The influence of intrinsic and extrinsic factors on protein-polyphenol interactions in dairy systems

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Abstract. The application of polyphenols in dairy products is becoming more widespread due to the rich protein content of dairy products reacting with polyphenols. Many studies have pointed to the positive effects of polyphenol-protein binding on dairy products. This article illustrated the mechanisms of how polyphenols react with proteins. The effects of the combination of the polyphenols and protein were described in terms of intrinsic and extrinsic factors, as well as the effects on food properties. Moreover, specific examples of the use of polyphenols in dairy products will also be proposed. Importantly, based on the reported findings, future processes that exploit and develop this interaction can be considered to enable the targeting of polyphenols and proteins in dairy products to secure a range of industrial benefits.

Keywords: Protein; polyphenols; protein-polyphenol interaction

1. Introduction

Global market demand for dairy products is increasing annually. Dairy products can be classified into the following types: 1. Liquid milk 2. Milk powder categories; 3. Condensed milk; 4. Milk fat; 5. Cheese; 6. Milk ice cream. The reason why dairy products are more and more popular is that they are rich in t nutrition, especially protein. Recognized around 120 years ago, there are two main families of protein in dairy products: whey protein and casein.

Casein accounts for the biggest ratio of milk; it is also used for dental care, prevention of osteoporosis and rickets, promotion of in vitro fertilization in animals, treating iron deficiency anemia, regulating blood pressure, neuritis of magnesium deficiency, and many other treatments, owing to its physiological impact, in particular the contribution to macronutrients (Ca, Mg) and micronutrients (Zn, Fe, Cu, Ni, Cr, Co, Mn, Se). Casein is primarily used in the food industry as a nutritional agent in solid foods. In food processing, it is also widely used as thickeners and emulsion stabilizers, and sometimes as a binding agent, a filling agent, and a carrier. Casein is particularly suitable for use in the production of cheese and ice cream (0.3% to 0.7%). αs1-, αs2-, β-and κ-casein are four different types of phosphorylated proteins and the casein fraction (the ratio is about：4.0:1.0:3.5:1.5), while 90% of them exist as colloidal complexes with a diameter of about 50-500 nm, called CMS (Clare et al., 2003; Dalgleish, 2011; Holt and Sawyer, 1993). And the characteristics of casein micelles have been widely investigated, although the internal structure of casein micelles remains controversial as to the internal structure of micelles of casein, it has been generally accepted that stability of the micelles is provided by the outer hair layer of κ-CN and that calcium phosphate in the micelles is present as colloidal nanoparticles (Shukla et al., 2009). The pyrrolidine ring of proline residues in casein provides multiple hydrophobic binding sites, providing strong conditions for strong binding of teicoplanin substrates (Luck et al., 1994).

Whey protein is a secondary product during the processing of casein and cheese production as well as has good processing properties and biological activity (Rebouillat and Ortegα-Requena, 2015). The high content of β-lactoglobulin in whey protein is difficult to hydrolyze by pepsin in its natural state, and its digestibility is altered by its interaction with polyphenols (Cao el al, 2017).

Plant components with several phenolic hydroxyl groups in their molecular structure can all be called polyphenolic compounds, the common ones being phenolic acids, flavonoids, tannins, and anthocyanins. Phenols are compounds found in plant foods that have potential health-promoting effects. It is found in common plants or food such as tea, cocoa beans, soya, vegetables, red wine, and fruits. They possess a broad array of biochemical attributes, including antioxidant, apoptotic, anti-aging, anti-cancer, cardiovascular protective, anti-inflammatory, enhanced endothelial activity, and angiogenic and cell proliferation inhibitory activities (Krekora et al., 2021). Common foods such as apples are rich in apple polyphenols, blueberries are rich in anthocyanins, and grapes are rich in resveratrol; onions, cauliflower, celery, and parsley are rich in flavonols and flavonoids; green tea is rich in tea polyphenols, coffee is rich in chlorogenic acid, dark chocolate is rich in cocoa polyphenols, etc. Tea

polyphenols are one of the natural antioxidants widely used in food processing, with good water solubility, among which epigallocatechin gallate (EGCG) has the highest content and the strongest antioxidant activity of polyphenolic monomers. Tea polyphenols are inherently bitter and astringent, and it is customary to combine tea and milk, which reduces the bitterness of tea and enhances the functional properties of milk such as antioxidant and emulsification. More and more products related to tea and milk are being launched in the market, mainly milk tea, matcha ice cream, matcha yogurt, and green tea cheese. The effect of polyphenols in tea on the functional properties of dairy products is inextricably linked to the interaction with dairy protein (Huang et al., 2002; Spencer et al., 1988).

