

# PROPERTIES AND APPLICATIONS OF POULTRY EGG AS ANTIOXIDANT FOOD: A REVIEW

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*Data de submissão: 19/12/2024*

*Data de aceite: 02/01/2025*

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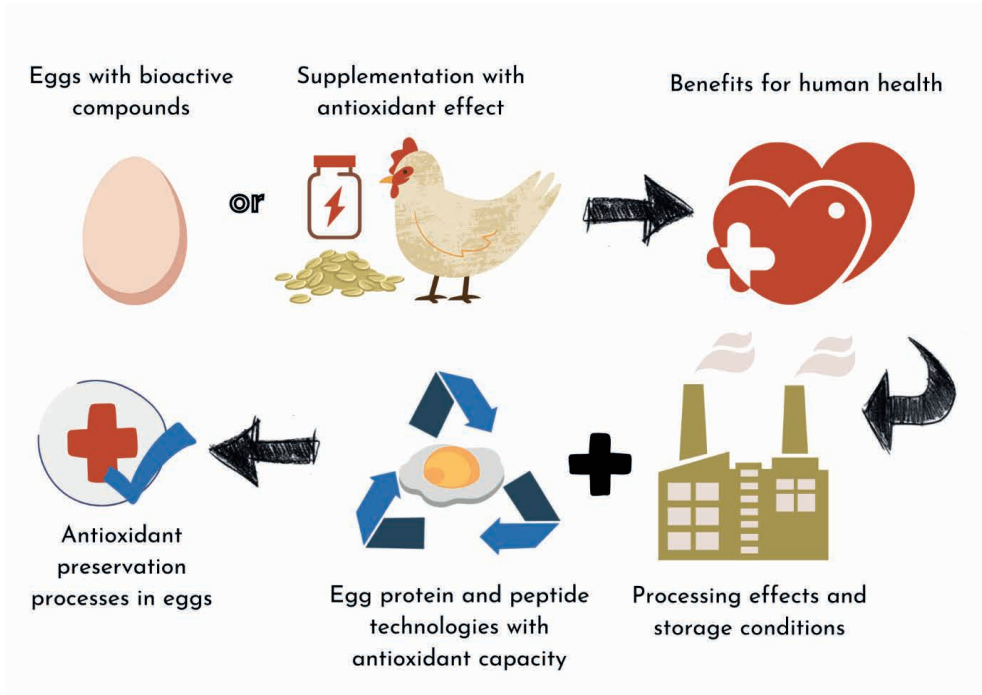
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consuming foods with natural antioxidant compounds, eggs can be an option for natural consumption and an alternative in the application of food matrices to replace synthetic antioxidants. This review describes the composition of eggs, compounds responsible for antioxidant properties, and studies of the processing and storage effects on antioxidant content in eggs. Based on recent literature on techniques to obtain, increase, and maintain antioxidants in eggs, protein hydrolysates from eggs are the most studied products and may be an alternative to synthetic antioxidants in human food. However, it is necessary to investigate bioavailability, digestibility, food safety, and other technologies to preserve the antioxidant properties of egg protein hydrolysates.

**KEYWORDS:** antioxidant peptides; egg quality; feed supplement; peptides hydrolysates; processing.

**ABSTRACT:** Antioxidants are molecules capable of inhibiting or delaying the oxidation of other molecules. Several foods contain bioactive compounds with antioxidant capacity, including eggs. Due to the population's growing interest in



## 1 | INTRODUCTION

Eggs are a source of protein with high nutritional value, accessible cost, and easy availability, which is important for maintaining a healthy diet [Tang et al., 2021]. Basically, an egg can be divided into three main components: shell, albumen, and yolk. The edible portion contains 74% water, 12% protein, 12% lipids, and less than 1% carbohydrates, vitamins, and minerals [Moreno-Fernández et al., 2020]. In addition to its nutritional value, many components present in the egg have health benefits, and it is used as functional food, which has increased the demand for the use of natural antioxidants in food preservation and human nutrition as health promoters. Peptides, vitamins A and E, carotenoids, phospholipids, and microminerals such as selenium, present in the egg, are known for their antioxidant properties [Tang et al., 2021; Moreno-Fernández et al., 2020; Lesniewski & Stangierski, 2018] and the role of these substances with anticancer, immunomodulatory, antimicrobial, and antihypertensive effects stands out [Bhat et al., 2021; Peñaranda-López et al., 2021; Réhault-Godbert et al., 2019].

