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Food Production, Processing and Nutrition (2023) 5:2
https://doi.org/10.1186/s43014-022-00121-0

Food Production, Processing and Nutrition

REVIEW

Open Access

Phenolic-protein interactions: insight from in-silico analyses – a review

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Abstract

Phenolic compounds are ubiquitous plant secondary metabolites that possess various biological activities and are known to interact with proteins, altering their structure and properties. Therefore, interactions between these compounds and proteins has gained increasing attention due to their potential benefits to human health and for exploitation by the food industry. Phenolic compounds and proteins can form complexes via covalent linkages and/or non-covalent interactions through hydrophobic, electrostatic, van der Waals forces and hydrogen bonding. This review describes possible mechanisms of phenol-protein complex formation, their physiological action and activities that are important in the food industry, and possible outcomes in the terms of molecular docking and simulation analysis. The conformational changes of the protein upon binding with polyphenols can lead to the folding or unfolding of the protein molecules, forming insoluble or soluble complexes. The concentration of polyphenols, their molecular weight and structure, ions/cofactors and conditions of the system determine the precipitation or solubilization of the complex, affecting their nutritional and functional properties as well as their bioactivities. In this regard, molecular docking and simulation studies of phenolic-protein interactions allows comprehensive virtual screening of competitive/non-competitive and site-specific/non-specific conjugation of phenolics with different protein targets and facilitates understanding the observed effects. The docking analysis of flavonoids with enzymes and milk proteins has indicated their potential application in producing nutraceuticals and functional foods. Thus, combining molecular docking and simulation studies with experimental techniques is vital for better understanding the reactions that take place during digestion to engineer and manufacture novel food ingredients with desirable pharmacological properties and as potential food additives.

Keywords: Polyphenols, Phenol-protein complexes, Molecular interactions, Molecular docking

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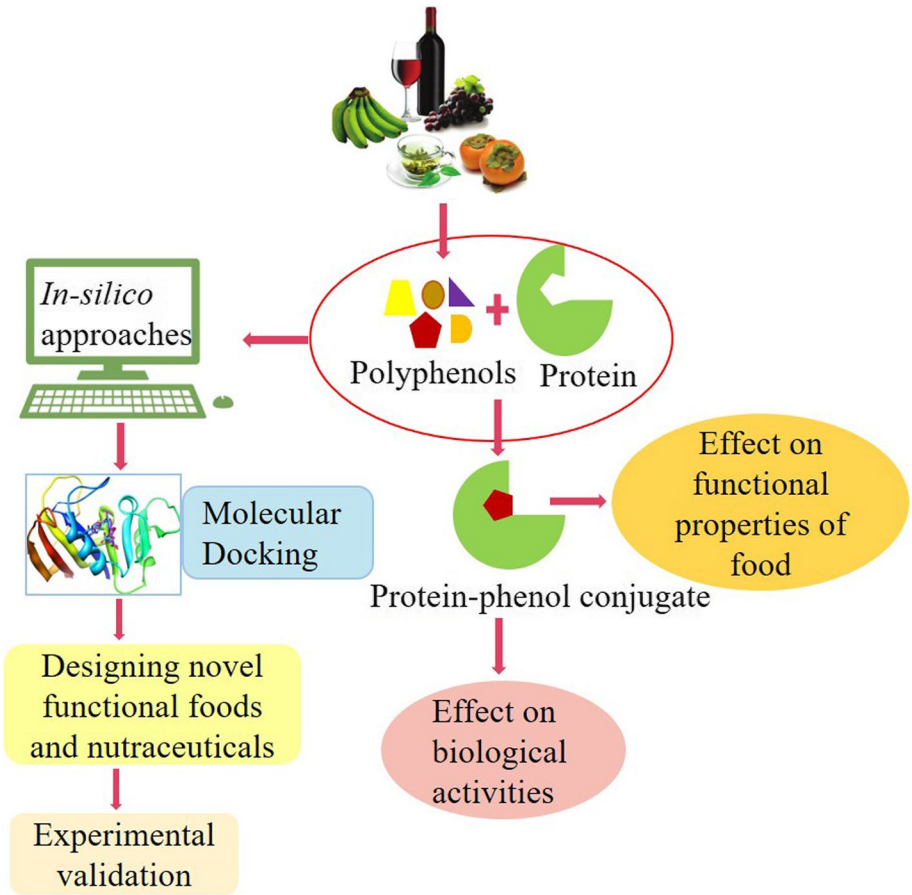
Graphical Abstract



Introduction

Food is a complex and heterogenous system containing different major and minor constituents. The major macromolecular components include proteins, polysaccharides, and lipids while minor food components are organic acids, pigments, aroma compounds, vitamins, and minerals, among others, which also provide the essential nutrients (Samant et al. 1993). These constituents individually or as complexes play critical functional roles in foods and in different bioactivities. Most scientific literature has documented the nutrient composition of foods, identification of novel compounds and their functional properties, and bioactivities. In the past decades, most studies had focused on determining the content of bioactive compounds and bioactivity of vegetables, legumes, grains, fruits, herbs, and seafood. At present, the attention has been drawn to the fundamental understanding of the behavior of food macromolecules to interpret bioactivities at the molecular level.

In this contribution, an overview of the main interactions between phenolics and proteins in foods including their effects on biological activities and food properties are presented. In addition, particular emphasis is given to understanding and predicting these interactions and outcomes in terms of molecular docking simulations with model systems containing selected phenolic compounds and proteins. The insights obtained at the molecular level will provide a fundamental framework for in-vitro studies from nutritional, functional and pharmaceutical perspectives.

Proteins are one of the primary macronutrients and functional components of the diet that determine food's textural, sensorial, and nutritional properties. According to the chemical structure, proteins are made up of 20 different amino acids that are linked together by peptide bonds in different combinations (Małecki et al. 2021). The linear sequence of amino acids within a protein is considered as the primary structure of the protein which drives the formation of the secondary structure through stable

folding patterns such as alpha helices and beta sheets. The folding is further driven by non-specific hydrophobic interactions that ultimately determine the protein's unique three-dimensional shape or tertiary structure and its function. The quaternary structure is a result of association of several protein chains into a specific spatial arrangement. Proteins undergo a wide range of structural and conformational changes through various complex interactions with other food components which provides numerous beneficial effects. Some of proteins have specific binding sites for other molecules that result in stable conformations upon binding and modulate the functioning of the system (Chen et al. 2021). In relation to the interactions between protein and phenolic compounds in food, proteins can form complexes with them, leading to changes in their structural, functional and nutritional properties which can broaden the range of functionalities achieved (Alu'datt et al. 2020; Ozdal et al. 2013).

Phenolic compounds are a major class of secondary metabolites in plants that are mainly derived from phenylalanine and tyrosine (Shahidi & Chandrasekara 2017). In this category, phenolic acids, namely hydroxybenzoic acid and hydroxycinnamic acid derivatives may be noted that are regarded as simple phenols consisting of a benzene ring substituted with hydroxyl groups. Meanwhile, polyphenols contain multiple rings with more than one phenolic group. Polyphenols can be classified as flavonoids and non-flavonoids according to their structures. The flavonoids include flavanones, flavanonols, flavones, flavonols, isoflavones, flavanols and tannins (hydrolysable and condensed) that are the most abundant and widespread dietary polyphenols (Fig. 1). The chemical structures of phenolic acids are given in Figs. 2 and 3. The different flavonoids differ greatly in their molecular structure based on the degree and pattern of glycosylation, hydroxylation, methoxylation and/or prenylation as shown in Figs. 4 and 5 (Guan et al. 2021). Most of them possess antioxidant activity, and hence they are frequently used as additives in functional dietary products (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021) in foods and biological systems. In addition, they affect flavor and nutritional quality of foods, plant growth and metabolism and act as substrates for oxidative browning reactions. They provide numerous beneficial health effects in humans by encompassing anti-inflammatory, antithrombotic, antibacterial, anti-allergic, antidiabetic and anticarcinogenic effects (Shahidi & Chandrasekara 2017).

Polyphenols are more stable under acidic conditions and the oxidative degradation of them frequently occurs during gastrointestinal digestion due to the nearly neutral pH condition in the small intestine. However, most polyphenols are too polar to penetrate through the intestinal membrane (Grgić et al. 2020) and most of them are present in food in the form of esters, glycosides, or polymers that cannot be absorbed in their native form. The membrane carriers must be involved in the transportation of phenolic compounds and they must be hydrolyzed by intestinal enzymes or by the colonic microflora before they can be absorbed (Manach et al. 2004). Therefore, the gastrointestinal stability and target delivery are

