Impact of polyphenols modification system on structure and function properties of egg whites proteins

تأثير نظام تعديل البوليفينول على البنية والخصائص الوظيفية لبروتينات بياض البيض

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ABSTRACT:

Eggs are a great source of many different proteins, and because egg whites foam so well, they are a key component in cakes. Many food products have made use of the interactions between polyphenolic chemicals and proteins, which affect the nutritional and functional qualities of food products. It is strongly suggested to explore and comprehend protein-phenolic interactions in order to increase and improve the quality and safety of food items, since many studies have been undertaken about the alteration of egg white proteins through the interaction of protein and polyphenols. The primary concern is the development of interactions between phenolic chemicals and proteins. Through their influence on the structure of proteins that are detected and measured using various techniques, these complexes may have a significant impact on the functional, nutritional, and shelf-life characteristics of proteins. This review's primary objective is to shed light on the interactions between polyphenols and the proteins found in egg whites, as well as how these interactions affect the structure, functionality, and overall character of the polyphenol-protein complex. Egg whites employ a few methods that can be used to investigate how proteins and other phenolic chemicals interact.

Key Words :egg whites, modification, polyphenols, structure , functional properties

المستخلص :

يعد البيض مصدرًا رائعًا للعديد من البروتينات المختلفة، ولأن بياض البيض رغوي بشكل جيد، فهو مكون رئيسي في الكعك. استفادت العديد من المنتجات الغذائية من التفاعلات بين المواد الكيميائية البوليفينولية والبروتينات، والتي تؤثر على الصفات الغذائية والوظيفية للمنتجات الغذائية. يُقترح بشدة استكشاف وفهم تفاعلات البروتين الفينولي من أجل زيادة وتحسين جودة وسلامة المواد الغذائية،

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حيث تم إجراء العديد من الدراسات حول تغيير بروتينات بياض البيض من خلال تفاعل البروتين والبوليفينول. الاهتمام الرئيسي هو تطوير التفاعلات بين المواد الكيميائية الفينولية والبروتينات. من خلال تأثيرها على بنية البروتينات التي يتم اكتشافها وقياسها باستخدام تقنيات مختلفة، قد يكون لهذه المجمعات تأثير كبير على الخصائص الوظيفية والغذائية ومدة صلاحية البروتينات. الهدف الأساسي لهذه المراجعة هو تسليط الضوء على التفاعلات بين البوليفينول والبروتينات الموجودة في بياض البيض، وكذلك كيفية تأثير هذه التفاعلات على البنية والوظيفة والطابع العام لمجمع بروتين البوليفينول. يستخدم بياض البيض بعض الطرق التي يمكن استخدامها لدراسة كيفية تفاعل البروتينات والمواد الكيميائية الفينولية الأخرى.

الكلمات المفتاحية: بياض البيض، التعديل، البوليفينول، التركيب، الخصائص الوظيفية

1. Introduction

Eggs are composed of three main parts: the shell, the white, and the yolk. The shell makes up 9.5%, the white 63%, and the yolk 27.5% of the egg (Cameron, 2020).

The chemical composition of eggs depends on the diet, age of the animal, season, genetic traits, and other factors. Kusum *et al.* (2018) found that the chemical composition of a whole chicken egg consists of 74% moisture, 12.8% protein, 11.8% fat, and small amounts of carbohydrates and minerals.

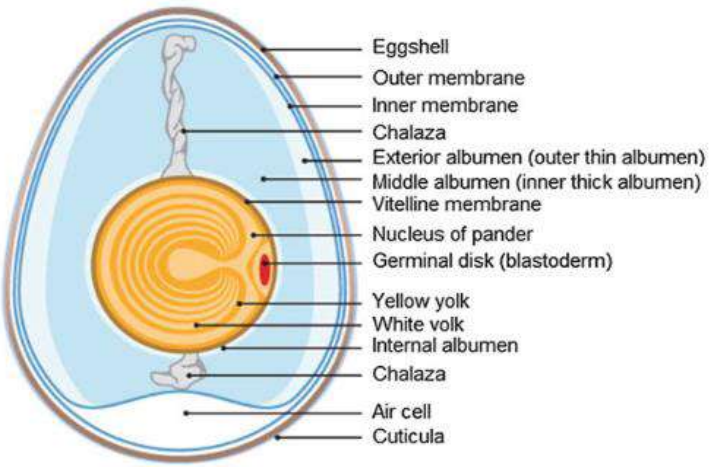

Figure (1) diagram of the structure of the egg, Cameron (2020)