Compounds with antioxidant power prevent, delay, and eliminate free radicals or other reactive species, preventing chain propagation in the metabolic system [Abeyrathne et al., 2022]. Oxidative damage caused by free radicals can lead to cell aging and predispose the individual to diseases such as atherosclerosis, diabetes, cancer, and liver cirrhosis [Abeyrathne et al., 2018]. Therefore, consuming antioxidant compounds is a positive action

in preventing these and other diseases and becomes essential for human health [Omri et al., 2019; Zaheer, 2017; De Mejia et al., 2020].

Many factors can modify the profile and number of substances with antioxidant capacity in eggs. Domestic or industrial processing and egg storage can change their characteristics, facilitate the subsequent protein enzymatic hydrolysis, and modify their antioxidant capacity [Liang et al., 2020; Liu et al., 2019].

In general, enzymatic hydrolysis or chemical modification to proteins is a common way to generate biologically active fragments and improve the antioxidant properties of proteins [Tu et al., 2020]. For example, ovotransferrin hydrolysates and its derived peptides have been demonstrated to exhibit stronger antioxidant activity than ovotransferrin pure [Wang et al., 2021]. Thermal processing can degrade egg yolk carotenoids and vitamin E [Zaheer, 2017], although, in these processes, proteins can conjugate with sugars and increase the antioxidant activity [Abeyrathne et al., 2018]. Another example is the freeze-drying process that maintains or improves the quality and extends the egg's shelf life [Katekhong & Charoenrein, 2017].

Based on this information, highlighting the importance of natural antioxidants in human health and food conservation, this work aimed to review the recent literature on the antioxidants present in eggs and which techniques are used to maintain, increase, and obtain them.

## **2 | ANTIOXIDANT COMPOUNDS IN EGGS**

Antioxidants are reducing substances that act to protect cells against oxidative damage. They can act in inhibiting chain reactions with iron and copper, in the interception of free radicals generated through cellular metabolism, and in repairing cellular damage caused by free radicals [Zhou et al., 2022]. Synthetic antioxidants are generally used in foods to delay oxidation reactions and thus preserve the nutrients of the food, increase the oxidative stability of food products, and consequently increase their shelf life [Benedé & Molina, 2020]. However, current trends indicate increased consumer interest in products without synthetic additives [Jain & Anal, 2017].

Despite not being considered an antioxidant food, eggs have several bioactive compounds such as proteins, peptides, phospholipids, vitamins A and E, selenium, and carotenoids that exhibit antioxidant activity [Escamila Rosales et al., 2023]. Some of these proteins have antioxidant properties by themselves. Still, peptide fragments derived from this exhibit a strong antioxidant activity by breaking the chain of free radical reactions and slowing down the rate of enzymatic oxidation [Nimalaratne & Wu, 2015].

## 2.1 Eggshell

The eggshell membrane is present in an egg between the albumen and the inner eggshell surface and contains about 62 proteins, such as collagen (type I, V, and X), glycoproteins, egg white proteins, and eggshell matrix proteins [Lee & Huang, 2019; Shi et al., 2021]. Eggshell membranes possess numerous biological functions, including antimicrobial, anti-inflammatory, anti-wrinkle, and antioxidant activities [Kulshreshtha et al., 2022].

Proteins that make up the eggshell membrane have been studied to produce peptide hydrolysates, which exhibit antioxidant activity by the methods of DPPH radical scavenging (2,2-diphenyl-1-picrylhydrazyl) and reducing power [Nimalaratne & Wu, 2015]. Eggshell and membrane were used as substrate for production of alkaline protease by *Bacillus altitudinis* GVC11 and showed significant activity of DPPH free radical scavenging [Nagamali et al., 2017]. Soluble eggshell membrane protein associated with polysaccharides extracted from algae exhibits inflammatory modulation activity and protection effect on epithelial cells damage [Shi et al., 2021].

## 2.2 Albumen and yolk proteins

Albumen is composed of more than 149 different kinds of proteins and is considered as an ideal source of this nutrient [Bhat et al., 2021]. The proteins present in albumen (, such as ovalbumin, ovotransferrin, lysozyme, cystatin, and ovomucin, and present in the yolk, such as phosvitin, have several biological effects (Table 01) [De Cesare et al., 2020; Quan & Benjakul, 2019; Yilmaz & Ağagündüz, 2020; Bhandari et al., 2020]. The antioxidant capacity of these proteins is due to their amino acid's constituents, such as tryptophan, tyrosine, phenylalanine, and nucleophilic amino acids containing methionine, sulfur, and cysteine, sequence, and molecular weight (MW) [Chen et al., 2022]. The peptides and amino acids can act as electron donors and can react with free radicals to convert them into stable products and terminate the radical chain reaction [Bazinet & Doyen, 2022].

