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Phenolic-Protein Interaction: Effects on Functional Properties of Phenolics and Advantages on Phenolic Delivery Platform Development

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Abstract- **The interaction between phenolics and proteins is one of the most research areas in food, pharmaceutical, and cosmetic fields since these interactions affect the functional properties of proteins and phenolics. By using this phenomena, organoleptic properties of phenolic compounds can be engineered, foaming and gelling properties of proteins can be increased, and also novel delivery platforms for phenolics can be designed. During the construction of phenolic-protein interaction, both covalent and non-covalent bonds occur. Formation of these bonds depends on the type of phenolic compounds and protein and also environmental conditions such as pH, temperature and ionic strengths. Understanding the exact mechanism behind these interactions are leading to generate tunable food, drug, and cosmetic products.**

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I. Introduction

Phenolic compounds, abundantly present in plants, account for one-third of the dietary phenols and are a large family of phytochemicals (Herrmann & Nagel, 1989). The phenolic compounds have numerous physiological functions, such as anti-inflammatory, anti-mutagenic, and antioxidant properties through phenolic compounds` potential to protect from oxidative stress (Lee et al., 2005; Altin, Gültekin-Özgüven & Ozcelik, 2018). The studies on animals and also human submits that phenolic compounds in daily diet have significant roles in protection from several diseases including certain types of cancers, cardiovascular diseases, and prevention of osteoporosis (Ali, H., 2012). Even insignificant structural differences in the location, number, or form of substituted groups can directly affect the bio-distribution, free concentration, and metabolism of phenolic compounds, and resulting affect to their bioactivity (Jaldappagari et al., 2013).

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Interactions between the different food compounds have been commonly identified and it is known to have influence on the biological, functional and, nutritional properties of food products. The interaction of phenolic compounds-proteins seems to be the most important one using different aspects. Phenolic acids could be interacted by non-covalently and covalently with proteins, and the interactions of protein-phenolic may affect the biological and functional properties of phenolics as well as proteins (Charlton et al., 2002). The bioactivity of a compound after the consumption is related to its bioaccessibility and bioavailability. Based on the type of interaction between phenolics-proteins can be increased or decreased their bioaccessibility and bioavailability. In the sensory aspect, organoleptic quality of food product such as color, bitterness, and astringency, is directly affected form phenolic-protein interaction. (De Freitas & Mateus, 2012; Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J., 2017).

In last decade, numerous studies are focusing on delivery of phenolics, since to contribute the functionality of phenolics is crucialsince they have several positive effects on health such as antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, and hepatoprotective effect. Protein-phenolic conjugates and protein-based nanoparticles are the most common studied techniques about a delivery of phenolics, which can be used in food, pharmaceutical, and cosmetic sector.

Therefore, understand the exact mechanism between phenolic-protein interaction not only leads to the observation of nutritional and sensory changes on foods but also enhance to develop novel strategies for functional foods and dietary supplement area. While the interactions can be analyzed considering several aspects, it still is a challenge for food analysis and researchers (Czubinski, J., & Dwiecki, K., 2017). To comprehend the roles of proteins and phenols in interaction, it is essential to designate the nature of the physicochemical and chemical interactions of the proteins-phenolic acids (Ali, H., 2012). The bonding types can be characterized by spectroscopic methods,

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microscopic methods, thermodynamic methods, bioinformatics methods, electrophoretic and chromatographic methods.

The goal of this review is to give researchers an overview of the currently used methods for identification of bonding type, negative and positive effects of interaction on food quality, bioaccessibility, bioavailability as well as to introduce the novel delivery strategies that base on phenolic-protein interaction.

II. Type of Bindings that Contributes to the Interaction

The phenolic-protein interaction is mainly contributed via non-covalent bonds, which are weaker than covalent bonds and they are always reversible. Among the covalent bonds, hydrogen bonds provide more stable complex than Van-der -Waals interactions, dipole-dipole interactions and hydrophobic interactions (Yuksel et al., 2010; Nagy et al., 2012; Jakobek 2015). While non-covalent bonds commonly occur in protein-phenolic interaction, in some case covalent interaction can also be formed (Gallo et al., 2013; El-Maksoud et al., 2018; Sui et al., 2018). To determine the phenolic-protein interaction is necessary for the biological activity of phenolics as well as proteins. There are several approaches to investigate this interaction such as, spectroscopic methods, microscopic methods, thermodynamic methods, bioinformatics methods, electrophoretic and chromatographic methods. Several studies focus on to investigate phenolic-protein interaction. Previous studies and their findings are summarized in Table 1. To obtain the excat result during determination of the binding type, different techniques are used together (Table 1).

