team found that the combined technique of high-speed shear misflow membrane separation could isolate and purify egg yolk immunoglobulin of different purities on a large scale. Its isolation and purification yielded IgY with 96.9% purity and a yield of 6.06 mg/mL egg yolk. This method could provide high-purity IgY while reducing processing time and saving cost, which is more friendly to the environment[35]. The isolation and purification of IgY enables efficient capture of specific antigens and provides a unique research tool in the field of food safety. Therefore, the isolation and purification of IgY are of paramount importance for improving the efficiency of antibody acquisition, reducing production costs, as well as advancing the field of biomedical and food safety research.

3.3 Preparation and isolation of bioactive peptides from eggs

The idea that egg whites and yolks are excellent sources of bioactive peptides is widely recognized in the fields of food science and nutrition. Bioactive peptides in egg whites and yolks can be used as ingredients in functional foods for the maintenance of human health, the prevention of disease, and the treatment of illness[11,36]. Most common of the methods to obtain bioactive peptides was enzymatic digestion or heat treatment. Commercial enzymes (protease, trypsin, pepsin, alkaline protease and papain, etc.) were processed to obtain peptides with relatively high-specificity and without denaturation of proteins and residual of organic reagents or toxic chemicals; therefore, it had been the primary method for the production of bioactive peptides[24,37]. Nevertheless, the enzymatic method required strict control of reaction temperature and pH,

with expensive cost and relatively low peptide production. Microbial fermentation was also the conventional method for the preparation of bioactive peptides, which was relatively inexpensive; however, there were disadvantages of insufficient peptide yield and specificity in this method, which was not suitable for widespread promotion[38]. Obtaining peptides by chemical hydrolysis was a simple and inexpensive method, except that extremely elevated temperatures and pH conditions could deteriorate the nutritional value of the protein hydrolysate, making it unsuitable to produce bioactive peptides[39]. Chemical methods are also used to hydrolyze proteins due to low cost and time savings. In an environment far from the isoelectric point, globular proteins unfold and expose hydrophobic groups. Peptide bonds in proteins tend to break in this environment, resulting in peptides and free amino acids. Liu et al.[40] noticed that treatment of egg yolk hydrolysate with 60% (*V*/*V*) ethanol concentration contributed to the generation and accumulation of iron-chelated peptides. Subsequently, by collecting different fractions were able to obtain egg yolk protein hydrolysate with iron chelating capacity up to 87.32%. Moreover, the obtained egg yolk hydrolysate had better thermal and alkali stability. However, there are still a number of limitations to chemical hydrolysis because natural proteins are complex and it is difficult to fully control the reaction process as well as the end-products obtained under denaturing conditions. Besides, chemicals may also lead to the decomposition of some of the active components of natural proteins[37]. With the continuous development of technology, there are more advanced processing technologies for the production of bioactive peptides, like high hydrostatic pressure processing, ultrasound, microwave-assisted extraction, ohmic heating, pulsed electric field, microwave-assisted extraction, and subcritical water hydrolysis[41]. The treatment of egg white proteins with pulsed electric fields after enzymatic digestion with alkaline enzymes was able to obtain enzymatic products with antioxidant activity 1.2 times higher than that of the control group[42]. As for basic research, purposeful bulk synthesis of active peptides with known amino acid sequences by efficient and convenient chemical synthesis methods had been becoming widespread[43].

The purification methods are commonly used to purify small molecules of bioactive peptides, such as ultrafiltration, chromatography (exclusion chromatography, ion-exchange chromatography, affinity chromatography, adsorption chromatography, etc.), and high-performance liquid chromatography[37]. A combination of several purification steps, including low molecular membrane filtration and reversed-phase high-performance liquid chromatography, was used to purify 16 antioxidant peptides from the enzymatic hydrolysate of egg white hydrolyzed by protease (protease isolated from *Aspergillus oryzae*). Among them, peptides with sequences AEERYP and DEDTQAMP (Ala-Glu-Glu-Arg-Tyr-Pro and Asp-Glu-Asp-Thr-Gln-Ala-Met-Pro) showed the highest antioxidant activity[44]. It has been well documented in previous studies that egg-derived peptides possess impressive biological activities; however, the variety of peptides available for practical applications is still very limited. Their high production costs, low yields, and the complexity of their isolation and purification processes have hindered their large-scale commercial production. In addition, unpurified protein hydrolysates have shown favorable antioxidant, anti-inflammatory, and antimicrobial properties[45]; therefore, the direct use of these hydrolysates to enhance the bioactivity of food products may be an effective alternative.

4 Activity of proteins and their peptides in eggs

4.1 Activity of proteins and their peptides in egg whites of poultry eggs

4.1.1 Anti-hypertensive effect and immune enhancing mechanism of OVA

As the most abundant protein in egg whites[46], the unique surface and thermal properties of OVA played a critical role in the foaming, emulsification and gelation properties of egg whites[47–48]. Many biological activities have been confirmed for OVA (Figure 2). Heat-denatured OVA had been demonstrated to stimulate serum production of interleukin (IL)-12, IL-17, and IL-10 in mice to diminish inflammation and enhance immunity[49]. Furthermore, selenium-modified OVA (Se-OVA) could effectively reduce the damage of cancer cells to immune tissues. On the one hand, Se-OVA improved the proliferation of T and B-lymphocytes. On the other hand, Se-OVA blocked the circulation of solid tumor cells through the mitochondrial pathway and accelerated the apoptosis of solid tumor cells[50]. It is surprising that the modified OVA also had a wound-healing-promoting effect. Silver ion cross-linked thiolate OVA resulted in 96.23% wound healing in scalded mice. The expression of transforming growth factor β in the serum of the mice was increased by 1.29 times compared to the control group. In addition, thiolate OVA reduced the levels of inflammatory cells and inflammatory factors and stimulated collagen production. It accelerated the wound healing process by promoting the growth and proliferation of fibroblasts, blood vessels, and granulation tissue at the wound site[51]. After being hydrolyzed, OVA was capable of exerting stronger biological activity. Researchers identified peptides with inhibition of angiotensin converting enzyme (ACE) and dipeptidyl peptidase-4 in OVA by using biological information[52]. RADHOF peptide, produced by the hydrolysis of OVA by chymotrypsin, was found to have the property of promoting vasodilation, which could effectively decrease blood pressure and reduce the probability of cardiovascular diseases[53]. OVA could also be employed as a carrier for nutrient delivery in the gastrointestinal tract. The nanocarriers constructed from OVA and polysaccharides effectively avoid the destruction and degradation of active substances (anthocyanins and curcumin) in the stomach through hydrogen bonding and electrostatic interactions[54–55]. Papain-hydrolyzed OVA was demonstrated to stimulate RAW264.7 macrophage activation and regulate the expression of cytokine IL-6 via the MAPK pathway. *In vitro* simulated digestion of OVA hydrolysate was able to increase the immunomodulatory activity, indicating that OVA hydrolysate could be used as an effective immunoenhancer in the functional food industry[56].

4.1.2 Mechanism of mineral absorption promotion and immune enhancement by OVT

The molecules of each OVT could bind to two Fe^{3+}, which could promote iron absorption and thus prevent iron deficiency anemia[57]. The biological activity of OVT as well as its peptides had been extensively studied (Figure 3). In a previous study by our team, we found that supplementation of 400 mg/(kg·d) of OVT for 30 days in rats with iron deficiency anemia significantly elevated serum iron and serum ferritin levels in the blood of rats[58].

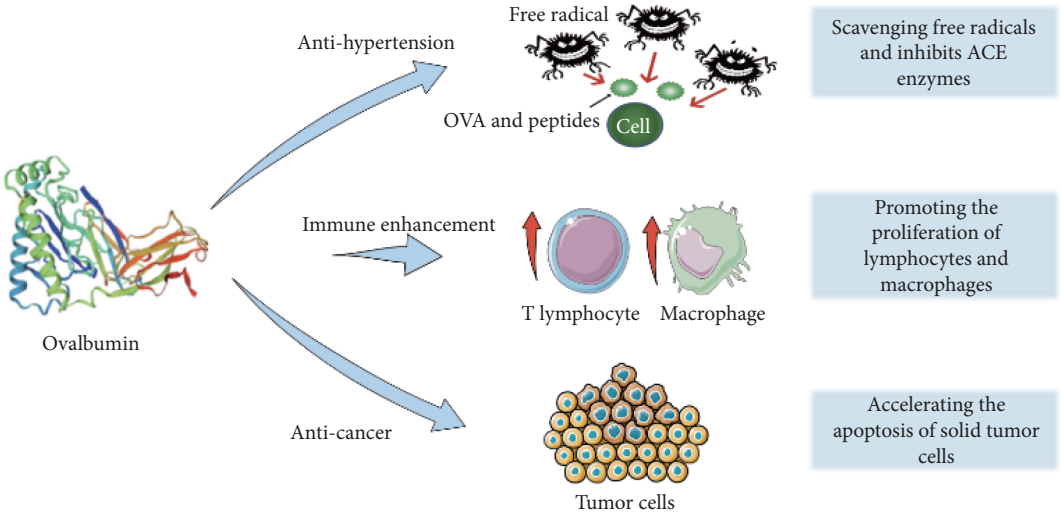

Figure 2 Blood pressure-lowering, immunity-boosting and anti-cancer properties of ovalbumin.

Moreover, the phosphorylated OVT could increase the solubility of calcium, thus promoting its absorption in the small intestine[59]. Other than promoting calcium absorption by the body, OVT also stimulated the proliferation and differentiation of osteoblasts and inhibition of osteoclast genesis *in vitro*, demonstrating that OVT was capable of preventing osteoporosis by mediating bone formation[60]. OVT promoted the maturation of intestinal dendritic cells in mice and exerted immunomodulatory effects against intestinal immunosuppressed mice by regulating the balance of Th1 and Th2 through the mitogen-activated protein kinase (MAPK) signaling pathway[61]. A recent study has investigated the effects of OVT on gastritis and the regulatory mechanisms on gastric epithelial cells (GES-1). Huang et al.[62] found that a low concentration of OVT (100 μg/mL) could significantly reduce the serum levels of inflammatory factors (IL-8, IL-6, and tumor necrosis factor α (TNF-α)). The protective effect of OVT on the stomach was also confirmed by the results of RNA sequencing. OVT might exert its anti-inflammatory effect through the MAPK and nuclear factor-kappa B (NF-κB) pathway. By preventing the activation of nuclear factors in the MAPK and NF-κB pathways, OVT down-regulated the secretion of the inflammatory factor IL-1, thus exerting an anti-inflammatory effect. As a natural protein, OVT has no toxic side effects on GES-1, making it a potential candidate for the treatment of gastritis[62].