Protein	Biological activity	References
<b>Ovoalbumin</b>	antihypertensive, immunomodulatory effects, antioxidant activity, metal chelator, antibacterial activity	[Garcés-Rimón et al., 2016]
<b>Ovotransferrin</b>	antimicrobial activity, antiviral, immunomodulatory effects, antihypertensive effects and anti-inflammatory properties, antioxidant activity.	[Jalili-Firoozinezhad et al., 2020]
<b>Lysozyme</b>	antibacterial properties, mainly Gram-positive bacteria, antiviral activity, anti-inflammatory agent, immunomodulator, anticancer, antioxidant activity, and immunostimulant.	[Wickramasinghe et al., 2021; Mumtaz & Ahmed, 2017; Singh & Ramaswamy, 2014]
<b>Cystatin</b>	cysteine protease inhibitors, prevent neurodegenerative diseases development, antimicrobial, antitumor, antioxidant activity, immunomodulatory	[Liao et al., 2018]
<b>Ovomucin</b>	Antibacterial and antiviral activity, antitumor, anti-inflammatory properties, anti-oxidation, immunomodulatory and, hypocholesterolemic action	[Farjami et al., 2021; Li et al., 2021]
<b>Phosvitin</b>	Antioxidant properties, high chelating power, antibacterial, immune-enhancing, melanogenesis-inhibitory, antigenotoxic, anti-elastase, and anti-hyaluronidase	[Zhao et al., 2023]

**Table 01.** Biological activities of albumen and yolk proteins.

### 2.3 Ovoalbumin

Ovalbumin is the most abundant protein in egg albumen, constituting more than half of it is proteins (52%) [Sheng et al., 2017]. It is a globular-shaped protein with 3 nm of diameter, formed by 354 amino acids and with 40 kDa of molecular weight [Yang et al., 2018]. It is isoelectric point is 4.5. It exhibits the gelling, foaming, and emulsifying properties of egg albumen. This albumen protein is the only with free sulfhydryl groups the processing exposed buried SH groups and form disulfide bonds through free sulfhydryloxidation and sulfhydryl-disulfide exchange [Gharbi & Labbafi, 2018].

Ovalbumin as well as ovotransferrin, lysozyme, ovomucin, and ovomucoid are commonly used to produce peptides with antioxidant activity [Abeyrathne et al., 2022]. The hydrolysis of ovalbumin using heat pretreatment followed by ultrasonication in duck albumen increases the antioxidant activities and emulsifying properties [Quan & Benjakul, 2019].

Covalent bonds can increase the antioxidant activities of ovalbumin. For example, mannose glycation after ultrasound pre-treatment at 600 W, increases antioxidant capacity and reduces the binding of immunoglobulins G and E, promising to produce hypoallergenic proteins [Yang et al., 2018]. Maillard reaction controlled, increased ovalbumin antioxidant activity when linked with galactomannan or dextran covalently [Abeyrathne et al., 2018]. Ovalbumin is linked with polyphenol rutin, there is an increase in antioxidant activity [Benedé & Molina, 2020]. Likewise, when bound to curcumin, it forms an amorphous complex with greater DPPH radical scavenging potential and reducing power than pure curcumin [Liu et al., 2019].

## 2.4 Ovotransferrin

Ovotransferrin is a monomeric glycoprotein formed by a single polypeptide chain of 666 amino acids, with 77.90 kDa of molecular mass. Is the most heat-labile protein in albumen. The isoelectric point (pI) is 6-6.5 [Gharbi & Labbafi, 2018]. Structurally it is folded into two globular lobes with an iron-binding site and interconnected by an alpha-helix of nine amino acid residues [Benedé & Molina]. Ovotransferrin belongs to the transferrin family, a group of iron-binding proteins that are widely distributed in various fluids. This protein exhibits many physicochemical properties like surface hydrophobicity, aggregation, solubility, foaming properties and emulsifying properties [Wang et al., 2021].

Ovotransferrin is an iron-binding protein. The main antioxidant mechanism of this protein is preventing metalcatalyzed lipid oxidation by chelating ionic irons and combining ovotransferrin with metal can improve the antioxidant capacity of ovotransferrin [Zhou et al., 2022].

The peptides from ovotransferrin had higher Fe-chelating activities than the native ovotransferrin [Rathnapala et al. 2021]. In this way, proteases, like papain, elastase and  $\alpha$ -chymotrypsin can hydrolyze ovotransferrin to generate bioactive peptides with strong antioxidant properties and Fe-chelating activity [Wickramasinghe et al., 2021].