Egg white proteins are composed of four layers: the outer light white, the outer thick white, the inner light white, and the chalaza layer. The chemical composition of egg white includes 92% water, 10% protein, 0.4-0.9% carbohydrates, 0.03% fat, and 0.5-0.6% ash. Albumins are soluble in water, Dissolve moderately in concentrated salt solutions and heat-denaturize. Globulins are insoluble in distilled water and saturated salt solutions, whereas glycoproteins are proteins linked to carbohydrates or carbohydrate derivatives. (Cameron, 2020).

The chemical composition of egg white represents 60% of the egg's weight, of which water is the main component, followed by carbohydrates, ash, and small amounts of fat (1%). Egg white is composed of 88% water and 11% protein (Erwanto and Sukarno, 2023).

Quan and Benjakul (2019) The egg white proteins (EWP) are made up of ovalbumin (OVA), ovotransferrin (OVT), ovomucoid (OVM), ovomucin (OVN), and lysozyme. OVA is a phosphoprotein that contains free sulfhydryl groups found in egg white proteins. Ovotransferrin includes fifteen disulfide bonds. Ovomucoid is a glycoprotein having nine disulfide linkages. Ovomucin is a very viscous glycoprotein. Lysozyme contains four disulfide links . The disulfide linkages and sulfhydryl groups found in egg white proteins combine to form a gel. Despite their low content, ovomucin's high viscosity induces gel formation. Table 1 demonstrates the content and characteristics of proteins found in chicken EWP.

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Table (1) content and percentages of egg white proteins, Guha *et al*. (2019)

Egg protein	Protein percentage %	Molecular weight
Major proteins		
Ovalbumin	54	٤٥
Ovotransferrin	12	٧٦
Ovomucoid	11	28
Ovomucin	٣٥	5500–8300
Lysozyme	34	14
Secondary proteins		
Ovoglobulin		
G2 globulin	٤٠	٤٥-٣٠
G3 globulin	40	—
Ovoinhibitor	15	٤٩
Ovoglycoprotein	10	24.4
Ovoflavoprotein	8	32
Ovomacroglobulin	5	769
Cystatin	0.5	12.7
Avidin	0.5	68.3

2. Modification proteins

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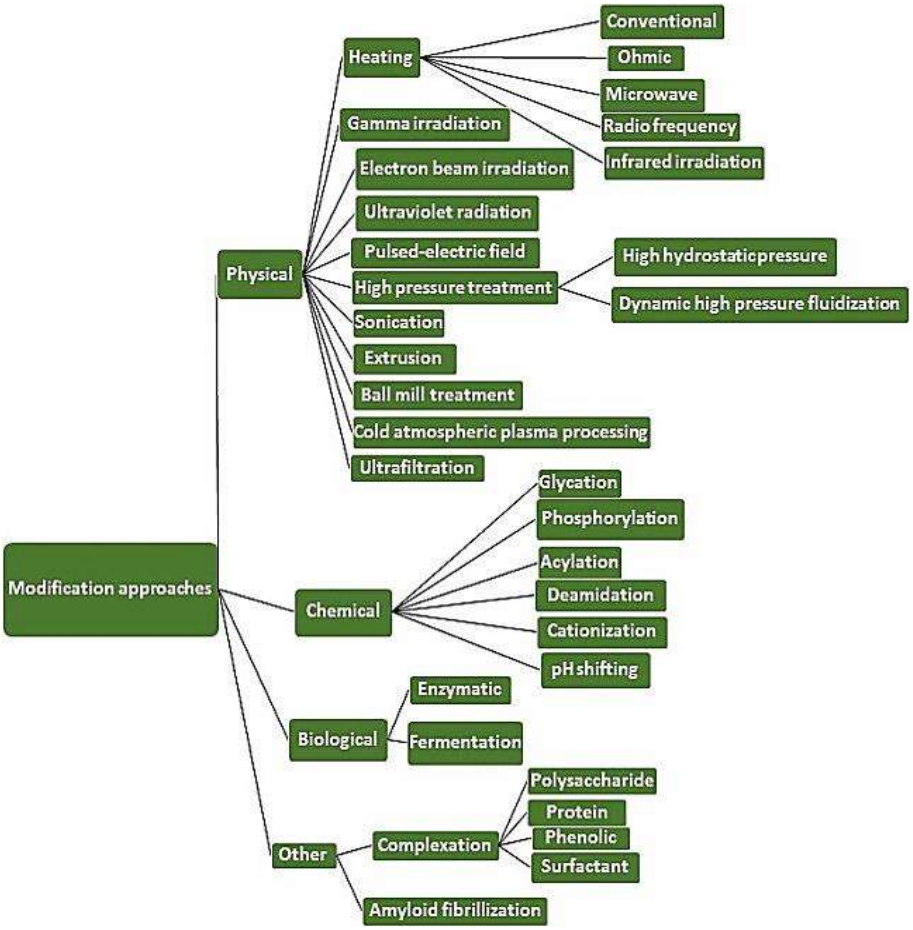
The term "protein modification" refers to changing the molecular structure or chemical groups of proteins in a specific way to improve their functions and biological activity. This provides modification with an opportunity to make them multi-functional components in the diet by changing their physical and chemical properties (Nasrabadi *et al*., 2021).