Ile-Arg-Trp, hydrolyzed OVT-derived peptide, inhibited the increase of intercellular adhesion molecule-I and cell adhesion molecule-I in blood vessels induced by NF-κB pathway, as well as exerted antioxidant, anti-inflammatory activities and improved the immune status of the organism[63]. GWN and GW, isolated from OVT, were capable to protect embryonic kidney cells from dasatinib-induced mitochondrial damage as cytoprotective antioxidant peptides in a dose-dependent manner. Moreover, the levels of ROS were effectively reduced by GWN and GW to protect the organism from oxidative damage[64]. As members of the transferrin family, OVT and lactoferrin possessed several similar functional properties (enhanced iron adsorption, immunomodulation, antioxidant, anti-microbial)[65]; therefore, it is promising that OVT could be substituted as a potential alternative to compensate for lactoferrin in the general environment where lactoferrin is in short supply.

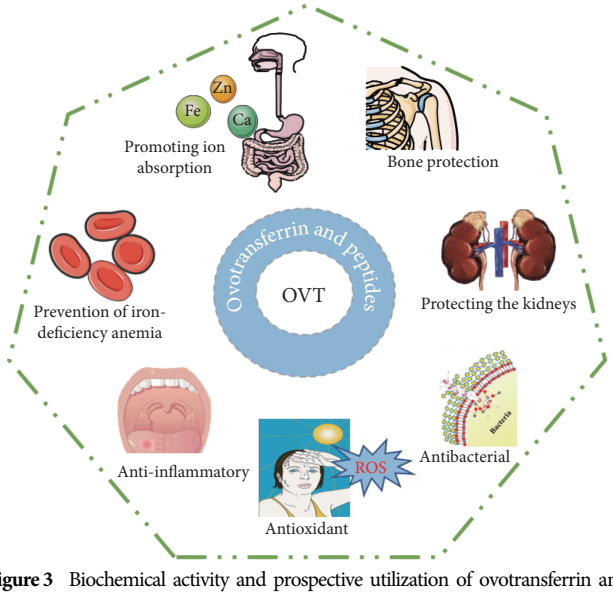

Figure 3 Biochemical activity and prospective utilization of ovotransferrin and its peptides.

4.1.3 *Antioxidant mechanism of OVMD*

OVMD, a complex protein belonging to the glycoprotein class, consists of stable α-helix and β-sheet in a spherical spatial structure. As a trypsin inhibitor, OVMD is not susceptible to digestion and decomposition in the stomach and intestine[66]. Therefore, OVMD could be employed to predict allergic reactions to eggs in humans. Measurement of IgE antibodies specifically bound to heated or untreated OVMD using an enzyme-linked immunosorbent assay was effective in predicting symptomatic egg allergic reactions[67]. However, it has been greatly limited in the development of OVMD's own bioactivity and its large-scale production in food products. Fortunately, the nutritional value of proteins could be significantly improved by enzymatic treatment. Hydrolysis of OVMD into small peptides eliminated its trypsin inhibitory activity and rendered it no longer allergenic. Excitingly, hydrolyzed OVMD also exhibited several biological activities. It was revealed that OVMD could be hydrolyzed by trypsin, pepsin, chymotrypsin, and papain to generate functional peptides with significant antioxidant activity

and ACE inhibitory activity[68]. Hydration of OVMD at 37 °C for 3 h was more likely to yield peptides with antioxidant-active amino acid sequences (Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu). In addition, the pepsin-treated OVMD hydrolysate displayed an inhibitory activity of about 80% against ACE[68–69], which had potential application in the prevention of cardiovascular diseases. Similarity, OVMD hydrolyzed by 1% papain or 1% alkaline protease produced peptides with strong ACE inhibitory activity. The products obtained by dual enzymatic hydrolysis (alkaline protease and trypsin) showed superior iron chelating ability and antioxidant activity than single enzymatic hydrolysis[69]. Whether single or dual enzymatic hydrolysis was employed to hydrolyze OVMD, hydrolysis products with different biological activities could be obtained. The hydrolysis products suitable for different application scenarios could be obtained by adjusting the treatment conditions. The further study of OVMD hydrolysates and their bioactive has provided a broad prospect for improving the high-value production of OVMD, and further research is expected to deepen the understanding of the properties of its hydrolysis products and promote its wider application in medicine, food and other fields.

4.1.4 Antimicrobial and regulatory organism inflammatory efficacy of OVMN

OVMN is a high molecular weight sulfated glycoprotein with more than 30% glycosylation, which is mainly found in the thick protein of egg white and could be categorized into α- and β-OVMN forms[70]. The adhesive nature of OVMN permitted it to effectively prevent the migration of microorganisms and protected against food poisoning associated with bacterial infections[71]. OVMN, as a prophylactic agent for bacterial gastroenteritis, could exert antimicrobial activity by disrupting the permeability of cell membranes of *Staphylococcus aureus, Staphylococcus* spp. and *Streptococcus mutans*, delaying the logarithmic phase of bacterial growth or shortening the stabilization phase[70]. Similarly, the sialic acid in OVMN could act as an antiviral agent, competitively binding to the recognition site of the avian influenza virus and inhibiting the infection of the host by the virus[72]. In terms of regulating inflammation, OVMN improved the immune status through stimulating the proliferation of macrophages and lymphocytes, which in turn promoted the synthesis of cytokines[73–74]. OVMN was homologous to the transmembrane glycoprotein Muc2 in the colonic mucus layer[71]. It could inhibit the production of TNF-α and IL-6 through a Toll-like receptor signaling pathway, effectively repairing the villi and intestinal barrier in mice with colitis. Supplementation of OVMN could increase the relative abundance of beneficial bacteria such as *Lactobacillus* and *Enterococcus faecalis* in the intestine of mice, which in turn increased the content of short-chain fatty acids and further promoted the proliferation of small intestinal epithelial cells and cupula cells, effectively ameliorating colitis (Figure 4)[74]. The enzymatic hydrolysate of OVMN could likewise protect the intestinal barrier and attenuate the inflammatory response. In the LPS-induced Caco-2 cell injury model, the hydrolyzed product of OVMN effectively alleviated the inflammatory injury and promoted the proliferation of Caco-2 cells[75]. The hydrolyzed product of OVMN was also proven to have powerful antioxidant effects, which might be used as an antioxidant in food processing[76]. The regulation of intestinal flora by OVMN effectively reduced body inflammation and enhanced body resistance. Meanwhile, the modulatory effect of OVMN on intestinal microbial homeostasis contributed to a greater understanding of the potential value of avian egg proteins in exerting immune health functions.

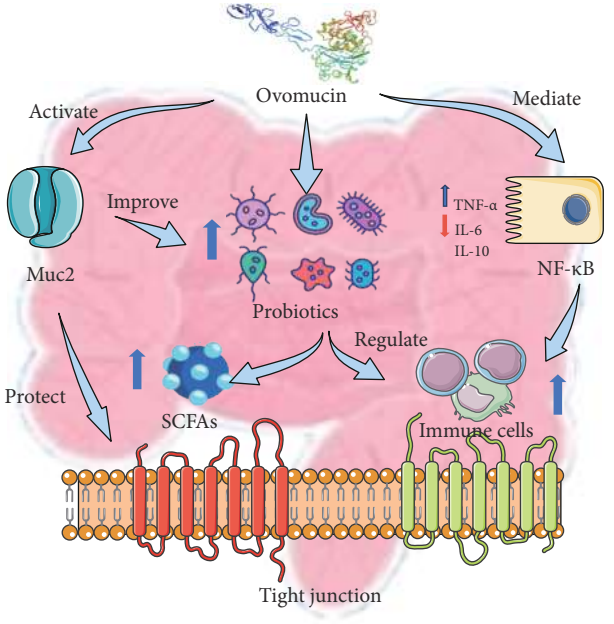

Figure 4 Protective effect of ovomucin on intestinal immunity and effect on intestinal flora in mice.

4.1.5 Antimicrobial effect and amelioration of immunodeficiency efficacy of LYZ

LYZ, a one-linked polypeptide formed by cross-linking four disulfide bonds (Figure 1), is also known as a cell membrane enzyme. It has been demonstrated that LYZ could hydrolyze the β-1,4 glycosidic bond between *N*-acetylcytidylic acid and *N*-acetylglucose in the peptidoglycan layer of the cell wall of Gram-positive bacteria, thus making the bacteria more susceptible to lysis[77]. Polypeptides with amino acid residues 15–21 and 98–108 in LYZ were shown to have antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*[78]. Furthermore, excellent inhibitory effect of LYZ on *Streptococcus pyogenes* made it possible to be applied in wound healing. LYZ promoted the growth of mouse fibroblasts, while also regulating cytokine levels and reducing inflammation[77]. Glycosylated LYZ could mediate the expression of TNF-α, IL-1β, and IL-8 in mice macrophage RAW264.7 cell line through JNF, ERK, and NF-κB pathways and improved the immunosuppression status of the organism[79]. LYZ has a high proportion of hydrophobic (43.4%) and basic (13.7%) amino acid residues, with the C-terminal residues of its peptide (Tyr and Trp) exhibiting excellent inhibitory activity against ACE as well as 1,1-diphenyl-2-acrylohydrazyl (DPPH) radical[80]. In addition, self-assembled nano-LYZ synthesized using the desolvation technique had exhibited certain anticancer activities. The nanostructured LYZ could kill 95% of human breast cancer cells by regulating the production of reactive oxygen species, effectively reducing tumor growth and preventing tumor metastasis[81]. As a potential therapeutic agent for static mechanical pain, LYZ could alleviate static mechanical pain by modulating the NRF1-Parkin-TACAN signaling pathway in sensory neurons. LYZ could stimulate the expression of Parkin in sensory neurons and reduce

TACAN membrane transport in sensory neurons. Furthermore, LYZ could moderate the level of inflammation in the body, while alleviating the static mechanical hypersensitivity response, to alleviating the static mechanical abnormal pain caused by nerve injury[82]. As a natural anti-infective substance with antiseptic effects, LYZ had antibacterial, antiviral, hemostatic, anti-swelling and analgesic effects, and accelerated tissue recovery functions[83]. Tablets containing LYZ were used to treat pharyngitis and mouth ulcers, in addition to being used as a preservative for foods such as wine, cheese and sausages. Moreover, LYZ had excellent inhibitory effect on human melanoma and had no toxic effect on normal keratinocytes[84]. Recently, increasingly researches had focused on fibrillated LYZ. Inhibitory effects of fibrillated LYZ against *Staphylococcus aureus* and *Escherichia coli* were remarkably stronger than those of non-fibrillated LYZ[85]. The excellent antimicrobial activity of fibrous LYZ not only provides a wide range of possibilities for its application in antimicrobial food packaging and antibiotic substitutes, but also offers potential opportunities for researchers to explore novel applications in medical, hygiene and sanitary products, as well as environmental hygiene. Its unique structure and bioactive properties open up a whole new direction for future scientific research and industrial innovation, and provide strong support for the establishment of a healthier and more sustainable production and lifestyle.