## 2.5 Lysozyme

Eggs are considered an abundant lysozyme source, containing about 0.3-0.4 g in albumen. Lysozyme is a polypeptide containing 128 amino acid residues with a molecular weight of 14.4 kDa and four disulfide bridges. It is isoelectric point (pI) is 10.7 and it is stabilized by four disulfite bonds [Gharbi & Labbafi, 2018]. This globular enzymatic protein is known for its antimicrobial properties, it acts by catalyzing the hydrolysis of specific polysaccharides contained in bacterial cell walls [Lesnierowski & Stangierski, 2018].

Lysozyme showed the highest reducing power, and the antioxidant properties of lysozyme increase when it is conjugated with other compounds. The conjugation with polysaccharides, for example, increases antioxidant activity in terms of chelating metal ions and DDPH radical scavenging, especially for guar gum [Mumtaz & Ahmed, 2017]. It increases antioxidant properties from 2.02% to 33.80% and 1.63 ascorbic acid equivalents (AAE) g<sup>-1</sup> (by reducing power) to 4.93 AAE g<sup>-1</sup> [Hamdani et al., 2018]. The same effect was observed when xanthan gum from *Xanthomonas campestris*, an anionic extracellular polysaccharide, was used for conjugation with egg lysozyme [Benedé & Molina, 2020]. Otherwise, the enzymatic hydrolysis of lysozyme yielded bioactive peptides with antioxidant capacity [Liao et al., 2018; Xiao et al., 2021].

## 2.6 Cystatin

Cystatin is a non-glycosylated cationic protein. Albumen contains the Type 2 cystatins, one of which is non-phosphorylated (pI 6.5, cystatin C1), whereas the other is phosphorylated (pI 5.6, cystatin C2). It is a small protein with a molecular weight of approximately 13 kDa, with about 115 amino acids and two disulphide bonds, but no carbohydrate [Lesnierowski & Stangierski, 2018].

Cystatin synthesizes and releases nitric oxide in macrophages, playing an important role in cellular antioxidant pathways, affecting the host's immune response through the cytokine network [De Cesare et al., 2020]. This protein has antimicrobial and anticancer effects and may also act against the development of Alzheimer's disease [Lesnierowski & Stangierski, 2018].

## 2.7 Ovomucin

Ovomucin is a mucin-like glycoprotein from albumen. It is composed of two subunits:  $\alpha$ -Ovomucin (molecular weight of 210 kDa) as more polymerized macromolecule and has long coiled regions and  $\beta$ -ovomucin that contains higher level of carbohydrates (molecular weight of 5290–8180 kDa) than  $\alpha$ -type. This protein is found in the thick and thin proteins of albumen, as well as in the frenulum and the outer layer of the yolk membrane and acts as a mechanical barrier for egg yolk against pathogens [Jalili-Firoozinezhad et al., 2020].

The main function of ovomucins is to maintain egg albumen structure and viscosity. However, ovomucin and ovomucin-derived peptides exhibit a lot of biological activities because of the existence of sialic acid groups. For example, ovomucin derivatives show antitumor activities inhibiting the formation of tumor blood vessel, antiviral and antibacterial activity, anti-inflammatory, immune regulation and, antioxidant activities [Tu et al., 2020].

The antioxidant activity of ovomucin is shown by free radical scavenging ability, ACE inhibitory activity, and metal ion chelating ability. And this property can be increased across technological processes. Hydrolysates of ovomucin in alkaline condition and mock digests have shown free radical scavenging activities [Tu et al., 2020]. Also, enzymatic hydrolysis of ovomucin with papain (OMPa) or alcalase (OMAl) produced peptides with high angiotensin-converting enzyme (ACE) inhibitory activity [Abeyrathne et al., 2018]. Treatments like heating under alkaline conditions and pulsed electric fields heating can enhance the antioxidant activity and iron-binding powder [Liu et al., 2019].

## 2.8 Phosvitin

Phosvitin is the main protein component from egg yolk granular fraction. This protein is composed of two fractions with molecular weights of 159 and 190 kDa and is the main phosphorus source in the yolk [Yılmaz & Ağagündüz, 2020]. It has a sequence of 216 amino

acid residues and 123 containing serine residues and most are phosphorylated. There are no methionine, tryptophan, or tyrosine amino acid present. This chemical characteristic of phosvitin confers better emulsifying properties than those of other proteins applied in food production. In addition, its structure with a short hydrophobic region and a large number of hydrophilic phosphoserines explains its ability to chelate cations with antioxidant power, as well as its amphiphilic action [Li et al., 2021].

The high chelating power with metals observed in phosvitin confers high antioxidant capacity of phosvitin against iron-induced oxidative damage. Approximately 95% of yolk iron is bound to phosvitin. Phosvitin is also effective against UV-induced lipid peroxidation, is commonly used in the food industry as an antioxidant [Xiao et al., 2020].