Scheme (1) Protein modification methods (Nasrabadi *et al*., 2021)

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2.3 Modification with phenols

Plant phenols: The term "phenol" is a chemical term that describes a benzene ring that carries one or more hydroxyl groups. The term "polyphenol" defines a natural product containing two benzene rings that carry one or more hydroxyl groups. Phenols are secondary metabolites of plants and form one of the most common and widespread plant groups. (Lattanzio,2013)

In general, phenolic compounds are classified into simple and multi-phenolic compounds. Simple phenols are those with only one phenol unit and a C6 typical structure, as seen in Figure 2. The organic group indicated by the letter R can be alkyl, alkenyl, aryl, etc.; or hydroxyl, alkoxy, amino, etc.

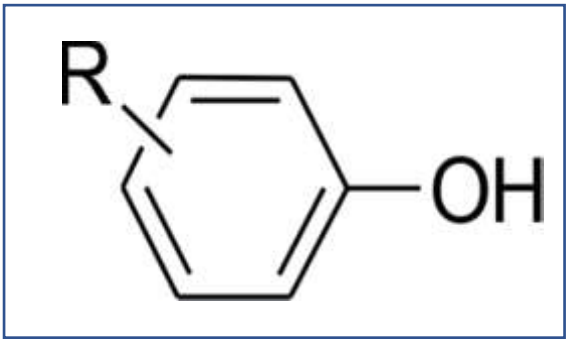

Figure (2) Structure of simple phenols

Phenolic compounds that have more than one phenol unit are considered polyphenols. Their structural formula contains C15. Flavonoids are one type of polyphenol, as shown in Figure (3). (AlMamari, 2021).

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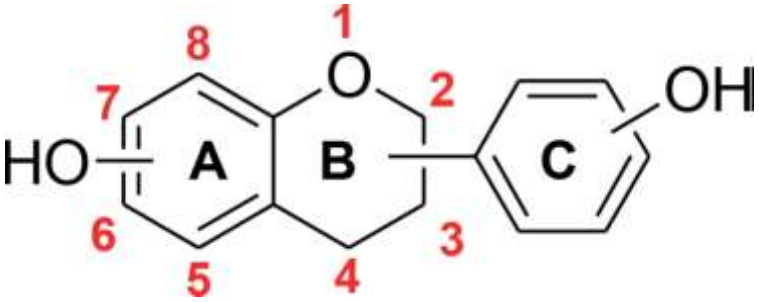

Figure (3) Structure of flavonoids

Table (2) Classification of phenolic compounds

1	Phenolic acid	١	Hydroxybenzoic acid derivatives						
			p-Hdroxybenzoic acid	Gallic acid	Vanillic acid	Syringic acid	Ellagic acid	Protocatechuic	Gentisic acid
		٢	Hydroxycinnamic acid derivatives						
			p-Coumaric acid	Caffeic acid	Ferulic acid	Sinapic acid		Chlorogenic acid	
2	Tannins	1	Hydrolyzable tannins						
			Gallotannins		Ellagitannins				
		2	Condensed tannins (Proanthocyanidins; Oligomer, polymers)						
			Dimers	Trimers	4-10mers	Polymers			
3	Flavonoids		Flavones	Flavonols	Isoflavones	Flavanones	Flavan-3-ol	Anthocyanidins	Chalcones
			Apigenin Luteolin *Chrysin *Rutin	*Quercetin *Kaempferol Myricetin* Isohamnetin*	Genistein* Curcumin* Daidzein* Glycitein* *Formononetin	*Naringenin *Hesperidin *Neohesperidin *Eriodictyol	Catechin(+)- (-)-Epicatechin (-)-Epicatechin-3-gallate (+)-Gallocatechin (-)-Epiallocatechin (-)-Epigallocatechin-3-gallate	*Cyanidin *Delphinidin *Pelargonidin *Peonidin *Petunidin *Malvidin	*Phloretin
4	Non-Flavonoids	1	Lignans						
			Pinoresinol	Matairesinol	Secoisolariciresino				
		2	Stilbene derivatives						
			Resveratrol	Pinosylvin	Piceid				

