



Milk in tea: exploring the chemistry and biological activities

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Abstract

Tea is a widely consumed beverage worldwide. Many countries have a daily practice of consuming black tea with milk. There has been an increase in the popularity of adding tea to dairy products. This review summarizes the effects of adding milk to tea on biological activities, the relationship between the polyphenols of tea and milk, and the possible applications of tea polyphenols in the dairy industry. Tea with milk has different health benefits than tea without milk, as it has been proven that proteins and polyphenols can have a strong interaction, which can decrease the availability of tea polyphenols. Milk proteins have the ability to transport and absorb catechins from tea through the digestive system. Consumers highly desire the maximum uptake of antioxidants from tea and proteins in milk without negatively impacting tea flavor. The relationships between milk protein and tea polyphenols could be favorably used in the dairy industry.

Keywords Catechins · Dairy industry · Health benefits · Milk proteins · Tea polyphenols

Introduction

Next to water, tea is the most widely consumed beverage worldwide. Tea is manufactured from the tender shoots of the tea plant (*Camellia sinensis*). It is reported that 76–78% of black tea, 20–22% of green tea, and about 2% of other types of tea are consumed in the world (Ociecek et al., 2023; Piyasena et al., 2024). Methylxanthines (theobromine, theophylline, and caffeine), polyphenols, proteins, carbohydrates, lignin, minerals, and amino acids, mainly tryptophan, L-theanine, and glutamic as well as organic acids, carotenoids, lipids, and vitamins, including E, C, and B, have been found in fresh tea leaves (Nelum et al., 2023; Skotnicka et al., 2011). Several chemical groups of polyphenols are present in tea leaves, and flavonoids are the predominant secondary metabolites, comprising up to 30% of the dry weight of harvestable tea leaves (see Fig. 1).

The total flavonoid composition in green tea is composed of 80% of catechin derivatives. Flavanols include epicatechin-3-gallate (ECG), (–)-epigallocatechin-3-gallate

(EGCG), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), gallic acid (GC), and (+)-catechin (C) (Hara et al., 1995; Piyasena et al., 2023a). Although tea has been used as a folk medicine for centuries, its therapeutic potential is still being investigated. The majority of its health benefits are due to the antioxidant properties in tea polyphenols, which are involved in a defense mechanism against oxidative damage to the body, thereby reducing the risk of developing non-communicable diseases, including diabetes, hyperlipidemia, cancer, etc., and also showing antimicrobial, antifungal activities, etc. (Modder and Amarakoon, 2002). Furthermore, biologically active compounds L-theanine, gamma-aminobutyric acid, and caffeine also play significant roles in the health benefits associated with tea consumption. Of the three xanthine molecules, including caffeine, theophylline, and theobromine in tea, caffeine is the most stable and prevalent alkaloid and is not altered during the oxidation process of tea manufacturing (Li et al., 2013; Wang et al., 2022). A non-protein amino acid, L-theanine, primarily originates from tea leaves and is also associated with soothing and relaxing properties of tea. In order to achieve its calming effect, there is an ability for L-theanine to cross the blood–brain barrier and interact with receptors (Wang et al., 2022).

Different types of tea are classified based on the preparation and the degree of oxidation during the processing of tea leaves. Plucking, withering, rolling, oxidation, and drying

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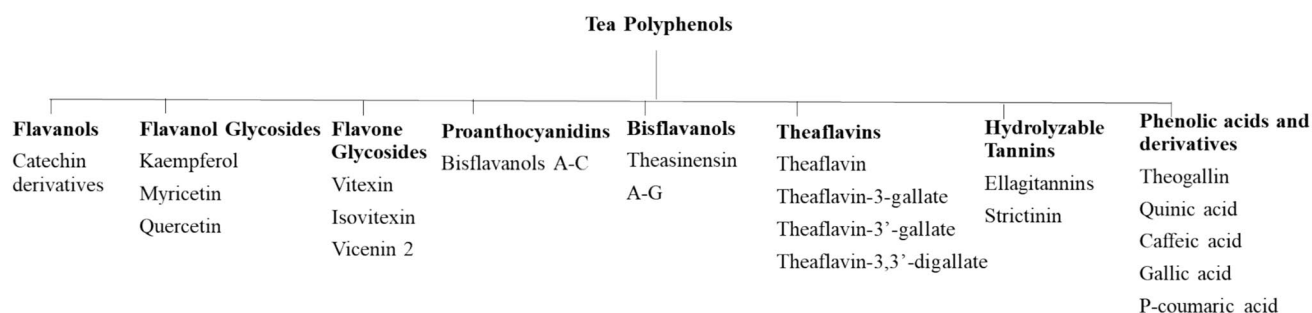


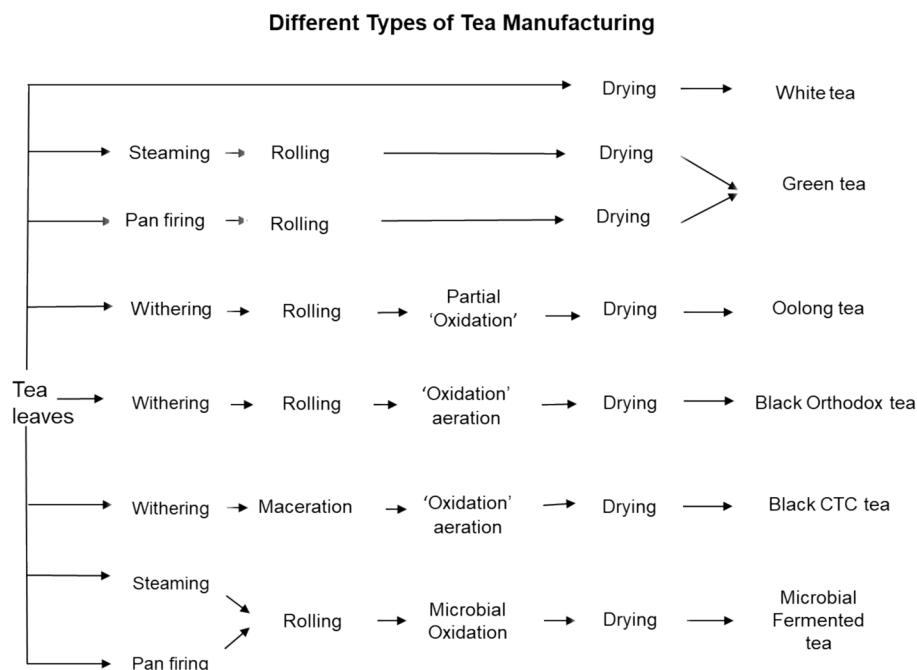
Fig. 1 Major phenolic compounds found in tea

are the basic steps of tea processing (see Fig. 2). Rolling and drying the leaves without oxidation results in green tea, whereas in black tea, it undergoes an oxidation step additionally (Hara et al., 1995). And also, oolong tea is obtained through partial oxidation. Enzymatic oxidation of tea polyphenols results in the formation of theaflavins and thearubigins. In fact, these compounds are responsible for the characteristic taste and astringency of black and oolong teas (Hara et al., 1995; Kottawa-Arachchi et al., 2022). White tea is a non-oxidized tea produced from the young buds of the tea plant. These young buds are shielded from sunlight during their growth to ensure a reduction in the formation of chlorophylls, thus giving the tea brew a white appearance (Piyasena et al., 2023b). Puerh tea is considered a unique tea since it undergoes a microbial fermentation (Modder and Amarakoon, 2002). However, the chemical diversity in tea due to the presence of compounds such as polyphenols, caffeine, amino acids, carbohydrates, purine alkaloids, and

vitamins has enabled a plethora of vital physiological properties and health benefits of tea.