The proteomic approach is one of the novel bioinformatic technique to identify the binding type. Gallo et al. (2013), investigated the type of interactions between cocoa polyphenols and milk proteins by proteomic technique. They characterized the interaction of β-lactoglobulin (β-Lg) with catechin and epicatechin, moreover identified the amino acid residue at the binding site. For this aim, they used the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) and the electrospray ionization tandem quadrupole/orthogonal-acceleration time-of-flight mass spectrometer (ESI-Q-TOF MS/MS). In these analyses, tryptic peptides of β-Lg have allowed the identification of the binding site as the free thiol group of cysteine and found that while polyphenols covalently bound with β-Lg, they interact with casein via non-covalent bonds. In another study, fluorescence, circular dichroism spectroscopy, and docking studies were used for characterization of the interaction between β-Lg and cyanidin-3-*O*-glucoside According to this study, the interaction was mainly contributed by both hydrogen bonding and the hydrophobic interaction (Cheng et al., 2017). Furthermore, after the binding of cyanidin-3-*O*-glucoside to β-Lg, the secondary structure of the β-Lg was changed in which the major structure of β-sheetincreased and the minor structure of α-helix decreased. The changes in secondary structure of proteins after the phenolic interaction also identified in different studies (Zhang et al., 2014; Al-Hanish et al., 2016; Jia et al., 2017). The significant reduction of α-helix and an increase of β-sheet and turn structures were determined in α-lactalbumin and β-Lg in the phenolic-protein complex (Zhang et al., 2014). Also, Jia et al. (2017) observed the conformational changes on the secondary structure of β-Lg, after phenolic compound attachment, via circular dichroism and Fourier transform infrared. According to their findings, after the binding of the phenolic compound, the surface hydrophobicity of β-Lg was changed. Thus α-helix to β-structures transition occurred. The non-covalent interactions between α-lactalbumin and epigallocatechin-3-gallate were determined by circular dichroism and Fourier transform infrared spectroscopy (Al-Hanish et al., 2016). Based on their findings, epigallocatechin-3-gallate caused the conformational changes in α-lactalbumin, which were inducing α-helix to β-structures transition.

Not only the non-covalent bonds but also the covalent bonds also occur in a phenolic-protein complex. Sui et al. (2018) demonstrated the conformational changes on soy proteins in the presence of anthocyanins. They used three-dimensional fluorescence and Fourier transform infrared spectroscopy to determine the interaction character. They reported that the covalent bonds occurred more abundant than non-covalent bonds in soy protein isolate-anthocyanin complex. The conformational changes on the secondary structure of soy protein isolate also detected as a decrease in β-sheets and an increase in β-turns and random coils.

III. Interaction Effect on Nutritional and Antioxidant Quality of Foods

The interaction of phenolics-proteins and starch may be one of the mostfundamental factors affecting the nutraceutical quality of the food products. There are several studies that is confirmed by earlier studies including phenolic–protein (Arts et al 2002; Świeca, Gawlik-Dziki, Dziki, et al., 2013), phenolic–phenolic (Gawlik-Dziki, 2012), and phenolic–starch interactions (Chai, Wang, & Zhang, 2013; Zhang, Yang, Li, & Gao, 2011, Swieca, M., et al., 2014).