2.3.1 Mechanism of protein-polyphenol complex formation

Phenolic compounds can form complexes by covalent or non-covalent bond interactions (hydrophobic, electrostatic, van der Waals, and hydrogen bonds). Proteins' structural changes upon binding with phenols can cause them to fold or unfold, forming insoluble or soluble complexes depending on the concentration

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of phenols, their molecular weight, structure, ionic factors/cofactors, and reaction conditions (solubility) of the compounds, which affect their nutritional and functional properties, as well as their biological activity. As a result, investigations have demonstrated that phenols have a considerable binding affinity to proteins through these interactions, therefore affecting their structure and functional capabilities. (Shahidi and Dissanayaka, 2023).

Protein-polyphenol interactions can be classified as either reversible or irreversible. Non-covalent bonds like as hydrogen bonds, hydrophobic bonds, and van der Waals forces are examples of reversible interactions; irreversible interactions occur when proteins and polyphenols bind via covalent bonding, When proteins attach to polyphenols, their structural characteristics change. Non-covalent interactions are the most common in nature. Non-covalent interactions are subject to environmental conditions like as temperature and pH variations, which affect protein and polyphenol binding. (Ozda *et al*., 2013).

2.3.2 Covalent interactions

Covalent interactions between proteins and polyphenols are typically mediated by C-N or C-S bonds, as illustrated in Figure 4. Phenolic compounds can produce a quinone radical from terminal amino acids by creating covalent bonds in the presence of oxygen or enzymes. (Harshadarai *et al*., 2022).

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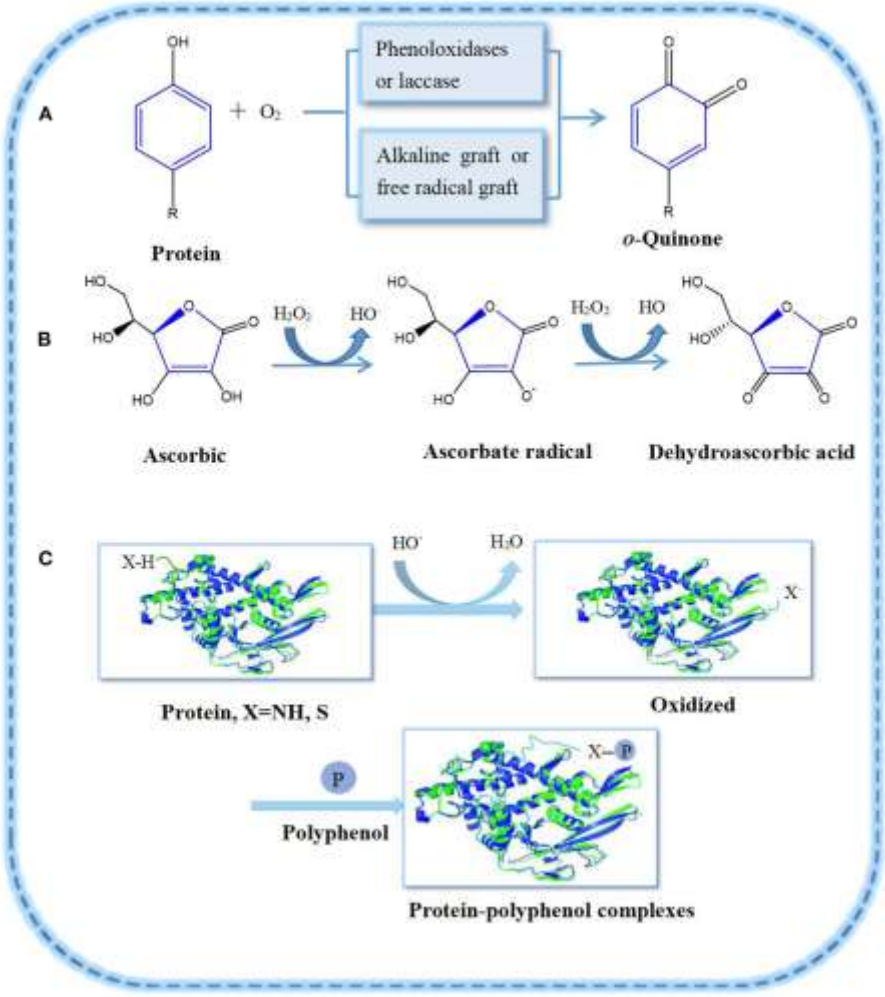