4.2 Biological activity of proteins and their polypeptides in poultry egg yolk

4.2.1 Antibacterial and immune enhancing mechanisms of IgY

IgY is composed of an Fc fragment (signal recognition and transmission) and two Fab fragments (antigen binding region)[86]. The structure and function of IgY in avian eggs are particularly like IgG in mammals[87]; otherwise, these two immunoglobulins are biologically distant from each other. Therefore, oral administration of IgY in mammals would not elicit a complementary response, making IgY an effective agent for the treatment of bacterial or viral infections in humans[88]. Sheng et al.[89] from our laboratory detected two potential *N*-glycosylation sites on IgY by nanoliter liquid chromatography-mass spectrometry and found that *N*-glycosylation could effectively maintain the structure of IgY and improve its stability in gastric juice. Subsequently, our group found that the Fab fragment in IgY could enhance the body immunity by blocking NF-κB and MAPKs pathways to regulate TLR4 and αvβ3 integrin-mediated inflammatory processes in the lipopolysaccharide-induced RAW264.7 macrophage[90]. Additionally, the gel containing 2% IgY showed excellent inhibition of caries-causing *Streptococcus pyogenes* and could effectively minimize the risk of caries[91]. In the presence of intestinal digestive enzymes, IgY was degraded by small molecule fragments (Fab and Fc). These fragments were absorbed by the intestine and enter the bloodstream, where they were capable of binding to the adhesion factors of specific pathogenic bacteria. This binding action prohibited the pathogenic bacteria from adhering to susceptible cells, while preventing their movement and travel, ultimately rendering the pathogenic bacteria pathogenic[92]. Similarly, it was effective that IgY could against oral diseases including periodontitis, gingivitis, and dental caries caused by bacteria like *Streptococcus pyogenes* and *Candida albicans*[92]. Continuous administration of tablets containing 72 mg of specific IgY for 8 weeks significantly reduced the frequency and severity of gingival bleeding, as well as the number of *Porphyromonas gingivalis* in patients with chronic periodontitis, which was effective in ameliorating periodontitis[93]. Numerous clinical trials have shown that oral IgY is effective in preventing and treating neonatal diarrhea and vomiting caused by rotavirus. Specialized IgY could prevent rotavirus from adsorbing to the body cells and enhance phagocytosis of macrophages by modifying the configuration of the virus surface[94]. Similarly, as a relatively readily available and inexpensive immunoglobulin, IgY has shown excellent therapeutic efficacy in eliminating the side effects of snake venom. After entering the human body, snake venom would bind to the receptors on the surface of human cells, and then enter the cells to produce toxic effects. Fortunately, IgY was capable of binding to snake venom and blocking its association to cellular receptors, thus preventing the venom from entering the cell and causing toxic effects. The specific IgY (96 μL) was able to neutralize 100% of the edema caused by *B. arietans* venom; meanwhile, the coagulation caused by *B. arietans* venom was also prevented 100% by the specific IgY[95]. These results demonstrate the potential of IgY as an alternative treatment for snake venom. Complementing the treatment and prevention of disease, IgY might also contribute to healthy weight loss by suppressing the activity of pancreatic lipase to augment the excretion of triglycerides[96]. Part of the biological activity of IgY had been presented in Figure 5.

Moreover, as a relatively high-stability immunoglobulin, IgY was capable of neutralizing SARS-CoV in VERO cells[97]. IgY could maintain activity in the upper respiratory tract of mice for several hours when formulated as an anti-SARS-CoV-2 nasal spray. The anti-SARS-CoV-2 IgY targeted stinging proteins and did not have toxic effects or any negative impact on rats 28 days after delivery[98]. In addition, it was capable of demonstrating a well-established safety profile in human clinical trials due to the distant relationship between anti-SARS-CoV-2 receptor binding domain IgY and IgG, and the absence of cross-reactivity. However, the cost of preparing specific anti-SARS-CoV-2 receptor binding domain IgY vaccine was relatively expensive and the production process was complicated, making it difficult to produce on a large scale[99]. With the deepening understanding of IgY, a relatively complete and unified technical model has been formed for IgY technology. IgY antibody-related products had formed a certain industrial base worldwide. Diagnostics and treatment of emerging acute pathogens might inspire the development of IgY products in the foreseeable future, based on breakthroughs in the development of IgY in diagnostic tests, pharmaceuticals, veterinary drugs, pet food, toothpaste and other areas.

4.2.2 Lipid regulation and glucose regulation mechanisms of lipoprotein

HDL is mainly composed of phospholipids, free cholesterol, cholesteryl esters, and apolipoprotein A-I[100]. HDL in yolk is capable of providing essential nutrients, including lipids, sugars, and proteins, to the embryo during its development[101]. HDL had been shown to chelate with Zn^{2+} and degrade during embryonic development, releasing Zn^{2+} into the developing embryonic system and preventing malformations during embryonic development by this pathway[102]. The extraordinary ability of HDL to remove atherosclerotic plaque made it a pivotal player in the treatment and prevention of cardiovascular disease[103]. Apolipoprotein A-I in HDL could regulate the metabolism of plasma lipoproteins and the reverse cholesterol transport by interacting with cell membrane

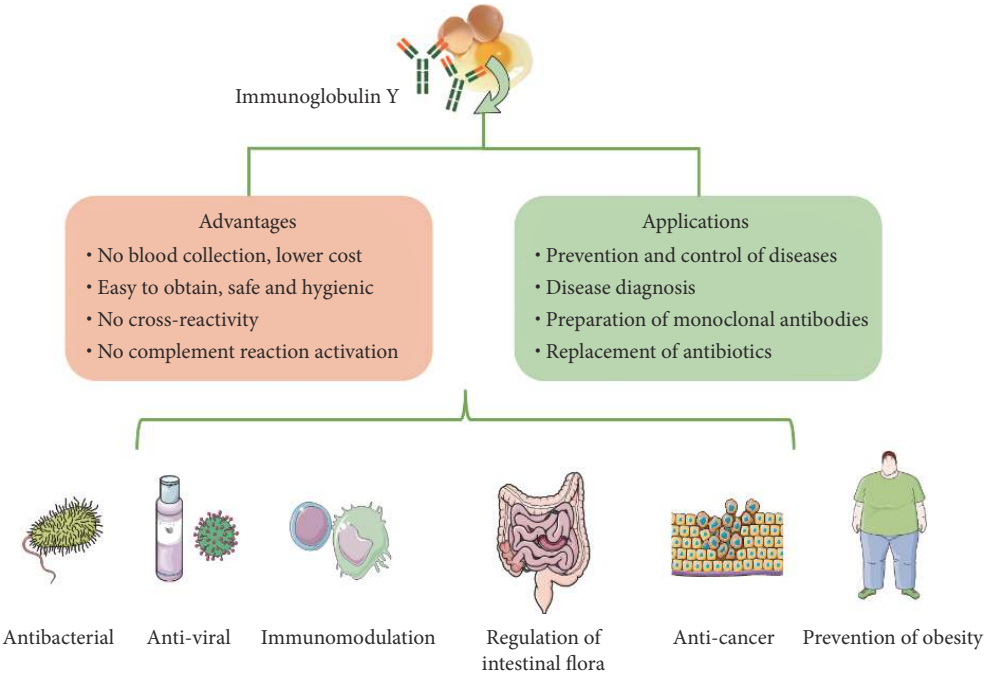

Figure 5 The bioactivities and potential applications of immunoglobulin Y.

receptors and activating lecithin-cholesterol acylase, thus lowering cholesterol in blood vessels[104]. Previous studies in our laboratory had revealed that HDL significantly decreased weight gain, abdominal adipose tissue accumulation, and the concentration of serum triglycerides in obese mice. In addition, HDL could raise the tolerance of the body to glucose and insulin while increasing the concentration of serum HDL cholesterol[105]. Egg yolk HDL exhibits a structure akin to human serum HDL, with a noted positive correlation observed between egg yolk intake and elevated serum HDL levels. Focusing on enhancing serum HDL function and considering HDL's documented antibacterial and antioxidant capabilities, investigating the impact of egg yolk HDL intake on human serum HDL metabolism holds significant importance. Subsequent studies on egg yolk HDL should further explore its structural attributes and potential bio-nutritional activities, thereby paving the way for future research directions in this domain.

Moreover, egg yolk HDL has more protein and less lipid compared to LDL, which allows it to have better affinity and loading capacity in binding bioactive compounds. It is possible that a nano-delivery system consisting of egg yolk HDL compounded with chitosan could potentially inhibit the release of curcumin in the stomach, resulting in the release of curcumin in the intestine[106].