The global tea market is considered one of the most rapidly growing industries at present, and with the rise of global interest and recognition, ample products are designed around tea. Currently, tea and tea extracts have been utilized as nutraceuticals, in the cosmetics industry, and in functional foods (Labbe et al., 2008; Wei et al., 2023). In some countries, black tea is consumed with considerable amounts of milk and sweeteners. However, adding milk to black tea is a very common practice, especially in the United Kingdom, where 98% of people consume tea with milk (Korir et al., 2014; Mao et al., 2021; Ryan and Petit, 2010). Not only milk is frequently utilized primarily with black tea, but it can also be incorporated into infusions of oolong, green, and various other herbal teas (Hertog et al., 1997). Bhagat et al. (2019) stated that, besides overcoming the astringent taste and bitterness of tea that tannins impart, incorporating milk, sugar,

Fig. 2 Schematic representation of different types of tea manufacturing



or both into tea is a common practice (Bhagat et al., 2019; Hertog et al., 1997). Additionally, incorporating milk into tea reduces the temperature of hot tea with the purpose of being consumed immediately (Bhagat et al., 2019). Recently, milky tea has become a popular beverage among young people in Asia, especially in Japan and China, and it is produced from infusions of black tea and milk (Lin et al., 2024; Mao et al., 2021). The most significant criterion for producing milk tea is the ratio of tea to milk, which defines the color and taste of milk tea (Yang et al., 2022). The proportion of milk added to tea, the composition of the milk, the type of tea, the brewing temperature, the process of adding milk to the tea, and the infusion method together influence the polyphenolic activity of the tea following the incorporation of milk into teas (Hertog et al., 1997; Rashidinejad et al., 2017). Approximately 76–78% of tea consumed globally is black tea; however, black tea is prepared with varying infusion times and amounts of tea, either in the form of tea leaves or tea bags. The preparation of the ideal cup of tea is still being discussed, and different opinions have been raised. However, the brewing techniques and consumption frequency of tea are also important (Kyle et al., 2007). A standard teabag is added to 170 mL of boiling water to make an English black tea infusion, which is then stirred for three minutes. Before adding milk, the tea bag is taken out and squeezed with a spoon. The required quantity of milk and sugar is added with consumer preference (van der Burg-Koorevaar et al., 2011). According to ISO 3103:2019, a standard black tea cup is prepared by weighing 2 g of black tea accurately, adding 100 mL of boiled water, and brewing for 5 min (ISO 3103:2019).

The color of the tea is considered to be one of the primary factors influencing consumers' food choices, in addition to the way that they assess the product's quality and taste (Mao et al., 2021). Yang et al. (2022) examined how the flavor of liquid milk tea differs with green tea, floral tea, oolong tea, dark tea, large-leaf yellow tea, and black tea. Tea (1 g) was brewed in water (50 mL, 80 °C) for 20 min and subsequently mixed with an equivalent ratio of milk to tea (1:1), and results revealed that the milk tea prepared from large-leaf yellow tea received a maximum score of 88.30 for the flavor. Yang et al. (2022) concluded that the color and taste of milk tea are primarily influenced by the tea-to-milk ratio as well as the polyphenol composition of tea (Yang et al., 2022).

Mao et al. (2021) explored how black tea infusion affected the color of milky tea prepared with non-dairy creamer. After precisely weighing 3 g of tea and placing it in a white porcelain pot, boiling water (150 mL, 100 °C) was added, and the mixture was infused for 5 min. The milky matrix (12 g of sucrose and 20 g of non-dairy creamer) was mixed with the hot tea infusion. Based on the findings, Mao et al. (2021) drew the conclusion that

tea color is determined by protein–polyphenol interactions that differ based on the type of tea, tea brewing conditions, and milk-to-tea ratio. And also, it is stated that the color of the liquid of milk tea is closely related to the pigments of tea and their interactions with protein–polyphenol. Tea polyphenols, such as theaflavins (golden yellow), thearubigins (orange-brown), and theabrownins (red-brown/dark brown), interact with milk proteins to form insoluble protein conjugates, changing the primary brown color of milk tea. Milk tea with low concentrations of theaflavins and thearubigins appears ivory, while those with higher concentrations become reddish yellow. Milk tea with low polyphenol contents appears lighter due to protein masking, while milk tea with high polyphenol content appears darker due to excess polyphenol remaining after protein masking (Mao et al., 2021; Wijegunawardhana et al., 2024). In addition to that, Mao et al. (2021) reported that the redness of milky tea depends on the concentration of theaflavins and EGCG and that the primary cause of the color formation is the interaction between polyphenols and proteins in milk (Mao et al., 2021).

In consideration of caloric intake based on adding milk to tea, it is reported that the tea brew itself has almost zero calorific value compared to the other beverages. If tea is consumed without milk, sugar, or other additives, there is a lack of calories (Modder and Amarakoon 2002; Piyasena et al., 2022). However, in general, milk contains more calories compared to tea brew. According to Pereira (2014), whole, low-fat, and skim milk have respective average calorie intakes of 62, 47, and 34 kcal (Pereira, 2014). Therefore, the increase in calorie intake is due to the addition of milk to tea infusion, as well as the type and amount of milk added, which determine the calorie intake.

According to the literature survey, the nutrient profile of teas is altered once milk or sugar is added to them, and the bioavailability of milk proteins and the tea polyphenols could be changed. As polyphenols combined with proteins through covalent and/or non-covalent interactions, proteins encounter modifications that could result in positive or negative effects (Bhagat et al., 2019; Lorenz et al., 2007). Adding milk to tea has the potential to decrease its health benefits, which is one of the main obstacles identified by several research groups (Rasheed 2019; Ruxton, 2008). As tea is consumed globally next to water, its health benefits raise significant issues for the public. The debate on the nutritional effects of adding milk to teas has been a topic of controversy for many years. There are currently few review publications that address this subject. Therefore, this review summarizes the effect of adding milk to teas in relation to positive and negative aspects of biological activities, the interactions between tea polyphenols and milk, as well as the possible applications of tea polyphenols in the dairy industry.

Interactions between tea polyphenols and milk

A number of investigations have demonstrated the beneficial and unfavorable interactions of incorporation into tea to milk (Arts et al., 2002; Korir et al., 2014; Sabouri et al., 2015). The irreversible and/or reversible relationships between milk proteins and tea polyphenols frequently result in structural alterations in protein structure (Aslandag et al., 2023). Hertog et al. (1997) stated that the unique cross-linking capacity of milk protein causes alterations in the nutritional profile of black tea (Hertog et al., 1997). Although numerous studies have been undertaken to study the relationship between tea polyphenols and protein and their bioactivity, there is a lack of in vivo or in vitro studies carried out to assess the impact of incorporating milk into tea on the biological activity and bioavailability of tea caffeine contents (Rashidinejad et al., 2017). It is reported that the protein structure of milk remains unaltered when interacting with caffeine (Madzharova and Weidner, 2024). However, some observational studies revealed that beverages containing caffeine have been linked to decreased bone mass and a higher possibility of fracture. Caffeine by itself clearly exhibits a very slight depressive effect on intestine absorption of calcium, as well as no impact on the 24-hour total excretion of calcium in the urine, as have been indicated by human physiological studies and controlled balance experiments. Skeletal fragility is undoubtedly associated with inadequate calcium intake, and high caffeine consumption is probably a marker of low calcium intake. Merely one or two tablespoons of milk could entirely offset the detrimental effect of caffeine on calcium absorption. Moreover, caffeine-containing beverages have an increased risk of osteoporosis in populations that consume substantially less calcium than is recommended. There is insufficient evidence to suggest that caffeine negatively affects bone health or the calcium economy in individuals who consume the daily calcium quantities currently prescribed (Barger-Lux and Heaney, 1995; Heaney, 2002).