Figure (4) Polyphenol oxidation and protein covalent interactions: (a) Polyphenol oxidation pathway, (b) Ascorbic acid oxidation, and (c) Protein-polyphenol binding interaction. (Li *et al*., 2021).

During the formation of a protein-polyphenol complex (PPCS), the polyphenol may react with amino acids on the proteins side chain (such as lysine and methionine), resulting in

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covalent connections between the protein and the polyphenol. (Quan *et al*., 2019).

Polyphenols can also be partially oxidized and coupled with proteins to produce complexes that enhance functional and antioxidant capabilities. (Liu *et al*., 2017).

Polyphenols can attached to proteins using both enzymatic and non-enzymatic mechanisms. Proteins interactions with free radicals generated by polyphenol oxidation are the mainstay of non-enzymatic. These techniques are thought to be superior to the conventional method for producing high-activity PPCs because they may increase the antioxidant activity of proteins more successfully (Gu *et al*., 2017; Liu *et al*., 2017).

You can also use enzymatic binding to create PPCs. Under basic conditions, oxidized tannin (TA) and catechin acid (CA) can covalently attach to the hydrolysis products of pig plasma proteins to establish complexes. This results in enhanced emulsifying and antioxidant capacity of the multi-peptides. (Chen *et al*., 2019).

Feng *et al.* (2018) Ovalbumin (OVA) has been found to form covalent complexes with antioxidants including Epigallocatechin (EGC), Catechin (C) and Epigallocatechin Gallate (EGCG). β-lactoglobulin-catechin (b-LG-CT), α-lactalbumin-CT, gelatin-gallic acid/catechin (gelatin-GA/CT), and lactoferrin-chlorogenic acid/epigallocatechin gallate/galic acid (LF-CA/EGCG/GA),were effectively prepared via this method, which also assessed the impact of polyphenol modifications on the structural and functional characteristics of these proteins. (Yi *et al*. 2016; Umile *et al*. 2009).

.2.3.3 Non-covalent interactions

As seen in figure 9, hydrogen bond ,hydrophobic interaction ,and electrostatic interaction are the main non-covalent mediators of interactions between protein and

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polyphenols (Le and Renard, 2012). The main factors influencing the non-covalent formation of complexes between proteins and polyphenols are hydrogen bonds and hydrophobic interactions.