4.2.3 Enhancement of bone density and antioxidant effect of PV

PV, a phospholipid protein, constitutes about 11% of yolk protein and is mainly composed of α-phospholipid protein and β-phospholipid protein[102]. Our team has conducted extensive research on PV and PV phosphorylated peptides (PPP), and discovered their prominent advantages in chelating metal ions, antioxidant activity, antibacterial activity, promoting biological mineralization, and activation[107]. PV bound with multiple phosphates and readily incorporates various cations such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+} in solution[108]. Glu-Asp-Asp-pSer-pSer, derived from PV, was capable of efficiently binding up to (468 ± 2.80) mg/g of calcium. Glu-Asp-Asp-pSer-pSer and Ca^{2+} were proven to be combined through electrostatic interactions. The addition of Ca^{2+} induced the folding of the peptide, resulting in the formation of ordered crystals. In addition, the bioavailability of Ca^{2+} bound to PPP had been shown to be superior to conventional calcium supplements such as calcium carbonate and calcium lactate. Thus, the combination of PPP with calcium as an ingredient in calcium supplements may contribute to more efficient absorption and utilization of calcium by the body[109]. Likewise, PPPs obtained by trypsin digestion promoted calcium absorption more effectively in comparison to commercially available casein phosphopeptides. Additionally, it was revealed that PV effectively controlled bone mineralization by regulating the expression of osteogenic protein-2 and osteoprotegerin mRNA in osteoblasts, leading to the amelioration of osteoporosis (Figure 6)[30,110]. Phosphorylated serine residues in PV could combine with Ca^{2+} to form soluble complexes which would reduce the loss of Ca^{2+} in the intestine. By facilitating intestinal absorption of Ca^{2+}, PV had promoted bone mineralization and elevated the bioavailability of calcium[111–113]. Moreover, it is observed that PV and its hydrolysis products exhibited superior antioxidant activity. PPPs were identified to reduce oxidative stress *in vitro* by modulating glutathione synthesis and antioxidant enzyme activity[114]. In a model of H_2O_2-induced Caco-2 cells, PPPs could upregulate the expression of antioxidant genes and promote the secretion of antioxidant enzymes, reducing oxidative stress and protecting cells from oxidative damage[114–115]. Lee et al.[116] found that PPP obtained by enzymatic hydrolysis (trypsin) after high temperature and light pressure pretreatment had the efficacy of reducing elastase and tyrosinase activities in B16F10 melanoma cells. In addition, melanin levels in melanoma cells decreased from 31.18% to 38.58% after the intervention of PPP. What's more, PPP also downregulated the expression of proteases associated with inflammation and secretion of NO. Therefore, PPP could be widely used as a potential anti-melanogenic, anti-elastase, and anti-inflammatory agent in human and skin care products. Moreover, the peptide Pt5-1c, derived from PV, has been shown to have multiple functions. Si et al.[109] demonstrated the ability of Pt5-1c to promote the proliferation of

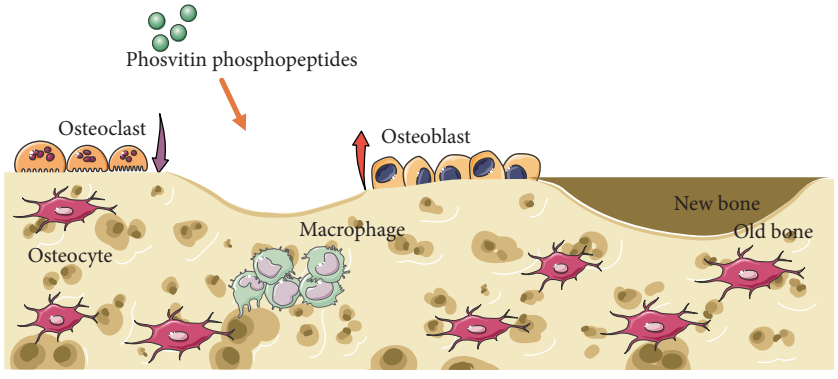

Figure 6 Schematic diagram of phosvitin phosphorylated peptide promoting osteoblast proliferation.

fibroblasts and to accelerate wound healing in mice by means of cellular and animal assays, respectively. In addition, Pt5-1c could stimulate the activation of fibroblasts into proto-myofibroblasts and myofibroblasts, thereby promoting the formation of granulation tissue as well as the remodeling of the extracellular matrix. Therefore, it can be considered that Pt5-1c might be a drug candidate with great potential to promote wound healing. Besides, Pt5-1c has other potentials. It has been found to be a therapeutic agent and adjuvant for the treatment of infectious diseases. Duan et al.[117] found that Pt5-1c had superior antimicrobial properties, rupturing bacteria by disrupting their outer walls and increasing the permeability of their membranes. It was also discovered that when combined with conventional antibiotics, Pt5-1c facilitated the entry of the antibiotics into the cells and demonstrated a satisfactory synergistic effect[117]. Currently, research on PV primarily focuses on its role in promoting mineral absorption in the body, and there is clear and direct evidence indicating that PV contributes to bone growth, thereby reducing the occurrence of osteoporosis. However, enhancing the biological availability of PV and precisely controlling its release, along with its peptides, in the intestine remain crucial focal points for researchers.

4.2.4 Egg yolk enzymatic digestion products

The application of enzymatically hydrolyzed egg yolks in food processing stems mainly from their unique flavor and excellent functional properties. Through the treatment of egg yolks with specific protein hydrolyzing enzymes, it can be broken down into peptides and amino acids that are smaller more digestible and absorbable by the human body. This process not only adds flavor to food, but also improves its nutritional value in many ways. The proteins in egg yolks are decomposed into smaller molecular fragments through enzymatic digestion. These molecules are highly biologically active and do not require the use of complex purification, making them superior ingredients in the production of functional foods. In addition, enzymatic digestion of egg yolks also contributes to enhancing the nutritional value of food products. During the enzymatic process, the enzymes can decompose large protein molecules, which originally cannot be digested and absorbed by the human body, into easily absorbed forms, thus improving the nutritional value of food[118]. The water-soluble peptides obtained after enzymatic digestion of egg yolk were effective in inhibiting the production of osteoclasts through *in vitro* experiment[119]. Similarly, it has been demonstrated that egg yolk hydrolysis products obtained by trypsin treatment had a remarkable impact on the differentiation and mineralization of osteoblasts in ovariectomized rats and resulted in a 9.1% enhancement of alkaline phosphatase activity[120]. In addition, PF201, an orally administered peptide extracted from egg yolk, was capable of reducing the occurrence of bone degeneration. The results of cellular assays demonstrated that PF201 promoted osteoblast proliferation and promoted bone formation in mice. Administration of D2, a metabolite of peptide PF201, equally accelerated bone healing in a mice fracture model and promoted the proliferation and differentiation of bone marrow mesenchymal stem cells[121]. By these, the potential of egg yolk hydrolyzed products in promoting bone growth as well as in combating osteoporosis was demonstrated. Hydrolyzed egg yolk powder was available everywhere and was indispensable for regulating the balance of bone metabolism[122]. In regions like China, Japan, and Korea, hydrolyzed egg yolk powder was commonly supplemented into milk, milk powder, and other products to promote bone growth and development in infants and children as a new resource food. In addition, it has been discovered that egg yolk hydrolysate could effectively chelate with ferrous ions and showed better scavenging ability for DPPH radicals, which could effectively delay the oxidative reactions of the organism, suggesting the potential utilization of egg yolk enzymatic products in slowing down aging[123]. There is a significant number of active peptides in enzymatic yolk. Marcet et al.[124] has identified eight peptides with anti-ACE activity in enzymatic yolk. Besides, the enzyme digestion product was obtained by hydrolyzing skimmed egg yolk powder with 1% pepsin (37 °C, pH 2, 4 h), followed by centrifugation and collection of the supernatant. The enzyme-digested egg yolk similarly had powerful inhibitory activity against *Staphylococcus aureus* and *Salmonella typhi*[125]. The two-step hydrolyzed egg yolk protein equally enhanced the immune activity of RAW264.7 and regulated the secretion of cytokines in macrophages. Enzymatic hydrolysis of egg yolk protein using trypsin and neutralizing enzymes yielded enzymatic products that were found to be responsible for the activation of RAW264.7 macrophages through the TLR-2/p38 and JNK pathways, increasing NO and TNF-α production as well as phagocytosis activity[126]. Research on the bioactivity of egg yolk hydrolysates is steadily increasing, which has the potential to facilitate the large-scale production and promotion of high-value poultry egg products. However, researchers should also focus on exploring the practical applications of poultry egg proteins and their peptides.

4.3 Activity of proteins and their polypeptides in poultry eggshell membranes

The eggshell membrane consists of 80%–85% organic and 10%–15% inorganic substrates[127]. In the organic matrix, 10% of the

components are collagen (type I, V and X) and 70% are minor proteins (bone bridging proteins, keratin, fibronectin, and glycoproteins)[128]. Besides, eggshell membranes contain bioactive components such as glycosaminoglycans, sulfated glycoproteins, hyaluronic acid, OVT, sialic acid, lysyl oxidase, LYZ, and *β*-*N*-acetylamino glucosidase[129]. There has been an emerging interest in developing and exploiting the functional activity of eggshell membranes as a by-product of eggs. Eggshell membranes are particularly suitable for repairing damaged connective tissue, owing to the similarity of the hyaluronic acid, keratin and collagen in eggshell membranes to the natural extracellular matrix. Li et al.[130] in our laboratory had revealed that a wound dressing made of eggshell membrane powder compounded with chitosan showed remarkable recovery effects on mouse wounds. The dressing shortened the wound healing cycle by inhibiting the inflammatory response and promoting cell proliferation at the wound site in mice. Apart from being used for wound restoration, the collagen in eggshell membranes could effectively repair, therapeutic, and healing skin burns[131–133]. Similarly, the proteins in egg shell membranes and their hydrolyzed peptides played a crucial role in antioxidant damage. The hydrolysis products of eggshell membrane, a powerful novel nutritional peptide, have demonstrated promising oxidative stress activity against Caco-2 cells could effectively scavenge hydroxyl radicals, superoxide anions[134]. Furthermore, nanoparticles that consisted of eggshell membrane proteins complexed with chitosan-rock polysaccharide could effectively inhibit the expression of the pro-inflammatory factor IL-6. Such nanoparticles might be exploitable for the protection of the prevention of intestinal barrier and treatment of inflammatory bowel disease[135]. Eggshell membrane-hydroxyapatite composition prepared using biomimetic mineralization technology not only facilitated the proliferation, adhesion, and spreading of MC3T3-E1 cells, but also promoted the expression of bone-related genes and proteins (dwarf-related transcription factor 2, type I collagen, and osteocalcin), which were anticipated to be excellent bone repair materials[136]. The global collagen peptides market size was expected to grow at a CAGR of 5.8% to reach $795 million by 2025, according to the findings of the market research firm *Markets and Markets* published in June 2020. Therefore, the high-value utilization of poultry egg by-products could be realized by carrying out series products such as poultry egg shell membrane organic calcium, egg membrane peptide solid beverage, and egg membrane wound healing composite membrane. Certainly, the development of these new products and technologies will likewise further expand the application prospects of shell films.