## 2.9 Phospholipids

Phospholipids (phosphorus-containing lipids) are present in cell membrane structure in all living species. Egg yolk is the richest source of phospholipids like lecithin. Lecithin is a component of the yolk granular fraction and produces about 68% of all phospholipids contained in it [Lesnierowski & Stangierski, 2018]. Lecithin is a group of phospholipids composed especially by phosphatidylcholine (~73.0%) and minor components such as phosphatidylethanolamine (PE), lysophosphatidyl choline [Zhao et al., 2023].

This phospholipid has a stronger antioxidant capacity than neutral lipids, and this relationship is positively correlated with unsaturation degree, with the greater saturation of the fatty acid side chains being inversely proportional to its antioxidant capacity [Muhammad et al., 2021]. Fractionation of lecithin-free egg yolk hydrolysates (LFEYH) peptides having different biological activities and functional properties an antioxidant, antimicrobial and angiotensin I-converting enzyme (ACE) inhibitory activities [Peñaranda-López et al., 2020].

## 2.10 Micronutrients

Egg micronutrients include fat soluble and water-soluble vitamins, minerals, and pigments. All micronutrients can be manipulated through the birds' diet. The egg albumen possesses high water-soluble vitamins (B1, B2, B3, B5, B6, B8, B9, and B12) while yolk contains all the vitamins (A, D, E, K, B1, B2, B5, B6, B9, and B12) except vitamin C (ascorbic acid) [Réhault-Godbert et al., 2019].

Vitamins A or retinol, C or ascorbic acid and E are known to have antioxidant action. Vitamin E, especially the more active form, alpha-tocopherol protects the cell membrane, prevents lipid peroxidation, and chelates reactive oxygen species (ROS). This mechanism of action occurs through the donation [Escamila Rosales et al., 2023]. Vitamin C, on the other hand, act against the superoxide radical anion,  $H_2O_2$ , the hydroxyl radical and singlet oxygen. Furthermore, can act synergistically with vitamin E by reacting with tocopheroxyl radical to regenerate its antioxidant [Muhammad et al., 2021].



About the inorganic compounds, egg albumen present phosphorus, calcium, potassium, sodium, copper, iron, magnesium, manganese, selenium, and zinc while egg yolk stands out of iron and zinc [Réhault-Godbert et al., 2019]. Some minerals, such as selenium and iodine, are often supplemented in poultry feed to increase its concentration and consequently its antioxidant potential. Selenium is component of selenoproteins such as glutathione peroxidases (GPx) and thioredoxin reductases (TrxR) and selenoprotein P many of these properties are involved in redox control [Muhammad et al., 2021].

The pigments like xanthophylls (lutein and zeaxanthin) and carotenes contribute to the yolks' yellow-orange color [Zaheer, 2017]. Carotenoids are fat-soluble compounds in general present in yolk due to the fat-soluble chemical characteristic, and the concentration is directly related to birds' diet composition [Omri et al., 2019]. Studies show that chickens reared in organic production systems exhibit higher content of carotenoids in egg yolks than chickens reared in conventional systems [Painsi et al., 2019]. The antioxidant action of carotenoids is attributed to double bonds presence that link with free radicals, protecting LDL cholesterol in the body against oxidative damage. However, it is important to consider that factors like high temperatures and UV light, oxygen, acid, transition metal, or interactions with radical and certain enzymes (mono- and dioxygenases, redox active metal ions) are responsible with a partial or complete loss of carotenoids bioactivity and reduction of antioxidant activity [Zaheer, 2017].