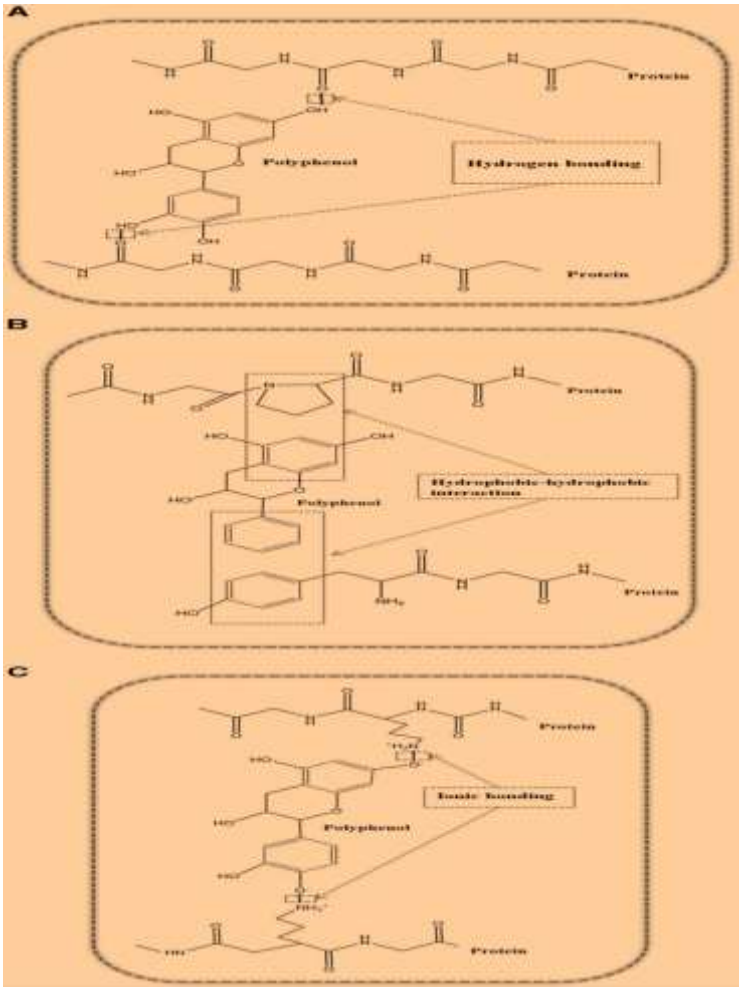

Figure (5) Non-covalent interactions between proteins and polyphenols include hydrogen (a), hydrophobic-hydrophobic interactions(b), and ionic bonds(c). (Le and Renard, 2012)

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2.3.4 Hydrogen Bonds

Polyphenols can be employed as hydrogen donors to produce hydrogen bonds (Buitimea et al., 2018) Polyphenols' OH group interact with the amine nitrogen and carboxyl oxygen groups of proteins. (Yildirim-Elikoglu and Erdem, 2022).

2.3.5 Hydrophobic Interactions

Surface hydrophobicity is a key indication of protein surface-related activities (Tang and Liu, 2013). The protein-polyphenol complex is formed primarily by the hydrophobic interaction between proteins and polyphenols. (Yuksel *et al*., 2010).

Staszewski *et al*. (2012) It should be mentioned that protein-polyphenol interaction is mostly mediated by hydrophobic forces. Furthermore, Some hydrophobic amino acids, like glycine and leucine, have been demonstrated to interact with the non-polar aromatic rings of polyphenols. (Ozdal *et al.*, 2013).

For example, The hydrophobic interaction between polyphenols and β-lactoglobulin (b-LG) altered the protein's secondary structure, resulting in increased stability. This trait is beneficial for stabilizing stable emulsions connected to PPCs. Protein structure can be altered by hydrophobic interactions with polyphenols. (Kanakis *et al.*, 2011).

2.3.6 Effect of Protein-Polyphenol Interaction on Protein Structure and Functional Properties

Changes in Protein Structural Properties

When proteins interact with polyphenols to form PPCs, their secondary, tertiary, and quaternary structures may change. Polyphenols modify protein secondary structure by transforming it to a turn, coil types include β-sheet, α-helical, β-turn, or random coil. (Hasni *et al*., 2011) .Ge *et al*. (2021) Analysis of the secondary structure of soluble soybean proteins in respect to tea polyphenols was conducted using circular dichroism (CD).

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Intermolecular interactions in proteins happen when the protein does not have random coils present any more and when the α-helix composition is augmented, due to the tea polyphenols' participation when forming a complex organization. Liu *et al*. (2015) was able to access the levels where LF was mixed with polyphenols while observing the structure by using absorbance values after UV/ Visible light absorption as seen in CD. The same effective spectral parameters of dark fluorescence spectroscopy can accordingly be employed to investigate the nature and dynamics of structural and dynamic changes induced by protein interactions with polyphenols.

The structures of lactoferrin-epigallocatechin gallate (LF-EGCG), lactoferrin-catechin acid (LF-CA), and gallic acid-lactoferrin (LF-GA) complexes were determined using fluorescence spectroscopy. It was shown that binding of the LF-polyphenols complex may have impacted an effect on tertiary structure of LF .

The UV absorption band caused by protein-phenol interaction resulted in an increase in the α-helix. Lactoferrin LF complexes with catechin acid (CA) or The intensity of the band increased somewhat from 210 to 230 nanometers when epigallocatechin gallate/gallic acid (EGCG) was added. The binding of LF with polyphenols can lead to an increase in the break-in α-helix with a parallel decrease in the random coil structure, indicating the effect of the binding on the restructuring of the polyphenols (Harshadrai *et al*.,2002) .