This article will deal with seven aspects. The first part explains the mechanism of interaction between polyphenol and protein. These include noncovalent interaction as well as covalent interaction. The second part describes methods that can be applied to detect polyphenol and protein complexes in dairy products delete. The third part assesses temperature, pH value, and chemicals how these extrinsic factors influence the complex formation of protein and polyphenols. The fourth part gives a brief discussionof the influence of intrinsic factors on the combinations of protein and polyphenol. The fifth part clarifies the influence of polyphenol-protein complexes on the functionality properties in the food system and how these can provide theoretical guidance for the analysis of the functional properties of dairy products. The last part provides a summary conclusion of the paper's findings.

2. The mechanism of interaction between proteins and polyphenols

Polyphenols interact with proteins in both covalent and non-covalent forms of binding. In contrast to non-covalent modifications, covalent modifications can irreversibly alter the function of the protein. In addition, polyphenol methylation blocks the hydroxyl group of reactivity and thus reduces covalent interactions between polyphenols and proteins (X. Sun et al., 2022).

2.1 Non-covalent interactions

Non-covalent interactions of polyphenols with protein proceed via hydrogen bonding, van der Waals forces, electrostatic interactions, and hydrophobic (Spencer et al., 1988). The nature of polyphenols determines the kinds of non-covalent reactions. For example, catechol is commonly extracted from tea, hydrogen bonding and hydrophobic phase binding to proteins: their hydroxyl groups are interacting with the polar groups of the peptide via hydrogen bonds; their hydrophobic substituents are bound to the protein by the hydrophobic interaction. After catechin is methylated, it can permeate and interact with the hydrophobic region of protein as its low polarity (Chen et al., 2021). Hasni and others (Hasni et al., 2011) covered that the β-lactoglobulin- polyphenols binding constants with α-CN and ß-CN, demonstrating that the β-CN complexes were more stable than α-CN, which was contributed to the presence of five phosphoserine residues in the hand ß-CN, which is more hydrophobic than α-CN. The hydrophilic κ-CN glycol-macropeptide is the key to the solubilization of the tea polyphenol-protein complex. The driving effect of tea polyphenols on the strong hydrophobic end of κ-CN makes the hydrophilic end of κ-CN The strong hydrophobic end of tea polyphenols and κ-CN makes the hydrophilic end of κ-CN less likely to fall off, enhancing the interaction between tea polyphenols and casein micelles was enhanced, which explains why the specific binding of EGCG to κ-CN restricts Haratifar et al (Haratifar and Corredig, 2014) further demonstrated that the native Ca2+ in cow's milk did not interact with EGCG and its enzymatic coagulation, but Ca2+ did not affect the interaction of casein micelles with EGCG. However, the stability of the casein micelles in combination with Ca2+ and tea polyphenols was less than that of the natural casein micelles.

2.2 Covalent interactions

The covalent association between polyphenols and proteins results from the oxidation and nucleophilic addition processes of polyphenols. Polyphenols produce quinones or semiquinones under enzymatic, oxidative, or thermal conditions. As a very highly reactive compound, quinone can further react with other quinones to form brown-black compounds, as well as in proteins, the lysine ε-amino group, cysteine sulfhydryl group, and tryptophan indole group form C-N or C-S covalent connections. The covalent bonding between the polyphenol-protein occurs in the conjugates between quinones or semiquinones and nucleophilic groups of protein, which is reversible or irreversible. The covalent binding products of tea polyphenols to proteins may contain the following 3 types, with amino or thiol side chains of amino acids; 2) reaction products conforming to bound dimers in addition to monomers; 3) monomers or oligomers acting as cross-links; and 4) a monomer or oligomer acting as a cross-link (Buitimea-Cantúa et al., 2018). The covalent forces of polyphenols and proteins have disulfide bonds in addition to peptide bonds, and these two are irreversible processes. The extent to which polyphenols covalently interact with proteins is dependent on the quantity of hydroxyl and aromatic groups in the polyphenol. It has been suggested that cinnamic acid (e.g. caffeic acid) derivatives made more covalent interactions with proteins than benzoic acid derivatives (e.g. gallic acid) (Chen et al., 2021; Le Bourvellec and Renard, 2012; Prodpran et al., 2012).