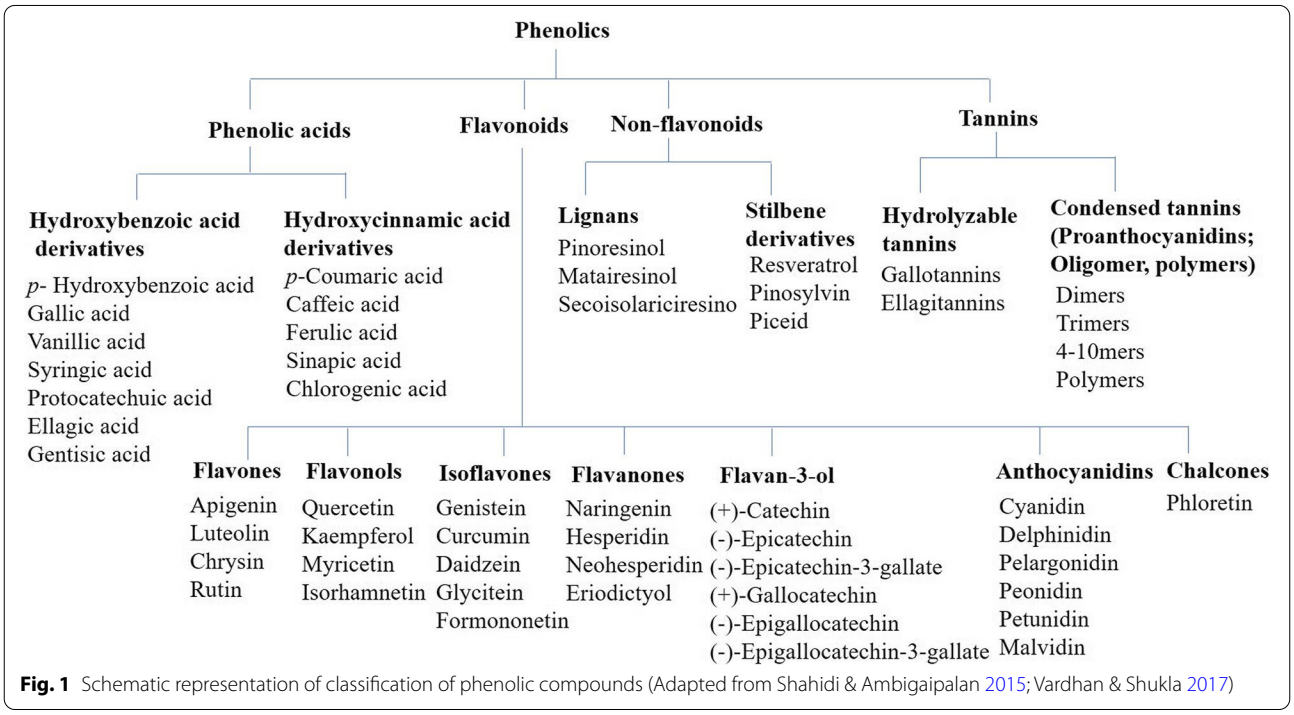


Fig. 1 Schematic representation of classification of phenolic compounds (Adapted from Shahidi & Ambigaipalan 2015; Vardhan & Shukla 2017)

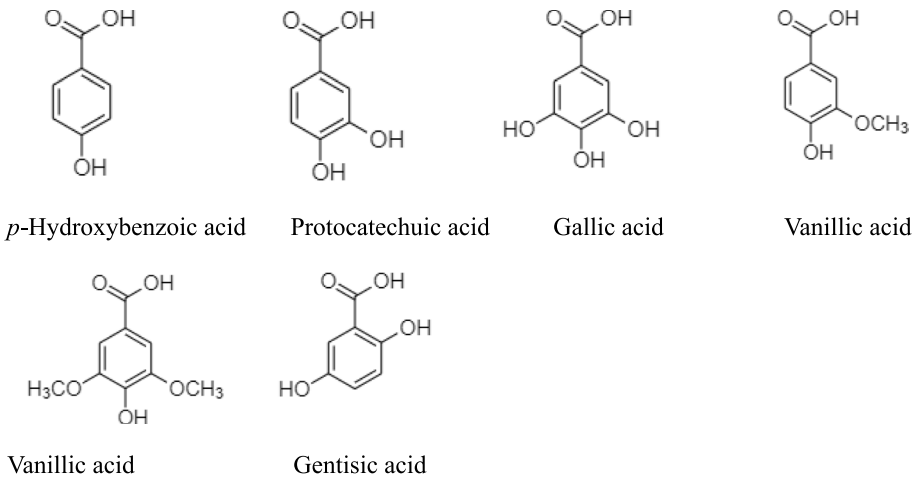


Fig. 2 Chemical structures of hydroxybenzoic acid derivatives

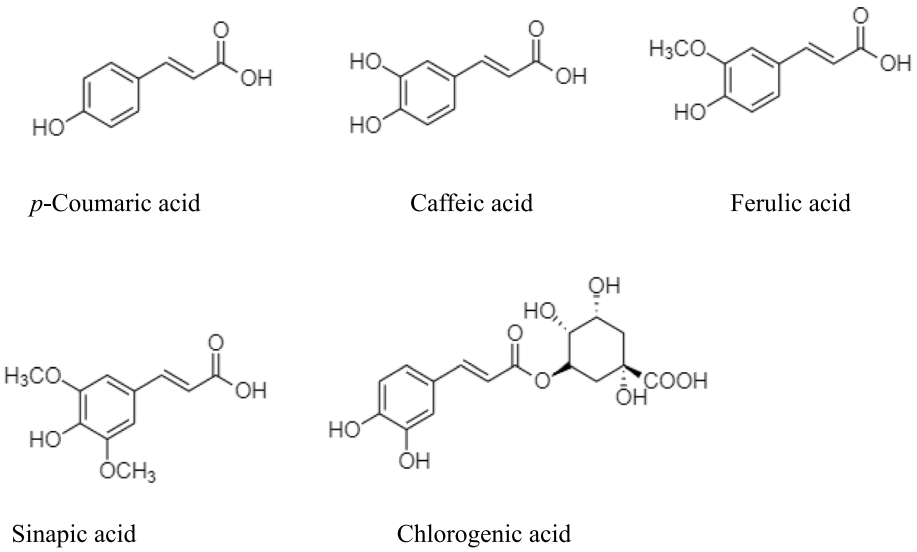


Fig. 3 Chemical structures of hydroxycinnamic acid derivatives

the key factors for phenolic acids and polyphenols to exert their biological activities inside the human body (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Minatel et al. 2017). The bioaccessibility and efficacy of phenolics that could generate different bioactivities are greatly affected by their interactions with other dietary food components encountered during food processing, storage and in the course of digestion in the gastrointestinal tract with enzymes, with blood plasma proteins, and with proteins in target tissues of organs in the human body (Guan et al. 2021; Minatel et al. 2017; Shahidi & Chandrasekara 2017; Rawel & Rohn 2010).

Many studies have shown that strong molecular interactions of polyphenols towards proteins consequently alter their structures and properties, significantly affecting the bioactivities that could generate beneficial effects on human health (Jiang et al. 2019). Better knowledge of molecular interactions between these two components and modification of physical and chemical properties of polyphenols would provide a way to improve their stability, cell uptake rate and effective target-specific delivery (Guan et al. 2021). Therefore, understanding these interactions at the molecular level during food processing, digestion and absorption in

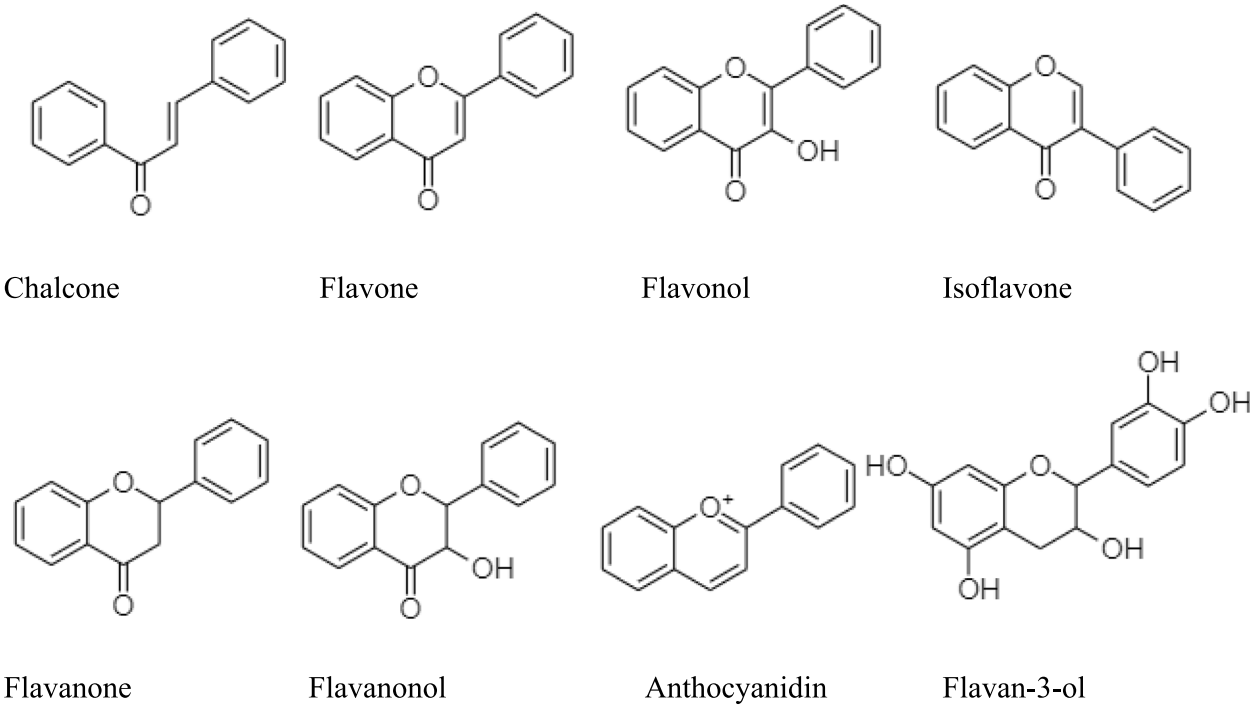


Fig. 4 Backbone chemical structures of flavonoids

humans are important in order to manufacture nutraceuticals with optimal bioactivity functionality.

The molecular interactions between phenolics and proteins

Plant phenolics may bind covalently or non-covalently with proteins and the type of interactions will depend on the mole ratio of phenolic/protein. Several phenolics could attach to one protein molecule or one phenolic may bind to several protein sites or protein molecules and hence the interactions are known to be multisite or multidentate, respectively (Prigent et al. 2003). Among the two kinds of interactions, evidences suggest that phenolics and protein mainly bind with each other through non-covalent bonding which are reversible interactions and the interaction is created by hydrophobic association which may subsequently be stabilized by hydrogen bonding (Prigent et al. 2003). Furthermore, interactions involving non-covalent bonds are susceptible to environmental factors such as temperature and pH, which affect the binding of protein and polyphenols (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Zhang & Zhong 2012). Hydrophobic interactions may occur between phenolic compounds and neutral amino acids of a protein, while hydrogen bonding may occur between the nitrogen or oxygen and hydroxyl groups of phenolic compounds with charged amino acids in the protein (Rawel & Rohn 2010). Many studies have shown that polyphenols possess strong bonding affinities towards protein via these interactions, consequently altering their structures and properties and significantly affecting the bioaccessibility and bioavailability of polyphenols (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021).