The concentration of the reactants could be the reason of the protein structural change or heat treatment may result in both chemical and physical changes **(**Al-Shibli *et al*.,2023) . The type of protein utilized, as well as the variations between laboratory and biological trials, all influence the protein-polyphenol interaction. As a result, The effect of polyphenols on proteins

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differs from instance to case: Interaction with polyphenols does not alter certain proteins, Others are affected by external circumstances, such as the concentration of the reaction solution. (Klaus *et al*., 2005).

Xue *et al.* (2021) investigated the enhancement of the physical and chemical properties of egg white proteins gel , the secondary structure, and the microscopic structure when modified with tea polyphenols and ultrasound. The modification was carried out with ultrasound at different powers from 0 to 360 watts. It was observed that with increasing ultrasound power, the soluble protein content increased. It was observed that ultrasound treatment causes a change in the molecular structure of the protein, which causes an increase in the solubility of the protein. Furthermore, the egg white protein's polarity increased when combined with polyphenols, resulting in enhanced water-protein interaction and an increase in disulfide bonds. The study showed that as ultrasound power increased, the levels of β-sheets and α-helices in egg via treated with tea polyphenols and ultrasound also increased. This resulted in enhanced gel stability and water preservation ability , peaking at 120 W ultrasound power. Therefore, using ultrasound and polyphenols to treat egg white protein gel is seen as an advanced technique that enhances the nutritional content of the protein present in egg whites

Budryn *et al*. (2014) utilized green coffee extract to change the proteins in egg whites. To examine how the inclusion of coffee polyphenols with protein effects the samples were heated to 110 and 180 °C for one hour. Samples with 3% green coffee extract demonstrated higher nutritional value and increased antioxidant activity compared to samples containing starch, sunflower oil, sucrose, and iron ions without coffee polyphenols.

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The addition of coffee polyphenols leads to the formation of covalent and non-covalent bonds. In addition, heating egg white protein added with coffee polyphenol led to partial hydrolysis of the protein. This is because the reaction occurs between amino acids and polyphenols in a faster way. In addition, heating the protein with coffee polyphenols shows high sensitivity to pepsin digestion.

2.3.7 Factors impacting interactions between proteins and polyphenols

The formation of protein-phenolic complexes is affected by various internal and external factors. Internal variables such as molecular weight, hydrophobicity, and the chemical composition of proteins and polyphenols, including the number and sequence of amino acids, are instances of internal factors.

Proteins and polyphenols establish a strong bond through hydrophobic interactions, which are heightened in the presence of basic amino acids., Functional groups such as glycosyl, hydroxyl, and methyl play a crucial role in the interaction. External factors like temperature, pH, and salt concentration affect the interaction between proteins and polyphenols. Shifts in temperature can impact the strength of non-covalent bonds and the structure of proteins, consequently influencing their interactions. High temperatures can cause proteins to change shape, reducing the ability of polyphenols to bind to them. The pH level, an external factor, also affects how soluble proteins and polyphenols are when they interacFor example, the polyphenols in tea and berries are highly sensitive to pH, especially at the isoelectric point of the protein.

A notably pH difference obserable in the non-covalent between of polyphenols in tea, coffee, and cocoa with β-

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lactoglobulin. The third outside factor is the strength of ions. Adding salt chloride to elevate the ionic strength lowers the interaction (Zhang et al. 2021)

3. Diagnosis of Modified Egg White Proteins

3.1 FT-IR spectroscopy.

Another function group of an aromatic which could be -nitrile, -hydroxyl, or -methyl is another of the group of various detectable functional groups found in a molecule.

the two techniques of the carbonyl investigation via FT-IR and spectroscopy are most defiantly that which we most often encounter.(Khan et al.,2018). FT-IR is considered by many as a powerful method because of its intricacy and therefore, communities may subject to its applications in researches. therefore, the way that the atoms interact with each other by integrating with some both covalent and noncovalent bonds; and also the interaction of one protein residue, which grows further to give the secondary structures of the macromolecule are formed. This is achieved in many different ways for instance by person that is a product that is involved in biodevices, or by creation of devices that can be used to measure concentrations of toxic substances.