3. Analytical methods for the interaction of polyphenolic compounds and proteins

The study of polyphenol-protein interactions can be carried out by both direct and indirect methods, and usually, a combination of methods is used in experiments to determine the reaction. The summarized approach is shown below in figure 1.

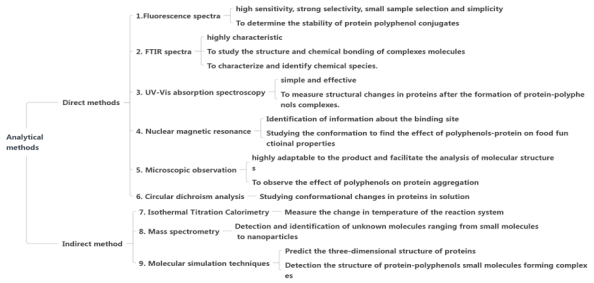

Fig.1 Analytical methods for the interaction of polyphenols with protein

3.1 Fluorescence spectra

In recent years, fluorescence spectroscopy has been frequently used to determine the stability of protein polyphenol conjugates, because of its high sensitivity, strong selectivity, small sample selection, and simplicity (Hemar et al., 2011; Wu et al., 2017). By fluorescence spectroscopy, it has been found that the best-selected docking positions of amino acid residues for different types of tea polyphenol-protein interactions are as follows in table 1 (Huang et al., 2002). Fluorescence spectroscopy can provide effective information on burst constants, the number of bond sites, and changes in entropy values, enthalpy change, and Gibbs free energy change, which has been widely used to make reliable speculations and judgments on the burst mechanism and the type of forces involved in the binding of small molecules and proteins (Yuan et al., 2017). A study has shown that polyphenol complexes formed with β-casein are more stable than those formed with α-casein by spectral data and binding energy (van de Langerijt et al., 2022). In addition, by analyzing turbidity, the addition of tea polyphenols to milk protein concentrates interactions with the hydrophobic region of casein micelles, thus stabilizing the structure of casein micelles and limiting thiocyanate dissociation, which can lead to a decrease in solution turbidity over time (van de Langerijt et al., 2022).

3.2 FTIR spectra

Fourier Transform Infrared spectroscopy has been widely used to study the chemical bonding and structure of molecules, and as a method for characterizing and identifying chemical substances. Infrared spectra are highly characteristic and can be used to do analytical identification by comparison with the infrared spectra of standard compounds (Zhu et al., 2006). By this analytical method, it is known that larger and bulkier polyphenols interfere more with the protein secondary structure since a large increase in ß -folding and a small increase in α-helix are observed when the two interact, and the conformational changes of ECG and EGCG are greater in polyphenol- ß -LG complexes than in C and EC complexes (Chanphai et al., 2018).

3.3 UV-Vis absorption spectroscopy

As an effective and simple way, UV-Vis absorption spectroscopy can measure structural changes in proteins after the formation of protein-ligand complexes. The combination of UV difference spectroscopy and fluorescence spectroscopy can determine whether the burst is static or dynamic. The combination of UV difference spectroscopy and fluorescence spectroscopy can determine whether the burst is static or dynamic. The UV absorption spectra of protein macromolecules generally have two. The UV absorption spectrum of a protein micro fraction generally has two characteristic absorption peaks, the one around 200 nm which represents the backbone structure of the protein, while the absorption peak near 280 nm represents aromatic amino acids, like tyrosine and tryptophan (Le Bourvellec and Renard, 2012). Phenolic compounds extracted from fruits such as apples and pears have been found to have antibacterial and antioxidant properties by this method (Fattouch et al., 2008).

3.4 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy uses the chemical shift of labeled carbon elements of the detected molecular structure to directly indicate whether binding interactions and associated conformational changes occur between polyphenols and proteins. Nuclear magnetic resonance spectroscopy allows precise identification of information about the binding site (Delius et al., 2017).

3.5 Microscopic observation

About the application of microscopic observation methods in the study of polyphenol-protein interactions, the main ones are atomic force microscopy, scanning electron microscopy, and laser confocal microscopy. These microscopic observation methods allow direct observation of polyphenol-protein interactions, are highly adaptable to the product, and facilitate the analysis of molecular structures (Li et al., 2019). By using the atomic force microscopy method, Liu et al (Liu et al., 2016b) observed that chlorogenic acid (CA) and EGCG, respectively, and lactoferrin both resisted the aggregation of lactoferrin upon heating, with chlorogenic acid lactoferrin binding being more hydrophobic and therefore more effective in preventing lactoferrin aggregation.