Covalent interactions

Covalent bonding causes irreversible interactions between polyphenol and protein molecules, usually via C-N or C-S linkage. The formation of quinones initiates it through enzymatic modification in the presence of oxygen or autoxidation under alkaline conditions. Polyphenol oxidases can catalyze the hydroxylation of monophenols into ortho-diphenols that readily oxidize into ortho-quinones. Since quinones are strong electrophiles, they undergo reactions with nucleophilic residues of protein or peptides (thiol, amino, guanidine, or imidazole) via Schiff-base (C=N) and Michael addition mechanisms (C–NH) and may induce protein crosslinking (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Liu et al. 2019). Both carbonyl groups of quinone can also react with lysine and form an imino-quinone adduct which can rearrange into an iminophenol (Yin et al. 2014; Li, He, et al. 2021; Li, Ritzoulis, et al. 2021). Besides, protein radicals which may be formed by free radical attack (hydroxyl radicals) could covalently bind to polyphenols at the ortho- or para-positions of their

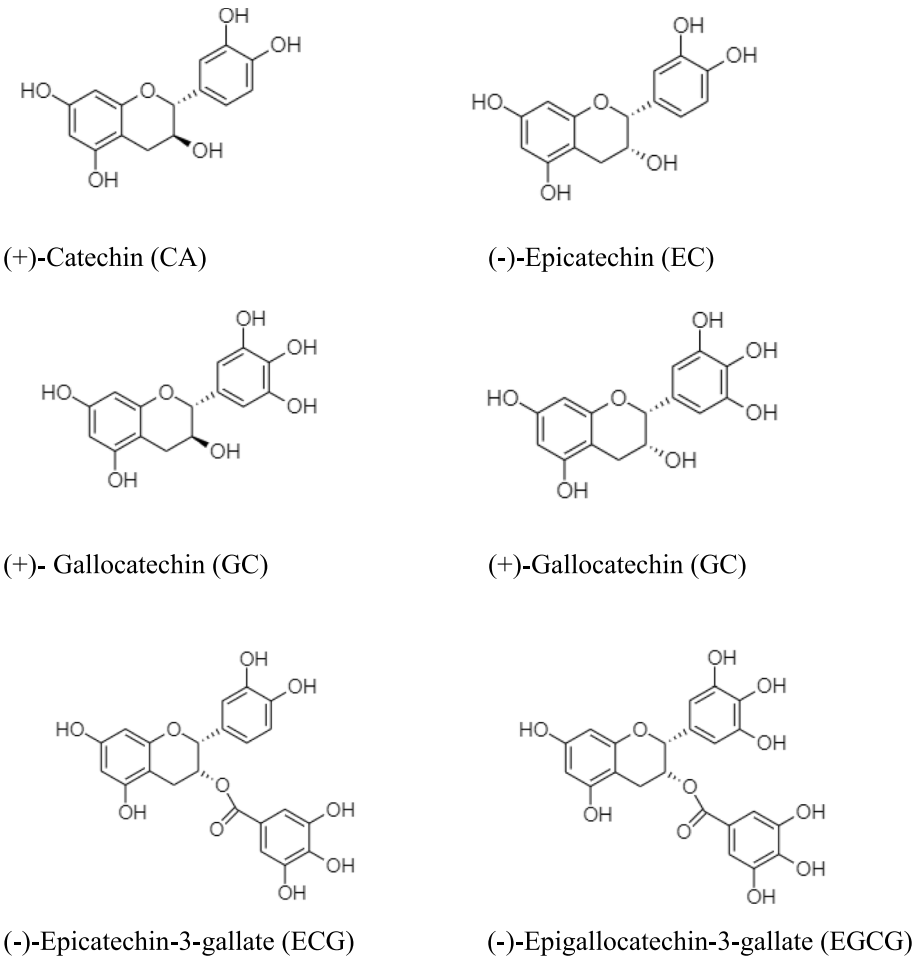


Fig. 5 Chemical structures of flavan-3-ols

hydroxyl groups or semiquinone radical intermediates derived from monophenols may attach to protein nucleophilic residues via covalent bonding as shown in Fig. 6 (Liu et al. 2017; Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Rawel & Rohn 2010).

The study on investigating chemical interactions between methyl caffeate, methyl dihydrocaffeate with thiols under radical oxidation conditions and their chemical structures determined by NMR analysis has shown the formation of 2′-monothiol adduct of caffeate and mono-, di- and trithiol adduct of dihydrocaffeic acid at the 2′,5′,6′ positions (Fujimoto & Masuda 2012). In a comparison of several catechins such as (−) -epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)- epigallocatechin gallate (EGCG), it was found that pyrogallol-type catechins (EGC and EGCG) have higher reactivity with protein thiols than catechol-type catechins ((EC and ECG). Further analysis has shown that only the pyrogallol structural motif on the B ring preferentially forms a B-ring quinone during autoxidation, consequently forming covalent bonds between catechins and protein thiols (Mori et al. 2010). Based on the molecular orbital calculations, this study has revealed that nucleophilic addition of the thiol can occur at the 2′-position on the quinone derivative (Fujimoto & Masuda 2012). Similar results were identified between enzymatically oxidized EGCG with cysteine, glutathione and formation of monoconjugates of these adducts as the major product (Sang et al. 2005).

Non-covalent interactions

Non-covalent interactions are usually reversible and occur via hydrophobic, hydrogen bonding, electrostatic, and van der Waals interactions. However, hydrogen bonding and hydrophobic interactions are the main forces involved in the non-covalent formation of complexes between proteins and polyphenols (Prigent et al. 2003; Li, He, et al. 2021; Li, Ritzoulis, et al. 2021). The major non-covalent forces involved in specific phenol-protein interactions can be determined by using

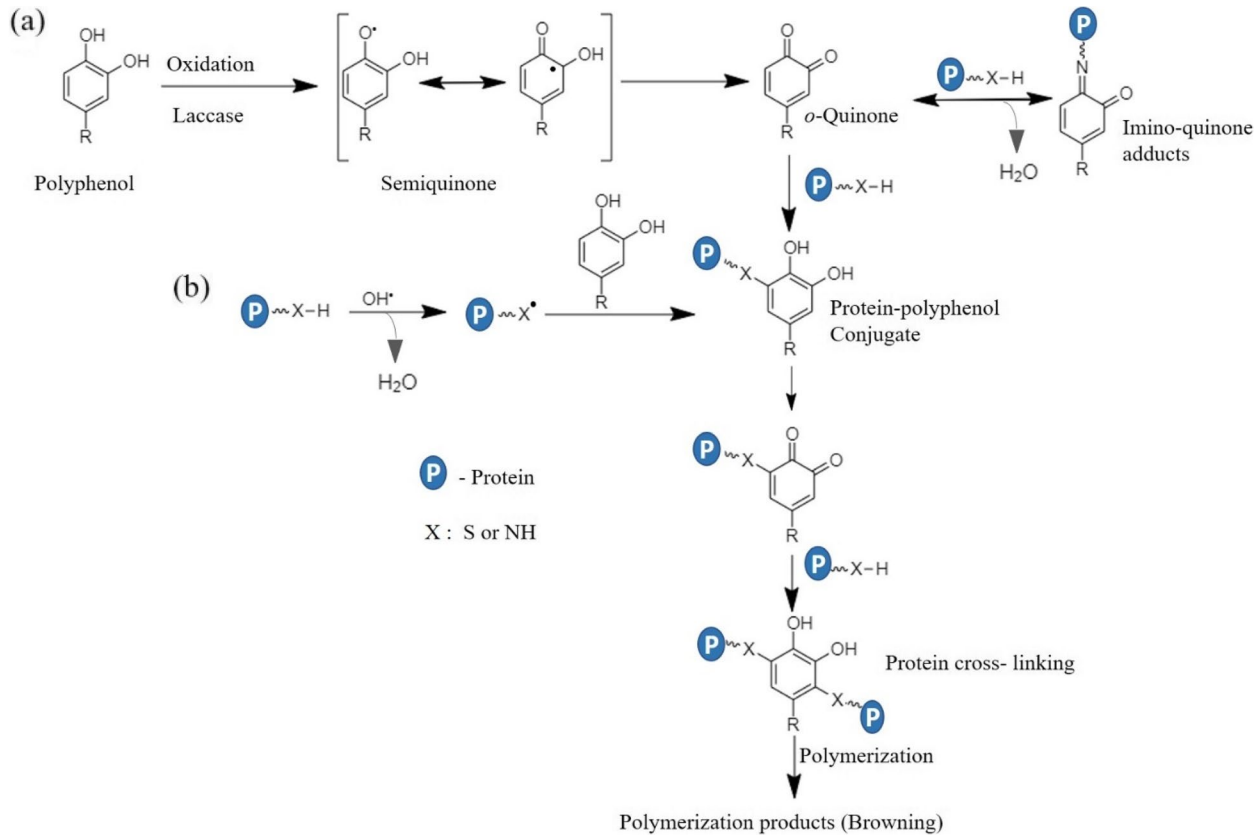


Fig. 6 Covalent bonding of polyphenols with protein. **a** Polyphenol-protein complexes formed by oxidation process - Michael addition and imino-quinone adducts. **b** Polyphenol-protein complexes formed by a free radical mediated process (Adapted from Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Liu et al. 2017; Rawel & Rohn 2010)

thermodynamic parameters, such as enthalpy change (△H) and entropy change (△S) (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Zhang & Zhong 2012).