Milk is a naturally occurring, nutrient-rich liquid that is fed to newborn mammals, including humans, in order to provide nutrition. Although humans can obtain milk from a variety of sources, such as buffalo, goats, cows, camels, sheep, etc., 85% of the milk produced by bovine cows is used in the dairy industry. In addition, 2.3–4.4% of protein, 85.5–88.7% of water, 4.8–4.9% of lactose, 2.4–5.5% of fat, and minerals, especially 0.2% of calcium phosphate, incorporated with casein micelles, and ~0.03% of vitamins are present in whole milk. Caseins are present in around 80% of milk proteins in bovine milk, and the primary protein types found in milk are caseins (β -casein, α S2-casein,

k-casein, and α S1-casein) and whey proteins, including a combination of globular proteins such as α -lactalbumin, immunoglobulin, serum albumin, and β -lactoglobulin. Caseins are found as casein micelles, which are primarily assembled via calcium phosphate bridging and protein–protein interactions. The micelles are 200 nm in diameter on average and have a hydrophilic surface layer with a hydrophobic interior. Research studies suggest that milk proteins have the ability to transport tea polyphenols by forming stable protein conjugates with large polyphenol molecules, thereby improving the bioavailability of dietary micronutrients. Additionally, it is reported that, due to their exceptional surface, biocompatibility, and biodegradability, owing to their high protein and fat content, as well as their ability to bind macro and small molecules and ions, milk proteins are thought to be natural carriers of bioactive molecules (Livney, 2010).

Many research studies reported that proteins and phenolic compounds form complexes by covalent bonds and/or non-covalent interactions, which include hydrogen bonding, van der Waals forces, hydrophobic interactions, and electrostatic interactions. Reversible interactions are carried out by non-covalent bonds, while irreversible interactions are generally the occurred covalent bonds (Aslandag et al., 2023; Han et al., 2011; Li et al., 2021; O'Connell and Fox, 1999; Yildirim-Elikoglu et al., 2018). For the formation of covalent bond, protein molecules and polyphenols interact irreversibly via covalent bonding, commonly through C-S or C-N or linkage (see Fig. 3). The process begins with the formation of quinones by enzymatic modification in the presence of oxygen or autooxidation under alkaline conditions. Due to their strong electrophilic characteristics, quinones react with nucleophilic residues of proteins or peptides via Schiff

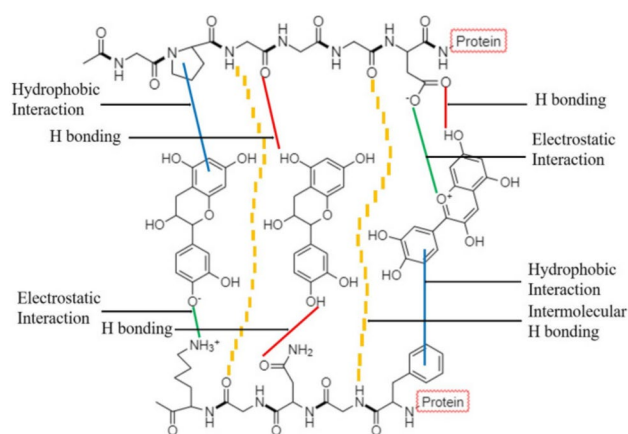


Fig. 3 Non-covalent interactions between polyphenols and proteins; the green line represents electrostatic interactions, the red line represents electrostatic interactions, and the blue line represents hydrogen bonding and hydrophobic interactions. (Adapted from Shahidi and Dissanayaka, 2023)

base (C=N) and Michael addition (C–NH) mechanisms, possibly resulting in protein crosslinking. Carbonyl groups of quinone react with lysine to form an imino-quinone adduct that rearranges into an iminophenol. Subsequently, protein radical resultant from free radical attacks (hydroxyl radicals) covalently link to polyphenols at the ortho- or para-positions of their hydroxyl groups. Additionally, semiquinone radical intermediates formed from monophenols attach to nucleophilic protein residues by forming covalent bonds (Shahidi and Dissanayaka 2023).

Non-covalent interactions are the most abundant type of interaction in nature of the two types, and environmental-related factors, including temperature and pH variations, could influence interactions involving non-covalent bonds (Aslandag et al., 2023; Han et al., 2011; Li et al., 2021; O’Connell and Fox 2001; Yildirim-Elikoglu and Erdem 2018). Hydrophobic, hydrogen bonding, electrostatic, and van der Waals interactions are non-covalent interactions that are generally reversible. The primary forces accountable for the non-covalent formation of complexes within proteins and polyphenols are hydrogen bonding and hydrophobic interactions. Hydrophobic interactions usually occur between hydrophobic amino acids and the aromatic ring of polyphenols (see Fig. 3). And also, hydrogen bonds among oxygen atoms in peptide bonds and hydroxyl groups in polyphenols, as well as electrostatic interactions (ionic bonds) between positively charged protein groups (e.g., lysine) and negatively charged hydroxyl groups of polyphenols (Shahidi and Dissanayaka, 2023). Yuksel et al. (2010) studied the interaction between green tea flavanoids and milk proteins has been investigated using the fluorescent probe binding method and isothermal titration calorimetry analysis. Findings revealed that hydrophobic interaction has been postulated as the source of the non-covalent relationships between tea phenolic constituents and proteins, which are retained via hydrogen bonding (Yuksel et al., 2010). In addition, the multiple spectroscopic analysis revealed that tea catechins bindings are through hydrogen bonding, hydrophobic interactions, and hydrophilic interactions (Chanphai et al., 2018).

Electrophoretic techniques are employed for an investigation into how colored tea polyphenols and milk proteins interacted in solution. The β and α -casein complexes of milk have been shown to form soluble casein-polyphenol complexes (Wijegunawardhana et al., 2024). Moreover, the spectroscopic analysis displayed that tea polyphenols have a weak binding ability with β -lactoglobulin. With catechin derivatives, binding ability increases as the number of hydroxyl groups increases. There are interactions between hydrophilic and hydrophobic in the complexation of polyphenol- β -lactoglobulin and the secondary structure of the protein can be altered after tea polyphenols interact with milk proteins. Molecular modeling studies exhibited that several amino acids participate in the formation of

polyphenol-protein complexation by hydrogen bonding. The conformation of β -lactoglobulin is altered by the existence of polyphenols, resulting in a stabilized protein structure at pH 7.4 (Hasni et al., 2011; Kanakis et al., 2011). In addition, polyphenolic ring stacking onto the planar hydrophobic surfaces dominates intermolecular binding. It is reinforced by several cooperative polyphenolic ring bindings. Affinities are unaffected by pH between 3.8 and 6.0 and deteriorate with increasing temperature. Self-diffusion rates of peptides with increasing polyphenol concentrations exhibit the increasing tendency of peptides to coat with polyphenol (Charlton et al., 2002). Whey proteins exist as dimers at ambient temperature and neutral pH; however, they breakdown into monomers at an acidic pH (Chanphai et al., 2018). The most prevalent complexes that form between casein and whey are called β -lactoglobulin- κ -casein complexes at pH 7 (Wijegunawardhana et al., 2024).