### **3 | TECHNOLOGIES FOR EGG PROTEINS AND PEPTIDES WITH ANTIOXIDANT CAPACITY**

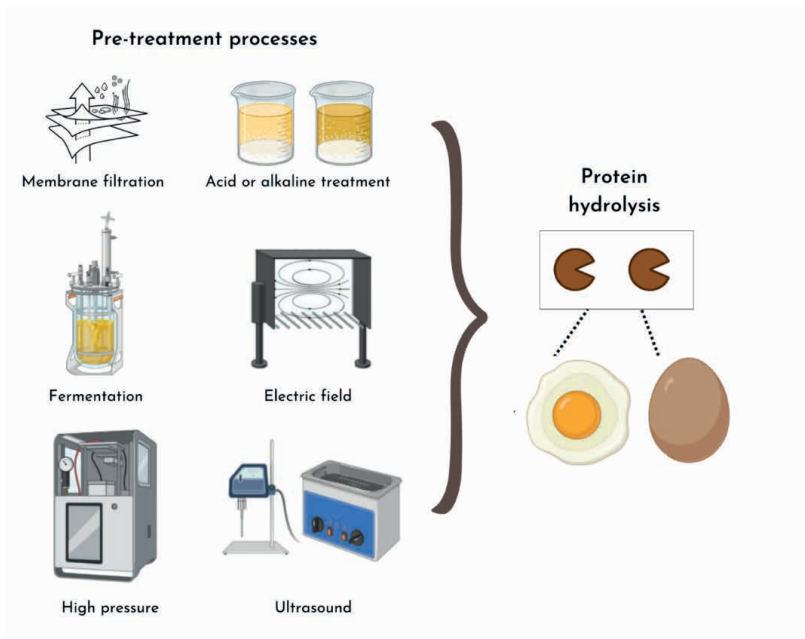
Although the egg is rich in proteins with antioxidant power, small peptides from these proteins possess greater bioactive potential since small peptides have greater accessibility of the functional side chain (R group) to reactive species and electron-dense peptide bonds [Benedé & Molina, 2020]. Smaller antioxidant peptides could exert better biological effects than proteins. Peptides from egg proteins hydrolysis can have antioxidant activity, being able to transfer electrons through metal chelation reaction and iron reduction power, and then increasing antioxidant power [Bhandari et al., 2020].

Many processes can be used for obtaining bioactive peptides as enzymatic hydrolysis with the use of enzymes derived from microbial (e.g. Alcalase, Neutrase, Flavourzyme), plant (e.g. papain) or animal (e.g. digestive enzymes, e.g. pepsin and trypsin), chemical hydrolysis or processing technologies like thermal or non-thermal treatments, for example [Stefanović et al., 2014; Zhang et al., 2019]. For example, proteases and peptidases can produce ovomucin hydrolysate with high bioactivity [Liu et al., 2018].

Enzymatic hydrolysis aims to improve the functional and nutritional properties of eggs, such as improving water solubility, increasing digestibility, reducing allergenic potential, and increasing antioxidant potential. Factors such as the type of enzymes and

the degree of hydrolysis can affect the performance of the hydrolysates formed and their bioactivity [Stefanović et al., 2014].

Some technological treatments have been applied to increase the decomposition of proteins, before or after enzymatic hydrolysis such as membrane filtration, acid, or alkaline treatment, high-pressure, heating, fermentation, electric field, or ultrasound [Farjami et al., 2021] (Figure 1).



**Figure 01.** Common pretreatment technologies for protein hydrolysates in eggs.

Ultrasound and high-pressure treatments improve the degree of hydrolysis of the egg albumen, releasing more peptides which increase the antioxidant properties [Gharbi & Labbafi, 2018]. Different studies using enzymatic hydrolysis and pre-treatment techniques combined with enzymes obtaining protein hydrolysates with peptides with high antioxidant potential (Table 02). For example, membrane technology can be used to selectively concentrate and/or fractionate these bioactive peptides [Zhang et al., 2019]. This technique can also improve antioxidant activity in the final hydrolysate [Xue et al., 2019]. The chromatography technique is used to purify the peptides, in addition to identifying and separating them [Johnny et al., 2022].