Generally, hydrophobic interactions occur between hydrophobic amino acids and the aromatic ring structure of polyphenols; hydrogen bonding between oxygen atoms of peptide bonds and hydroxyl groups of polyphenols and electrostatic interactions or ionic bonding between positively charged groups of proteins, such as the lysine, and negatively charged hydroxyl groups of polyphenols (Fig. 7). The mechanism of interactions has been elucidated by identifying the haze forming proteins' specific affinity for polyphenols (Asano et al. 1982) and tannin-protein complex (Le Bourvellec & Renard 2012; Oh et al. 1980). According to these studies, it has been shown that haze forming proteins that are rich in proline residues are responsible for the specific binding with polyphenols. These findings also suggest that open and flexible structure of a protein can facilitate the entry of polyphenols into them by maximizing the available binding surface to form hydrophobic interactions and hydrogen bonds. Due to the folded structure of the globular protein, only surface-exposed aromatic residues are involved in hydrophobic association with polyphenols (Le Bourvellec & Renard 2012; Murray et al. 1994). The pyrrolidine ring of proline cannot form intramolecular and intermolecular hydrogen bonds with oxygen atoms of peptide bonds and it allows these free oxygen atoms to bind with hydroxyl groups of polyphenols via hydrogen bonding (Asano et al. 1982). Since charges of proteins are mainly influenced by pH, the electrostatic interactions are pH-specific (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; le Bourvellec & Renard 2012; Oh et al. 1980).

Polyphenolic-protein interaction mechanism

The binding of polyphenol alters the three-dimensional structure of protein molecules (Bandyopadhyay et al. 2012). This was particularly characterized by the in-vitro investigation of the interactions between the human salivary proline-rich protein IB5 and a wine and tea tannin model; complexation of tea polyphenol (EGCG) with β-lactoglobulin as well as with α-casein, and β- casein. One study showed that IB5 undergoes an unfolded to folded structure upon binding with EGCG and the folding

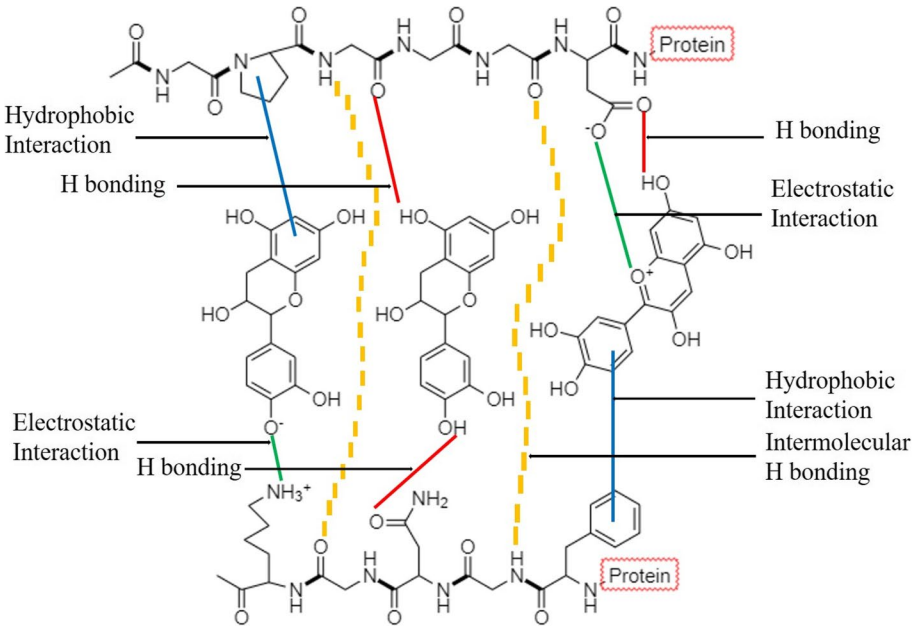


Fig. 7 Major non-covalent interactions between polyphenols and proteins; green, red and blue lines indicate electrostatic interactions, hydrogen bonding and hydrophobic interaction, respectively (Adapted from Le Bourvellec & Renard 2012; Li, He, et al. 2021; Li, Ritzoulis, et al. 2021)

is favored by multiple hydrogen bonds between IB5 and hydroxyl groups of polyphenols where more compact structures occur as the number of tannins bound to the protein increases. In addition, they have proposed that different folding structures may form with the binding of individual tannins or the binding of aggregated tannins. Once tannins aggregate on proteins via hydrophobic interactions, which are the primary interactions, the complex associates further via secondary interactions; hydrogen bonding which acts as a linker between several proteins and triggers the formation of insoluble aggregates as shown in Fig. 8 (Bandyopadhyay et al. 2012; Canon et al. 2011).

This mechanism is mainly associated with the astringency arising from precipitation of polyphenol-protein complexes, an important protective mechanism in animals that consume polyphenols (Charlton et al. 2002). It was demonstrated that polyphenols can self-associate via π-π stacking and form colloids which can influence their affinity for proteins. The study based on the role of proanthocyanidin monomers versus oligomers in wine turbidity indicated that proanthocyanidin monomers self-associate with a high affinity constant to form micelles at low critical micelle concentration (CMC) whereas self-association of oligomers occurs with a low affinity at a high CMC to form small micelles which are stabilized by charge repulsion between particles, suggesting that procyanidin oligomers are fully available to interact with saliva proteins (Pianet et al. 2008). Therefore, the formation of colloidal particles depends on their critical aggregation concentration or critical micelle concentration and the conditions of the medium. In the presence of low concentrations of polyphenols they act as single molecules that can interact individually with proteins, while under increasing concentrations of polyphenols above the critical aggregation concentration, they might interact with proteins as an aggregate or peptides become increasingly coated with polyphenols provide cooperative polyphenol bridges causing the peptides to dimerize and form an insoluble complex (Charlton et al. 2002; Pianet et al. 2008; Poklar Ulrih 2017; Zanchi et al. 2007).

The binding affinity of polyphenols to protein is size-dependent and increases with the molecular size of polyphenols. The structural analysis of tea polyphenols with milk β-lactoglobulin (βLG) indicated a significant increase in protein β-sheets structures and a minor increase in protein α-helical structures by reducing its random coil structures upon binding with high concentration of polyphenols. This eventually results further folding of the protein (Kanakis et al. 2011) and is indicative of protein structural stabilization in the presence of tea polyphenols. Similarly, lactoferrin (LF)-polyphenol conjugates formed with epigallocatechin gallate, chlorogenic acid and gallic acid revealed an increase in the content of α-helices while reducing other secondary structures. Further, a higher radical scavenging activity in the conjugates than only LF in the increasing order of chlorogenic acid–LF > EGCG–LF > gallic acid– LF

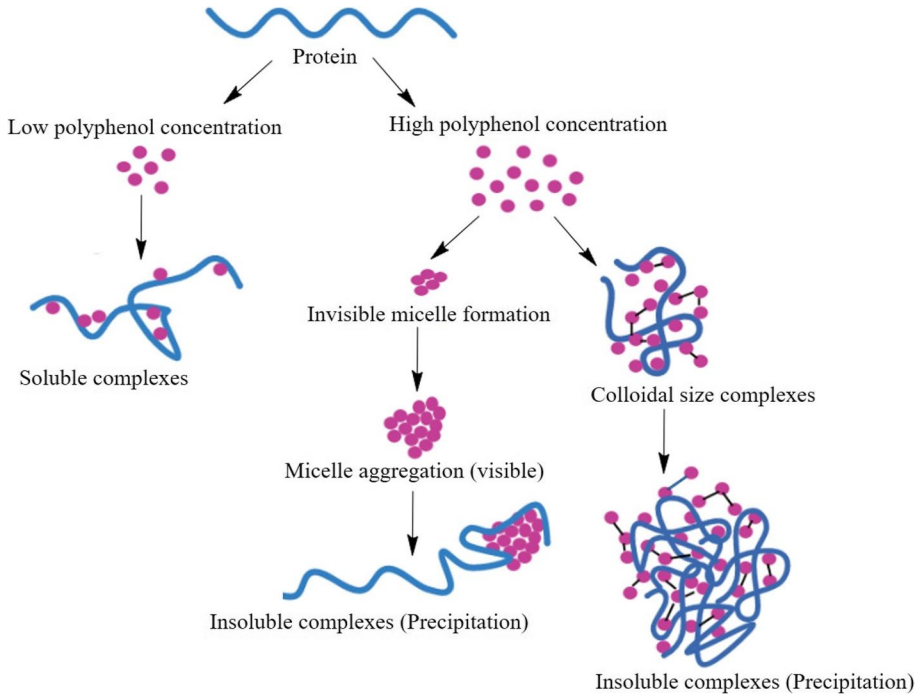


Fig. 8 Schematic representation of possible polyphenolic-protein interaction mechanism (Adapted from Canon et al. 2011; Charlton et al. 2002)

conjugates was observed (Liu et al. 2015). The hydrophobic association between a galloyl ring and the pyrrolidine ring and the complex stabilization by secondary hydrogen bonding were investigated between salivary proline-rich proteins (PRP) and tannins in the oral cavity. The association between different PRP-tannin complexes can compete kinetically with dissociation of the complexes in the presence of a high concentration of tannin molecules, thereby leading to higher orders of complexation and eventual precipitation (Murray et al. 1994).

In contrast to previous observations, the same method of analysis of milk α-casein and β-casein with tea polyphenols resulted in the reduction of α-helix with an increase in random coil and turn structure of the protein due to a further protein unfolding. The protein affinity towards polyphenol is higher with β-casein than α-casein as β-casein is more hydrophobic (Hasni et al. 2011). Similarly, βLG–polyphenol conjugates formed with EGCG and catechin via covalent bonding resulted in a slight decrease in α-helical and β-sheet contents, causing the protein to become more unfolded. Compared with these two conjugates, EGCG showed a greater reactivity and binding strength with free amino, thiol and tyrosine residue groups of the βLG than catechin (Liu et al. 2018). The same structural changes were observed in covalent binding of βLG with chlorogenic acid (CGA). A decrease of a-helix and β-sheet with the corresponding increase of β-turn and random coil was indicated, resulting to an unfolded structure (Fan et al. 2017). The structural analysis of in-vitro digested soy protein isolate (SPI) and black rice anthocyanins conjugates linked covalently demonstrated secondary structural changes of the protein with a reduction in the content α-helix and β-sheets. The tertiary structure of SPI was found to be less compact after conjugation with anthocyanins due to the unfolding of polypeptide chains (Jiang et al. 2019). It is well demonstrated that these structural changes could play a major role in the effect of milk on the antioxidant capacity of tea polyphenols (Chanphai et al. 2017; Hasni et al. 2011; Kanakis et al. 2011).