5 Conclusion

Part of poultry egg proteins have been used for medical applications on an industrial scale owing to their affordability, accessibility, nutritional richness, and accessibility to digestion. A daily intake of poultry eggs is a simple and effective strategy to prevent metabolic diseases and atherosclerosis. Peptide has become another vital object of life science research object after gene with the rapid development of biotechnology. At present, peptide products derived from poultry egg proteins have been widely used in numerous fields such as medicine, food, health care products, cosmetics, and biological materials. It is evident that peptide-based functional foods would occupy an essential market pattern in the future health industry. Administration of complete poultry egg hydrolysates is likely to yield combined biological effects compared to individual isolated peptides from poultry eggs. However, there are few studies on intact poultry egg hydrolysates *in vitro* and *in vivo*. Therefore, focusing the research on the active functions played by the whole hydrolysate of poultry egg proteins in organisms will contribute to further increasing the value of poultry eggs and provide novel insight into the prevention and treatment of chronic diseases.

Author contributions

Xiaomeng Li: formal analysis; investigation; resources; writing - original draft. **Minquan Xia**: investigation; writing - review & editing. **Qi Zeng**: investigation; writing - review & editing. **Haoyang Sun:** investigation; writing - review & editing. **Xi Huang**: writing - review & editing. **Dong Uk Ahn:** writing - review & editing. **Mohamed Salama:** writing - review & editing. **Fayez Khalaf Mourad:** writing - review & editing. **Zhaoxia Cai**: conceptualization; funding acquisition; project administration; resources.

Conflict of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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References

[1] D. S. Chauhan, R. Prasad, R. Srivastava, et al., Comprehensive review on current interventions, diagnostics, and nanotechnology perspectives against SARS-CoV-2, Bioconjug. Chem. 31 (2020) 2021–2045. https://doi.org/10.1021/acs.bioconjchem.0c00323.

[2] S. Gerber, M. O'Hearn, S. M. Cruz, et al., Changes in food security, healthfulness, and access during the coronavirus disease 2019 pandemic: results from a National United States Survey, Curr. Devel. Nutrit. 7 (2023) 100060. https://doi.org/10.1016/j.cdnut.2023.100060.

[3] T. R. Pakki, N. Mariana, M. L. Tampubolon, et al., Side effects after mRNA COVID-19 vaccine as a booster in health workers, Iran. J. Public Health 51 (2022) 2504–2509. https://doi.org/10.18502/ijph.v51i11.11167.

[4] R. Thirumdas, A. Kothakota, R. Pandiselvam, et al., Role of food nutrients and supplementation in fighting against viral infections and boosting immunity: a review, Trends Food Sci. Technol. 110 (2021) 66–77. https://doi.org/10.1016/j.tifs.2021.01.069.

[5] Z. Wang, Y. Liu, L. Wei, et al., What are the risk factors of hospital length of stay in the novel coronavirus pneumonia (COVID-19) patients? A survival analysis in southwest China, PLoS ONE 17 (2022) e0261216. https://doi.org/10.1371/journal.pone.0261216.

[6] C. L. McIntyre, A. Temesgen, L. Lynch, Diet, nutrient supply, and tumor immune responses, Trends Cancer 9 (2023) 752–763. https://doi.org/10.1016/j.trecan.2023.06.003.

[7] S. Basak, J. Gokhale, Immunity boosting nutraceuticals: current trends and challenges, J. Food Biochem. 46 (2022) e13902. https://doi.org/10.1111/jfbc.13902.

[8] K. Kolbe, Mitigating climate change through diet choice: costs and CO_2 emissions of different cookery book-based dietary

options in Germany, Adv. Clim. Chang. Res. 11 (2020) 392–400. https://doi.org/10.1016/j.accre.2020.11.003.

[9] X. H. Lv, X. Huang, B. Ma, et al., Modification methods and applications of egg protein gel properties: a review, Compr. Rev. Food Sci. F. 21 (2022) 2233–2252. https://doi.org/10.1111/1541-4337.12907.

[10] X. Li, Z. Li, X. Zhang, et al., Restoration of immunity by whole egg was superior to egg white or egg yolk in a cyclophosphamide-induced immunocompromised mouse model, Food Biosci. 50 (2022) 102013. https://doi.org/10.1016/j.fbio.2022.102013.

[11] S. Jalili-Firoozinezhad, M. Filippi, F. Mohabatpour, et al., Chicken egg white: Hatching of a new old biomaterial, Materials Today 40 (2020) 193–214. https://doi.org/10.1016/j.mattod.2020.05.022.

[12] R. Huopalahti, R. López-Fandiño, M. Anton, et al., Bioactive Egg Compounds. Springer Berlin Heidelberg, 2007, pp. 99–102.

[13] E. D. N. S. Abeyrathne, H. Y. Lee, D. U. Ahn, Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents: a review, Poultry Sci. 92 (2013) 3292–3299. https://doi.org/10.3382/ps.2013-03391.

[14] C. Guerin-Dubiard, M. Pasco, D. Molle, et al., Proteomic analysis of hen egg white, J. Agric. Food Chem. 54 (2006) 3901–3910. https://doi.org/10.1021/jf0529969.

[15] Y. Cui, X. Li, M. Lu, et al., Role of polysaccharide conjugation in physicochemical and emulsifying properties of egg phosvitin and the calcium binding capacity of its phosphopeptides, Food Funct. 10 (2019) 1808–1815. https://doi.org/10.1039/c8fo02464b.

[16] H. Sugino, M. Ishikawa, T. Nitoda, et al., Antioxidative activity of egg yolk phospholipids, J. Agric. Food Chem. 45 (1997) 551–554. https://doi.org/10.1021/jf960416p.

[17] X. He, J. Wang, Y. Wang, et al., Quantitative lipidomic analysis of egg yolk, yolk granule, and yolk plasma, J. Food Compos. Anal. 115 (2023) 104880. https://doi.org/10.1016/j.jfca.2022.104880.

[18] J. Zheng, T. Bu, L. Liu, et al., Naturally occurring low molecular peptides identified in egg white show antioxidant activity, Food Res. Int. 138 (2020) 109766. https://doi.org/10.1016/j.foodres.2020.109766.

[19] S. Arena, G. Renzone, A. Scaloni, A multi-approach peptidomic analysis of hen egg white reveals novel putative bioactive molecules, J. Proteomics. 215 (2020) 103646. https://doi.org/10.1016/j.jprot.2020.103646.

[20] C. J. Sun, J. N. Liu, W. B. Li, et al., Divergent proteome patterns of egg albumen from domestic chicken, duck, goose, turkey, quail and pigeon, Proteomics 17 (2017). https://doi.org/10.1002/pmic.201700145.

[21] S. Arena, A. Scaloni, An extensive description of the peptidomic repertoire of the hen egg yolk plasma, J. Agric. Food Chem. 66 (2018) 3239–3255. https://doi.org/10.1021/acs.jafc.8b01183.

[22] T. A. E. Ahmed, H. P. Suso, M. T. Hincke, In-depth comparative analysis of the chicken eggshell membrane proteome, J. Proteomics 155 (2017) 49–62. https://doi.org/10.1016/j.jprot.2017.01.002.

[23] J. Haginaka, Chromatographic separations and analysis: protein and glycoprotein stationary phases, in: Reference module in chemistry, molecular sciences and chemical engineering, 2022, Elsevier.

[24] C. Chang, T. Lahti, T. Tanaka, et al., Egg proteins: fractionation, bioactive peptides and allergenicity, J. Sci. Food Agric. 98 (2018) 5547–5558. https://doi.org/10.1002/jsfa.9150.

[25] X. M. Jiang, T. Diraviyam, X. Y. Zhang, Affinity purification of egg yolk immunoglobulins (IgY) using a human mycoplasma protein, J. Chromatogr. B 1012 (2016) 37–41. https://doi.org/10.1016/j.jchromb.2016.01.012.

[26] L. Q. Sun, H. X. Lu, J. Y. Wang, et al., Electroseparation of lysozyme from egg white by electrodialysis with ultrafiltration membrane, Sep. Purif. Technol. 317 (2023) 123710. https://doi.org/10.1016/j.seppur.2023.123710.

[27] S. Ji, D. U. Ahn, Y. Zhao, et al., An easy and rapid separation method for five major proteins from egg white: successive extraction and MALDI-TOF-MS identification, Food Chem. 315 (2020) 126207. https://doi.org/10.1016/j.foodchem.2020.126207.

[28] X. Ma, R. Liang, X. Yang, et al., Simultaneous separation of the four major allergens of hen egg white, J. Chromatogr. B 1152 (2020) 122231. https://doi.org/10.1016/j.jchromb.2020.122231.

[29] J. Zhang, J. Sun, Y. Liu, et al., Separation and purification of phosvitin phosphopeptides using immobilized metal affinity nanoparticles, J. Chromatogr. B. 893/894 (2012) 121–126. https://doi.org/10.1016/j.jchromb.2012.02.046.

[30] X. Zhang, X. Huang, M. Ma, Role of phosphorylation of phosvitin in the phase transformation of mineralization, Int. J. Biol. Macromol. 101 (2017) 712–718. https://doi.org/10.1016/j.ijbiomac.2017.03.158.

[31] B. Lei, J. Wu, Purification of egg yolk phosvitin by anion exchange chromatography, J. Chromatogr. A 1223 (2012) 41–46. https://doi.org/10.1016/j.chroma.2011.12.023.

[32] H. Y. Lee, E. D. N. S. Abeyrathne, I. Choi, et al., Sequential separation of immunoglobulin Y and phosvitin from chicken egg yolk without using organic solvents, Poultry Sci. 93 (2014) 2668–2677. https://doi.org/10.3382/ps.2014-04093.

[33] E. M. Redwan, A. A. Aljadawi, V. N. Uversky, Simple and efficient protocol for immunoglobulin Y purification from chicken egg yolk, Poultry Sci. 100 (2021) 100956. https://doi.org/10.1016/j.psj.2020.12.053.

[34] M. R. Almeida, F. Ferreira, P. Domingues, et al., Towards the purification of IgY from egg yolk by centrifugal partition chromatography, Sep. Purif. Technol. 299 (2022) 121697. https://doi.org/10.1016/j.seppur.2022.121697.