The performances of green and black tea catechins in milk are more complex than individual catechins (Bourassa et al., 2013; Rashidinejad et al., 2017; Ryan and Petit, 2010; Ye et al., 2013). It has been claimed that the interactions between milk protein and flavan-3-ol (see Fig. 4) are dependent on the structures of both proteins and polyphenols, which can be changed by gastrointestinal digestion and may be milk, as well as the bio accessibility of flavan-3-ol, especially through small intestinal and gastric digestion, based on experimental results (Moser et al., 2014; Xiao et al., 2011). And also, greater affinity for proteins is correlated with increased hydrophobicity of flavonoids, and an overall stronger affinity is demonstrated by the existence of a gallic

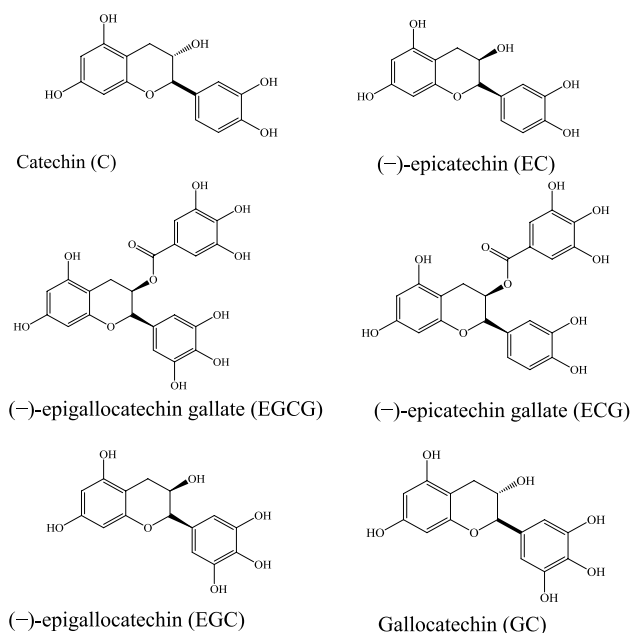


Fig. 4 Major catechins found in tea

acid ester on the C-ring (Bohin et al., 2012; Rashidinejad et al., 2017). Furthermore, temperature, ionic strength, and pH of the solution impact these polyphenol-protein interactions. The degree of hydrophobic interactions has been altered by changes in the pH or ionic strength of the medium (Bandyopadhyay et al., 2012).

Ye et al. (2013) carried out a comparative investigation on the interactions between green (pH 5.82) incorporated with milk (pH 6.60) and black tea polyphenols (pH 5.18) incorporated with milk (pH 6.50) using ultracentrifugation separation followed by Fourier transform infrared spectroscopy and UV–vis spectroscopic methods. EGCG exhibited greater binding affinities for the milk proteins than those with EGC, C, and EC (Ye et al., 2013). Chanphai et al. (2018) reported the comparison of the loading efficiencies and structure–activity relationships of catechins with milk proteins. With larger polyphenolic compounds, EGCG > ECG > EC > C, polyphenol–protein conjugates are more stable and effective at loading (Chanphai et al., 2018). Xie et al. (2013) investigated the impact of milk on the bio accessibility of green tea catechins following gastrointestinal digestion, and intestinal absorption of catechins was then verified using an *in vitro* model of Caco-2 cells. Results revealed that gallate catechins, such as EGCG and ECG, have a high affinity to bind milk proteins, whereas EC and EGC have a lower affinity. And also, catechins (see Fig. 4) remained stable and exhibited high sensitivity to intestinal digestion during gastric incubation. Milk may boost the biological availability of catechins by improving their trans-epithelial absorption in green tea (Xie et al., 2013). Polymerized tea polyphenols, including thearubigins and theaflavins found in black tea, exhibit a greater tendency to bind milk proteins. This binding reduces the number of free hydroxyl groups, which can impact the antioxidant activity of tea polyphenols (Dubeau et al., 2010). And also, whey proteins predominantly bind and transport smaller tea polyphenol molecules, whereas casein micelles are more likely to interact with highly polymerized polyphenols (Ye et al., 2013).

Some studies have found that milk proteins are beneficial for the intestinal transportation of catechins derived from green tea as well as their absorption (Bhagat et al., 2019). The improvement in catechin bio accessibility and antioxidant capacity is found to be positively linked with the affinity for binding of catechins to proteins (Qie et al., 2021). It is stated that having black tea every day results in a modest absorption of soluble oxalate; nevertheless, drinking tea with milk frequently results in minimal oxalate absorption from tea (Savage et al., 2003). Furthermore, the introduction of polysaccharide and oligosaccharide stabilizers improves the stability of polyphenol–protein complexes (Song et al., 2023). The amount of L-theanine is mainly affected by the tea brewing period. However, minimal quantities of sugar

and milk have no significant impact on L-theanine content, and the milk content increased, resulting in a significant drop in measurable L-theanine levels (Keenan et al., 2011). In conclusion, hydrophobic and hydrophilic interactions are the two primary factors that trigger the interaction between tea polyphenols and proteins. The strength of these binding forces depends on the structure of the tea polyphenol and protein molecules, as well as the proportion of them in a specific medium. Furthermore, galloylated polyphenolic constituents have a greater affinity for protein binding than non-galloylated constituents. Tea polyphenols bind proteins by hydrogen bonding, hydrophilic, and hydrophobic interactions, with β -casein exhibiting the most stability, followed by α -casein and β -lactoglobulin. A partial destabilization of protein structure results from polyphenol binding, which modifies the secondary structure of proteins by increasing the β -sheet and α -helix for β -lactoglobulin and decreasing the β -sheet and α -helix structures for α - and β -caseins (Aslandag et al., 2023; Chanphai et al., 2018; Yuksel et al., 2010). Heating causes the covalent bonds, whereas hydrogen bonding forms the non-covalent linkages (Leenan et al., 2000).

Interactions between proline and tea polyphenols

Numerous studies have demonstrated the significant binding capacity that exists between proline-rich milk proteins, such as casein, and the hydroxyl groups of tea polyphenols. It has been documented that hydroxyl groups of catechins are strongly favored by proline (O'Connell and Fox, 2001). Besides, it is reported that the interaction between polyphenols and salivary proline is the primary cause of the mouthfeel and astringency of tea. The formation of polyphenol/peptide complexes causes astringency, which serves as a defense mechanism in animals that ingest polyphenols (Charlton et al., 2002). It has been reported that the saliva of humans is a complicated physiological secretion composed of various protein types, the most prevalent being proline-rich proteins, which are responsible for numerous processes. As polyphenols and proline-rich salivary proteins interact, they generate complexes that are insoluble and break down the salivary film that lubricates the oral cavity, thereby decreasing the lubricity of the tissues in the mouth and causing astringency (Bandyopadhyay et al., 2012). Furthermore, it is stated that proteins in saliva may serve as scavenger molecules to decrease the absorption of tannins in the intestine (Morzel et al., 2022). Catechins which are attached to milk proteins have lowered availability for interactions with salivary proteins, causing to a decrease in astringency (Kardum and Glibetic, 2018). It is reported that polyphenols form thermodynamically beneficial complexes with proteins

containing a high proline concentration and polyphenols with a galloyl moiety (Shahidi and Dissanayaka, 2023).

Polyphenols exhibit numerous negative impacts on animals, including sequestration of dietary iron and inhibiting digestive enzymes. Proline binds in polyphenols, causing astringency and thus effectively preventing their entry to the gastrointestinal tract (Charlton et al., 2002). Furthermore, as the hydrophobic character of casein is due to the electrostatic interactions of casein micelles, there is a slightly high charge in casein, including many prolines and a few cystine residues. The formation of β -casein micelles and their stability is influenced by temperature, pH, and salt concentration. And also, the hydrophilic N-terminal component of β -casein is extremely charged at pH 6.6, which is the pH of natural milk. On the other hand, a lengthy component at the C-terminus is primarily hydrophobic and displays merely no charges (Li et al., 2019). Considering that the micelle is hydrophobic, caseins exhibit a tendency to bind to other proteins as well as certain ligands (Aslandag et al., 2023; Korir et al., 2014; Rashidinejad et al., 2017). It is suggested that, compared to simple phenolic structures, larger and more complicated phenolics show better affinity for proline-rich proteins (Baxter et al., 1997). Moreover, it is reported that casein has an affinity for the interaction of polyphenols via noncovalent and covalent interactions and forms a complex (Bourassa et al., 2013; Dubeau et al., 2010; Rashidinejad et al., 2017; Ye et al., 2013). It is reported that galloylated monomers (EGCG, EGC, and ECG) bind to proline-rich proteins more strongly than non-galloylated monomers (EC and C). The results additionally suggest that higher proline concentration in the protein chain enhances the binding of tea polyphenols. Furthermore, it is stated that polyphenol binding alters the three-dimensional configuration of protein molecules (Bandyopadhyay et al., 2012). The phenolic–protein interactions influence the activity of compound and exert antagonistic or synergistic effects depending on the structure of compounds, temperature, pH, physiological status, and method and food processing conditions (Shahidi and Dissanayaka, 2023).