Physiological relevance of phenol-protein interactions

The interactions of phenolics with proteins plays an important role in their physiological function in the human body. Digestion of phenolic compounds begins in the oral cavity, where phenolics are released from the food matrix and come in contact with enzymes present in the saliva upon grinding food into smaller pieces. It has been proven that interaction between salivary proline-rich proteins and tannins in the oral cavity cause precipitation as the number of tannin molecules binding to the salivary proline-rich protein (PRP) increases (Murray et al. 1994). In addition, a study based on quercetin and

genistein has found that glucosides of flavonoids can be hydrolyzed by β-glucosidase from residual oral bacteria and shed oral epithelial cells to release aglycones. Moreover, the experiment showed that consumption of a diet containing quercetin and genistein could act as a potent inhibitor of oral cancer cell proliferation by inhibiting PI3-kinase and tyrosine kinases, respectively (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Walle et al. 2005).

The interaction of polyphenols with salivary albumin and mucin has been proven to improve their solubility in the oral cavity and substantially increases their availability as potent antioxidants, stickiness to oral surfaces and prolonged retention in the oral cavity. This will cause a tingling sensation after consumption of red wine, cinnamon extracts or causing staining of teeth as these food sources are rich in proanthocyanidins. Therefore, the steady slow release of sticky antioxidant polyphenols could make them more available as effective antioxidants and might be beneficial by attenuating the toxic effects induced by combination of oxidants and pro-inflammatory agonists. This is an important finding for further investigation in drug synthesis (Ginsburg et al. 2012). The correlation of the enzyme activity with binding of phenolic acids was suggested by reacting α-amylase, trypsin and lysozyme with ferulic acid, caffeic acid, chlorogenic acid, gallic acid, quinic acid, and *p*-benzoquinone. Further, the study revealed that *p*-benzoquinone has a strong influence on the hydrolytic activity of α-amylase and proteolytic activity of trypsin as *p*-benzoquinones are able to form quinones. Ferulic acid showed the lowest reactivity and the activity of the enzyme was decreased with increasing pH. This investigation could be of much importance in macerating enzymes in the production of juice (Rohn et al. 2002).

Oxidation, degradation or transformation of polyphenols frequently occurs during their gastrointestinal digestion, resulting in potential enhancement or reduction in the bioactivity of polyphenols. Most phenolic compounds are released from food particles during gastric digestion and some can be absorbed in the stomach in their free form, such as phenolic acids (Grgić et al. 2020). The majority of unabsorbed polyphenols in the upper digestive tract pass into the colon, and are then metabolized by a series of digestive enzymes (Bohn 2014). An in-vitro study that investigated the impact of individual digestion stages on polyphenols in apples indicated that approximately 65% of total amount of polyphenols was released during the gastric phase whereas less than 10% occurred during intestinal digestion (Bouayed et al. 2011).

Most polyphenols are present in food in the form of esters, glycosides, or polymers and in their native form, they have low absorption in the small intestine. They are subjected to hydrolysis by intestinal enzymes or the colonic microflora (Manach et al. 2004) and then pass through the small intestine epithelium by passive diffusion based on their lipophilicity (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021). The pH increases from around 2–4 to approximately 7, allowing the activation of enzymes secreted by the pancreas and bile aiding in the digestion of apolar components and may result in water-soluble micelles (Grgić et al. 2020).

During absorption, polyphenols are conjugated by methylation, sulfation, and glucuronidation in the small intestine and later in the liver (Manach et al. 2004). Glucuronidation and sulfation convert polyphenols into hydrophilic conjugates whereas methylation produces slightly hydrophobic conjugates and significantly impacts polyphenols' bioavailability (Liu & Hu 2009). These circulating conjugated derivatives of phenols are extensively bound to human serum albumin (Manach et al. 2004). It was found that hydrophobic forces and hydrogen bonding are involved in the binding interaction between polyphenols and plasma proteins. Due to this reversible binding to plasma proteins, polyphenols and their metabolites rapidly exchange between free and bound forms within the circulation. The glycation of type 2 diabetes plasma proteins (TPP) has shown a lower non-covalent binding affinity for dietary polyphenols than polyphenol interaction with healthy human plasma proteins (HPP). Further analysis has indicated that the binding affinity difference between HPP–polyphenol and TPP–polyphenol is high with lipophilic polyphenols and in both cases, the affinity decreases slightly with increasing the number of hydroxyl groups in the polyphenols. This study could be used to understand how glucose or glycated plasma proteins affect the binding process of drugs and nutrients (Xie et al. 2012). The remaining unconjugated compounds are regenerated with the help of microbial enzymes from the intestines before re-absorption and the remaining unabsorbed components are removed in the feces (Grgić et al. 2020).

Potential bioactivities

Ovotransferrin (OTF), represents 12–13% of the total egg white. In the preparation of ovotransferrin-catechin conjugates using radical grafting and alkaline, catechin covalently binds to lysine (residues 327) and glutamic acid (residues 186) in ovotransferrin, thus improving its antioxidant activity. Therefore, the grafting of OTF with catechin is an effective means to enhance the oxygen radical scavenging capacity of the protein (You et al. 2014). Similarly, it was found that covalently linked conjugates using soy protein isolate (SPI) and black rice anthocyanins exhibit higher antioxidant capacity after gastric and intestinal digestion (Jiang et al. 2019). In contrast, the covalent attachment of quercetin to bovine serum albumin (BSA) reported

a decline in the total antioxidant activity after in-vitro digestion. None of the derivatives' antioxidant power was reached to the equivalent amount of free quercetin (Rohn et al. 2004). Besides, microencapsulating polyphenols by protein-derived materials have been applied to reduce their degradation during gastrointestinal digestion, and increase their levels and assimilation in the intestine. For instance, whey protein-based microcapsules loaded with an anthocyanin rich bilberry extract showed that antioxidant capacity could be preserved by interactions between whey proteins and bilberry extract compounds than non-encapsulated anthocyanin until the point of action is reached (Li, He, et al. 2021; Li, Ritzoulis, et al. 2021; Betz et al. 2012).

The effect of covalent interaction between polyphenol and protein on allergenic capacity was studied with control βLG and βLG-EGCG, βLG-CA conjugates binding to immonoglobin E (IgE) antibodies. A reduction of allergenic capacity was found in conjugates than in the control, and βLG–EGCG exhibited a low capability of IgE binding than βLG–CA. It was suggested that the polyphenol-binding site in βLG directly masks the region where IgE binds, preventing IgE from accessing the protein appropriately due to the secondary structural changes in βLG that affect the conformation of IgE binding region after conjugation with EGCG and CA (Liu et al. 2018). Based on these observations, a new possibility of producing hypoallergenic foods via covalent interaction with polyphenols was demonstrated.

Dietary polyphenols have been widely claimed to inhibit inflammatory responses and are becoming potential therapeutic agents for inflammatory diseases. Binding of resveratrol with lectin; a sugar-binding protein, exhibited an effective synergetic activity with 2:3 ratio of lectin to resveratrol. It has been suggested that resveratrol binds in the rigid β-sheets through H-bonds and hydrophobic interaction with amino acids and acts as free radical scavenger and reduces the inflammatory action through the inhibition of many pro-inflammatory events (Rocha et al. 2015). The oral administration of resveratrol-loaded zein nanoparticles to mice induced a suppression of tumor necrosis factor alpha (TNF-α) which is an inflammatory cytokine produced during acute inflammation. Furthermore, week-long oral administration of this polyphenol-based compound has shown a high oral bioavailability and was able to diminish the endotoxic shock induced in mice and protected from inflammatory symptoms (Penalva et al. 2015).

The anti-cancer activity of polyphenols can be enhanced through conjugation with proteins. The effect of curcumin on tumor suppression was extensively studied in-vitro as well as in immunocompromised mice. In-vitro studies found that curcumin occupies the ATP-binding pocket of tyrosine regulated kinase 2 (DYRK2) by inhibiting the action of phosphorylation of ATP-dependent protease complex, especially 26S proteasome. This reduces 26S proteasome activity, leading to impaired cell proliferation while inducing apoptotic cell death and reducing tumor growth. In addition, in-vivo analysis in immunocompromised mice having breast cancer revealed that curcumin treatment significantly reduces its tumor burden (Banerjee et al. 2018). The investigation of the in-vitro effects of catechin-lysine (CA:Lys) complex indicated a selective anti-migratory effects in breast, pancreatic and colorectal cancer cell lines compared to non-cancer cell lines. The complex has a pro-apoptotic impact in all cancer cell lines and induces a concentration-dependent decrease in cancer cell proliferation (Silva et al. 2019). The study aimed at screening the potential anti-cancer activity of bovine serum albumin (BSA)-catechol revealed that they can decrease the viability of cancer cells by approximately 80–85% at a concentration of 100 μg of conjugates. In contrast, the free polymers or phenolics did not affect the viability of cancer cells (Rai et al. 2018). The results suggest that these types of biological activities strongly depend on the quantity of conjugated phenolic moiety on the biopolymer backbone (Rai et al. 2018; Von Staszewski et al. 2012). Similar observation occurred in nano-complexes, built using β-lactoglobulin (βLG)-green tea polyphenols and caseinomacropeptide (CMP)-green tea polyphenols conjugates (Von Staszewski et al. 2012). They exert a better antiproliferative activity on some particular tumor cell lines in a concentration-dependent manner. Similarly, nanoencapsulated EGCG in casein micelles of skim milk significantly decreased the proliferation of HT-29 cancer cells, while there was no considerable effect with free EGCG. The complexes also demonstrated that nanoencapsulation might not reduce their bioavailability (Haratifar et al. 2014). In addition, the extensive analysis of three flavonoids from fingerroot plant (*Boesenbergia rotunda*) including cardamonin, pinocembrin, and pinostrobin have shown a strong non-competitive inhibitory action on beta-site amyloid precursor protein cleaving enzyme 1 (BACE1). Here, cardamonin presented the most potent inhibition against BACE1 among other two flavonoids (Youn & Jun 2019). Hence, this might be a promising chemopreventive modality for Alzheimer's disease (AD); a neurodegenerative disorder due to the formation of beta-amyloid where BACE1 is essential (Zhao et al. 2007).