[35] M. Xia, C. Liu, D. U. Ahn, et al., Large-scale isolation and purification of yolk immunoglobulin with different purity levels via a combination technique based on high-speed-shear crossflow membrane separation, Food Hydrocoll. 140 (2023) 108618. https://doi.org/10.1016/j.foodhyd.2023.108618.

[36] S. Moreno-Fernández, M. Garcés-Rimón, M. Miguel, Egg-derived peptides and hydrolysates: a new bioactive treasure for cardiometabolic diseases, Trends Food Sci. Technol. 104 (2020) 208–218. https://doi.org/10.1016/j.tifs.2020.08.002.

[37] Y. H. Zhang, J. Bai, W. N. Jiang, et al., Promising hen egg-derived proteins/peptides (EDPs) for food engineering, natural products and precision medicines, Res. Vet. Sci. 128 (2020) 153–161. https://doi.org/10.1016/j.rvsc.2019.11.011.

[38] C. Raveschot, B. Cudennec, F. Coutte, et al., Production of bioactive peptides by *Lactobacillus* species: from gene to application, Front. Microbiol. 9 (2018) 2354. https://doi.org/10.3389/fmicb.2018.02354.

[39] N. Wisuthiphaet, S. C. Napathorn, Optimisation of the use of products from the cane sugar industry for poly(3-hydroxybutyrate) production by *Azohydromonas lata* DSM 1123 in fed-batch cultivation, Process Biochem. 51 (2016) 352–361. https://doi.org/10.1016/j.procbio.2015.12.009.

[40] Y. Liu, Z. Wang, A. Kelimu, et al., Novel iron-chelating peptide from egg yolk: Preparation, characterization, and iron transportation, Food Chem. X 18 (2023) 100692. https://doi.org/10.1016/j.fochx.2023.100692.

[41] S. K. Ulug, F. Jahandideh, J. Wu, Novel technologies for the production of bioactive peptides, Trends Food Sci. Technol. 108 (2021) 27–39. https://doi.org/10.1016/j.tifs.2020.12.002.

[42] S. Y. Lin, Y. Guo, Q. You, et al., Preparation of antioxidant peptide from egg white protein and improvement of its activities assisted by high-intensity pulsed electric field, J. Sci. Food Agr. 92 (2012) 1554–1561. https://doi.org/10.1002/jsfa.4742.

[43] W. Z. Zhao, D. Zhang, Z. P. Yu, et al., Novel membrane peptidase inhibitory peptides with activity against angiotensin converting enzyme and dipeptidyl peptidase IV identified from hen eggs, J. Funct. Foods 64 (2020) 103649. https://doi.org/10.1016/j.jff.2019.103649.

[44] C. Nimalaratne, N. Bandara, J. P. Wu, Purification and characterization of antioxidant peptides from enzymatically hydrolyzed chicken egg white, Food Chem. 188 (2015) 467–472. https://doi.org/10.1016/j.foodchem.2015.05.014.

[45] E. D. N. S. Abeyrathne, X. Huang, D. U. Ahn, Antioxidant, angiotensin-converting enzyme inhibitory activity and other functional properties of egg white proteins and their derived peptides: a review, Poultry Sci. 97 (2018) 1462–1468. https://doi.org/10.3382/ps/pex399.

[46] I. Kumar, B. Yaseen, C. Gangwar, et al., Ovalbumin mediated eco-friendly synthesis of silver oxide nanoparticles and their antibacterial and antifungal studies, Mater. Today 46 (2021) 2330–2334. https://doi.org/10.1016/j.matpr.2021.04.403.

[47] L. Sheng, G. Y. Tang, Q. Wang, et al., Molecular characteristics and foaming properties of ovalbumin-pullulan conjugates through the Maillard reaction, Food Hydrocoll. 100 (2020) 105384. https://doi.org/10.1016/j.foodhyd.2019.105384.

[48] G. Hu, Z. Batool, Z. Cai, et al., Production of self-assembling acylated ovalbumin nanogels as stable delivery vehicles for curcumin, Food Chem. 355 (2021) 129635. https://doi.org/10.1016/j.foodchem.2021.129635.

[49] P. Rupa, L. Schnarr, Y. Mine, Effect of heat denaturation of egg white proteins ovalbumin and ovomucoid on $CD4^+$ T cell cytokine production and human mast cell histamine production, J. Funct. Foods 18 (2015) 28–34. https://doi.org/10.1016/j.jff.2015.06.030.

[50] N. Sun, A. Teng, Y. Zhao, et al., Immunological and anticancer activities of seleno-ovalbumin (Se-OVA) on H22-bearing mice, Int. J. Biol. Macromol. 163 (2020) 657–665. https://doi.org/10.1016/j.ijbiomac.2020.07.006.

[51] X. Fu, Y. Chen, G. Hu, et al., A novel antibacterial hydrogel based on thiolated ovalbumin/gelatin with silver ions to promote wound healing in mice, Int. J. Biol. Macromol. 253 (2023) 127116. https://doi.org/10.1016/j.ijbiomac.2023.127116.

[52] B. Ma, X. Fu, P. Zhu, et al., Allergenicity, assembly and applications of ovalbumin in egg white: a review, Crit. Rev. Food Sci. (2023). https://doi.org/10.1080/10408398.2023.2202774.

[53] N. Zhou, Y. Zhao, Y. Yao, et al., Antioxidant stress and anti-inflammatory activities of egg white proteins and their derived peptides: a review, J. Agric. Food Chem. 70 (2022) 5–20. https://doi.org/10.1021/acs.jafc.1c04742.

[54] X. Zhang, Q. Zeng, Y. Liu, et al., Enhancing the resistance of anthocyanins to environmental stress by constructing ovalbumin-propylene glycol alginate nanocarriers with novel configurations, Food Hydrocoll. 118 (2021) 106668. https://doi.org/10.1016/j.foodhyd.2021.106668.

[55] Q. Zeng, W. Zeng, Y. Jin, et al., Construction and evaluation of ovalbumin-pullulan nanogels as a potential delivery carrier for curcumi, . Food Chem. 367 (2022) 130716. https://doi.org/10.1016/j.foodchem.2021.130716.

[56] H. Y. Cho, J. E. Lee, J. H. Lee, et al., The immune-enhancing activity of egg white ovalbumin hydrolysate prepared with papain via MAPK signaling pathway in RAW264.7 macrophages, J. Funct. Foods 103 (2023) 105487. https://doi.org/10.1016/j.jff.2023.105487.

[57] X. Wang, Z. Wei, C. Xue, The past and future of ovotransferrin: physicochemical properties, assembly and applications, Trends Food Sci. Technol. 116 (2021) 47–62. https://doi.org/10.1016/j.tifs.2021.07.003.

[58] E. R. Werner, C. D. Arnold, B. L. Caswell, et al., The effects of 1 egg per day on iron and anemia status among young malawian children: a secondary analysis of a randomized controlled trial, Curr. Devel. Nutrit. 6 (2022) nzac094. https://doi.org/10.1093/cdn/nzac094.

[59] S. H. Moon, J. H. Lee, D. U. Ahn, et al., *In vitro* antioxidant and mineral-chelating properties of natural and autocleaved ovotransferrin, J. Sci. Food Agric. 95 (2015) 2065–2070. https://doi.org/10.1002/jsfa.6921.

[60] N. Shang, J. Wu, Egg white ovotransferrin attenuates RANKl-induced osteoclastogenesis and bone resorption, Nutrients 11 (2019) 2254. https://doi.org/10.3390/nu11092254.

[61] G. Zhu, J. Luo, H. Du, et al., Ovotransferrin enhances intestinal immune response in cyclophosphamide-induced immunosuppressed mice, Int. J. Biol. Macromol. 120 (2018) 1–9. https://doi.org/10.1016/j.ijbiomac.2018.08.058.

[62] Y. Huang, S. Chen, Y. Yao, et al., Ovotransferrin inhibits TNF-α induced inflammatory response in gastric epithelial cells via MAPK and NF-κB pathway, J. Agric. Food Chem. 71 (2023) 12474–12486. https://doi.org/10.1021/acs.jafc.3c00950.

[63] K. Majumder, S. Chakrabarti, S. T. Davidge, et al., Structure and activity study of egg protein ovotransferrin derived peptides (IRW and IQW) on Endothelial inflammatory response and oxidative stress, J. Agric. Food Chem. 61 (2013) 2120–2129. https://doi.org/10.1021/jf3046076.

[64] K. S. Bhullar, F. Ashkar, J. Wu, Peptides GWN and GW protect kidney cells against Dasatinib induced mitochondrial injury in a SIRT1 dependent manner, Food Chem. Mol. Sci. 4 (2022) 100069. https://doi.org/10.1016/j.fochms.2021.100069.

[65] J. K. Mann, T. Ndung'u, The potential of lactoferrin, ovotransferrin and lysozyme as antiviral and immune-modulating agents in COVID-19, Future Virol. 15 (2020) 609–624. https://doi.org/10.2217/fvl-2020-0170.

[66] Y. Li, J. Ding, S. Zhang, et al., Pulsed electric field-assisted alcalase treatment reduces the allergenicity and eliminates the antigenic epitopes of ovomucoid, J. Agric. Food Chem. 71 (2023) 3424–3432. https://doi.org/10.1021/acs.jafc.2c07824.

[67] H. Ando, R. Movérare, Y. Kondo, et al., Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy, J. Allergy Clin. Immunol. 122 (2008) 583–588. https://doi.org/10.1016/j.jaci.2008.06.016.

[68] E. D. N. S. Abeyrathne, H. Y. Lee, C. Jo, et al., Enzymatic hydrolysis of ovomucoid and the functional properties of its hydrolysates, Poultry Sci. 94 (2015) 2280–2287. https://doi.org/10.3382/ps/pev196.

[69] E. D. Abeyrathne, H. Y. Lee, C. Jo, et al., Enzymatic hydrolysis of ovomucin and the functional and structural characteristics of peptides in the hydrolysates, Food Chem. 192 (2016) 107–113. https://doi.org/10.1016/j.foodchem.2015.06.055.

[70] Q. Xu, X. Li, Y. Lv, et al., Effects of ultrasonic treatment on ovomucin: structure, functional properties and bioactivity, Ultrason. Sonochem. 89 (2022) 106153. https://doi.org/10.1016/j.ultsonch.2022.106153.