The chemical structure of phenolic compounds is the enhancive reason for interaction with major food components. In other words, a hydroxyl group and carboxylic acids give rise to increasing of phenolic interaction with proteins, carbohydrates, lipids, and

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minerals (Bravo, 1998; Alu'datt, Rababah, Ereifej, Brewer, & Alli, 2013; Escarpa & Gonzalez, 2001). As mentioned before, phenolic acids and proteins bring about two main types of interactions (covalent or non-covalent) that result in two kinds of precipitation of proteins. First one is multisite interactions, that is, several phenolics bind to one protein molecule and the second one is multidentate interactions, that is, one phenolic binds to several protein sites or protein molecules. A study by Rawel, Meidtner, and Kroll (2005) stated that the non-covalent binding of phenolic compounds does not affecton the secondary structure of the proteins while may induce to distinct alteration in the tertiary structure of proteins. However, it has been indicated that the covalent binding of phenolics compounds may affect both tertiary and secondary structures of proteins (Kroll et al., 2003). Moreover, these interactions may also end in changes in the thermal stability, solubility, and digestibility of food proteins (Kroll et al., 2003; Ozdal et al., 2013). S. Wu et al. (2018) have studied on 71 phenolic acids, and the derivatives of these phenolics were chosen to estimate the binding affinity with β-lactoglobulin. According to their study, the potential mechanisms for increased binding affinity is the inclusion of the hydrogen bond in the interaction between the β-lactoglobulin and phenolic acids. The interaction between β-lactoglobulin–phenolic acid has enhanced antioxidant activity when compared tothat of the phenolic acids alone (S. Wu et al., 2018). This study gives perspective for understanding the relationship between the chemical structure of phenolic acids and the affinity for β-lactoglobulin.

Most of the nutritional concern of phenolics focus on their side effects caused by the capability of phenolics to conjugate and precipitate protein, lipids, minerals and carbohydrates resulting in reducing food digestibility (Bravo, 1998). On the other hand, several studies indicated that regarding the bio-functional impact of the naturally occurring interactions between phenolics with food constituents such as antioxidant effects become inadequate (Bravo, Saura-Calixto, and Goni, 1992; Alu'datt, Rababah, Ereifej, Brewer, et al., 2013).

IV. Interaction Effect on Organoleptic Quality of Foods

Polyphenol compounds are currently present in multiform beverages and food products. Several properties of phenolic compounds are using in the food industry. Antioxidant properties of phenolic compounds have been utilized in the food industry to stabilize cysteine proteases and to prolong shelf-life of processed products (Howell, 2005). Also, phenolic compounds including anthocyanins, β-carotene, riboflavin, and curcumin are using as natural food colorants in beverage products (Mortensen, 2006). These compounds have poor solubility and limited chemical stability which is challenging as a natural colorant in food products and beverages (Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J., 2017). On the other hand, phenolic-protein interaction is using to maintain the stability of phenolic-colorants. The interaction between protein and phenolic compounds is initially starting with a hydrophobic effect and is stabilized by hydrogen bonding (Oh, Hoff, Armstrong, & Haff, 1980; Siebert, 1999). Thus, different research groups are studying on improvement of the stability of phenolic-colorants by using phenolic-protein interaction phenomena. Chung et al. (2015) proved that when heated whey protein isolates are added to model beverage systems containing ascorbic acid, it is improved the color stability of anthocyanins in the beverage. Ascorbic acid has a strong effect to tint ofthe anthocyanins in these systems. (Mercadante & Bobbio, 2007; Poei-Langston & Wrolstad, 1981). Recently, Chung and co-workers studied beverage products containing ascorbic acid. They examined the effect of polypeptides and amino acids on the color stability of anthocyanins. Also, the product containing ascorbic acid and an amino acid is two times more convenient regarding to the average half-life of the anthocyanin than alone ascorbic acid added the product (Chung C. et al., 2017).

Astringency sensation and bitterness are another important sensory attribute for wine. Interaction and precipitation of proline-rich proteins, especially, salivary proteins by tannins is estimated to be the general explanation of astringency onset (Charlton et al., 2002; De Freitas & Mateus, 2012; Haslam & Lilley, 1988). Soares et al. (2018) found evidence that the interaction of proline-rich proteins by tannins is disparate when the proteins are present simultaneously or alone. However, protein-phenolic interaction and co-protein interaction via tannins may be responsible and need to be more investigated.