Investigating the interaction between EGCG and α-glucosidase through kinetics analysis, fluorescence spectra, Fourier transform infrared (FT-IR) spectrometry and molecular docking studies have shown a significant inhibitory activity of EGCG on α-glucosidase in a reversible and non-competitive manner. The inhibitory

rate was proportional to the concentration of EGCG and its inhibition was higher than that of the positive control acarbose where IC_{50} values were 19.5 ± 0.3 and 278.7 ± 1.1 μM, respectively. It was also found that EGCG could enhance glucose uptake and promote GLUT4 translocation to plasma membrane via PI3K/AKT signaling pathway in L6 skeletal muscle cells, indicating its antidiabetic activity. The EGCG conjugates have demonstrated their possible use as natural α-glucosidase inhibitors with potential applications in the development of functional food (Xu et al. 2019).

It was suggested that EGCG can be protected against chemical degradation or oxidation by nanoencapsulation with proteins, mainly through hydrophilic and hydrophobic interactions. With the aim of delivering EGCG, the nanoparticles were engineered by conjugating βLG–chlorogenic acid (CGA). The conjugation resulted in a higher retention rate of EGCG in βLG–CGA than in βLG alone. Under simulated gastric digestion, in-vitro release of EGCG from βLG–CGA nanoparticles was slower and less than that from BLG nanoparticles. This indicates the potential use of βLG–CGA conjugate as a nutraceutical to protect EGCG in the stomach and its prolonged releasing in the intestine with enhanced stability (Fan et al. 2017). Furthermore, stabilization and immobilization of palladium (Pd) nanoparticles was improved by EGCG grafted collagen fibers (CF). Further analysis demonstrated that the particle size of Pd and catalyst activity depended on the grafting degree of EGCG on CF. The heterogeneous Pd nanoparticle catalysts prepared through this method can be easily recovered, repeatedly used, and stored for 2 months in air with efficient catalytic activity. Therefore, EGCG-grafted CF could be a good stabilizer to synthesize similar heterogeneous metal nanoparticle catalysts that prevent the leaching of active metal species during their recycling process (Wu et al. 2009). Moreover, cross-linking of gelatin-chitosan blends with phenolic compounds was synthesized by laccase catalysis to develop bioactive hydrogel dressings. It was found that laccase initiates phenolic compounds' oxidation and forms quinones that can conjugate with amino residues, resulting in a stabilized biopolymer network. In addition, enzymatically assembled hydrogels prepared with polyphenol cross-linking exert wound healing activity by inhibiting deleterious wound enzymes and bacterial growth, where the inhibition activity is primarily dependent on the amount of the released phenolic compounds (Liu et al. 2019; Rocasalbas et al. 2013). Generally, it has been proven that interactions of polyphenols with protein can exert different biological activities that can generate potential health benefits.

Potential effects on the food industry

The interaction between polyphenols with proteins such as enzymes plays an important role in the food industry. For instance, a novel coating material for preserving fresh seafood has been prepared by conjugation of chlorogenic acid (CGA) to a well-known hydrolysate of collagen, gelatin. The results indicated that gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) are more susceptible than gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*). The inhibition activity increases with the concentration of CGA which resembles the activity of free CGA against bacteria and indicates that CGA could bind to the outer membrane of bacteria, disrupting and permeabilizing the cell membrane and causing the leakage of cytoplasm macromolecules and eventually cell death (Lou et al. 2011). The study implies that the antibacterial activity of CGA in CGA-Gel is retained during conjugation and its possibility for application as coating preservative for seafood against bacteria (Fu et al. 2017).

Diets high in fat and red meat and partially oxidized foods are contributory risk factors due to lipid peroxidation. The addition of red wine polyphenols to the meat cutlets indicated a dramatic reduction in the accumulation of malondialdehyde (MDA) in human tissues, proposing that one of the reasons might be the interaction between red wine polyphenols and proteolytic enzymes in the gastrointestinal tract. Therefore, the consumption of polyphenol-rich fruits, vegetables, and derived beverages during the meal seems to reduce these risk factors and provide important protective benefits for health (Gorelik et al. 2008). It has been suggested that protein-polyphenol conjugate may provide better protection for easily oxidized lipophilic nutraceuticals in emulsion-based systems than the protein alone. Covalent binding of βLG with catechin prepared by a free radical method has been used to improve the chemical stability of β-carotene in nanoemulsions. The results indicated that the retention rates of β-carotene in nanoemulsions were approximately two times higher in βLG − catechin conjugates than βLG alone with no phase separation or creaming after 30 days of storage at 50 °C (Yi et al. 2015). Similarly, engineered catechin-egg white protein (CA-EWP) conjugates-based nanoemulsions had a high physicochemical stability of β-carotene in emulsions than in non-conjugates. Therefore, the proteins modified with polyphenols can be widely used as novel food-grade antioxidant emulsifiers and stabilizers for β-carotene that are applicable in bioactive compounds encapsulation delivery systems (Gu et al. 2017).

In comparing fish oil emulsions with ovalbumin (OVA) and covalently bonded catechin-OVA conjugates, fish oil emulsions coated by conjugates demonstrated higher

storage stability against lipid oxidation with less viscosity. Stable conjugates displayed significant potential in inhibiting lipid oxidation in fish oil emulsions than non-conjugates of OVA through their higher antioxidant activity and accumulation at the lipid-water interfacial (Feng et al. 2017). Supporting evidence was provided by the studies based on cuttlefish skin gelatin modified with 5% oxidized tannic acid. It was able to inhibit the formation of thiobarbituric acid reactive substances (TBARS) in the emulsion more effectively than with non-conjugated gelatin throughout the 12 days of storage. Due to its enhanced antioxidative activity with no detrimental effect on emulsifying properties, gelatin-oxidized tannic acid conjugates could be used to inhibit lipid oxidation of menhaden oil-in-water emulsion effectively, and the efficacy was dose-dependent (Aewsiri et al. 2009).

Usually, edible films are prepared from natural proteins, which are good polymeric substances and are environmentally friendly. However, they exhibit low mechanical and barrier properties and are more prone to microbial growth, discoloration and short shelf-life. Especially, these protein films combined with phenolic compounds have been developed as they can act as cross-linkers between individual protein molecules. They are able to reduce the free space in the polymeric matrix, resulting in high mechanical and barrier properties of protein-based films, extending food material's shelf life and improving its quality (Hassan et al. 2018). Films prepared using cold water fish gelatin with different concentrations of caffeic acid and ferulic acid under alkaline and aerobic conditions have shown the highest effect in decreasing solubility, water vapor permeability, and oxygen permeability with caffeic acid-gelatin conjugate, indicating that caffeic acid was more effective compared with ferulic acid. The results clearly demonstrate the enhancement of safety of biodegradable packaging by improving their barrier properties that are greatly influenced by the type of phenolic compound used for the conjugation (Araghi et al. 2015).

In addition to the natural pigments that give the food color, the polyphenol-quinone reaction with or without amino acid residues can result in different colored complexes ranging from deep brown, yellow, to green. During alkaline extraction of proteins from sunflower extraction meal (SEM) lead to green discoloration as a result of the reaction between nucleophiles (free NH_2 groups) in SEM protein with chlorogenic acid quinones present in the meal (Bongartz et al. 2016). It was found that reactions between quinones produced from polyphenols and thiol groups of amino acid residues result in discoloration, condensation reactions between unbound quinones in the absence of amino acid residues result in a dark brown color. A mixture of brown quinone condensation products and thiol protein-bound quinones can result in light brown to yellow colors. Therefore, the colored complexes formed by amino acid residues bound quinones could be used as natural pigments in food (Keppler et al. 2020).

Polyphenols are mainly responsible for the astringency and "mouthfeel" of tea and wine through their interactions with basic salivary proline-rich proteins (Charlton et al. 2002). Besides that, these conjugates are assumed to be involved in reducing astringency and bitterness, thus enhancing the food flavor. Peroxidases oxidize the polyphenols present in cocoa beans and the enzyme activity increases during the fermentation and drying of the beans. It could be presumed that modification of amino acids upon binding of oxidation products of phenolic compounds could reduce the astringent and bitter taste, thus also contributing to the flavor of cocoa (Rawel et al. 2019). In addition, the color and the aroma of foods can be manipulated by controlling the Maillard reaction and subsequent downstream reaction products in food systems. Quinones are readily formed in polyphenol-containing foods during processing and storage and can react with amines which are starting materials of the reaction to form either benzoquinone imines or amine−quinone adducts via Michael addition. The modification of amines by oxidized polyphenols, quinones will eventually inhibit the Maillard reaction or non-enzymatic browning (Lund & Ray 2017). It was found that epicatechin (EC) has a remarkable inhibitory effect on the Maillard derived compounds. The addition of EC to ultrahigh-temperature (UHT) processing of bovine milk inhibited key aroma compound generation during the UHT processing with no significant increase in perceiving the bitter taste with a lower level than 0.2% EC. Therefore, the effect of these natural polyphenol-protein conjugates for reducing off-flavor formation has gained major attention in food processing (Colahan-Sederstrom & Peterson 2005; Li et al. 2008; Lund & Ray 2017). Moreover, a series of studies based on the interactions between CGA with sunflower and canola seed proteins have been found to improve plant proteins' nutritional and functional quality (Rawel & Rohn 2010).

As reported, the phenolic-proteins interactions could be used to modify conventional food products. This may also enhance interesting food properties, including nutritional value and bioactivities.