Addition of milk to tea and its bioactivity

Numerous clinical, *in vivo*, and *in vitro* investigations have shown the health benefits associated with tea in different mechanisms of action (Modder and Amarakoon, 2002). Furthermore, the calming, stimulating, and mood-improving effects of drinking tea are well-known factors that contribute to relaxation. Some epidemiological studies have discovered that regular tea consumption is associated positively with psychological stress and depression (Lange et al., 2022; Piyasena et al., 2024). In addition, milk proteins hold significant importance in the availability of

catechins and polyphenols. Therefore, this interaction could be beneficial for drinking tea with and without milk (Van der Burg-Koorevaar et al., 2011). Some studies reported that the influence of milk incorporated into black tea on blood response showed that catechins in green and black teas are quickly absorbed and that milk does not impart catechin bioavailability (van het Hof et al., 1998).

What effects on cancer?

It is reported that in clinical and preclinical research studies, the polyphenols of green tea are utilized in prostate cancer chemoprevention. When added to milk, the EGCG found in green tea remains bioactive and inhibits the growth of colon cancer cells at high polyphenol concentrations. Encapsulated tea polyphenols are more bioavailable and have been found to be enhanced in their anticancer action. It has been observed that milk phospholipids and milk proteins can be used to encapsulate tea polyphenols (Chanphai et al., 2018). Weisburger et al. (1997) examined the impact of tea, or tea with milk, on rat models of cancer, including colon and breast. The results revealed that tea reduced the volume and multiplicity of mammary gland tumors; additionally, milk and tea had a stronger protective effect. Black tea itself reduced breast cancer, whereas black tea incorporated into milk provided more defense against cancers, including breast and colon. And also, full-fat milk had an additional additive impact on the antioxidant properties (Weisburger et al., 1997).

A population-based study assessed the impact of tea, coffee, and additional ingredients on the incidence of endometrial cancer. This study has not provided positive evidence that drinking tea or coffee can significantly lower the risk of endometrial cancer. However, adding sugar or honey to tea increases risk, and adding milk to tea lowers risk, which are interesting but need to be confirmed in more population-based research (Bandera et al., 2010). In addition, the potential *in vitro* antimutagenic impact of green and black teas was investigated using an *in vitro* gastrointestinal model, which included conditions in the human digestive tract, and results revealed that after milk was added to tea, its antimutagenic properties have been hindered. Once whole milk, semi-skimmed milk, and skim milk were added to black tea, the maximal inhibition of antimutagenic properties demonstrated with black tea was lowered by 22, 42, and 78%, respectively. The antimutagenic properties of green tea have been demolished by over 90% in whole and skim milk, and more than 60% inhibition was exhibited in semi-skimmed milk (Krul et al., 2001).

Antioxidant activity

Numerous investigations have been conducted to assess the influence of milk proteins on the antioxidant activity of teas. After being added to milk, tea polyphenols are found to have a masking and non-masking effect. It has been investigated that black tea polyphenols by themselves have the highest antioxidant activity. Some studies indicated that the antioxidant activity of black tea is lowered by incorporating black tea into milk (Hertog et al., 1997; Langley-Evans, 2000; Otemuyiwa et al., 2017). Langley-Evans (2000) reported that consuming tea with milk decreased the antioxidant activity of the whole blood in comparison with consuming only tea (Langley-Evans, 2000). Korir et al. (2014) revealed that tea with milk and sweeteners remarkably reduced the antioxidant capacity of tea, depending on the concentration. Additionally, it is stated that the antioxidant capacity of tea incorporated into milk ranged from 0% (v/v) to 2% (v/v) without a significant difference. And also, as milk concentration increased above 2% (v/v), the antioxidant activity declined considerably. It is stated that the tea with milk decreased the overall antioxidant capacity by 7–25% and also the antioxidant capacity of black tea was remarkably lower than that of green tea (Korir et al., 2014). Moreover, it is revealed that tea with milk lowers its free polyphenol content due to its binding with milk caseins, as measured by micellar electrokinetic chromatography (Kartsova and Alekseeva, 2008). The impact of milk on the antioxidant potential of English breakfast teas, Darjeeling, and green teas utilizing three complementing tests: voltammetry, lipid peroxidation inhibition, and ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) were evaluated. The findings revealed that milk reduced the antioxidant potential of all types of teas examined (Dubeau et al., 2010). It is discovered that microencapsulation of extracts of purple tea with milky tea resulted in high antioxidant activity in milky tea (Farrell et al., 2024).

Imran et al. (2017) reported that the solvents and time intervals had a substantial impact on the antioxidant potential of black tea. The ethanolic extract of black tea showed the highest antioxidant activity compared to black tea brew, and the antioxidant metrics of the black tea extracts were decreased with milk (Imran et al., 2017). Ryan and Petit (2010) stated that the antioxidant capacity of tea has been considerably declined by skimmed milk in comparison with whole and semi-skimmed milk. Besides, adding all types of milk, including skimmed, semi-skimmed, and whole milk, reduces the overall antioxidant content of black tea (Ryan and Petit, 2010). The research study conducted by Serafini et al. (1996) stated that the antioxidant capacity in humans was declined by incorporating

black tea into milk compared to the consumption of black tea alone (Serafini et al., 1996). According to ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and CAA (cellular antioxidant activity) assays, milk incorporating into black tea and milk and sugars incorporating into black tea reduced the potential antioxidant capacity of black tea by 30 and 45%, respectively. The FIC (ferrous iron chelating) method produced the opposite result (Soultani et al., 2024).

Leenen et al. (2000) examined both black and green tea with and without milk on plasma antioxidant activity in humans. Blood samples were collected at baseline and at numerous intervals for up to 2 h, followed by tea consumption and also plasma was investigated for total catechins and antioxidant activity. The standard addition of 10–15% of milk exhibited no impact on the antioxidant capacity of plasma in humans (Leenen et al., 2000). Reddy et al. (2005) studied the impact of black tea with milk on antioxidant activity in male adult volunteers, and the findings suggested that black tea with milk may not adversely affect the antioxidant activity (Reddy et al., 2005). It is reported that soy milk with tea exhibited either remarkably higher antioxidant capacity or no alteration compared to tea incorporated into semi-skimmed bovine milk (Hurrell et al., 1999; Ryan and Sutherland, 2011). It is stated that the bioavailability of flavonoids has not been affected by adding milk to tea, as confirmed by the study on 18 healthy volunteers (Hollman et al., 2001). Stanner (2007) studied the evidence of the bioavailability and activity of tea polyphenols using a clinical trial, and the findings revealed that while some reported a considerable decrease in the biological availability and activity of tea polyphenols, others observed no alteration (Stanner 2007). Additionally, it is reported that polyphenols could greatly increase the antioxidant activity of β -lactoglobulin; in fact, the greatest antioxidant capabilities have been found in the β -lactoglobulin-EGCG complex (Man et al., 2024).

Simanjuntak et al. (2017) described that as oxygen atoms from the amino acid side chains of milk proteins oxidize tea polyphenols in alkaline pH, cross-linking of tea proteins forms stable quinone reactive, which reacts with sulfhydryl groups of milk proteins to affect the capacity of polyphenol electron donation and decline the antioxidant activity of tea. Therefore, it is proposed that the antioxidant capacity of tea incorporated into skim milk has remarkably declined (Simanjuntak et al., 2017). Some research studies have exhibited that tea with milk has lower antioxidant potential because of the development of complexes that are not absorbed between tea polyphenols and milk proteins. Also, it is reported that polyphenols are embedded in many components, such as carbohydrates, lipids, and proteins, and protein–polyphenol interaction is the most detrimental to polyphenol bioavailability (Charlton et al., 2002; Dubeau et al., 2010).