[71] A. Tu, X. Zhao, Y. Shan, et al., Potential role of ovomucin and its peptides in modulation of intestinal health: a review, Int. J. Biol. Macromol. 162 (2020) 385–393. https://doi.org/10.1016/j.ijbiomac.2020.06.148.

[72] Q. Xu, Y. Shan, N. Wang, et al., Sialic acid involves in the interaction between ovomucin and hemagglutinin and influences the antiviral activity of ovomucin, Int. J. Biol. Macromol. 119 (2018) 533–539. https://doi.org/10.1016/j.ijbiomac.2018.07.186.

[73] T. Oguro, K. Watanabe, H. Tani, Morphological observations on antitumor activities of 70 kDa fragment in α-subunit from pronase-treated ovomucin in a double grafted tumor system, Food Sci. Technol. Res. 6 (2000) 179–185. https://doi.org/10.3136/fstr.6.179.

[74] T. Aobai, W. X. Chao, C. Hongwang, et al., Ovomucin ameliorates intestinal barrier and intestinal bacteria to attenuate DSS-induced colitis in mice, J. Agric. Food Chem. 69 (2021) 5887–5896. https://doi.org/10.1021/acs.jafc.1c00865.

[75] J. E. Yi, J. Zhao, J. P. Wu, Egg ovotransferrin derived IRW exerts protective effect against H_2O_2-induced oxidative stress in Caco-2 cells, J. Funct. Foods 39 (2017) 160–167. https://doi.org/10.1016/j.jff.2017.10.012.

[76] O. K. Chang, G. E. Ha, G. S. Han, et al., Novel antioxidant peptide derived from the ultrafiltrate of ovomucin hydrolysate, J. Agric. Food Chem. 61 (2013) 7294–7300. https://doi.org/10.1021/jf4013778.

[77] W. Krzysciak, A. Jurczak, J. Piatkowski, et al., Effect of histatin-5 and lysozyme on the ability of *Streptococcus mutans* to form biofilms *in vitro* conditions, Postepy. Hig. Med. Dosw. 69 (2015) 1056–1066. https://doi.org/10.5604/01.3001.0009.6575.

[78] Y. Mine, F. P. Ma, S. Lauriau, Antimicrobial peptides released by enzymatic hydrolysis of hen egg white lysozyme, J. Agric. Food Chem. 52 (2004) 1088–1094. https://doi.org/10.1021/jf0345752.

[79] Y. M. Ha, S. H. Chun, S. T. Hong, et al., Immune enhancing effect of a Maillard-type lysozyme-galactomannan conjugate via signaling pathways, Int. J. Biol. Macromol. 60 (2013) 399–404. https://doi.org/10.1016/j.ijbiomac.2013.06.007.

[80] S. Rao, J. Sun, Y. Liu, et al., ACE inhibitory peptides and antioxidant peptides derived from *in vitro* digestion hydrolysate of hen egg white lysozyme, Food Chem. 135 (2012) 1245–1252. https://doi.org/10.1016/j.foodchem.2012.05.059.

[81] S. Mahanta, S. Paul, A. Srivastava, et al., Stable self-assembled nanostructured hen egg white lysozyme exhibits strong anti-proliferative activity against breast cancer cells, Colloid. Surfaces B 130 (2015) 237–245. https://doi.org/10.1016/j.colsurfb.2015.04.017.

[82] X. L. Zhang, Y. Lei, Y. B. Xiao, et al., Hen egg lysozyme alleviates static mechanical pain via NRF1-Parkin-TACAN signaling axis in sensory neurons, Neuroscience 502 (2022) 52–67. https://doi.org/10.1016/j.neuroscience.2022.08.010.

[83] L. Pellegrino, A. Tirelli, A sensitive HPLC method to detect hen's egg white lysozyme in milk and dairy products, Int. Dairy J. 10 (2000) 435–442. https://doi.org/10.1016/S0958-6946(00)00065-0.

[84] P. Attri, N. K. Kaushik, N. Kaushik, et al., Plasma treatment causes structural modifications in lysozyme, and increases cytotoxicity towards cancer cells, Int. J. Biol. Macromol. 182 (2021) 1724–1736. https://doi.org/10.1016/j.ijbiomac.2021.05.146.

[85] Z. Wei, S. Wu, J. Xia, et al., Enhanced antibacterial activity of hen egg-white lysozyme against *Staphylococcus aureus* and *Escherichia coli* due to protein fibrillation, Biomacromolecules 22 (2021) 890–897. https://doi.org/10.1021/acs.biomac.0c01599.

[86] X. Zhou, Y. Wang, D. U. Ahn, et al., An easy and simple separation method for Fc and Fab fragments from chicken immunoglobulin Y (IgY), J. Chromatogr. B 1141 (2020) 122011. https://doi.org/10.1016/j.jchromb.2020.122011.

[87] X. Zhang, R. A. Calvert, B. J. Sutton, et al., IgY: a key isotype in antibody evolution, Biol. Rev. Camb. Philos. Soc. 92 (2017) 2144–2156. https://doi.org/10.1111/brv.12325.

[88] W. Lee, A. Syed Atif, S. C. Tan, et al., Insights into the chicken IgY with emphasis on the generation and applications of chicken recombinant monoclonal antibodies, J. Immunol. Methods 447 (2017) 71–85. https://doi.org/10.1016/j.jim.2017.05.001.

[89] L. Sheng, Z. He, Y. Liu, et al., Mass spectrometry characterization for *N*-glycosylation of immunoglobulin Y from hen egg yolk, Int. J. Biol. Macromol. 108 (2018) 277–283. https://doi.org/10.1016/j.ijbiomac.2017.12.012.

[90] X. Zhou, D. U. Ahn, M. Xia, et al., Fab fragment of immunoglobulin Y modulates NF-kappaB and MAPK signaling through TLR4 and alphaVbeta3 integrin and inhibits the inflammatory effect on R264.7 macrophages, J. Agric. Food Chem. 69 (2021) 8747–8757. https://doi.org/10.1021/acs.jafc.1c03330.

[91] E. W. Bachtiar, A. Afdhal, R. Meidyawati, et al., Effect of topical anti-S*treptococcus mutan*s IgY gel on quantity of *S. mutans* on rats' tooth surface, Acta Microbiol. Imm. H. 63 (2016) 159–169. https://doi.org/10.1556/030.63.2016.2.2.

[92] C. L. Leiva, M. J. Gallardo, N. Casanova, et al., IgY-technology (egg yolk antibodies) in human medicine: a review of patents and clinical trials, Int. Immunopharmacol. 81 (2020) 106269. https://doi.org/10.1016/j.intimp.2020.106269.

[93] S. V. Nguyen, M. T. H. Nguyen, B. C. Tran, et al., Evaluation of lozenges containing egg yolk antibody against *Porphyromonas gingivalis* gingipains as an adjunct to conventional non-surgical therapy in periodontitis patients: a randomized controlled clinical trial, J. Periodontol. 89 (2018) 1334–1339. https://doi.org/10.1002/Jper.18-0037.

[94] X. T. Wang, L. J. Song, W. P. Tan, et al., Clinical efficacy of oral immunoglobulin Y in infant rotavirus enteritis systematic review and meta-analysis, Medicine 98 (2019) e16100. https://doi.org/10.1097/MD.0000000000016100.

[95] V. B. Gyawu, C. K. Firempong, J. A. Hamidu, et al., Production and evaluation of monovalent anti-snake immunoglobulins from chicken egg yolk using Ghanaian puff adder (*Bitis arietans*) Venom: isolation, purification, and neutralization efficacy, Toxicon. 231 (2023) 170180. https://doi.org/10.1016/j.toxicon.2023.107180.

[96] M. Hirose, T. Ando, R. Shofiqur, et al., Anti-obesity activity of hen egg anti-lipase immunoglobulin yolk, a novel pancreatic lipase inhibitor, Nutr. Metab. 10 (2013) 70. https://doi.org/10.1186/1743-7075-10-70.

[97] H. Wang, Q. Zhong, J. Lin, Egg yolk antibody for passive immunization: status, challenges, and prospects, J. Agric. Food Chem. 71 (2023) 5053–5061. https://doi.org/10.1021/acs.jafc.2c09180.

[98] L. R. Frumkin, M. Lucas, C. L. Scribner, et al., Egg-derived anti-SARS-CoV-2 immunoglobulin Y (IgY) with broad variant activity as intranasal prophylaxis against COVID-19, Front. Immunol. 13 (2022) 899617. https://doi.org/10.3389/fimmu.2022.899617.

[99] S. A. El-Kafrawy, A. T. Abbas, C. Oelkrug, et al., IgY antibodies: the promising potential to overcome antibiotic resistance, Front. Immunol. 14 (2023) 1065353. https://doi.org/10.3389/fimmu.2023.1065353.

[100] X. Gao, S. Yuan, High density lipoproteins-based therapies for cardiovascular disease, J. Cardiovas. Dis. Res. 1 (2010) 99–103. https://doi.org/10.4103/0975-3583.70898.

[101] N. Xiao, Y. Zhao, Y. Yao, et al., Biological activities of egg yolk lipids: a review, J. Agric. Food Chem. 68 (2020) 1948–1957. https://doi.org/10.1021/acs.jafc.9b06616.

[102] D. Groche, L. G. Rashkovetsky, K. H. Falchuk, et al., Subunit composition of the zinc proteins alpha- and beta-lipovitellin from chicken, J. Protein Chem. 19 (2000) 379–387. https://doi.org/10.1023/a:1026487414167.

[103] S. Eftekhar, H. Parsaei, Z. Keshavarzi, et al., The prevention and treatment effects of egg yolk high density lipoprotein on the formation of atherosclerosis plaque in rabbits, Iran. J. Basic Med. Sci. 18 (2015) 343–349.

[104] D. M. DiMarco, A. Missimer, A. G. Murillo, et al., Intake of up to 3 eggs/day Increases HDL cholesterol and plasma choline while plasma trimethylamine-*N*-oxide is unchanged in a healthy population, Lipids 52 (2017) 255–263. https://doi.org/10.1007/s11745-017-4230-9.

[105] Z. Yu, C. Mao, X. Fu, et al., High density lipoprotein from egg yolk (EYHDL) improves dyslipidemia by mediating fatty acids metabolism in high fat diet-induced obese mice, Food Sci. Anim. Resources 39 (2019) 179–195. https://doi.org/10.1016/j.fbio.2019.100492.