By this method we can know what the amide I range is most likely the one that is changed because the other regions only point out that this area is more affected by environmental conditions. alterations in secondary structure**.**

Seczyk et al. (2019) used FT-IR spectroscopy to identify alterations in protein conformation resulting from the interaction of phenolic compounds with albumins and globulins, both types of proteins present in white beans. The specimens were examined in the area linked to changes in protein secondary structure, spanning from 1480 to 1700 cm^{-1}.

According to Zhou *et al*. (2019), there was a identifitly in the structural, chemical, and physical characteristics of the surimi gel (fish

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protein concentrate) when the amount of polyphenol-rich tea extract (0.2%, 0.4%, 0.6%, and 0.8%) used to incubate egg whites were varied.

The polyphenol treatment changed the secondary structure of egg white, which caused in a decrease in the α-helix structure and an increase in the β-folding structure, as identified by FT-IR analysis. Through the enhancement of the β-folding structure and decreased of the α-helix structure, FT-IR analysis was able to determine the polyphenol treatment of egg white. In addition, it was found that the strength of the surimi gel network and its ability to holding water were noticeably enhanced when polyphenols were added to the egg whites.

3.2 Scanning Electron Microscopy (SEM)

The process of scanning electron microscopy involves scanning samples using a concentrated electron beam in order to create picture of the samples. The atoms in the samples converse with the electrons to give details about the surface composition and topography (Moslim, 2022).

In their study, Johny *et al*. (2021) found that when they connected through examination coagulated egg white proteins that had been hydrolyzed utilizing scanning electron microscopy and contrasted them to non-hydrolyzed control samples, the microscopic structure of the hydrolyzed proteins revealed to be spongy and elastic, in contrast to the control samples, which had not undergone enzymatic treatment, demonstrated a rigid structure in the shape of a sheet with a glassy surface.

3.3 Fluorescence spectroscopy

Proteins' fluorescence emission intensity is primarily because of the fluorophores tryptophan, phenylalanine, and tyrosine. Ovaalbumin is the main protein located in egg whites and has an importent effect on the intensity of fluorescence emission. Utilizing fluorescence spectroscopy is capable of identify changes in the structure of the protein that emits

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fluorescence (Deseta, 2023). Differences in the composition of thick egg white were found using fluorescence spectroscopy following being exposed to different temperatures. After being subjected to treatment at a lower temperature of 30 °C, the fluorescence intensity of the egg white increased in comparison to when it was thick and untreated. This treatment promted the protein structure being released, exposing the internal groups, as mention by (Luo *et al*., 2022)

4. Phenolic Compound : Application in Protein Modification

Proteins are prefect polymeric materials regarding the production of edible films. Proteins interact with phenolic compounds to form cross-links that improve the mechanical and barrier properties of the produced films. For example, Liu et al. (2019) used oxidized phenolic components (tannic and caffeic) from green tea extract to study the mechanical and barrier properties of gelatin film with turmeric. The results revealed an improvement in tensile strength, reduced water vapor permeability, solubility, and elongation at break of the gelatin and turmeric layers in water. The Films also demonstrated increased antioxidant activity (Al-Hilifi *et al*.,2023), as well as superior mechanical and barrier qualities. As a result, they can be utilized in food packaging to increase its shelf life.

Feng *et al.* (2023) explained that the interaction of polyphenols with proteins has multiple food and medical applications. The interaction of soy protein with polyphenols enhances the strength of the gel network. Tea polyphenols can be applied as an excellent egg white product gel model. Gelatin gel treated with gallic acid polyphenols enhances antioxidant and antimicrobial activity, which can be used in dietary systems. The alcoholic extract of sumac fruits was used as a rich source of natural antioxidants and also for its great potential in improving meat quality (Hashim and Fadhil,2021).

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Deseta *et al*. (2023) studied the possibility of producing antifungal films from nano-sized egg white protein with polyphenols. Antifungal properties, high packaging efficiency, transparent yellow color, low flexibility, and brittleness characterized the manufactured films.

Liu *et al*. (2023) also showed that polyphenols can be used as a preservative due to their antioxidant properties. They also work to strengthen films due to their high ability to bind polymers. In addition, adding polyphenols to protein changes the digestibility of protein-coated fat droplets, which is beneficial in controlling gastric digestion.

Al-Ansari (2018) studied sponge cake's qualitative and physicochemical properties using different concentrations of sweet bean powder when stored for different periods. It was found that the addition of sweet bean powder has an effective effect on the sensory properties of color, size, and pulp freshness, as well as the effect on the storage properties of the manufactured cake.

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