In conclusion, when the OH group of polyphenol binds to a protein, it forms a complex, and the utilization rate is likely to be lower than when it is a free polyphenol. The counter-argument to this may be related to the lower antioxidant activity of black tea in the mixture of black tea and milk. In addition, as a similar example, the antioxidant potential of black tea is lowered due to the development of complexes that are not absorbed into the body, and it has been mentioned that protein–polyphenol interaction is a factor that interferes with the bioavailability of tea polyphenols.

Effect of sugar on antioxidant activity

According to the literature survey, there are inconsistent findings regarding the antioxidant activity of tea after adding sugar. Korir et al. (2014) reported that tea with milk, honey, and sucrose reduces the antioxidant ability of tea, with a decrease in antioxidant activity being more noticeable in tea with milk. Although antioxidant activity is relatively lower than that of the tea, the antioxidant activity of honey is significantly higher than that of sugar and stevia. Interestingly, *Stevia rebaudiana* Bertoni (stevia), which is sweeter than sugar, exhibited no significant effect on the antioxidant potential of tea with or without milk (Korir et al., 2014). Meanwhile, honey has been associated with tea that exhibited greater antioxidant potential than that of other sweeteners due to the presence of some chemical constituents, including catalase, chrysin, vitamin C, pinocembrin, and pinobanksin (Korir et al., 2014). Oolong tea with sugar had no impact on the antioxidant activity; nevertheless, the addition of sweetener resulted in an 18 and 14% improvement in ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and FIC (ferrous iron chelating) assays, respectively. The antioxidant capacity of black tea was reduced by 12–30% when milk and sugar were added (Sharma et al., 2008). Whereas employing the CAA (cellular antioxidant activity) approach, the sweetener reduced the antioxidant potential of tea by 8% (Soultani et al., 2024; Wipatanawin et al., 2015). Meanwhile, Sharma et al. (2008) stated that black tea demonstrated the strongest antioxidant activity subsequent to black tea with sugar and black tea with milk and sugar. Further Sharma et al. (2008) reported that milk and sugar enhanced and stabilized the antioxidant potential of black tea (Sharma et al., 2008). Additionally, Bartoszek et al. (2018) carried out a research study to ascertain the effects of adding milk, sugar, honey, and lemon on the antioxidant properties of tea. While adding honey increased the tea infusion's overall antioxidant capacity, adding sugar, milk, and lemon juice to tea had no apparent impact on the tea infusion's antioxidant qualities (Bartoszek et al., 2018). In addition to that, Otemuyiwa et al. (2017) studied seven brands of tea samples and in vitro simulated

digestion modeling stomach and small intestine were carried out on tea infusion with or without sugar, and phenolic content and antioxidant activity were analyzed. The results revealed that black tea possessed the highest polyphenolic content and antioxidant activity. The in vitro available polyphenolic compounds were significantly reduced when sugar was added to black tea and significantly increased when milk was added (Otemuyiwa et al., 2017). It has been reported that in a rat model, there was a 2.5- to threefold greater absorption rate of EGCG and EGC when consuming green tea with sugar and ascorbic acid compared to consuming green tea only (Bohn 2014). Furthermore, it is reported that the antioxidant content of tea remained unchanged with the addition of sugar to oolong tea (Wipatanawin et al., 2015). Further research studies into the impact of sugars on polyphenol absorption are necessary to provide evidence that the hypothesis on polyphenol absorption may be enhanced by sugars.

Cardiovascular diseases

Epidemiological research studies discovered that regular consumption of black tea reduces the risk of having cardiovascular disease, as well as black tea considerably improving endothelial function in humans; however, black tea with milk entirely eliminates this improvement (Lorenze et al., 2007; Modder and Amarakoon, 2002). It has been discovered that regular black tea consumption improves endothelial function and blood pressure by increasing nitric oxide. Black tea exhibits favorable effects on vascular function as measured by flow-mediated dilatation, which is immediately reversed by adding milk to tea (Ahmad et al., 2018). Ahmad et al. (2018) conducted a randomized controlled crossover clinical trial to determine the impact of regular consumption of black tea on vascular function and blood pressure in healthy volunteers. Regular consumption of black tea has been linked to lower blood pressure. Black tea with milk modifies the acute and short-term effects on vascular function and blood pressure in healthy young women and men. Black tea with milk considerably reduced this positive effect, resulting in a slight but considerable increase in blood pressure (Ahmad et al., 2018). Tea with milk is not related to an increased risk of ischemic heart disease in middle-aged Welsh males. However, several epidemiological research studies suggest that tea consumption is inversely related to the incidence of coronary heart diseases in older Dutch men (Keli et al., 1996). Stanner (2007) evaluated the study on the influence of tea with milk on dietary health benefits in Great Britain. The study involved a crossover design in which 16 postmenopausal healthy women were assigned 500 mL of black tea, skimmed milk, or boiled water. The findings of the study were inconsistent with previous studies, making it

unable to come up with a definite hypothesis on the influence of milk and tea on cardiovascular health (Stanner, 2007).

Negative effect on diabetes

According to Anderson and Polansky (2002), polyphenols found in tea may also boost insulin action. The major insulin-potentiating effect of oolong tea and green teas was attributed to EGC, gallo catechin gallate, EGCG, and ECG, and in black tea, the activity has been detected along with theaflavins, tannins, and EGCG. Several known tea components have been demonstrated to improve insulin levels, with EGCG having the most activity, followed by ECG, polyphenols, and theaflavins. Epicatechin, caffeine, and catechin had negligible insulin-enhancing effects. Adding 2% milk (5 g) per tea cup reduced insulin-potentiating activity by one-third, whereas adding 2% of 50 g of milk per cup reduced insulin-potentiating activity by approximately 90%. Soy milk and nondairy creamers have similarly reduced insulin-producing activity (Anderson and Polansky, 2002).

Antimicrobial activities

A computational analysis found that theaflavin derivatives in black tea may serve as potential inhibitors against SARS-CoV-2 target proteins (Gogoi et al., 2021). Black tea and its oxidized compounds, theaflavins, significantly inhibited the delta, kappa, alpha, and gamma variant viruses of SARS-CoV-2. Interestingly, adding milk to black tea completely prohibited alpha and delta strains. This may cause a suppressive effect exerted by milk casein, and it is stated that further clinical investigations are necessary to evaluate this effect (Ohgitani et al., 2021a, b). Nakashio et al. (2023) studied adding milk canceled the anti-SARS-CoV-2 activity of black tea since casein binds to theaflavin derivatives (Nakashio et al., 2023). Besides, it is reported that sugar, lime juice, and whey protein powder have not been associated with the anti-SARS-CoV-2 activity of black tea (Ohgitani et al., 2021a, b).

It has been found that tea exerted antibacterial activity against *Enterococcus faecalis* (MTCC 439), *Salmonella typhi* (MTCC 531), *Pseudomonas aeruginosa* (MTCC 1035), *Shigella flexneri* (MTCC 1457), *Staphylococcus aureus* (MTCC 87), and *Staphylococcus epidermidis* (MTCC 435) to various extents, and after adding milk and sugar, the antibacterial activity remains unchanged. Abd Allah et al. (2012) stated that tea and tea with milk demonstrated a remarkable antibacterial action against *Lactobacillus* sp. and *Streptococcus mutans*. Hence, it is recommended that moderate consumption of tea and tea with milk is an excellent natural measure for alleviating dental caries. There

is strong evidence that the bioactive components of tea and milk can prevent the proliferation of *Streptococci* and *Lactobacilli* agents (Abd Allah et al., 2012; Singh Arora et al., 2009).