3.6 Circular dichroism analysis

Circular dichroism spectroscopy, which is caused by adsorption differences between left and right circular polarization optical, is a valuable technique for studying protein conformational changes in solution (Niu et al., 2019). The principle is that it can reveal small changes in protein structure. Far-UV Circular dichroism spectroscopy reflects the characteristics of the secondary structure of proteins and can therefore be readily used to estimate (Micsonai et al., 2018).

3.7 Isothermal Titration Calorimetry

As an analytical and calorimetric technique, it uses one of the reagents to titrate against the other, with the temperature change of the reaction system measured as the amount of titrant added is altered. Kilmister et al (Kilmister et al., 2016) used isothermal titration calorimetry to study the interaction of proanthocyanidins

isolated and purified from cocoa beans with bovine serum albumin. The interaction between proanthocyanin oligomers of different molecular weights and proteins was investigated using a proanthocyanin and protein precipitation model (Harbertson et al., 2014). It was shown that the larger molecular weight proanthocyanins had a greater surface area and greater opportunity for protein-tannin-protein cross-linking than the smaller molecular weight molecules, and when a ternary complex has formed a precipitate was generated. However, this method does not identify the specific site of tannin and globular protein binding, as the binding of the two occurs randomly in the hydrophilic and hydrophobic regions of the surface of the protein. This type of non-specific bonding divides the combined isotherm into two separate parts, where there is a distinct binding constant and ΔH for each binding site. In addition, the ΔS values of the interactions are susceptible to systematic errors, so the isothermal titration technique needs to be further improved for the study of interactions between mixtures that are not homogeneous like forwarding anthocyanins.

3.8 Mass spectrometry

As a direct technique, mass spectrometry can detect and identify ranging from small molecules to unknown molecules in nanoparticles. In the research about interactions between small molecules and protein, mass spectrometry is the detection of the vehicle products of small molecule-protein complexes that have been ionized, enabling the sensitive, rapid identification of the sites where small molecules and proteins bind (Lim and Lord, 2002). Gallo et al (Gallo et al., 2013) found no stable adducts between individual caseins and neither catechin/epicatechin by MALDI-TOF-MS and identified the binding site of cocoa polyphenols to α-lactalbumin as the free thiol moiety of cysteine.

3.9 Molecular simulation techniques

Molecular simulation techniques are convenient and accurate for studying the structural and functional relationships of biological macromolecules. It can be used not only to predict the three-dimensional structure of proteins and the structure of protein-ligand small molecules forming complexes (Wang et al., 2019). It can also be used to simulate the binding of proteins to small ligand molecules, determine the site of binding and understand the energy changes during the binding process.

4. Influence of extrinsic factors on complex formation

For most proteins and polyphenols, the complexation reactions they produce are highly dependent on the external environment. Changes in temperature, pH, and inorganic salts can have both negative and positive effects on binding. The effects of external conditions on the binding of polyphenols to proteins in dairy products are as follows in table 1.

Table 1 Overview of studies describing the effect of changes in external conditions on polyphenol protein binding.

condition	polyphenols-protein	Effect of changing conditions	Experimental methods	reference
temperature	β-Lg–EGCG	Intermolecular Van der Waals forces at elevated temperatures and electrostatic effects above 85C°.	Circular dichroism; MALDI-TOF-MS; Fluorescence spectroscopy	(Qie et al., 2020)
	Curcumin-CMS	Heat promotes the binding of curcumin and protein	fluorescent labelling	(Rahimi Yazdi and Corredig, 2012)
	LF-CA,LF-EGCG	Heating causes the LF-CA and LF-EGCG solutions to change from dark green and brown to clear, and produces insoluble aggregates.	FTIR and circular dichroism	(Liu et al., 2016b)
pH	Tea polyphenols-EWP	Treatment with tea polyphenols significantly improved the emulsification activity and stability of EWP under neutral conditions.	fluorescence spectra	(J. Sun et al., 2022)
Chemicals	Lactoferrin chlorogenic acid	When glucose is added to the system, the ternary coupling formed is able to increase the stability of the complex.	Mass spectrometry	(Niu et al., 2019)