In respect of food processing, there are only limited studies to confirm the effects of phenolic-protein interactions. Indeed, for the food industry, the interaction is exploited as refining and clarification treatments to improve haze stability (Cosme, Ricardo-da-Silva, & Laureano, 2008). There are few studies on improving textural properties via the phenolic-protein interaction. Wu, Clifford, and Howell (2007) reported that the forming and the gelling potential of egg albumin protein could be significantly increased by addition of the instant green tea. In another study, green tea powder added to weat dough to enhance viscous/elastic modulus and the stability of wheat dough. (Wang et al., 2015). Having obtaining protein-phenolic interaction between fruit extract and soy protein isolate in a gluten-free rice noodle, improved noodle quality, and the dough is achieved (Lee et al., 2016). Lately, Song & Too (2017) the quality of rice-substituted fried noodles is

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enhanced via interaction of pea protein isolate with green tea extract to the supported both antioxidant activity and network.

Beyond that, presence of phenolic compounds at a nutritional daily intake had no adverse impact on protein digestibility. However, the high dose consumption of a phenolic extract may appear destructive to humans who consume low protein amount. Hence, consuming polyphenol supplements needs to be given to the benefit/risk balance with dedicated care (Dufour, C. et al., 2018).

V. Interaction Effect on Bioaccessibility/Bioavailability of Phenolics

To demonstrate the bioactivity of a compound on human health, it should have a sufficient bioaccessibility and bioavailability. While bioaccessibility refers to the (%) undecomposed (bioactive) fraction of a compound after gastro-intestinal digestion, bioavailability represents the (%) metabolized fraction of a bioactive compound. Both bioaccessibility and bioavailability of phenolic compounds can be improved, impeded or unaltered when co-delivered with other foods like rich in protein, carbohydrate, fat or fiber (Yang, Koo, Song, & Chun, 2011). The bioaccessibility has been currently demonstrated to be affected by the co-digestion of phenolics with various food components (McDougall, Dobson, Smith, Blake, & Stewart, 2005; Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006; Ribnicky et al., 2014; Sengul, Surek, & Nilufer-Erdil, 2014).

There is currently a natural affinity between polyphenols and proteins (Bandyopadhyay, Ghosh, & Ghosh, 2012). Thereby, protein-rich food matrices can be stabilized and concentrate anthocyanins and also other phenolics (Roopchand, Kuhn et al., 2012; Roopchand, Grace et al., 2012; Roopchand et al., 2013). Grape and blueberry polyphenol-enriched protein matrix have been studied for oral administration, and the hypoglycemic effect obtained in mice indicates that the phenolic-protein complex is bioactive. (Roopchand et al., 2013). In another study, it is reported that anthocyanins are conserved by the sorption to defatted soy flour while transiting through the upper gastrointestinal tract by permitting huge amounts to be gained to the colon (Ribnicky D. M. et al., 2014). Pineda-Vadilloet al. (2016) studied on protein-rich products enriched with grape extracts. As a result, they showed that the food matrix has no affect on the antioxidant activity; while the antioxidant capacity was steady during the oral and gastric phases, it considerably increased during the intestinal phase of digestion. There is also another study, that supports the approach of the non-covalently polyphenols-proteins interaction which are hydrolyzing during digestion, and there was no effect on the absorption of polyphenols and proteins (Budryn, G., & Nebesny, E., 2013). These studies indicated that becoming complex with the protein matrix does not affect the bioaccessibility of anthocyanins/polyphenols negatively (Budryn, G. & Nebesny, E. 2013; Ribnicky D. M. et al., 2014; Pineda-Vadillo et al., 2016). In the previous*in vivo* models, it was demonstrated that the bioavailability of milk and coffee can reduce when they are consumed together (Duarte & Farah, 2011). The different inferences among various studies may be the potential of some phenolics to be complex with digestive enzymes and food matrix. The previous study of Rohn et al. (2002) confirmed that the activity of selected digestive enzymes such as trypsin and α-amylase was a decline in the case of protein-phenolic interaction. Thereby, the interaction has caused to the antioxidant activity of phenolic compounds reduce as it cannot leave the protein-phenolic complex. However, the interaction of phenolic-protein occur only some functional groups and the un-interacted part still can be show activity (Rohn, Rawel, & Kroll, 2002; Alminger, M. et al., 2014). Indeed, being complex with polyphenols is known to reduce protein digestibility via protecting proteins from enzyme degradation or through interaction with digestive enzymes.