Technologies	Experimental conditions	Main conclusions	References
Enzymatic hydrolysis	<ul style="list-style-type: none"> <li>Alkaline protease</li> <li>pH 9.1 to 44 °C</li> <li>Enzyme dosage 21,000 U/g of substrate</li> </ul>	Identified MWCOIV peptide with AA, reducing power and high potential for scavenging -OH radicals and singlet oxygen	[Quan et al., 2020]
	<ul style="list-style-type: none"> <li>Two enzymatic hydrolysis steps:</li> <li>5% Alacase pH 11.0 and 49 °C for 4 h</li> <li>5% flavoring protease pH 7.0 53 °C for 4 h</li> </ul>	Identified VYLPR peptide with AA, inhibiting lipid peroxidation and LDH intracellular activity and regulation of relative enzymes	[Zimoch-Korzycka & Jarmoluk, 2015]
	<ul style="list-style-type: none"> <li>Bromelain from Pineapple Crown (30 CDU/ml)</li> <li>Incubation time (up to 24 h in 35 °C)</li> </ul>	24 h of hydrolysis formed bioactive with high antioxidant response and elimination of DPPH, ABTS, peroxy and superoxide radicals.	[Wang et al., 2018]
Combination of techniques	<ul style="list-style-type: none"> <li>Ultrasound (33-38 kHz) and heating (22-53 °C) as pre-treatment (15-59 min)</li> <li>Enzymatic hydrolysis with proteases (pH solution 7.0-10.0)</li> </ul>	Ultrasound pre-treatment followed by alcalase at pH 9.25 had changes in peptide composition and greater AA	[Stefanović et al., 2014]
	<ul style="list-style-type: none"> <li>5 proteases 9 U/mg with different dosages (0-15 U/mg)</li> <li>Sodium sulfite (Na<sub>2</sub>SO<sub>3</sub>) (0-49 mM)</li> <li>Incubation time (2-10 h)</li> </ul>	Peptides identification with AA from Na <sub>2</sub> SO <sub>3</sub> (38 mM) and alkaline protease (12U/mg) at pH 8.0 at 53 °C for 4h	[Zhao et al., 2019]
	<ul style="list-style-type: none"> <li>Trypsin hydrolysis (up to 120 min)</li> <li>High pressure (HP) (329–529 MPa) (5–15 min)</li> </ul>	Increased degree of hydrolysis and AA with release of bioactive peptides in HP for 5 min	[De Cesare et al., 2020]
	<ul style="list-style-type: none"> <li>Hydrolysis with the intracellular protease enzyme of <i>Lactobacillus plantarum</i> 18 °C in 120 rpm in different pH (4.0-8.0)</li> <li>Incubation time (6-70 h)</li> </ul>	Hydrolysates with bioactive properties, AA and angiotensin I convert enzyme inhibition after fermentation, 34 h at pH 8.0	[Quan & Benjakul, 2019]
	<ul style="list-style-type: none"> <li>Pulsed electric fields (1.4-1.7 kV/cm) and heating (59 and 80 °C for 10 min) as pre-treatment with different pH (4.0-9.0)</li> <li><i>Vitro</i> gastrointestinal hydrolysis (35 °C in 125 rpm)</li> </ul>	Pulsed electric fields and heating (80°C) to pH 4.0 increased anti-inflammatory and AA activity of ovomucin hydrolysates	[Liu et al., 2019]

**Table 02.** Studies to obtain egg protein hydrolysates and peptides with antioxidant activity (AA).

Egg bioactive peptide fractions show a promising future in human health, mainly because they are easily absorbed into the intestine and enter the bloodstream intact [Liao et al., 2018]. Lee et al. (2017) reported that ovotransferrin hydrolysates showed stronger cytotoxic activities against human cancer cell lines and the authors claim that the hydrolysates of ovotransferrin have great potential for use as a food ingredient with antioxidant and anticancer activities.

Furthermore, hydrolysates are interesting products in food application from a technological point of view [Moreno-Fernández et al., 2020]. Zimoch-Korzycka and Jarmoluk (2015) used lysozyme from white egg hen with 2000 U/mg activity to produced biologically active edible hydrosols for applied to the surface of food products. The results showed that the addition of lysozyme significantly increases antioxidant activity in meat samples with hydrosols. Duck albumen hydrolysate-epigallocatechin gallate (DE)-conjugate retard lipid oxidation of fish tofu during the storage by lowered increases in peroxide value and thiobarbituric acid reactive substances [Quan & Benjakul, 2020].

However, there are few studies with trials in humans, although in vitro studies have shown high potential in health and the low commercial application of egg-derived hydrolysates or peptides that exhibit antioxidant capacity is due the lack of scalable production processes, the few digestibility and bioavailability studies, and the absence of clinical trials that probe their potential health benefits [Wang et al., 2018].

#### **4 | EFFECTS OF DOMESTICAL OR INDUSTRIAL PROCESSING AND STORAGE CONDITIONS ON EGG ANTIOXIDANTS**

Thermal treatment, such as cooking, dry heat, spray drying or pasteurization are processes usually employed in the food industry to enhance sensory quality and extend shelf life of eggs and eggs derivates [Chen et al., 2022]. In domestical environments, eggs are usually processed before consumption and during preparation of different products. This processing affects their functional properties, microbial quality, shelf-life, and protein digestibility [Bhat et al., 2021].

Processing and storing eggs in natura or in liquid form affects the total antioxidant capacity, which can increase or decrease [Tang et al., 2021]. Thermal processing leads to oxidation, degradation and leaching of vitamin C, vitamin E, and phenolic compounds, which reduce free amino acid content and antioxidant activity [Nimalaratne et al., 2016].

Boiling, frying and microwave heating tends to modify the nutrients in eggs. For example, cooking reduces the amount of nutrients like polyunsaturated fatty acids, selenium, and vitamins A and E, especially in hard-boiled eggs [Réhualt-Godbert et al., 2019]. In this way, Chen et al. (2022) studied different thermal processing of egg white hydrolysate, and the treatment at 63 °C in a water bath for 18 min was the best condition to preserve and increase the antioxidant activity.