Methods of determination

The interaction between phenolics-protein conjugates and their conformational changes upon binding can be determined by spectroscopic measurements, thermodynamic, electrophoretic, chromatographic and bioinformatics analyses (Czubinski & Dwiecki 2017). Several

techniques such as fluorescence spectroscopy, circular dichroism (CD) spectroscopy, dynamic light scattering (DLS), Fourier transform infrared (FT-IR) spectroscopy, chromatography, isothermal titration calorimetry (ITC), and nuclear magnetic resonance (NMR), electrospray ionization mass spectrometry (ESI-MS), X-ray diffraction and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) methods have been used to understand the reversible and irreversible association between these two components (Bandyopadhyay et al. 2012; Buitimea-Cantúa et al. 2018). In addition to these in-vitro chemical methods, computational methods have been used widely as they allow cost and time effective elucidation techniques regarding the biological and biochemical roles of protein, and subsequent changes upon ligand binding, analyzing target structures for possible binding and predicting active sites and biochemical function of newly sequenced proteins and their interacting components (Poklar Ulrih 2017; Roche et al. 2015).

Therefore, protein-polyphenol interactions can be predicted, improved or stabilized through rational design and engineering, followed by a range of in-vitro chemical or cell-based assays and in-vivo models. Moreover, these automated methods are able to determine interactions and fundamental biochemical processes between phenolic-protein conjugates on a large scale. The molecular docking studies are one of the virtual screening methods that are extensively used to interpret these interactions (Allahdad et al. 2019; Guan et al. 2021; Mohammadi & Moeeni 2015; Petsko & Yates 2011).

Molecular docking as a powerful method for examining phenolic-protein interactions

Molecular docking is an in-silico computational tool that can be used to predict the preferred orientation, affinity, and interaction of a phenolic compound in the binding site of a protein. This method allows for comprehensive screening of different protein targets selected from the structures available in Protein Data Bank (Czubinski & Dwiecki 2017; Chen & Zhi 2001). The phenolic-protein docking process involves two basic steps: prediction of the phenolic compound (ligand) conformation, its position and orientation within the sites of the protein (receptor) and assessment of the binding affinity (Meng et al. 2012). Therefore, programs based on different algorithms such as AutoDock 4.0, AutoDock Vina and DOCK 6 can generate different possible adduct structures that are ranked and grouped using a scoring function in molecular docking studies (Czubinski & Dwiecki 2017).

In terms of the potential modes of action for phenolic compounds with protein, a blind docking approach with novel protein or site-specific docking with known target pocket of the protein can be performed. According to the results obtained, the lowest binding energy value indicates a favorable and stable polyphenol-protein complex while other negative values of binding energy indicate the spontaneous or possible binding of the polyphenol ligand towards the protein. In addition, docking of the phenolic compounds can be carried out both in the absence and in the presence of ions/cofactors. This will allow to compare the affinity of the polyphenol molecule to the protein target in both conditions and to understand whether the ligand was bound to a functionally active molecule or not (Czubinski & Dwiecki 2017; Hassan et al. 2017). Providing predictions for the binding free energy, force type, binding site, and functional groups of phenolics involved in the interactions with amino acid residues have been studied in several polyphenol-protein conjugates, to demonstrate the potential multi-target effects of phenolic compounds and evaluate their in vivo physiological or pharmacological activities (Guan et al. 2021; Lacroix et al. 2018).

The molecular docking experiments for analyzing the interactions of *trans*-resveratrol and curcumin with bovine α-lactalbumin (α-LA) exhibited that their interactions are spontaneous and strong because of the negative value of the binding free energy. In curcumin-bovine α-LA conjugates, the most stable conformation with the lowest binding energy binds in the vicinity of the Trp-118 with two hydrogen bonds formed with Gln-117 and Glu-113 of the protein. In trans-resveratrol-bovine α-LA conjugates, two hydrogen bonds formed with Gln-54 and Ser-47 contribute to building the most preferred complex in the cleft region or the vicinity of the cleft region between Trp-60 and Trp-104. According to the comparison of these data, higher measured values for the binding free energy was in binding curcumin to bovine α-LA, providing the suggestion that curcumin and trans-resveratrol are appropriate additives in the food industry and drug formulation (Mohammadi & Moeeni 2015).

One of the studies determined the putative interaction of quercetin, quercitrin, and rutin with βLG using molecular docking studies and molecular dynamics (MD) simulation. The results indicated that quercetin and quercitrin bind to the internal cavity of βLG with one hydrogen bonding (Lys-69) and three hydrogen bonding (Pro-38, Lys-69, and Ile-71) interactions, respectively. Rutin binds at the entrance of the internal cavity of βLG with four hydrogen bonds (Lys-70, Ile-71, Asn-88, and Ser-116) because of its large structural volume. The lower binding constant (Ka) value with high binding energy for βLG-rutin complex was observed due to its binding to the entrance of the internal cavity, concluding that hydrogen bonding plays a dominant role in ligand bonding than hydrophobic interactions. Further analysis suggested that the structure of the ligand-binding site

remained approximately rigid during the simulation and favorable complex formation occurred in quercetin with βLG with no conformational change upon their binding (Sahihi et al. 2012). Blind docking of βLG with tea polyphenols such as catechin (C), epicatechin (EC) and epigallocatechin gallate (EGCG) indicated that several amino acid residues in the proteins are engaging with each polyphenol to form a network of H-bonds. The docking results show that C binds at the central cavity of βLG, implying a stable conformation of the protein (Al-Shabib et al. 2020; Kanakis et al. 2011) whilst EC and EGCG are in the vicinity of the internal cavity (Kanakis et al. 2011). Further analysis of βLG-C complex through molecular docking and simulation studies confirmed that the complex is principally stabilized by hydrophobic interactions with amino acid residues Pro-38, Leu-39, Val-41, Val-43, Ile-56, Leu-58, Ile-71, Ile-84, Val-92, Phe-105 and Met-107 (Al-Shabib et al. 2020). However, comparing the binding energy of βLG with C, EC and EGCG shows that more stable polyphenol–protein complexes are formed with EC and EGCG than CA as they can form more hydrogen bonds (Kanakis et al. 2011).

Moreover, docking studies on βLG with 3,4-dihydroxybenzoic acid, gallic acid, syringic acid, caffeic acid, ferulic acid, and chlorogenic acid conjugation has shown that cinnamic acid derivatives exhibit a stronger binding affinity with βLG than benzoic acid derivatives. The binding affinity decreased in the order of caffeic acid > chlorogenic acid > ferulic acid > syringic acid > 3,4-dihydroxybenzoic acid > gallic acid. Molecular dynamics (MD) simulations between these phenolic acids and βLG show that the protein accommodates the ligand in its hydrophobic central cavity, interacting mainly via hydrophobic forces. The nature of interactions as well as binding affinity depend on the type of phenolic acids along with their degree of hydroxylation, methylation and steric hindrance, which could be applicable in developing nutraceuticals or functional food products containing these compounds (Li et al. 2020). The nature of interactions and formation of thermodynamically favorable conformation were studied by docking and MD simulations of ferulic acid with the dimer and monomer forms of βLG. The results showed that the preferred binding site in the dimer form lies at the interface of the two monomers whereas it lies within the calyx shaped β-barrel of monomer form of βLG which exhibits the highest binding affinity. In two cases, the complexes were stabilized by hydrogen bonding and hydrophobic interactions. The binding of ferulic acid in monomer form reflects that the ligand entering at the calyx region of βLG following non-covalent interactions with the surrounding residues is essential in stabilizing the ligand within the receptor. This kind of information could be highly relevant to the food industry to enhance associative interactions for the development of novel bioactive compounds (Abdollahi et al. 2020).

The in-silico elucidation of the antidiabetic mechanism of EGCG with α-glucosidase indicated the binding of EGCG at the site close to the active site pocket of α-glucosidase and forms a stable complex with five intermolecular hydrogen bonds, which are the main forces between EGCG and α-glucosidase. It was identified that the binding site between α-glucosidase and EGCG was different from acarbose, a competitive inhibitor against α-glucosidase and there was no interaction between EGCG and the catalytic residues of Glu-277 and Asp-352. The docking studies illustrated that EGCG is a non-competitive inhibitor for α-glucosidase, providing the sense that EGCG might act as a promising antidiabetic agent (Xu et al. 2019).

Docking validation of potential human protein targets of apple polyphenols and possible mechanisms of chemoprevention in colorectal cancer revealed that some selected antioxidants can form stable complexes at cavities different from the active site. For instance, the binding of chlorogenic acid (CGA) to GTPase H-ras occurs at the allosteric site of the enzyme instead of the canonical binding site. In the presence and absence of ions/cofactors, direct docking analysis has been carried out to understand if the ligand is bound to a functionally active molecule and identify potential and stable protein-ligand interactions with selected targets. In this study, the lowest binding energy or the most stable complex corresponds to the interaction between quercitrin and hypoxanthine-guanine phosphoribosyltransferase enzyme. In addition, it was found that KDM1A gene coding lysine demethylase 1A and hyperin prefers binding whereas gene GAMT coding guanidinoacetate *N*-methyltransferase is the preferred target for hyperin, isoquercitrin, phloridzin and rutin present in apple. These observations clearly support the hypothesis that polyphenolic compounds act synergistically with proteins, providing cumulative effects on nucleotide metabolism and methyltransferase enzymes similar to the action of anti-cancer drugs (Scafuri et al. 2016).

Youn and Jun (2019) investigated the potential inhibitory action of cardamonin, pinocembrin, and pinostrobin on beta-site amyloid precursor protein cleaving enzyme1 (BACE1) as natural products-based therapy for Alzheimer's disease (AD). Molecular docking analysis illustrated a non-competitive inhibitory activity for all these three compounds with reference to resveratrol used as the positive control. According to the binding energies, the most stable conjugates among proposed complexes were cadamonin-BACE1 which showed the strongest and effective inhibition. However, it was suggested

that hydrophobic interaction between this compound involving the stabilization as cardamonin does not form any hydrogen bonds with BACE1. Stable conformations formed between P-glycoprotein and the flavonoids were analyzed through docking simulations to predict their possibility to pass the blood brain barrier. The computational analysis indicated that these flavonoids from *B. rotunda* may be promising AD preventative agents, and extensive examination needs to be carried out through in-vitro assays to support these predictions.