Other biological activities

It is reported that tea is a strong stimulator of gastric acid, which could be lowered by incorporating milk into tea (Dubey et al., 1984). And also, tea consumption has not been linked to reflux symptoms or erosive esophagitis. Consuming tea incorporated into milk or sugar had no effect on reflux symptoms or erosive esophagitis (Wei et al., 2019). Studies on the digestive problems when drinking tea and milk together have not been reported. However, black tea increased the gut microbiota, which plays important roles in physiological and pathological processes of the host (Gao et al., 2020). Research data have indicated an increase in α -diversity of the gut microbiota due to black tea, while β -diversity of the gut microbiota is also modulated by it. The effects of black tea on gut microbiome are complex in healthy people; thus, it offers a novel perspective on the relationship between black tea, gut microbiota, and health (Gao et al., 2020). In addition, tea polyphenols have the potential to affect the gut microbiota by creating a favorable environment for beneficial bacteria, which in turn lowers inflammation and oxidative stress, protecting the liver (Xu et al., 2023). The absorption of tea polyphenols begins at the small intestine. But they have low bioavailability, i.e., they do not readily absorb into the bloodstream. A large proportion of unabsorbed tea polyphenols eventually move to the colon and strongly interact with gut microbiota, which is bidirectional. Tea polyphenols can improve the composition of gut microbiota by increasing the population of beneficial bacteria (Yılmaz et al., 2020).

There was a reversed J-shaped relationship between consumption of tea and the occurrence of acute kidney diseases, indicating that slight to moderate consumption of tea with milk is appropriate for a balanced diet (Liu et al., 2023). It has been revealed that Fe (iron) in milk reacts with tea polyphenols and forms insoluble complexes that limit polyphenol and iron absorption in the intestine (Abd Allah et al., 2012). Additionally, the intake of milk incorporated into tea has been reported to have a substantial effect on declining skin wrinkles and roughness in elderly people via reducing lipid peroxidation (Bhagat et al., 2019). In addition, consumption of milk with tea reduces the effect of green tea catechins on diet-induced thermogenesis (Hursel and Westerterp-Plantenga, 2011).

Compared with the individual elements, phenolic-protein interactions improve biological activities as well as the bioavailability of such polyphenols. In consideration of the

digestion of polyphenols, it is stated that during digestion, complexes of polyphenol-protein undergo breakdown (Van der Burg-Koorevaar et al., 2011). Moreover, it is reported that polyphenols are more stable in acidic environments and are commonly degraded oxidatively during gastrointestinal digestion due to the almost neutral pH of the small intestine. However, the majority of polyphenols are too polar to pass across the intestinal membrane, and also the majority of them are found in food as polymers, glycosides, or esters that are not absorbed in their native form. Prior to being absorbed, phenolic compounds must be digested by intestinal enzymes or the colonic microflora, and the membrane carriers must be engaged in their transportation. Thus, the gastrointestinal stability and target delivery are the main factors for polyphenols to perform their bioactivities in the human body. Phenolic compounds interact with the gastrointestinal tract with enzymes, blood plasma proteins, and proteins in target tissues of organs in the human body, which has a significant impact on their bioavailability and effectiveness in producing various bioactivities. Oxidation, degradation, or transformation of polyphenols usually takes place during their gastrointestinal digestion, the consequences being potential improvement or reduction in the biological activity of polyphenols. In addition, polyphenols have been microencapsulated utilizing substances derived from proteins to raise their concentrations and absorption in the intestine and decrease their breakdown during gastrointestinal digestion (Shahidi and Dissanayaka, 2023). According to in vitro research studies, it is reported that bile salts substantially solubilize salivary protein–tannin complexes; however, the degree of tannin polymerization determines solubility under gastric conditions. Moreover, the interactions between salivary proteins and polyphenols may impact the digestive process. Polyphenols attach to and inhibit salivary amylase, resulting in influences on starch digestion. Some salivary proteins hinder tannin-induced reduced protein digestibility. This may be due to the tannins, prior to their interacting with digestive proteases, binding with polyphenols (Morzel et al., 2022). Besides, tea polyphenols form complexes with milk proteins (α - and β -caseins and β -lactoglobulin), which sterically inhibit accessibility of pepsin and lead to improved protein stability throughout digestion (Cirkovic Velickovic et al., 2018). The specific mechanisms of breakdown and any negative impacts of protein–polyphenol complexes are still poorly understood in the literature, in spite of the fact that the formation and transport of polyphenols with proteins is a well-established phenomenon. Absorption of most phenolic components generally occurs mainly in the small intestine. Although animal and plant proteins have low influence on the biological availability of polyphenols, in vitro studies indicate that milk proteins may increase the intestinal absorption of polyphenols from tea (Kardum and Glibetic 2018). The interactions between digestive enzymes and

polyphenolic compounds, as well as hydrolysis processes, have not been fully investigated, and further research is required for this perspective.

Impact of substitution with soy milk instead of milk

Plant-based milk is frequently promoted as a viable, healthy, and animal-friendly substitute, which is derived from the water extraction of grains, legumes, or nuts and is totally free of any substances that are derived from animals (Haas et al., 2019). As compared to market shares, soy milk is the most popular plant milk worldwide (Haas et al., 2019). The nutritional benefits of soymilk, including its superior quality, readily digested proteins, plenty of vital fatty acids, and lack of cholesterol, have made it a popular beverage in Asia and are progressively gaining acceptance in Europe (Ge et al., 2021). As soymilk is appropriate for those who are intolerant to lactose or easily allergic, it is also used as a promising alternative to cow's milk (Ge et al., 2021). Demand for dairy substitutes is rising as a result of issues caused by diets high in cholesterol, concerns about the environment, lactose intolerance, and milk allergies. Plant-based milk serves the same functions as regular milk and has an analogous appearance and taste (Haas et al., 2019). Though plant-based milk replacements are acceptable as a substitute for milk, they vary greatly in their composition of nutrients, such as providing less vitamin B₁₂ and calcium than milk. Soy milk has approximately ten times less calcium than cow's milk (Haas et al., 2019). Fortified soy-based substitutes, including calcium, vitamin B₂, and B₁₂, can successfully substitute for milk and dairy products in the diet (Taeger and Thiele, 2024). The relationship between tea polyphenols and soybean protein isolate can change the protein confirmations and the size of protein aggregates (Ge et al., 2021). To preserve the overall antioxidant properties of the black tea infusion, soy milk could be a good substitute for semi-skimmed bovine milk (Ryan and Sutherland, 2011).