Regarding the content of vitamins in liquid eggs subjected to different cooking methods, fried eggs (118 °C for 2.5 min) followed by eggs cooked in a microwave (180 W for 4 min) showed higher levels of vitamins A and E when compared to baked eggs (180 °C for 18 min.) [Tang et al., 2021]. The longer heating time associated with the high heating temperature causes oxidative damage to the vitamins present in the egg.

Cooking also leads to sizeable conformational protein changes, although it does not significantly affect their amount. With cooking, the egg yolks oxygen-radical scavenging

capacity is significantly reduced, which is associated with the free aromatic amino acids, lutein, and zeaxanthin, and affects yolk lipids. On the other hand, cooked eggs after simulated gastrointestinal digestion indicated that peptides derived from ovalbumin exhibit more antioxidant activity [Wang et al., 2018]. Thus, to obtain greater retention of nutritional components and antioxidant compounds in eggs, the ideal would be the consumption of poached or boiled eggs, where the albumen is cooked (to inactivate antinutritional factors and potential pathogenic bacteria), while the yolk remains essentially raw (to preserve most vitamins, lipids, micronutrients, and some antioxidants molecules) [Réhault-Godbert et al., 2019].

Some processes can positively affect egg proteins and peptides, preserving the antioxidant capacity. Some examples of these process are low temperature, addition of natural antioxidants, use of electric field, modified atmosphere, high pressure [Wang et al., 2018]. The activity of some antioxidants, such as ovalbumin, can increase under glycosylation with glucose under heat moisture treatment, which increases DPPH radical-scavenging activity and Trolox equivalent antioxidant capacity assay [Benedé & Molina, 2020]. Ovalbumin was glycosylated with glucose and maltose mixture under heat moisture treatment at 120 °C for 20 min and the results showed stronger browning intensity as well as reducing power and DPPH scavenging activity than other samples (ovoalbumin glycosylated with lactose or soluble starch) [Zhao et al., 2019]. Liu et al. (2019) investigated the effects of pulsed electric fields and heating at different pH on ovomucin-depleted albumen's antioxidant and anti-inflammatory activity after *in vitro* gastrointestinal hydrolysis. The results showed that pulsed electric fields and heating (80 °C for 10 min) at pH 4 enhanced the antioxidant activity of the whole hydrolysates, chemically determined using DPPH and ORAC assays.

Another example is the use of electron beam irradiation. This technology changes the albumen protein and increases hydrophobic amino acids on the surface of the protein and breakage of disulfide bonds in sulfhydryl groups, these reacted with free radicals, consequently increasing the antioxidant activity [Liu et al., 2019]. A colder light-free environment also shows good conservation of carotenoids in whole eggs, eggshells, and liquid eggs. High pressure and ozone increase carotenoid concentration in liquid eggs. Regarding what happens with carotenoids during the preparation process, boiling eggs for 10 minutes increased the level of carotenoids in eggs, probably due to the bioavailability of cell destruction and isomerization effects [Painsi et al., 2019]. Atmospheric modification experiments demonstrate that storing eggs in a hydrogen-modified atmosphere slows down oxidation processes. These results showed that egg quality has a prolonged shelf life when eggs are stored under these conditions [Wang et al., 2022]. Albumen powder produced from dry heat treatment showed excellent antioxidant properties and thermal stability, in addition to increasing digestibility [Wang et al., 2018].

The antioxidant properties of proteins can be enhanced by forming covalent conjugates or non-covalent complexes with polyphenols [Jing et al., 2020]. Phenolic compounds can be used to reduce malonaldehyde production during storage and increase the shelf life of egg powders [Matumoto-Pintro et al., 2017].

It is noteworthy that most processing technologies, such as those already mentioned, can modify other functional and nutritional properties of eggs, improving digestibility and reducing egg proteins allergenicity [Chen et al., 2022]. Combined heat treatment and high-pressure processing (HPP) can partly inactivate antinutrients like avidin [Bhat et al., 2021].

## 5 | CONCLUSIONS

Egg antioxidant capacity has been proven in several studies, and it concludes that it can be an alternative to synthetic antioxidants in foods. The main obtained egg antioxidant compounds were protein hydrolysates and peptides. Therefore, studies on the preservation of the antioxidant activity of these products must be further investigated since depending on the technology used in a food process, it can reduce antioxidant power. However, some improvements are needed to continue investigating, such as digestibility and bioavailability in clinical trials, after processing and during storage, and safety for potential food applications. Likewise, researching the technologies that can maintain egg quality to its antioxidant property, as well as its effects and how to preserve it until consumption so that it is viable to produce such compounds on a large scale.

## 6 | ACKNOWLEDGEMENTS

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for granting a scholarship (302408/2022-3)

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