Identification of flavonoids that can regulate sirtuin 6 (SIRT6) activities is considered promising therapeutics for age-related diseases including cancer, diabetes, neurodegenerative diseases and metabolic disorders. Therefore, molecular docking has been carried out to discover their binding sites on SIRT6, and to identify principle interactions occurring on the enzyme active site with inhibitors and activators. The results have shown that the cleft present in enzyme forms the pocket for acetylated sirtuin substrate and for NAD^+ cofactor, which requires the activation of the enzyme. Furthermore, they reported that activator compounds bind to a site outside of the cleft by forming interactions with a loop near the acetylated peptide substrate binding site. The activators may induce conformational changes in the loop upon binding to the putative activator site, improving acetylated substrates' binding. Contrarily, the binding site of the majority of inhibitors was situated close to the binding site of known sirtuin inhibitors which can restrict NAD^+ binding. Among different flavonoids, catechins with galloyl moiety exhibit a greater inhibitory activity by partially occupying the acetylated sirtuin substrate's binding site and allowing more interactions with the binding pocket. It was also identified that kaempferol can act as a potent dual modulator due to its ability to bind multiple sites and form similar interactions with the same amino acid residues where the known activators and inhibitors interact with. An in-silico based mutation analysis was performed to examine the impact of activator site's residues Gly-156, Asp-185, Trp-186, Glu-187 and Asp-188 on the action and/ structure of SIRT6. Extensive docking analysis revealed that different flavonoids could alter SIRT6 activity in a structure-dependent manner (Guan et al. 2021; Rahnasto-rilla et al. 2018).

Troxerutin (TX), a bioflavonoid, has been shown to exhibit anti-neoplastic and anti-cancer activities. Assessing the modulatory action of TX on transcription factors such as IKKβ, Nrf2 and Keap-1 through docking studies illustrated that TX interacts with the active sites of proteins by forming hydrogen bonds and π-cation interaction. TX with IKKβ conjugate has shown the most stable complex with the lowest binding energy compared to binding with other transcription factors, implying that high binding affinities are accompanied with hydrogen bond interactions. Based on the combination of docking analysis with immunoblotting and immunocytochemistry analysis, it was predicted that the formation of stable conjugates might result in a conformation change of transcription factors, followed by inactivating the expression of oncogenes and hence can be deemed as a potent drug in anti-cancer therapy (Thomas et al. 2018).

The structure-based virtual screening of flavonoids indicated that most flavonoids can bind to the acetylated lysine (KAc) binding site of BD1 of Brd4 receptor molecule and can act as novel natural bromodomain inhibitors. Flavonoids were found to occupy the active site, forming hydrogen bonds between the acetyl carbonyl oxygen and the amino group of the receptor's conserved Asn-140 amino acid residue. Quercetin binds in a similar manner and shows the highest binding affinity while illustrating that these ligands may prevent the binding of Brd4 to acetylated lysines on nucleosome histones and inhibit RNA Polymerase II mediated transcription elongation. Moreover, the blocking of KAc site may inhibit the binding to the Myc gene resulting in a low level expression of the Myc gene, leading to low proliferation of cancer cells (Raj et al. 2017).

Investigation of inhibitory effects of anthraquinones on tyrosinase shows that they enter the active site of tyrosinase in the form of one molecule and competitively inhibit the activity of tyrosinase. The majority of interactions were electrostatic forces and hydrophobic interactions compared to hydrogen bonds due to the presence of aromatic rings in the structure of anthraquinones. Further analysis revealed that the inhibitory effect on tyrosinase activity was accomplished by acting on histidine residues bound to copper ions rather than chelating them. It was suggested that the binding of anthraquinones at the active site of tyrosinase results in conformational changes of secondary structure and prevented the entry of substrates, inhibiting the tyrosinase activity and in turn regulating the melanogenesis (Zeng et al. 2020). Similar molecular docking analyses were carried out to evaluate the synergetic effect of quercetin, cinnamic acid and ferulic acid on tyrosinase enzyme. Quercetin located at the hydrophobic pocket of the enzyme formed strong hydrophobic interactions and showed slightly higher binding affinity compared to binding with cinnamic and ferulic acids. Furthermore, computational docking simulations showed that quercetin, cinnamic acid and ferulic acid bind with different sites on tyrosinase in a non-competitive manner, exhibiting their ability to express synergistic action in inhibiting the tyrosinase activity (Yu et al. 2019).

The preferred binding sites of phenolics on scallop gonad protein isolates (SGPIs) were identified and

visualized by molecular docking simulation studies. Vitellogenin and β-actin, as the main crystal structures of SGPIs have been analyzed with EGCG to evaluate their ability to form stable complexes. It was found that EGCG inserts into the hydrophobic central cavity of vitellogenin or β-actin and stabilizes the conjugate via hydrogen bonds, van der Waals and hydrophobic interactions. Besides, interaction of these proteins with EGC and ECG resulted in decreasing order of SGPIs-binding capacity of phenolics as EGCG > ECG > EGC, indicating that binding affinities mainly rely on the number of –OH groups present in the ligand molecule. In the process of interactions, hydrogen bond and van der Waals forces dominated between SGPIs and EGCG while hydrophobic interaction forces were dominant in SGPIs-ECG complex. Therefore, a comprehensive theoretical understanding of the induced effect of these phenolic compounds on the structure and function of SGPIs could be used to accomplish desired practical aspects (Han et al. 2021).

Investigation on binding sites and binding affinities of catechin derivatives for bovine serum albumin (BSA) showed that ester catechins (EGCG and ECG) possess high binding affinities than non-ester catechins (EGC and EC) as they can form more hydrogen bonds (Yu et al. 2020). Further, interaction of EGCG with BSA by molecular operating environment (MOE) suite docking simulation program has indicated that EGCG interact with both Trp − 134 residue; at drug-binding site I by π–π stacking and Trp-213 residue; on the molecular surface of BSA (Ikeda et al. 2017). These results suggest that substitution at the C-3 position or galloyl moiety of catechins determines their binding affinities against serum albumin. Together, these simulation studies support the idea of formation of tea cream and controlling the performance of tea beverage products by introducing tea polyphenols (Yu et al. 2020). In studying the molecular nature of interactions between β-casein and *p*-coumaric acid, computational docking has been done to identify the location of their specific binding site and to obtain the thermodynamically stable conformation of β-casein with *p*-coumaric acid. Modeling outcomes have shown that the ligand molecule interacts with amino residues within the core of β-casein receptor molecule forming a hydrogen bond between hydroxyl group of *p*-coumaric acid and the carbonyl group of the peptide backbone of Ile-27 (Kaur et al. 2018).

Several studies have shown that docking algorithms are capable of identifying putative phenolic-protein binding sites of novel conjugates with their binding affinities, analyzing interactions and conformations that can be used to recognize and interpret their potential activities from practical perspectives. Moreover, it demonstrated that polyphenols with galloyl moiety bind most strongly to extended proteins with a high proline content to form thermodynamically favorable complexes. The degree of hydroxylation, methoxylation and steric hindrance of the polyphenol mainly determine its binding affinity for the protein molecule and structural conformation of the compound.

Conclusion

Complexation of phenolic compounds with proteins via covalent and/or non-covalent bonding entails changes in proteins that could result in favorable or unfavorable properties. The phenolic-protein interactions affect the compound's activity and exert synergistic or antagonistic effects depending on the type and structure of compounds, molecular weight, concentration, pH, temperature, cofactors, method and food processing conditions, and physiological status. These conjugates are able to improve biological activities such as antioxidant, anti-inflammatory, anti-allergic and anti-cancer activities and bioavailability of polyphenols compared to individual components. In addition, they can also act as food preservation agents against microorganisms and lipid oxidation, emulsions for delivery of nutraceuticals, edible films for food packaging and drug releasing modulators and may also act as natural food color, flavor and texture modifier. It has been demonstrated that the underlying mechanism of these actions and interactions at the molecular level can be extensively investigated, specifically using molecular docking approaches with various analytical techniques. It can be concluded that polyphenols bind most strongly to extended proteins with a high proline content and polyphenols with galloyl moiety to form thermodynamically favorable complexes, by modulating their activities. As shown in earlier studies, molecular docking programs are able to successfully predict the binding modes between protein and polyphenols in a large scale. Therefore, the docking studies provide scientific basis to determine putative binding sites, stable conformations of conjugates and identifying promising compounds that can be developed further, to accomplish desired functional and health aspects. However, more in-depth studies are needed for a comprehensive understanding of the interaction between phenolic compounds and proteins and factors that affect their binding affinities for enhancing and expanding the application potential of different phenolic compounds in the food and pharmaceutical industries.

Abbreviations

AD: Alzheimer's disease; BACE1: Beta-site amyloid precursor protein cleaving enzyme 1; BSA: Bovine serum albumin; CA: (+)-Catechin; CGA: Chlorogenic acid; CMC: Critical micelle concentration; EC: (−) -epicatechin; ECG:

(−)-epicatechin gallate; EGC: (−)-epigallocatechin; EGCG: (−)- epigallocatechin gallate; EWP: Egg white protein; HPP: Human plasma proteins; MD: Molecular dynamics; MDA: Malondialdehyde; MOE: Molecular operating environment; PRP: Proline-rich proteins; SEM: Sunflower extraction meal; SGPIs: Scallop gonad protein isolates; SPI: Soy protein isolate; TBARS: Thiobarbituric acid reactive substances; TPP: Type 2 diabetes plasma proteins; UHT: Ultrahigh-temperature; α-LA: α-lactalbumin; βLG: β-lactoglobulin.

Acknowledgements
Not applicable.

Authors' contributions
The study was conceptualized by FS and, CDS participated in literature survey, data collection and drafting the manuscript. The finalization of the draft with editing, providing suggestions and extensive revising was done by FS. Authors read and approved the final manuscript.

Funding
We are grateful to the National Science and Engineering Research Council (NSERC) of Canada for financial support.

Availability of data and materials
All data generated are included in the references of this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Author Dr. Fereidoon Shahidi is editor-in-chief of ***Food Production, Processing and Nutrition*** and he was not involved in the journal's review of, or decisions related to this manuscript..

Received: 2 October 2022 Accepted: 7 December 2022
Published online: 03 January 2023

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