4.1 Temperature

Temperature not only determines the rate of chemical reactions but also affects the nature of the molecule itself. Thus, for protein and polyphenol systems, temperature plays a very important role, mainly affecting non-covalent interaction. The hydrogen bonding force decreases or even disappears with increasing temperature, while the hydrophobic force increases. When the temperature is over 60 C° , hydrogen bonding and vander Waals forces were would emerge; when the temperature is over 85 C°, the electrostatic interaction occurs in ß-Lg-EGCG complexes (Qie et al., 2020). Yazdi et al found that heating promotes the binding of curcumin and protein in

milk by a fluorescent labeling method using changes in fluorescence intensity and static burst constants. When titrated using curcumin, unheated milk casein had the lowest maximum fluorescence intensity binding constant. The different values of binding constants for heated milk and heated casein suggest that the binding sites for curcumin and casein are increased upon heating, possibly even on casein micelles. The binding constant values for casein in heated milk and unheated casein indicate that the heated whey protein aggregates bind to casein micelles and thus have an increased affinity for curcumin (Rahimi Yazdi and Corredig, 2012).

Researchers also indicated that the interaction of tea polyphenols with milk proteins at the same temperature increases with the increasing concentration of tea polyphenols. When the temperature reached 65 C°, some of the proteins in whey protein thermally denatured so that their globular structure folded back to stretch, exposing more clusters of hydrophobic groups and providing more sites for the binding of tea polyphenols. Therefore, the binding degree at this temperature will be significantly higher than 25 C° and 95 C°. In addition, heat treatment also affects fluorescence intensity, with high temperatures showing a slight increase followed by a decrease compared to the fluorescence intensity at 25C°. When the temperature is increased from room temperature to 65C°, the thermal induction has less effect on the structural stretch and affinity of milk proteins, while when it is then increased to 95C°, the temperature has a greater effect on the protein structure (Wang et al., 2017).

The denaturation of natural LF during heating above 60 degrees increases the hydrophobicity of the ionic strength and reduces the electrostatic repulsion of the protein, the aggregation of the protein and thus producing a turbid solution. At a pH of 7.0, free polyphenols can interact with LF to form suspended particles, and the increased hydrophobic interaction during heat treatment enhances the interaction between the two. Liu et al found that solutions of LF-CA and LF-EGCG couples were dark green and brown under neutral conditions and became clear after heating at ninety degrees. The investigators directly observed extensive segregation in the LF-CA and LF-EGCG mixture, which increased with heating time and produced many insoluble aggregates. By atomic force microscopy, only a few small aggregates were found in the LF-EGCG coupling after heat treatment, while almost no aggregates were found in the LF-CA coupling. This indicates that the lf-polyphenol couples were able to maintain good stability during the heat treatment due to the high charge, space effect, and hydrophobicity of the polyphenols (Liu et al., 2016b).

4.2 PH

The effect of PH on the binding of polyphenols and proteins is more complex and can be broadly divided into three areas. Firstly, pH affects the structure of the protein. For example, when at neutral pH, β-lactoglobulin presents as a dimer, while several amino acid residues are exposed for complexation with phenolic compounds for electron transfer to obtain antioxidant activity several amino acid residues with phenolic compounds and electron transfer for antioxidant activity. In contrast, under acidic conditions, the formation of β-Lg octamers leads to reduced antioxidant activity, which is reduced by the exposure of protein reactive moieties, due to reduced exposure of protein reactive groups. In contrast, when it is in an extremely alkaline environment, the protein undergoes partial unfolding, leading to the unfolding of the peptide chain to expose more binding sites and enhance binding to polyphenols (Liu et al., 2016b). Moreover, the pH value determines the amino acids bound to the polyphenols in the protein: cysteine with a lower pKa compared to the amino group in lysine (8.33 vs. 10.28) also reacts rapidly over lysine in the moderately basic conditions in which autoxidation occurs. (Keppler et al., 2020). The second is the impact of pH value on the polyphenol structure. pH modifies the properties and binding affinity of the polyphenols, thus affecting the anti-oxidative activity of the protein-phenol complex. Polyphenols are readily oxidized under alkaline conditions, resulting in covalent bonding with proteins, and the strength of covalent binding increases with increasing pH (de Morais et al., 2020). Finally, pH also has a large effect on the complexes themselves. Under acidic conditions, the forces between polyphenols and proteins tend to be non-covalent. The electrostatic repulsive forces are weakest and the non-covalent interactions between polyphenols and proteins are strongest as pH values approach and fall below the protein isoelectric point (Hagerman and Butler, 1981).