Tea polyphenols in dairy industry

In recent decades, there has been a major focus on developing food products with improved nutritional and physical characteristics. Therefore, the fortification of food items with diverse biologically active components has attracted greater attention, with dairy products being of specific significance due to their high consumption ratios. Food technologists have made numerous attempts to develop functional food formulations enhanced with polyphenolic components since these compounds have a more favorable bioavailability when consumed with

food than as supplements. Protein–phenolic interactions affected the performance of both proteins and phenolic components, resulting in significant differences in end product properties (Aslandag et al., 2023). Moreover, milk proteins in the dairy industry appear to be alternative delivery vehicles for certain tea polyphenols as well. The characterization and assessment of milk protein–phenolic relationships in functional dairy product formulas are critical for the design of processes and final food product quality (Aslandag et al., 2023; Han et al., 2011; Yildirim-Elikoglu and Erdem 2018). In consideration of all these aspects, the incorporation of tea polyphenols into dairy products has recently received more interest. It is stated that the catechins in green tea have a relationship with both fat and proteins in milk (Rashidinejad et al., 2016). It is reported that tea polyphenols are very essential in improving the nutritional and functional properties of yogurt (Feng et al., 2024). Furthermore, encapsulated tea polyphenols with calcium alginate and collagen hydrolysate beads have been utilized successfully to enhance the precipitation of milk proteins for fermentation into fortified set yogurt. Rashidinejad et al. (2016) examined two green tea catechins, C and EGCG, for their behavior in a model milk system. The pH and temperature were changed to mimic the cheese-making process. The results revealed that both the total polyphenol content and antioxidant activity were significantly increased when two distinct concentrations (250 and 500 ppm) of either C or EGCG were added to whole milk (Rashidinejad et al., 2016). And also, it is observed that extract of green tea enhanced the flavor of yogurt and milk as well as increased the metabolic rate of lactic acid bacteria in yogurt (Amirdivani and Baba, 2015; Marhamatizadeh et al., 2013). Furthermore, green tea extract incorporated into the heated milk sample had a more noticeable effect on the renneting process (Han et al., 2011). The viability of lactic acid bacteria throughout the storage period has not been affected by the incorporation of green tea polyphenols into yogurt. According to a study by Rogalska et al. (2024), bioactive substances were 91.58% protected after encapsulated tea polyphenols were added to yogurt. Also, the yogurt fermentation procedures have not been influenced by the addition of tea polyphenols to the milk environment (Rogalska et al., 2024). Yuksel et al. (2010) stated that the concept that milk proteins could cross-link is important since it may have an impact on the textural characteristics of milk products, including cheese and yogurt. Improved milk products with desirable texture characteristics can be developed by using the cross-linking effect of green tea flavonoids on milk proteins (Yuksel et al., 2010). The structural variations of the tea polyphenols have a great impact on their affinity for binding with milk proteins. A typical characteristic of milk proteins is their tendency

for cross-linking, which affects both nutritional value and texture. Temperature, pH, and concentrations of proteins and polyphenols affect the extent to which proteins bind to tea polyphenols (Yuksel et al., 2010).

The cheese curds have been functionally enhanced with single phenolic compounds such as EGCG, catechin, homovanillic acid, flavones, hesperetin, and tannic acid, in addition to natural raw substances such as dried cranberry powder, green tea extract, and grape extract. Cheese curds, including polyphenolic constituents at a concentration of 500 ppm, demonstrated considerable free radical scavenging action. Adding biologically active polyphenols to cheese curd enhanced its nutritional value (Han et al., 2011). Interestingly, Sabouri et al. (2015) revealed that it is possible to use sodium caseinate emulsions as EGCG carriers, and the physico-chemical characteristics of the emulsions are being impacted by the complexes that form at the interface (Sabouri et al., 2015). In addition, an appropriate amount of oat milk added to particular quantities of green tea extract could improve the bioavailability of tea polyphenols and the stability of the oat milk tea system (Qin et al., 2023). Likewise, tea polyphenols are incorporated into the production of biodegradable films and food adhesives, as well as the dispersion of materials in the food industry (Cheng et al., 2024; Wang et al., 2023; Xue et al., 2024; Yi et al., 2024). These findings are important for the advancement of new dairy-based foods with tea polyphenols. In terms of the uses of polyphenols in the fortified food sector, tea polyphenols could be used to improve the nutritional and physical characteristics of food products.

The Maillard reaction is commonly observed in the food processing industry, and it contributes significantly to the color and flavor of foods. It is a non-enzymatic browning reaction that occurs between reducing sugars and amino groups of amino acids during heat treatment (Piyasena et al., 2022, 2023b). Heat treatment is critical in the production of dairy products since it reduces the nutritional content of milk by producing harmful Maillard reaction products, which have been linked to oxidative stress and inflammatory reactions, resulting in diseases. It is reported that tea polyphenols exhibit high inhibitory activities on the Maillard reaction products compared to cysteine hydrochloride and vitamin E in brown fermented milk, which has a brown color and burnt flavor. Polyphenols in tea reduce the Maillard reaction by trapping α -dicarbonyl molecules or reacting with Strecker aldehydes, which undergo oxidation by benzoquinone, consequently decreasing the Maillard reaction (Li et al., 2023a). In addition, it has been reported that the two-step sterilized milk that contains tea polyphenols has a long shelf life (Li et al., 2023a). It is stated that tea polyphenols could be utilized as additives that reduce the formation of Maillard reaction products in brown fermented yogurt and brown fermented milk while retaining color and

flavor (Li et al., 2023b). Milk incorporated into tea extracts enhanced the stability of heat as well as coagulation time and increased the alcohol stability of milk (O'Connell and Fox, 2001). Moreover, green tea is frequently used in limited quantities due to its low bioavailability and unpleasant taste. Flavonoids in green tea could be used to regulate the Maillard browning reaction in the dairy industry (Schamberger and Labuza, 2007). The relationship between milk protein and tea polyphenols could be favorably used in the dairy industry to regulate the formation of Maillard reaction products as well as enhance the nutrition of food products and increase their shelf life.

Despite the diverse traditional tea drinking practices, people are becoming more mindful of the positive health effects of both tea polyphenols and milk proteins; however, there is adequate data to justify any suggestions for the optimal milk-to-tea ratio. Moreover, the importance of milk fat has been overlooked, despite the fact that milk contains fat globules that could potentially interact with tea catechins. It is emphasized that further research is necessary since different tea polyphenols may have varied effects on milk fat globules (Rashidinejad et al., 2017). There are inadequate studies on the bioavailability of alkaloids and L-theanine in tea with milk. Prior studies have mostly concentrated on interactions between proteins and polyphenols using in vivo methods. Further clinical trials are essential to confirm the relationship between tea polyphenols and milk proteins, as well as their potential health benefits. More research into calorie intake as a result of adding milk to tea is important. Furthermore, subsequent investigations ought to concentrate on developing novel delivery mechanisms that efficiently transfer tea polyphenols to their intended molecules. Consequently, it is reported that the stability of flavonoids during storage could be enhanced via interactions with milk proteins. It is recommended to employ polyphenol-milk beverage models to deliver fruit phenolic compounds (Kardum and Glibetic 2018).

Tea polyphenols have been linked to various pharmacological and beneficial effects on health, as well as improving the nutritional value of food products. Although many Asian cultures prefer tea without any added ingredients, the custom of milk being incorporated into tea is prevalent. Furthermore, consumers desire to obtain maximum benefits from tea polyphenols and milk proteins without compromising on the flavor of tea. The molecular structure of proteins determines the bonding affinity of tea polyphenols, and proteins also exhibit an increased binding affinity for tea polyphenols as their molecular size increases. Polymerized tea polyphenols in black tea have a greater tendency to bind milk proteins. According to the experimental evidences, it is concluded that tea polyphenols are capable of binding to proteins either covalently or noncovalently and form complexes which depends on the concentration of protein and tea

polyphenols. In addition, non-covalent bond is sensitive to environmental variables like pH and temperature, and these variables have an impact on protein and polyphenol binding. A number of investigations have demonstrated that tea polyphenols have substantial bonding affinities towards proteins through these interactions, which alter their structures as well as characteristics and have a major impact on their bio-accessibility and bioavailability. Milk proteins have the ability to transport tea polyphenols by forming stable protein conjugates with polyphenol molecules, thereby improving the bioavailability of dietary micronutrients. However, there is a discrepancy in the evidence regarding the impact of tea with milk on antioxidant and other biological activities. The type of tea and its composition, the method of tea brewing and preparation of tea-milk infusion, the type and quantity of milk, as well as the biological assays employed to determine the biological activities could account for this discrepancy. In addition, the relationships between milk protein and tea polyphenols could be favorably used in the dairy industry to regulate the formation of Maillard reaction products, enhance the nutritional contents of food products, and increase their shelf life. However, further research is necessary to fully comprehend how tea polyphenols interact with proteins in order to improve applications in the food and pharmaceutical sectors.

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