VI. Delivery Techniques based on Phenolic-Protein Interaction

Phenolic compounds are highly unstable bioactive compounds due to exposure to degradation by light, soluble oxygen or enzymes. The basic goal of delivery techniques is to protect the phenolic compounds from adverse environmental conditions. Protein-based delivery techniques are one of the novel strategies from this area, and there are numerous studies about this subject. The delivered phenolic compounds, as well as the delivery system, is summarized in Table 2. These strategies can mainly divide into two groups; (i) protein-phenolic conjugates and (ii) protein-based nanoparticles.

a) Protein-phenolic conjugates

Protein-phenolic conjugates can be used to enhance antioxidant activity of a protein as well as its stability (Frazier et al., 2010; Wu et al., 2011; El-Maksoud et al., 2018) or to reduce the degradation level of delivered phenolic compounds. Since the non-covalent interactions are reversible, the stable structure cannot be obtained by non-covalent bonds. Besides, these types of interactions lead to alterations on protein and phenolic compound structure, hence their functionality and nutritional value are changed (Mehanna et al., 2014; Ozdal et al., 2013). Conversely, conjugates are constructed with covalent bonds. Hence stable structure can be obtained. El-Maksoud et al. (2018) were produced covalently bond protein-phenolic conjugates via carbodiimide cross linker chemistry. The

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phenolic compound that they used in conjugate was caffeic acid, and β-lactoglobulin was selected for protein part of conjugates. They reported that the conjugates showed better water solubility than native β-lactoglobulin and non-covalently bond β-lactoglobulin-caffeic acid complex. Moreover, the thermal stability of β-lactoglobulin significantly was increased with this conjugate.

In the view of nutraceutical delivery aspect, protein-phenolic conjugates offer several advantages. Liu et al. (2016) investigated the physicochemical properties of β-carotene emulsions stabilized via chlorogenic acid–lactoferrin–glucose/polydextrose conjugates. They indicated that the produced conjugate offered better emulsifying properties such as the physicochemical stability of β-carotene emulsions can be conserved during the freeze–thaw treatment. Besides, chemical stability of β-carotene in the emulsions against ultraviolet light exposure can enhanced by the conjugate. Therefore, they suggested that the conjugates containing protein, polyphenol and carbohydrates could be a smart building block for delivery systems (Liu et al., 2016). In another study, the chemical stability of curcumin to degradation at physiological pH and of resveratrol to degradation under ultraviolet irradiation conditions was obtained by zein-epigallocatechin gallate conjugates (Liu et al., 2018).

b) Protein-based nanoparticles

Zein and gliadin are the prolamine-type proteins which generally occur in in cereals such as corn and wheat, respectively. The four major components of zein are α, β, γ and δ-zein (Hu & McClements, 2015). Since both zein and gliadin contain the high amount of non-polar amino acids in their primary structure, they are soluble in aqueous ethanol solution (60–90%), but insoluble in water (Rombouts et al., 2009; Shukla & Cheryan, 2001). Because of their highly hydrophobic nature, these proteins can be easily converted into spherical colloidal nanoparticles, which are effective delivery agents for phenolic compounds (Chen, Zheng, McClements, & Xiao, 2014). Previous studies reported that protein-based nanoparticles are suitable and effective delivery agents for different phenolic compounds (Table 2). During the formation of protein-based nanoparticles with phenolics, non-covalent interactions such as electrostatic interaction, hydrogen bonding, and hydrophobic interactions were involved in the structure (Dai et al., 2018). On the other hand, these interactions mainly depend on the type of protein and phenolic compounds. Joye et al. (2015) studied on binding ability of resveratrol to the zein and gliadin. They assumed that hydrogen bonds are the main force that contributed to the interaction between resveratrol and zein. However, the hydrophobic interactions constructed the resveratrol-gliadin interaction. In another study about curcumin delivery by zein-nanoparticles, are indicated that hydrogen bonds between the phenolic hydroxyl groups in curcumin and the carbonyl group in amide bonds in zein were attributed to the formation of protein-based nanoparticle with polyphenol (Dai et al., 2017; Sun et al., 2017). These nanoparticles are not only protecting the related phenolic compounds form adverse environmental conditions, beyond that they support the controlled released of the phenolics. Liang et al. (2017) reported that the controlled release property of epigallocatechin gallate was improved by zein/chitosan nanoparticles and according to Sun et al. (2017), controlled release of curcumin during in vitro digestion, can be obtained by zein-shellac composite colloidal particles. In another study, in vitro release of curcumin as well as its stability are improved by zein nanoparticles (Dai et al., 2018). The authors suggested that curcumin might bind to zein in tyrosine residue. Since, the aromatic side groups and double bonds in zein molecules could absorb UV light (Luo et al., 2013), zein nanoparticles enhance the stability of curcumin against UV light. The zein-lecithin composite nanoparticles also improved the stability of curcumin against UV irradiation, high ionic strength and thermal treatment (Dai et al., 2017).