4.3 Chemicals

The formulation and characteristics of binary couples including protein-polyphenols or polysaccharide-protein complexes have been studied extensively. Much research has been carried out on protein-polyphenol formation and properties. The properties of protein-polyphenol complexes are also altered when other chemicals are present. For example, when using external forces to add polysaccharides to form polysaccharide-protein-polyphenol ternary couples, the properties are also altered. Liu et al (Liu et al., 2016a) chose lactoferrin as the surface-active protein and dextrose as the hydrophilic polysaccharide to form ternary couples with chlorogenic acid and confirmed the existence of the complexes by electrophoresis and mass spectrometry. Compared to chlorogenic acid-lactoferrin, the (chlorogenic acid-lactoferrin)-dextran physical mixture, both have the same thermal behavior, while the conjugate of (Cα-LF)-DEX has a significantly higher thermal transition temperature. This indicates that the thermal reaction caused by the ternary conjugate can increase the denaturation temperature of the protein while contributing to the thermal stability of lactoferrin in high-temperature products. This experiment also investigated the average emulsion droplet size and found that the size of the droplet in complexes was smaller in the presence of DEX and that the stable emulsion of the complex had the best stability.

Salt ions can significantly affect the reaction between polyphenols and proteins. Often, inorganic salts can promote the occurrence of turbid precipitation of complexes. For example, when trivalent AL+3 or Ca+2 is

present in the system, they can both form ligand bonds with proteins, and an abundance of phenolic hydroxyl and carboxyl groups associated with polyphenols, further enhancing the covalent interaction. Van de Langerijt et al found by turbidity and analytical rational analysis that the addition of thiocyanate to milk protein concentrate leads to dissociation of casein micelles and a decrease in solution turbidity over time, also an increase in the surface hydrophobicity of casein micelles and overall transmittance. However, when tea polyphenols were added, the reduction of turbidity was inhibited. Thus, in a polyphenol-protein bound system, the addition of salt ions can cause dissociation of the complexes, which in turn leads to a decrease in the turbidity of the solution (van de Langerijt et al., 2022). The proteins can also be covalently modified by the addition of organ Sulphur, such as allyl sulfides and isothiocyanates. Isothiocyanates unfold proteins, altering their hydrophobicity, exposing more binding sites, and perhaps enabling enhanced binding to polyphenols (Keppler et al., 2020).

In addition, some experiments have pointed to a process whereby the protein content of the supernatant rises and then falls and then increases again with increasing concentrations of ethanol in gelatin-tannic acid mixtures. This process also represents a decrease followed by a decrease and then an increase in the content of the polyphenol-protein complex. When the concentration of ethanol in the solution reaches 4%, probably due to the greatest difference between the pH of the solution and the protein isoelectric point, resulting in a greater electrostatic repulsion of the protein with the complex cross-linking pull, the least amount of complex is produced. And when the alcohol concentration rises to 6%, the amount of polyphenol-protein complexes is greatest (Huang et al., 2003).

5. Effect of intrinsic factors on protein-polyphenol interactions

5.1 Types as well as structures of polyphenols

The size, shape(flexibility), and structure of the polyphenol molecule itself(the number of gallic acyl groups and hydroxyl groups it carries), etc., can affect its reaction with proteins. There was a significant difference between the complexes of natural and pure polyphenols bound to milk proteins. When sodium caseinate solution was bound to the phenolic compound, the retention time of different phenolic compound-protein complexes was different. When sodium caseinate interacted with pure phenolic substances: the EGCG-protein complex eluted faster, indicating a strong binding effect of both; tannic acid as a large molecule bound to sodium caseinate more strongly than EGCG; catechin mixed with sodium caseinate, the sodium caseinate molecule changed slightly; similarly, hesperidin and homovanillic acid with sodium caseinate interaction was relatively weak. And when natural polyphenols (i.e., cranberry extract, green tea extract, grape extract) elute in combination with sodium caseinate a new major peak is generated, indicating that the polyphenols in the crude extract are highly differentiated from casein. The interaction of natural polyphenols with casein was less than that of pure polyphenol compounds (Han et al., 2019). Additionally, methylation, methoxylation, hydrogenationglycosylation as well as gallic acylation modifying flavonoids affect the interaction of polyphenols with milk proteins. Xiao et al (Xiao et al., 2011) showed that methoxylationmethylation and glycosylation were detrimental to the reaction of polyphenols with milk proteins: hydroxylation of benzene rings A and B increased the affinity of flavonoids for milk proteins, but the effect was not significant: hydrogenation of benzene ring A hydrogenation, the affinity of flavanones for milk proteins was increased: randomization of the C=C.