[106] C. J. Andersen, C. N. Blesso, J. Lee, et al., Egg consumption modulates HDL lipid composition and increases the cholesterol-accepting capacity of serum in metabolic syndrome, Lipids 48 (2013) 557–567. https://doi.org/10.1007/s11745-013-3780-8.

[107] W. Liu, M. Zhao, S. Li, et al., Advances in preparation and bioactivity of phosvitin phosphopeptides, J. Future Foods 2 (2022) 213–222. https://doi.org/10.1016/j.jfutfo.2022.06.003.

[108] G. Taborsky, Interaction of cytochrome c and the phosphoprotein phosvitin. Formation of a complex with an intact 695-mmicron absorption band, Biochemistry 9 (1970) 3768–3774. https://doi.org/10.1021/bi00821a016.

[109] K. Si, T. Gong, S. Ding, et al., Binding mechanism and bioavailability of a novel phosvitin phosphopeptide (Glu-Asp-Asp-pSer-pSer) calcium complex, Food Chem. 404 (2023) 134567. https://doi.org/10.1016/j.foodchem.2022.134567.

[110] B. Jiang, Y. Mine, Preparation of novel functional oligophosphopeptides from hen egg yolk phosvitin, J. Agric. Food Chem. 48 (2000) 990–994. https://doi.org/10.1021/jf990600l.

[111] X. Zhang, X. Huang, M. Ma, Phosphorylated serine clusters of phosvitin plays a crucial role in the regulatory mineralization, Int. J. Biol. Macromol. 115 (2018) 1109–1115. https://doi.org/10.1016/j.ijbiomac.2018.04.130.

[112] X. Zhang, Q. Jia, M. Li, et al., Isolation of a novel calcium-binding peptide from phosvitin hydrolysates and the study of its calcium chelation mechanism, Food Res. Int. 141 (2021) 110169. https://doi.org/10.1016/j.foodres.2021.110169.

[113] M. Zhao, S. Li, D. U. Ahn, et al., Phosvitin phosphopeptides produced by pressurized hea-trypsin hydrolysis promote the differentiation and mineralization of MC3T3-E1 cells via the OPG/RANKL signaling pathways, Poultry Sci. 100 (2021) 527–536. https://doi.org/10.1016/j.psj.2020.10.053.

[114] X. Xu, S. Katayama, Y. Mine, Antioxidant activity of tryptic digests of hen egg yolk phosvitin, J. Sci. Food Agric. 87 (2007) 2604–2608. https://doi.org/10.1002/jsfa.3015.

[115] D. Young, F. Nau, M. Pasco, et al., Identification of hen egg yolk-derived phosvitin phosphopeptides and their effects on gene expression profiling against oxidative stress-induced Caco-2 cells, J. Agric. Food Chem. 59 (2011) 9207–9218. https://doi.org/10.1021/jf202092d.

[116] J. E. Lee, B. J. An, C. Jo, et al., The elastase and melanogenesis inhibitory and anti-inflammatory activities of phosvitin phosphopeptides produced using high-temperature and mild-pressure (HTMP) pretreatment and enzyme hydrolysis combinations, Poultry Sci. 102 (2023) 102680. https://doi.org/10.1016/j.psj.2023.102680.

[117] H. Duan, X. Zhang, Z. Li, et al., Synergistic effect and antibiofilm activity of an antimicrobial peptide with traditional antibiotics against multi-drug resistant bacteria, Microb. Pathogenesis 158 (2021) 105056. https://doi.org/10.1016/j.micpath.2021.105056.

[118] S. Tang, X. Zhou, M. Gouda, et al., Effect of enzymatic hydrolysis on the solubility of egg yolk powder from the changes in structure and functional properties, LWT-Food Sci. Technol. 110 (2019) 214–222. https://doi.org/10.1016/j.lwt.2019.04.070.

[119] H. K. Kim, S. Lee, andK. H. Leem, Protective effect of egg yolk peptide on bone metabolism, Menopause 18 (2011) 307–313. https://doi.org/10.1097/gme.0b013e3181f31b1f.

[120] N. Xiao, Y. Zhao, W. He, et al., Egg yolk oils exert anti-inflammatory effect via regulating Nrf2/NF-kappaB pathway, J. Ethnopharmacol. 274 (2021) 114070. https://doi.org/10.1016/j.jep.2021.114070.

[121] Y. Kitaura, U. Nakamura, C. Awada, et al., Orally administrable peptides derived from egg yolk promote skeletal repair and ameliorate degenerative skeletal disorders in mouse models, Regen. Ther. 21 (2022) 584–595. https://doi.org/10.1016/j.reth.2022.11.002.

[122] Y. Liu, S. Lu, J. Meng, et al., Complexation between egg yolk protein hydrolysate, phytic acid and calcium ion: binding mechanisms and influence on protein digestibility and calcium uptake, LWT-Food Sci. Technol. 184 (2023) 114986. https://doi.org/10.1016/j.lwt.2023.114986.

[123] Z. J. Bao, Y. Zhao, X. Y. Wang, et al., Effects of degree of hydrolysis (DH) on the functional properties of egg yolk hydrolysate with alcalase, J. Food Sci. Technol. 54 (2017) 669–678. https://doi.org/10.1007/s13197-017-2504-0.

[124] I. Marcet, J. Delgado, N. Díaz, et al., Peptides recovery from egg yolk lipovitellins by ultrafiltration and their *in silico* bioactivity analysis, Food Chem. 379 (2022) 132145. https://doi.org/10.1016/j.foodchem.2022.132145.

[125] T. Pimchan, F. Tian, K. Thumanu, et al., Isolation, identification, and mode of action of antibacterial peptides derived from egg yolk hydrolysate, Poultry Sci. (2023) 102695. https://doi.org/10.1016/j.psj.2023.102695.

[126] J. H. Lee, J. E. Lee, H. D. Paik, Immunomodulatory activity of egg yolk protein hydrolysates prepared by novel two-step hydrolysis: a study of mechanism and stability after *in vitro* digestion model, Poultry Sci. (2022) 101802. https://doi.org/10.1016/j.psj.2022.101802.

[127] K. Kaweewong, W. Garnjanagoonchorn, W. Jirapakkul, et al., Solubilization and identification of hen eggshell membrane proteins during different times of chicken embryo development using the proteomic approach, Protein J. 32 (2013) 297–308. https://doi.org/10.1007/s10930-013-9487-0.

[128] M. K. Sah, S. N. Rath, Soluble eggshell membrane: a natural protein to improve the properties of biomaterials used for tissue engineering applications, Mater. Sci. Eng. C-Materi. Biol. Appl. 67 (2016) 807–821. https://doi.org/10.1016/j.msec.2016.05.005.

[129] G. J. Ahlborn, D. A. Clare, B. W. Sheldon, et al., Identification of eggshell membrane proteins and purification of ovotransferrin and beta-NAGase from hen egg white, Protein J. 25 (2006) 71–81. https://doi.org/10.1007/s10930-006-0010-8.

[130] X. Li, Z. Cai, D. U. Ahn, et al., Development of an antibacterial nanobiomaterial for wound-care based on the absorption of AgNPs on the eggshell membrane, Colloid. Surfaces B 183 (2019) 110449. https://doi.org/10.1016/j.colsurfb.2019.110449.

[131] N. Xiao, X. Huang, W. He, et al., A review on recent advances of egg byproducts: preparation, functional properties, biological activities and food applications, Food Res. Int. 147 (2021) 110563. https://doi.org/10.1016/j.foodres.2021.110563.

[132] J. Hohlfeld, A. D. Roessingh, N. Hirt-Burri, et al., Tissue engineered fetal skin constructs for paediatric burns, Lancet 366 (2005) 840–842. https://doi.org/10.1016/S0140-6736(05)67107-3.

[133] Z. Y. Cheng, S. H. Teoh, Surface modification of ultra thin poly (epsilon-caprolactone) films using acrylic acid and collagen, Biomaterials 25 (2004) 1991–2001. https://doi.org/10.1016/j.biomaterials.2003.08.038.

[134] Y. Shi, J. Kovacs-Nolan, B. Jiang, et al., Antioxidant activity of enzymatic hydrolysates from eggshell membrane proteins and its protective capacity in human intestinal epithelial Caco-2 cells, J. Funct. Foods 10 (2014) 35–45. https://doi.org/10.1016/j.jff.2014.05.004.

[135] M. C. Lee, Y. C. Huang, Soluble eggshell membrane protein-loaded chitosan/fucoidan nanoparticles for treatment of defective intestinal epithelial cells, Int. J. Biol. Macromol. 131 (2019) 949–958. https://doi.org/10.1016/j.ijbiomac.2019.03.113.

[136] X. Chen, L. Zhu, W. Wen, et al., Biomimetic mineralisation of eggshell membrane featuring natural nanofiber network structure for improving its osteogenic activity, Colloid. Surfaces B 179 (2019) 299–308. https://doi.org/10.1016/j.colsurfb.2019.04.009.

[137] K. Tonguc Altin, N. Topcuoglu, G. Duman, et al., Antibacterial effects of saliva substitutes containing lysozyme or lactoferrin against *Streptococcus mutans*, Arch. Oral Biol. 129 (2021) 105183. https://doi.org/10.1016/j.archoralbio.2021.105183.

[138] Y. Yao, T. Liu, N. Wu, et al., An efficient, scalable and environmentally friendly separation method for ovoinhibitor from chicken egg white, LWT-Food Sci. Technol. 127 (2020) 109367. https://doi.org/10.1016/j.lwt.2020.109367.

[139] W. Jianping, Eggs as functional foods and nutraceuticals for human health, Royal Society of Chemistry, 2019.

[140] F. GENG, X. HUANG , M. H. MA, Hen egg white ovomacroglobulin promotes fibroblast migration via mediating cell adhesion and cytoskeleton, J. Sci. Food Agric. 96 (2016) 3188–3194. https://doi.org/10.1002/jsfa.7498.

[141] Y. Kishimoto, C. Taguchi, E. Saita, et al., Additional consumption of one egg per day increases serum lutein plus zeaxanthin concentration and lowers oxidized low-density lipoprotein in moderately hypercholesterolemic males, Food Res. Int. 99 (2017) 944–949. https://doi.org/10.1016/j.foodres.2017.03.003.