VII. Conclusion

The interaction between phenolic compounds and proteins is an important phenomena since it affects the functionality, biological activity and nutritional quality of protein and phenolics. The interaction is contributed with both non-covalent and covalent bonds that depend on the type of protein and phenolics as well as the environmental conditions. Depending on the bonding type, there occur conformational changes in protein structure. There are several techniques for determining the bounding type such as spectroscopic methods, microscopic methods, thermodynamic methods, bioinformatics methods, electrophoretic and chromatographic methods. The delivery of phenolics in the desired system can be done by novel agents which are constructed with proteins. Indeed, the bonding type is important to select the novel delivery strategies. If the protein functionality is important in delivery system, then the covalent bonds are crucial to eliminating the structural changes. But if the controlled released of phenolic is desired, the non-covalent bonds are wanted.

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Table 1: Bounding type of protein-polyphenol interaction, the interaction factors and determination methods.

Polyphenol	Protein	Determination Method	Type of Interaction	Effect of Interaction	Reference
Green tea flavanoids	Milk proteins	Fluorescent probe binding method Isothermal titration calorimetry	Hydrophobic interactions between catechin and β-casein.	Protein surface hydrophobicity was decreased by the hydrophobic binding between milk proteins and GT flavanoids	Yuksel et al., 2010
Pelargonidin	Dairy proteins: β-lactoglobulin Caseinate	Fluorescence spectroscopy	Hydrophobic interactions between pelargonidin-β-lactoglobulin Hydrogen bonding between pelargonidin-caseinate	the structural conformation of the milk proteins effect the binding process	Arroyo-Maya et al., 2016
Chlorogenic acid Ferulic acid	β-lactoglobulin	Fluorescence spectroscopy	Hydrogen bonding and Van der Waals interactions between β-	The secondary structure of β-lactoglobulin	Jia et al., 2017

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Epigallocatechin-3-gallate		Molecular modeling study	lactoglobulin and chlorogenic acid, β-lactoglobulin and ferulic Acid Hydrophobic interaction between β-lactoglobulin and epigallocatechin-3-gallate	changed from α-helix to β-structures transition	
Black rice anthocyanins	Soybean protein isolate	Three-dimensional fluorescence Fourier transform infrared spectroscopy	Covalent interactions between soybean protein isolate and black rice anthocyanins	Changes in the secondary structure of soybean protein isolate with a decrease in β-sheets and an increase in β-turns and random coils.	Sui et al., 2018
Proanthocyanidins	Wheat gluten proteins	Attenuated total reflectance – Fourier transform infrared spectroscopy	Hydrophobic interactions between gluten and proanthocyanidins	reduced gluten solubility in urea decreased surface hydrophobicity of glutenins	Girard et al., 2018
Chlorogenic acid Caffeic acid Ferulic acid Coumalic acid	α-lactalbumin β-lactoglobulin	Fourier transform infrared spectroscopy Fluorescence spectroscopy Circular dichroism	Interact with the C=O and C-N groups of the whey protein structural subunits	Conformational changes of whey protein: reduction in the amount of α-helix increase the β-sheet and turn structures	Zhang et al., 2014
Caffeic acid Gallic acid	Trypsin	Fluorescence spectroscopy Molecular modeling studies	Hydrogen bonding between trypsin and phenolic acids	inhibit trypsin by altering the enzyme conformation	Liu et al., 2017
Gallic acid Epigallocatechin gallate	Whey proteins	Isothermal titration calorimetry	Hydrophobic interaction between whey proteins and phenolics	structural changes, modify the surface activity, and enhance the foaming properties of whey proteins.	Cao et al., 2018
Resveratrol	Whey proteins: Lactoferrin holo-lactoferrin apo-lactoferrin Whey protein isolate: β-lactoglobulin α-lactalbumin-rich fractions	Fluorescence spectroscopy	Dipole–dipole and Van der Waal interactions between whey proteins and resveratrol	Interaction did not affect the secondary structure of the proteins	Hemar et al., 2011
Epigalocatechin-3-gallate	bovine α-lactalbumin	Fluorescence quenching analysis Circular dichroism Fourier transform infrared spectroscopy spectra	Hydrophobic interactions between a-lactalbumin and epigalocatechin-3-gallate	Conformational changes in α-lactalbumin inducing-helix to β-structures transition	Al-Hanish et al., 2016
Chlorogenic acid	Sunflower protein isolate	Isothermal Titration Calorimetry analyses	Hydrogen bonding between Sunflower protein isolate and chlorogenic acid	Enhanced the interfacial and emulsifying properties of sunflower proteins	Karefyllakis et al., 2017
Cyanidin-3-O-glucoside	β-lactoglobulin	Fluorescence spectroscopy Circular dichroism spectroscopy	Hydrophobic interaction between β-lactoglobulin and cyanidin-3-O-glucoside	alterations of the secondary structures of β- lactoglobulin, with a decrease in α-helix, and an increase in β-sheet	Cheng et al., 2017