5.2 Types and structures of proteins

One of the major contributing variables impacting non-covalent interactions is the amino acid makeup and structure of proteins. Proline-rich proteins are prone to hydrogen bonding and hydrophobic interactions with polyphenols. Firstly, proline-rich proteins usually have good molecular flexibility, for example, the special structure of gelatin greatly increases the chance of peptide bonds forming hydrogen bonds with polyphenols; secondly, the carbonyl group linked to the tertiary amine group of proline is an active hydrogen acceptor compared to primary and secondary amines; thirdly, the exposed hydrophobic level of the pyrrolidine group of protein proline residues is a good hydrophobic binding site, which is easy to interact with the benzene or pyran ring of polyphenols interact with the benzene or pyran ring of polyphenols (Murray et al., 1994). Except for proline, arginine has an irregular hydrophobic side chain and can form hydrogen bonds with polyphenols, the positively charged guanidine group it carries being the hydrogen donor. In contrast, aromatic amino acids such as tryptophan and tyrosine are good hydrophobic interacting groups. Researchers found that both (+)-catechin and tannins had higher binding constants to α-amylase (proline and tryptophan-rich) than to the globulin BSA. cyanidin anthocyanins tend to bind proteins with irregular and helical conformations than proteins with small and tightly folded structures (myoglobin, lysozyme, BSA) (Freitas and Mateus, 2002; Hagerman and Butler, 1981).

In milk, the main proteins are whey and casein. However, it is mostly casein that binds to polyphenols in milk. The amount of protein elution was influenced by the binding of polyphenols and proteins. Therefore, the effect of different phenolic compounds on the binding of the complexes can be determined by the elution amount of the complexes. Some studies have shown that according to whey protein isolate (WPI) aggregation size does not change by mixing with pure monophenolic compounds. It indicates that phenolic compounds do not bind to WPI (Han et al., 2019). The main caseins in dairy products includeβ-lactoglobulin, α-casein, and β-casein. Using infrared spectroscopy, people have characterized complexes and get the order of stability of polyphenol binding to different caseins: ß -casein > α-casein > ß- LG (Chanphai et al., 2018).

6. Functional properties of food systems containing protein-polyphenol complexes

This section will describe the interaction of polyphenols with proteins in food properties. This will also be illustrated using practical examples of specific dairy products, which will help the development of new products.

Table 2. The effect of protein-polyphenol complexes on functional properties of dairy products

changes	application	polyphenols	protein	reference
Improvement of foaming	butter,ice cream; cheese	Tea polyphenols	b-lactoglobulin	(Rodriguez et al., 2015)
smell	cream	Phenol, cresol, guaiacol and 3-methylindole	whey protein	(Ramshaw and Roberts, 1990)
color	Milk Tea	Theaflavin	milk protein	(Huang et al., 2002)
Viscosity textural properties	yogurt	Tea Polyphenols, Pu-erh Tea Extract	yogurt protein	(Najgebauer-Lejko et al., 2020)
Gel mechanism	Skimmed milk	Tannic acid	yogurt protein	(Han et al., 2019)
Increased viscosity and elasticity	dairy product	Tea Polyphenols	b-lactoglobulin	(von Staszewski et al., 2012)
Improves antioxidant activity	Milk	Chlorogenic acid	Whey protein	(Serafini et al., 2003)
Promotes the growth of lactobacillus bulgaricus	Soy Milk	Tea Polyphenols	Whey protein	(Zhao and Shah, 2014)
Improved protein digestibility	dairy product	phenolic acid	Black bean protein	(Yan et al., 2019)

6.1 Mouth feel

The formation of whey protein particles is known to be an important factor in the taste of milk that replaces fat droplets in the production of dairy products. The production of such particles is influenced by a mix of heat and shear stress activities. The aggregation of the proteins themselves may also lead to an effect on the texture of the particles in the food. In turn, both covalent and non-covalent binding of polyphenols and proteins improve to some extent the cross-linking and emulsification of proteins as well as the foaming properties. Among the dairy products, for example, butter, ice cream, whipped cream, mousse, cheese, etc, are aerated foods. There has been a rising trend in recent years to favor the soft and dense texture of small bubbles. The capacity of the foaming agent to immediately reduce surface tension and adsorb at the water interface are important elements in foam production. Researchers have known that whey proteins in dairy products, such as β-lactoglobulin and caseinomacropeptide (CMP), have good foaming properties. For β-lactoglobulin, the foam stability is better than that of CMP, but the foaming ability is weaker. Rodriguez et al (Rodriguez et al., 2015) investigated the use of tea polyphenol-protein interactions to influence the interfacial ability of proteins, which in turn were able to improve foaming. Experimental results using foam spillover as a function of polyphenols: the addition of polyphenols significantly increased the high stability of the foam for both β-LG and CMP proteins. There was no significant difference between the foam made from CMP, however, the addition of polyphenols leads to the faster liquid drainage of the foam made from β-LG-polyphenol nanoparticles. The synthesis of β-LG and CMP-polyphenol nanoparticles appeared to be particularly effective at preventing foam from falling. In addition to its effect on foaming behavior, the covalent modification of proteins by polyphenols can prevent the hardening of food texture.