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		Molecular docking studies	Hydrogen bonding between β-lactoglobulin and cyanidin-3-O-glucoside		
Rosmarinic acid	bovine milk whey protein: α-Lactalbumin, β-Lactoglobulin and Lactoferrin	Fourier transform infrared spectroscopy Differential scanning calorimetry	Hydrophobic interactions hydrogen bonding dipole–dipole interactions between bovine milk whey protein and rosmarinic acid	physical and reversible interactions	Ferraro et al., 2015
Cocoa phenolics: Catechin Epicatechin	β-lactoglobulin	Proteomic techniques	Covalent and non-covalent bonding between β-lactoglobulin and cocoa phenolics	decreasing the in vitro antioxidant activity of polyphenols	Gallo et al., 2013

Table 2: Delivery techniques based on polyphenol-protein interaction

Technique			Delivered bioactive compound	Function	Reference
Protein-polyphenol conjugate	Type of Polyphenol compound	Type of Protein			
	Caffeic acid	β-Lactoglobulin	β-Lactoglobulin	surface-active agents with exceptional antioxidant properties	El-Maksoud et al., 2018
	Chlorogenic acid	Lactoferrin	β-carotene	effective emulsifiers to stabilize β-carotene emulsions	Liu et al., 2016
	Epigallocatechin gallate	Zein	Curcumin and Resveratrol	improve the stability and bioaccessibility of curcumin and resveratrol	Liu et al., 2018
Encapsulation	Type of delivery particle				
	Zein/chitosan nanoparticles		Epigallocatechin gallate	Increase the antioxidant activity of epigallocatechin gallate	Liang et al., 2017
	Zein/rhamnolipid complex nanoparticles		Curcumin	Increase the stability and in vitro bioaccessibility of curcumin	Dai et al., 2018
	Zein nanoparticles and gliadin nanoparticles		Resveratrol	rationalize ingredient selection and production of protein nanoparticles and microparticles for encapsulation,	Joye et al., 2015
	Pectin coated caseinate/zein nanoparticles		Curcumin	Increase the antioxidant activity of curcumin	Chang et al., 2017
	Zein/lecithin composite nanoparticles		Curcumin	improved the physicochemical stability of Curcumin	Dai et al., 2017
	Zein-shellac composite colloidal particles		Curcumin	controlled release of curcumin in simulated gastrointestinal fluids.	Sun, et al., 2017
	Emulsion system				
	O/W emulsion Stabilizer: Dairy proteins: whey and sodium caseinate plant-derived proteins: soy and pea		Lycopene	improve the physicochemical stability and bioavailability of lycopene	Ho et al., 2017

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