6.2 Smell

Polyphenols affect the odor of dairy products. The endogenous phenolic compounds come from the milk produced by different species of animals and vary in structure and concentration, capable of producing different flavored cheeses. For example, the odor of galactic cheese can be improved by specific phenolic compounds, giving it a good appetizing effect, and Lopez reports that conjugated phenolic compounds have a more significant effect on the flavor of dairy products than free phenolic compounds. In dairy products, most of the compounds are conjugated phenolic compounds, with only small amounts of free phenolic compounds. Any heat-treated dairy product is likely to contain free phenolic compounds, which are released mainly through heat treatment and enzymatic digestion. In addition, endogenous phenolic compounds, especially phenol, cresol, guaiacol, and 3-methylindole, have a beneficial effect on the flavor of the cream. Traditionally, people smoke the cheese directly in oil and the smoked cheese contains phenolic compounds such as guaiacol and eugenol. In addition, when cheese is left in clouds of pine for long periods, the cheese can be high in phenols and terpenes. All these phenolics can improve the organoleptic properties of the cheese. The addition of polyphenols such as vanillin, tea polyphenols, tea aromatic smokers, and pecan fructose to ice cream and Bulgarian yogurt also improves the flavor, however, the mechanism by which this affects the flavor has not yet been discovered. However, Walker and manning suggested that the presence of phenolic compounds in whey powders, milk concentrates, milk proteins, and skimmed milk powders is a Maillard reaction that has a negative impact on the flavor of the product (Mcsweeney, 2010; Ramshaw and Roberts, 1990; Urbach, 1997).

6.3 Color formation

The reaction of polyphenol-quinones with amino acid residues can generate colors ranging from dark brown,

and yellow to green (Keppler et al., 2020). The color produced is usually related to the previous reaction. In the absence of amino acid residues, the unbound quinones between condensation reactions produce a dark brown color. The quinone discolors when it reacts with the thiol group of an amino acid residue, and the light brown to yellow color is the brown quinone condensation product in solution with the thiol protein-bound quinone. It is possible to use the combination of polyphenols and proteins to improve the milk color of dairy products (Keppler et al., 2020). For example, lactoferrin, chlorogenic acid, and LF-epigallocatechin-3-gallate are apparent under neutral conditions. However, LF-CA and LF-EGCG complexes produce dark green and brown samples, which can be used as natural colors in food and beverage goods (Liu et al., 2016b). Huan et al (Huang et al., 2002) found that the total amount of theaflavin in the different levels of fermentation of Keemun increases and then decreases with time. Compared to theaflavin4, after the re-addition of milk,theaflavin1, theaflavin2, and theaflavin3, the cream color correlation of the solutions was more significant. In addition, the researchers compared the different theaflavin fractions in Kenyan black tea and Qi Hong, and when the proportions of polyphenols were the same, the milk color quality was the same. These results suggest that the phenolics vary in different plants and that the type of polyphenol correlates differently with the color quality of the milk. Colour intensity can be achieved by using oxidized polyphenols to induce more thiol groups rather than amino groups (Li et al., 2016, p.).

7. Conclusion

Polyphenols and proteins, as well as their interactions, have long been studied. The different environmental elements involved in processing hamper their utilization in food even further. Moreover, both can have an impact on other nutritional constituents in dairy products, as well as the functional qualities of the dairy product itself, both favorably and adversely. The majority of current study into them has been conducted in model systems. Other elements must be considered if a certain feature of its reaction is to be used in practice. As a result, the researcher can begin with the desired reaction of the two and prevent interference from other substances with the reactant. To operate in practice, the observable function must be analyzed as a whole in conjunction with other molecular actions in the system. As a result, there is still significant potential for the functional characteristics of the two reactions on the product, and more in vivo or in vitro tests or holistic integrated methodologies are required to validate the biological impacts as well as the nutritional increase.

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